

Improving the sensory and nutritional quality of fresh meat

Related titles:

Lawrie's meat science Seventh edition

(ISBN 978-1-84569-159-2)

Lawrie's meat science has established itself as a standard work for both students and professionals in the meat industry. Its basic theme remains the central importance of biochemistry in understanding the production, storage, processing and eating quality of meat. At a time when so much controversy surrounds meat production and nutrition, *Lawrie's meat science* provides a clear guide which takes the reader from the growth and development of meat animals, through the conversion of muscle to meat, to the point of consumption. The seventh edition includes details of significant advances in meat science which have taken place in the last eight years, especially in areas of eating quality of meat and meat biochemistry.

Meat processing: Improving quality

(ISBN 978-1-85573-583-5)

Meat is both a major food in its own right and a staple ingredient in many food products. This major collection summarises key developments in research, from improving raw meat quality and safety issues to developments in meat processing and specific aspects of meat product quality such as colour, flavour and texture. Part I considers the various aspects of meat quality. Part II discusses how these aspects of quality are measured and Part III reviews the range of new processing techniques that have been deployed at various stages in the supply chain.

Microbiological analysis of red meat, poultry and eggs

(ISBN 978-1-84569-059-5)

These food products are, or have been, major global causes of foodborne human disease and are also susceptible to microbial growth and spoilage. Therefore monitoring their safety and quality remains a concern. With the recent development of more preventative, risk-based approaches to food safety control, microbiological testing of foods now has a more significant role to play in food safety management. With chapters written by international experts, this collection reviews the key issues in this dynamic area of food microbiology.

Details of these books and a complete list of Woodhead's titles can be obtained by:

- visiting our website at www.woodheadpublishing.com
- contacting Customer Services (e-mail: sales@woodheadpublishing.com; fax: +44 (0) 1223 893694; tel.: +44 (0) 1223 891358 ext.130; address: Woodhead Publishing Ltd, Abington Hall, Granta Park, Great Abington, Cambridge CB21 6AH, England)

Improving the sensory and nutritional quality of fresh meat

**Edited by
Joseph P. Kerry and David Ledward**



**CRC Press
Boca Raton Boston New York Washington, DC**

WOODHEAD PUBLISHING LIMITED

Cambridge New Delhi

Published by Woodhead Publishing Limited, Abington Hall, Granta Park,
Great Abington, Cambridge CB21 6AH, England
www.woodheadpublishing.com

Woodhead Publishing India Pvt Ltd, G-2, Vardaan House, 7/28 Ansari Road, Daryaganj,
New Delhi – 110002, India

Published in North America by CRC Press LLC, 6000 Broken Sound Parkway, NW,
Suite 300, Boca Raton, FL 33487, USA

First published 2009, Woodhead Publishing Limited and CRC Press LLC

© 2009, Woodhead Publishing Limited

The authors have asserted their moral rights.

This book contains information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. Reasonable efforts have been made to publish reliable data and information, but the authors and the publishers cannot assume responsibility for the validity of all materials. Neither the authors nor the publishers, nor anyone else associated with this publication, shall be liable for any loss, damage or liability directly or indirectly caused or alleged to be caused by this book.

Neither this book nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming and recording, or by any information storage or retrieval system, without permission in writing from Woodhead Publishing Limited.

The consent of Woodhead Publishing Limited does not extend to copying for general distribution, for promotion, for creating new works, or for resale. Specific permission must be obtained in writing from Woodhead Publishing Limited for such copying.

Trademark notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation, without intent to infringe.

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library.

Library of Congress Cataloging in Publication Data

A catalog record for this book is available from the Library of Congress.

Woodhead Publishing ISBN 978-1-84569-343-5 (book)

Woodhead Publishing ISBN 978-1-84569-543-9 (e-book)

CRC Press ISBN 978-1-4200-7790-2

CRC Press order number WP7790

The publishers' policy is to use permanent paper from mills that operate a sustainable forestry policy, and which has been manufactured from pulp which is processed using acid-free and elemental chlorine-free practices. Furthermore, the publishers ensure that the text paper and cover board used have met acceptable environmental accreditation standards.

Typeset by Ann Buchan (Typesetters), Middlesex, England

Printed by T J International Limited, Padstow, Cornwall, England

Contents

Contributor contact details xv

Preface xxi

Part I Understanding meat quality

1 Trends in meat consumption and the need for fresh meat and meat products of improved quality 3
M. D. Aaslyng, Danish Meat Research Institute, Denmark

1.1 Introduction 3

1.2 Eating meat for pleasure 6

1.3 Eating meat for nutrition 9

1.4 Variability in meat and meat products 11

1.5 Future trends 12

1.6 Acknowledgement 13

1.7 References 13

2 Biology and regulation of carcass composition 19
P. L. Greenwood, NSW Department of Primary Industries, Australia, and F. R. Dunshea, University of Melbourne, Australia

2.1 Introduction 19

2.2 Patterns of growth of carcass tissues 20

2.3 Biology of carcass tissue development and growth 22

2.4 Consequences of prenatal and postnatal growth and development for carcass composition and meat quality 29

2.5 Influences of metabolic modifiers on carcass characteristics 37

2.6 Genotypic influences on carcass composition 42

2.7	Future perspectives	46
2.8	Sources of further information and advice	47
2.9	References	47
3	Fresh meat texture and tenderness	61
	<i>D. A. King, T. L. Wheeler, S. D. Shackelford and M. Koohmaraie,</i> <i>US Meat Animal Research Center, USA</i>	
3.1	Introduction	61
3.2	Muscle constituents and structure contributing to tenderness variation	62
3.3	Antemortem factors affecting meat tenderness	65
3.4	Postmortem technologies affecting meat tenderness	71
3.5	Laboratory tenderness assessment	74
3.6	On-line tenderness prediction	77
3.7	Conclusions	78
3.8	Sources of further information and advice	79
3.9	References	79
4	Meat color	89
	<i>R. A. Mancini, University of Connecticut, USA</i>	
4.1	Introduction	89
4.2	Myoglobin chemistry	90
4.3	Antemortem factors affecting meat color	94
4.4	Laboratory analysis of meat color	96
4.5	Postmortem factors affecting meat color	99
4.6	Product enhancement	102
4.7	New developments and new areas of research	103
4.8	Future directions	104
4.9	Conclusion	104
4.10	Sources of further information and advice	104
4.11	References	104
5	Flavour development in meat	111
	<i>J. S. Elmore and D. S. Mottram, University of Reading, UK</i>	
5.1	Introduction	111
5.2	Flavour formation in meat	112
5.3	Dietary effects on meat flavour	122
5.4	Other pre-slaughter factors affecting meat flavour	127
5.5	Post-slaughter factors affecting meat flavour	131
5.6	Off-flavours in meat	133
5.7	Laboratory analysis of meat aroma compounds	134
5.8	Future trends	138
5.9	Sources of further information and advice	139
5.10	References	139

6	Fresh meat water-holding capacity	147
	<i>E. Huff-Lonergan, Iowa State University, USA</i>	
6.1	Introduction	147
6.2	Water-holding capacity defined	148
6.3	Inherent factors in postmortem muscle that influence water-holding capacity	148
6.4	Ante- and early postmortem factors that influence water- holding capacity	153
6.5	Future trends	156
6.6	Sources of further information and advice	156
6.7	References	157
7	The nutritional quality of meat	161
	<i>H. K. Biesalski and D. Nohr, University of Hohenheim, Germany</i>	
7.1	Introduction	161
7.2	Macronutrients in meat	162
7.3	Meat micronutrients	166
7.4	Laboratory analysis of the nutritional quality of meat	171
7.5	Future trends	172
7.6	Conclusions	172
7.7	Sources of further information and advice	173
7.8	References	173
8	Sensory evaluation of fresh meat	178
	<i>M. G. O'Sullivan and J. P. Kerry, University College Cork, Ireland</i>	
8.1	Introduction	178
8.2	Sensory evaluation of meat colour	179
8.3	Sensory evaluation of meat flavour	183
8.4	Sensory assessment of meat tenderness	188
8.5	Future trends	192
8.6	References	193

Part II Improving the quality of fresh meat: genetic and genomic technologies

9	New insights into the biology of meat quality from genomic and proteomic perspectives, with particular emphasis on beef	199
	<i>A. M. Mullen, L. Pannier and R. Hamill, Ashtown Food Research Centre, Teagasc, Ireland</i>	
9.1	Introduction	199
9.2	Genetic markers	201
9.3	Functional genomics	206
9.4	Proteomics	210
9.5	Summary	214
9.6	Acknowledgements	215
9.7	References	215

10 Genetic and genomic approaches to improving pork quality	225
<i>M. T. Cairns, NUI Galway, Ireland</i>	
10.1 The importance of genetic and genomic approaches in improving pork quality	225
10.2 Progress with identifying genes responsible for the meat quality traits in pigs	227
10.3 Functional genomics and improving pork quality	233
10.4 Proteomics and improving pork quality	235
10.5 Quantitative trait loci analysis and improving pork quality	236
10.6 Future trends	239
10.7 Sources of further information and advice	241
10.8 References	241
11 Genetic and genomic approaches to improving sheep meat quality	249
<i>S. C. Bishop and E. Karamichou, The Roslin Institute and R(D)SVS, University of Edinburgh, UK</i>	
11.1 Introduction	249
11.2 Genetic variation in sheep meat quality	250
11.3 Genes impacting on meat quality	254
11.4 Quantitative trait loci approaches to improving meat quality	256
11.5 The contribution of functional genomics	258
11.6 Future trends	260
11.7 Acknowledgements	261
11.8 References	261
12 Use of meat quality information in breeding programmes	264
<i>G. Simm, N. Lambe, L. Bünger, E. Navajas and R. Roehe, Scottish Agricultural College (SAC), UK</i>	
12.1 Introduction	264
12.2 Issues affecting the inclusion of meat quality information in breeding programmes	265
12.3 Breeding programme design to include meat quality (MQ) goals	268
12.4 Techniques for measuring meat quality	281
12.5 Future trends	286
12.6 Sources of further information and advice	287
12.7 References	287
13 Genetic-based diagnostic tools for predicting meat quality	292
<i>W. Barendse, CSIRO Livestock Industries, Australia</i>	
13.1 Introduction: the need for better methods to predict meat quality	292
13.2 Developing genetic-based diagnostic tests for predicting meat quality	295

13.3	Current status of development and future potential	298
13.4	Future trends	303
13.5	Sources of further information and advice	305
13.6	Acknowledgements	305
13.7	References	305

Part III Improving the quality of fresh meat: production strategies

14	Optimising the nutritional profile of beef	321
	<i>K. Nuernberg, Research Institute for the Biology of Farm Animals, Germany</i>	
14.1	Introduction: the potential to improve the nutritional profile of beef	321
14.2	Optimising the nutritional profile of beef	323
14.3	Optimising the quantity of vitamins and micronutrients in beef	331
14.4	Future trends and conclusions	332
14.5	References	333
15	Optimising the nutritional and sensorial profile of pork	342
	<i>J. Mourot, INRA, France</i>	
15.1	Introduction	342
15.2	Pork composition	343
15.3	The sensorial qualities of pork	344
15.4	Effects of breeding factors on meat sensorial and nutritional qualities	345
15.5	Orientation of pig production	346
15.6	Conclusions	353
15.7	References	354
16	Using antioxidants and nutraceuticals as dietary supplements to improve the quality and shelf-life of fresh meat	356
	<i>M. N. O'Grady and J. P. Kerry, University College Cork, Ireland</i>	
16.1	Introduction	356
16.2	Factors affecting fresh meat quality and shelf-life: appearance (colour), lipid oxidation and microbiology	358
16.3	Chemistry and structure of vitamin E	361
16.4	Chemistry and structure of green tea catechins	366
16.5	Chemistry and structure of grape seed extract and bearberry compounds	369
16.6	Chemistry and structure of oregano and rosemary compounds	372
16.7	Conclusions	376
16.8	References	377

17 Organic meat quality	387
<i>A. Braghieri and F. Napolitano, Università degli Studi della Basilicata, Italy</i>	
17.1 Introduction	387
17.2 The quality of organic meats as compared to	392
conventional products	
17.3 Safety and healthiness of organic meat	401
17.4 Future trends	406
17.5 Sources of further information and advice	407
17.6 Acknowledgement	408
17.7 References	408
18 Improving the quality of meat from ratites	418
<i>K. W. McMillin, Louisiana State University Agricultural Center, USA and L. C. Hoffman, Stellenbosch University, South Africa</i>	
18.1 Introduction	418
18.2 Ratite meat industries	419
18.3 Body and carcass quality traits	420
18.4 Influences on composition and quality development	422
18.5 Raw chilled ratite meat characteristics	426
18.6 Value-added products from ostrich meat	437
18.7 Future trends	439
18.8 Conclusions	440
18.9 Sources of further information and advice	440
18.10 References	441
19 Improving the meat quality of venison and other exotic game	447
<i>L. C. Hoffman, Stellenbosch University, South Africa and K. W. McMillin, Louisiana State University Agricultural Center, USA</i>	
19.1 Introduction	447
19.2 Improving meat quality by means of the production system ...	450
19.3 Transport, lairage and slaughtering techniques	459
19.4 Post-mortem intervention to improve the meat quality	460
19.5 Improving or maintaining the meat quality post-mortem	463
19.6 Value-added products as a means to improve the quality	
attributes of exotic meats	467
19.7 Future trends	468
19.8 References	469

Part IV Improving the quality of fresh meat: processing strategies

20 Automated grading of beef carcasses	479
<i>P. Allen, Ashtown Food Research Centre, Teagasc, Ireland</i>	
20.1 Introduction	479

20.2	The purpose of carcass grading	480
20.3	Carcass grading based on visual assessment	481
20.4	Development and application of automated methods: Video Image Analysis (VIA)	482
20.5	Future trends	489
20.6	Sources of further information and advice	490
20.7	References	490
21	Determining the lean content of pork carcasses	493
	<i>C. Pomar and M. Marcoux, Agriculture and Agri-Food Canada, Canada, M. Gispert and M. Font i Furnols, IRTA, Spain and G. Daumas, IFIP Institut du Porc, France</i>	
21.1	Introduction	493
21.2	Determination of carcass lean yield	494
21.3	On-line determination of carcass composition and lean yield . .	497
21.4	Current technologies available to accurately determine carcass composition and lean yield	502
21.5	Limits of current technologies for estimating carcass composition and carcass value	506
21.6	Future trends	510
21.7	Conclusions	512
21.8	References	513
22	New methods for analysis of factors affecting meat eating quality	519
	<i>V. H. Segtnan, K. I. Hildrum and J. P. Wold, Nofima Food, Norway</i>	
22.1	Introduction	519
22.2	Meat industry needs for on-line spectroscopic analysis	520
22.3	Selected on-line spectroscopic techniques for meat	521
22.4	Problems and pitfalls in on-line spectroscopic analysis	532
22.5	Sources of further information and advice and future trends . .	536
22.6	References	537
23	Chilling and freezing of meat and its effect on meat quality	539
	<i>S. J. James and C. James, University of Bristol, UK</i>	
23.1	Introduction	539
23.2	Effect of chilling and freezing on meat tenderness and texture	544
23.3	Effect of chilling and freezing on drip production	548
23.4	Effect of chilling and freezing on meat colour and appearance	550
23.5	Future trends	554
23.6	Sources of further information and advice	555
23.7	References	555

24	Carcass interventions and meat tenderness	561
	<i>M. M. Farouk, E. Wiklund and K. Rosenvold, AgResearch MIRINZ, New Zealand</i>	
24.1	Introduction	561
24.2	Whole-carcass interventions to improve tenderness	562
24.3	Ageing of meat to improve tenderness	566
24.4	Novel technologies to improve tenderness	571
24.5	Processing techniques to improve tenderness of	
	individual muscles/cuts	573
24.6	Future trends	577
24.7	Sources of further information and advice	577
24.8	References	578
25	Sensory and quality properties of packaged meat	585
	<i>M. G. O'Sullivan and J. P. Kerry, University College Cork, Ireland</i>	
25.1	Introduction	585
25.2	Packaged meat	586
25.3	Colour changes and packaged meat	589
25.4	Lipid oxidation and packaged meat	591
25.5	Catalysis of lipid oxidation	593
25.6	Tenderness and packaged meat	595
25.7	Future trends	597
25.8	References	598
26	Characterizing muscle properties to develop muscle-specific intervention strategies and improve meat cuts for the consumer . .	605
	<i>C. R. Calkins, University of Nebraska, USA and D. D. Johnson, University of Florida, USA</i>	
26.1	Introduction	605
26.2	Overview of US beef muscle profiling projects	606
26.3	Methods	617
26.4	Optimization	621
26.5	Future trends	625
26.6	Sources of further information and advice	626
26.7	References	626
27	Animal welfare and meat quality	628
	<i>J. Hartung, B. Nowak and A. C. Springorum, University of Veterinary Medicine, Hanover, Germany</i>	
27.1	Introduction	628
27.2	Definition of animal welfare	629
27.3	Meat quality traits	631
27.4	Impact of housing and management on meat quality	633
27.5	Impact of transport and lairage on meat quality	635

27.6	Impact of stunning on animals and meat condition.	637
27.7	A risk assessment approach for animal welfare and meat quality in slaughter animals.	641
27.8	Conclusions	642
27.9	Future trends	643
27.10	References	643

Contributor contact details

(* = main contact)

Editors

Dr J. P. Kerry
Department of Food and Nutritional
Sciences
University College Cork
Ireland
E-mail: joe.kerry@ucc.ie

Professor Emeritus D. A. Ledward
University of Reading
UK
E-mail: Ledwarddav@aol.com

Chapter 1

Dr M. D. Aaslyng
Danish Meat Research Institute
Maglegårdsvej 2
DK-4000
Roskilde
Denmark
E-mail: mas@danishmeat.dk

Chapter 2

Dr P. L. Greenwood*
NSW Department of Primary
Industries
Beef Industry Centre of Excellence
University of New England
Armidale
NSW 2351
Australia
E-mail: paul.greenwood@dpi.nsw.gov.au

Professor F. R. Dunshea
Department of Agriculture and Food
Systems
Graduate School of Land and
Environment
University of Melbourne
Parkville
VIC 3052
Australia
E-mail: fdunshea@unimelb.edu.au

Chapter 3

D. A. King,* T. L. Wheeler, S. D.
Shackelford and Dr M.
Koohmaraie

USDA-ARS
US Meat Animal Research Center
PO Box 166
Clay Center
NE
USA

E-mail: andy.king@ars.usda.gov
koohmaraie@email.marc.usda.gov

Chapter 4

Assistant Professor R. A. Mancini
University of Connecticut
Dept. of Animal Science
Storrs
CT 06269
USA

E-mail: richard.mancini@uconn.edu

Chapter 5

Dr J. Stephen Elmore* and Professor
Donald S. Mottram
Department of Food Biosciences
University of Reading
Whiteknights
Reading
RG6 6AP
UK

E-mail: J.S.Elmore@reading.ac.uk

Chapter 6

Professor E. Huff-Lonergan
Professor of Animal Science
Iowa State University
2372 Kildee Hall
Ames

IA 50011-3150
USA

E-mail:
ELONERGA@IASTATE.EDU

Chapter 7

Professor H. K. Biesalski* and
Professor D. Nohr
Department of Biological Chemistry
and Nutrition
University of Hohenheim
Garbenstrasse 30
70593 Stuttgart
Germany

E-mail: biesal@uni-hohenheim.de
nohr@uni-hohenheim.de

Chapter 8

M. G. O'Sullivan* and Dr J. P. Kerry
Department of Food and Nutritional
Sciences
University College Cork
Ireland

E-mail: maurice.osullivan@ucc.ie

Chapter 9

Dr Anne Maria Mullen*, Dr Liselotte
Pannier and Dr Ruth Hamill
Meat Technology Department
Ashtown Food Research Centre
Teagasc
Ashtown
Dublin 15
Ireland

E-mail: anne.mullen@teagasc.ie

Chapter 10

Dr M. T. Cairns
National Centre for Biomedical
Engineering Science/Martin Ryan
Institute (NCBES/MRI)
University Road
NUI Galway
Ireland

E-mail: michael.cairns@nuigalway.ie

Chapter 11

Professor S. C. Bishop* and E.
Karamichou
The Roslin Institute and R(D)SVS
University of Edinburgh
Midlothian
EH25 9PS
UK

E-mail:

Stephen.Bishop@Roslin.ed.ac.uk

Chapter 12

Professor G. Simm,* N. Lambe, L.
Bünger, E. Navajas and R. Roehe
Sustainable Livestock Systems
Group
Scottish Agricultural College (SAC)
Sir Stephen Watson Building
Bush Estate
Penicuik
EH26 0PH
UK

E-mail: Geoff.Simm@sac.ac.uk
Nicola.Lambe@sac.ac.uk

Chapter 13

Dr W. Barendse
CSIRO Livestock Industries

Queensland Bioscience Precinct
306 Carmody Road
St. Lucia 4067
Queensland
Australia

E-mail: Bill.Barendse@csiro.au

Chapter 14

Dr K. Nuernberg
Research Institute for the Biology of
Farm Animals
Wilhelm-Stahl-Allee 2
D-18196 Dummerstorf
Germany

E-mail: knuernbg@fbn-dummerstorf.de

Chapter 15

J. Mourot
INRA
UMR 1079 Systèmes d'Elevage
Nutrition Animale et Humaine
F-35590 Saint Gilles
France

E-mail:

jacques.mourot@rennes.inra.fr

Chapter 16

Dr M. N. O'Grady* and Dr J. P.
Kerry
Department of Food and Nutritional
Sciences
University College Cork
Cork
Ireland

E-mail: Michael.OGrady@ucc.ie

Chapter 17

Dr A. Braghieri* and Professor F.
Napolitano
Dipartimento di Scienze delle
Produzioni Animali – Università
degli Studi della Basilicata
Viale dell'Ateneo Lucano 10
85100 Potenza, Italy

E-mail: ada.braghieri@unibas.it
fabio.napolitano@unibas.it

Chapter 18

K. W. McMillin
School of Animal Sciences
Louisiana State University
Agricultural Center
Francioni Hall
Baton Rouge
Louisiana 70803-4210, USA

Dr L. C. Hoffman*
Department of Animal Sciences
Stellenbosch University
PO Box X1
Matieland
7602
South Africa

E-mail: lch@maties.sun.ac.za

Chapter 19

Dr L. C. Hoffman*
Department of Animal Sciences
Stellenbosch University
PO Box X1
Matieland
7602
South Africa

E-mail: lch@maties.sun.ac.za

K. W. McMillin
School of Animal Sciences
Louisiana State University
Agricultural Center
Francioni Hall
Baton Rouge
Louisiana 70803-4210,
USA

Chapter 20

Dr Paul Allen
Ashtown Food Research Centre
Teagasc
Ashtown
Dublin 15
Ireland

E-mail: paul.allen@teagasc.ie

Chapter 21

Dr C. Pomar* and M. Marcoux
Dairy and Swine Research and
Development Centre
Agriculture and Agri-Food Canada
Sherbrooke
Québec
Canada
J1M 1Z3

E-mail: pomarc@agr.gc.ca

M. Gispert and M. Font i Furnols
IRTA
Finca Camps i Armet
17121 Monells
Spain

G. Daumas
IFIP Institut du Porc
La Motte au Vicomte
BP 35104
35651 Le Rheu Cedex
France

Chapter 22

Vegard H. Segtnan,* Dr Kjell Ivar
Hildrum and Jens Petter Wold
Nofima Food, Matforsk AS
N-1430 Ås
Osloveien 1
Norway

E-mail: jens.petter.wold@nofima.no
vegard.segtnan@nofima.no
kjell.ivar.hildrum@nofima.no

Chapter 23

Dr S. J. James* and Dr C. James
Food Refrigeration & Process
Engineering Research Centre
(FRPERC)
University of Bristol
Churchill Building
Langford
Somerset
BS40 5DU, UK

E-mail: steve.james@bristol.ac.uk
chris.james@bristol.ac.uk

Chapter 24

Dr M. M. Farouk,* Dr E. Wiklund
and Dr K. Rosenvold
AgResearch MIRINZ
East Street
Private Bag 3123
Hamilton 3240
New Zealand

E-mail:
mustafa.farouk@agresearch.co.nz

Chapter 25

M. G. O'Sullivan* and Dr J. P. Kerry

Department of Food and Nutritional
Sciences
University College Cork
Ireland

E-mail: maurice.osullivan@ucc.ie

Chapter 26

Professor C. R. Calkins*
University of Nebraska
Department of Animal Science
A213 AnS
Box 830908
Lincoln
NE 68583-0908
USA

E-mail: ccalkins1@unl.edu

Dr D. D. Johnson
University of Florida
Department of Animal Science
PO Box 110910
Gainesville
FL 32611
USA

E-mail: dwainj@unl.edu

Chapter 27

Professor J. Hartung*, Dr B. Nowak
and A. C. Springorum
Institute of Animal Hygiene
Welfare and Behaviour of Farm
Animals
University of Veterinary Medicine
Hanover Foundation
Bünteweg 17p
30559 Hanover, Germany

E-mail: Joerg.Hartung@tiho-hanover.de
Bernhard.Nowak@tiho-hanover.de

Preface

In most societies meat is the centre point of many meals and failure of this key, expensive ingredient to live up to expectation is obviously undesirable and has been a major stimulus for food research for many decades. However, in spite of the considerable resources that have been expended on this research, the ability of the meat industry to consistently deliver meat of a uniform high quality cannot be guaranteed. Although individual consumers may have different criteria as to what constitutes the perfect steak/roast, it is generally accepted that in most circumstances, for whole/non-comminuted meats, a tender juicy product that is full of flavour yet is wholesome, of high nutritional value and attractive to the eye is desired (though individual consumer preferences for these properties, especially flavour, are readily apparent). If meat is to be further processed, then the manufacturer will pay a premium for meats that have the desired technological properties in addition to their organoleptic characteristics.

In recent years our understanding of the scientific basis of the quality attributes of meat has become more detailed, providing new approaches to the control of eating and technological quality. This book attempts to bring together current views, from distinguished scientists from a wide range of disciplines, on this complex area.

The book is divided into four parts, dealing specifically with our understanding of what exactly is meat quality and how genetic and genomic technologies, as well as production and processing strategies, can be used to improve meat quality. Thus the eight chapters in Part I deal with trends in meat consumption, factors influencing carcass composition and current views on factors affecting the texture, colour, flavour, nutritional value and technological properties of meats. The five chapters in Part II consider the latest genetic and genomic approaches to improving the quality of beef, pork and sheepmeat, and how such information can be used in breeding programmes and as diagnostic tools in the prediction of quality. In Part III the six chapters deal with how production strategies can be used to improve the quality of meats (beef, pork and sheepmeat), with particular mention being made of the very effective means available to optimise the nutritional profile of the major meat-producing animals. The use of dietary additives to improve fresh meat quality and the effect of organic production systems on quality are also discussed, as is the important relationships between animal welfare considerations and meat quality. The final eight chapters (Part IV) discuss how processing strategies can be used to improve quality. Two chapters deal with the use of on-line or non-invasive

techniques to assess the lean meat content of carcasses, and other factors such as colour, texture, pH and flavour that are important in determining meat quality. Other chapters deal with the characteristics of individual muscles and how such information can be used to meet consumer requirements for different qualities, the effects of chilling, freezing and thawing on quality, and how additional technologies such as electrical stimulation, hot boning and methods of suspension can be used to improve tenderness. Two further chapters describe how feeding dietary antioxidants and antimicrobials can effectively enhance the sensory properties and shelf-life of meat, and how modern packaging technologies may also assist in improving such properties. Whole chain approaches to specifically improve the flavour of beef, or to improve meat quality generally, are also described in some detail.

We acknowledge that, though written by experts, this book does not give the definitive answers to all the key questions as to how we can improve the sensory and nutritional quality of fresh meat. However, we trust it will be a valuable source of information to those both new to and already conversant with the area, and will also stimulate researchers to make further advances in this complex field of study.

Joseph P. Kerry
David Ledward

Part I

Understanding meat quality

1

Trends in meat consumption and the need for fresh meat and meat products of improved quality

M. D. Aaslyng, Danish Meat Research Institute, Denmark

Abstract: Meat is one of our main food items and most recipes are named after the meat ingredients. During the last fifty years, meat consumption has increased by approximately 50% in both USA and Europe. Meat is eaten due to its culinary status (we like meat) but also due to its nutritional value. This chapter focuses on the meat consumption pattern and on the factors important for both the gastronomic and nutritional value of meat.

Key words: meat consumption, eating quality, nutritional quality, meat, pork, beef, poultry.

1.1 Introduction

Meat is one of our main food items. Looking in a cook book, most recipes are named after the meat ingredients and meat is in general seen as a central part of the meal (Horowitz, 2006). Already by the Middle Ages, eating meat was seen as a sign of prosperity and wealth (Anon, 2007), and following the increased wealth in the community, the amount of meat eaten increased (Horowitz, 2006). From the 1950s to the 1990s meat consumption per inhabitant increased by approximately 50% in both the USA and Europe (Fagt *et al.*, 1992; Horowitz, 2006), and also in the last ten years increasing meat consumption has been seen (Breadsworth *et al.*, 2004). In the southern part of Europe, meat has now overtaken fish as the main protein source and actually more meat is eaten today in countries around the Mediterranean compared with the northern part of Europe, which by tradition used to be heavily meat eating (Marques-Vidal *et al.*, 2006; Naska *et al.*, 2006). Even though some people regard meat negatively (Holm *et al.* 2000) 98–99% of the

4 Improving the sensory and nutritional quality of fresh meat

population eat meat regularly (Garemo *et al.*, 2007; Guenther *et al.*, 2005), even though newer surveys show a gradual decrease in meat consumption in the last years, at least in Denmark (Fagt *et al.*, 2002).

The main meat types are beef, poultry (chicken and turkey), and pork. In an investigation in the USA of what kind of meat was eaten in a two-day period in 1994–1996, beef was the most frequently eaten meat, followed by chicken, with pork the least popular. However, increase in meat consumption in the USA is most noticeably an increase in consumption of chicken (Sloan, 2003), and today this type of meat might be the most popular. Also, in Norway the consumers prefer white to red meat (Kubberød *et al.*, 2002a).

However, behind these facts about meat consumption, variations are present. It is reported in many countries that boys and men eat more meat compared with girls and women (Cosgrove *et al.*, 2005; Guether *et al.*, 2005; Lyhne, 2005). Also the type of meat that the two genders prefer, differ. Men prefer red meat while women eat more white meat and even take a dislike to red meat (Kubberød *et al.*, 2002a, 2006). Also the attitude towards meat is more positive for men compared with women (Kubberød *et al.*, 2002b).

An age difference in meat consumption pattern also exists. Young consumers eat less meat and prefer, to a large degree, white meat to red meat compared with more adult consumers (Aaslyng *et al.*, 2007b; Koizumi *et al.*, 2001; Kubberød *et al.*, 2002a); and families with children eat more meat than families without children (Groth *et al.*, 2003). It also seems that more educated consumers eat less meat (Groth *et al.*, 2003). In general, young well-educated women are among the least meat-eating consumers while adult, less educated men are among the most meat-eating.

The cutting of the meat has an influence on its popularity. Minced meat is often preferred, as the chance of success in cooking is high (Holm *et al.*, 2000). A Swedish survey showed that 98% of children eat minced meat at least once a week (Garemo *et al.*, 2007). In Denmark, a geographic difference is seen regarding which form of pork is mostly eaten. In a city close to the capital, whole meat pieces, such as chops or roasts, are often bought, while far from the capital, minced meat is more often chosen (unpublished data).

Which meat-based meal is the most popular differs during a lifetime. In Sweden, 83% of children had sausages at least once a week (Garemo *et al.*, 2007), and also in Denmark sausages are mainly eaten by children. [Figure 1.1](#) shows the popularity of five meals, depending on the consumer's age. The results are from a survey in which consumers indicated which meals they had eaten in the past week. Please note that this procedure (having more pork meals than chicken meals to choose between) means that chicken seems most popular, even though pork is eaten more often.

Beef bolognaise and other stews, and chicken, are the two most popular meals, independent of age. Sausages are very popular up to 14 years of age, at which point their popularity drops dramatically. However, from 25–34 years they regain their popularity and are the fourth most popular meal. This corresponds to the period in which many people have small children – who eat sausages! Whole beef roasts and

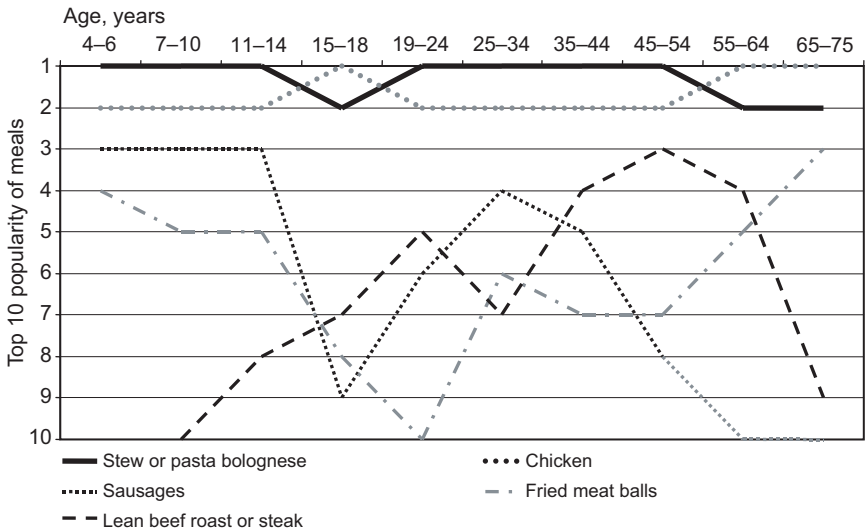


Fig. 1.1 The popularity of five selected meals in Denmark (National Danish Dietary Survey, 2000–2002; Fagt, Pers. Comm., 2007).

steaks are most popular with consumers from 35–65 years old, after which they drop again. This last decrease can be due either to a reduced chewing capacity or to a reduction in income and thereby in the amount of disposable money for buying food.

The way in which meals with meat are composed can be either very traditional or more varied. When pork is served as a whole roast in Denmark, the accompaniments are often very traditional, including potatoes, gravy, pickled red cabbage and occasionally salad. In contrast, pork chops are served with a much larger variety of accompaniments. The basic accompaniment is mainly potatoes and sometimes rice, while pasta is seldom served. In addition to this, salad, bread and a large variety of vegetables both hot and cold are served with the chops (Aaslyng *et al.*, 2007a). The same pattern seen for chops has been seen for beef steaks. They are most often served with potatoes, combined with a large variety of vegetables and salad (unpublished data).

In this way, some cuts seem much more traditional in the way they are served compared with others, in which the serving can be more varied. It is not established how these traditions are kept or broken. Who decides what is trendy? Chefs experiment with meals – not only the cooking of the meat, in which trends using low cooking temperatures for long times, often combined with a low internal temperature, have arrived in the last years – but also in the way the whole meal is composed. However, these trends are seldom converted directly to ordinary consumers. Meals prepared in the home are more focused on taste, convenience,

health and nutrition. The meals must be easy to shop for and easy to prepare after a long day at work. Also, a high success rate in cooking is important as we all prefer food of good taste. To achieve this, well-known recipes are often chosen and inspirations for new meals are sought from cook books and food magazines. These meals are often made for special occasions and, if they are a success, transferred fully or partly to everyday meals. (Andersen, Pers. Comm., 2007).

But why do we eat meat and what are the trends in meat-eating habits? These questions are important in order to understand the consumption of this food group. We can eat food for several reasons. Pleasure (we actually like meat) and nutrition (meat is a good protein source with several nutritionally beneficial factors) are two of the main reasons for including meat in meals.

1.2 Eating meat for pleasure

Eating meat for pleasure means we like meat. For steaks, chops or roasts, three sensory attributes are of major importance for the hedonic value of the meat: Tenderness, juiciness, and flavour (both the presence of fried flavour and absence of off-flavours) (Aaslyng *et al.*, 2007b; Bryhni *et al.*, 2003; Miller *et al.*, 2001). Which attribute is considered the most important depends on factors such as geographic location (Aaslyng *et al.*, 2007b) and on the level of the other attributes (Huffman *et al.*, 1996; Killinger *et al.*, 2004). With tender meat, variations in juiciness or flavour have a larger impact on preference compared with less tender meat, in which increased tenderness is of more importance (Miller *et al.*, 2001). However, these three parameters can only be assessed on cooked meat. When buying meat, we have to guess the eating quality from the appearance. The appearance of the raw meat is therefore very important for the consumer.

1.2.1 Appearance

The appearance of meat at the point of buying is important for choice. For pork, colour and subcutaneous fat (fat layer) are the two main drivers of acceptability. The consumer associates colour with freshness while a thin fat layer is associated with health. Overall, both dark and light meat colour can be preferred, depending on the country and consumer segment within the country (Ngapo *et al.*, 2007). As an example, most consumers in Australia and Poland prefer light coloured pork chops while the opposite is true for Germany and Taiwan. For all countries, a low fat cover is preferred by most consumers. High marbling of pork chops was only important for most consumers in Australia and Ireland. For the rest of the 21 investigated countries, marbling was not important for choice of chop (Ngapo *et al.*, 2007). In beef, it has been seen that, at the moment of purchase, consumers prefer steaks with low marbling, but when actually tasting the meat, they prefer steaks with a higher degree of marbling (Jeremiah *et al.*, 1992; Madsen, Pers. Comm., 2007)

1.2.2 Tenderness

Tenderness has often been described as the most important factor for high eating quality, especially in beef. Consumers can distinguish between tender and tough beef steaks, whereas variations in tenderness in the middle groups are more difficult to separate (Sivertsen *et al.*, 2002). It has also been shown that a certain level of tenderness is crucial to be acceptable (Huffman *et al.*, 1996) and that tenderness of beef is so important for consumers, they are actually willing to pay more for tenderness (Boleman *et al.*, 1997). An increased preference with increasing tenderness has also been reported in pork (Aaslyng *et al.*, 2007b).

Tenderness of meat can be due to many different factors and there are many ways to achieve tenderness (Aaslyng *et al.*, 2004a). Ageing is a well known way of increasing tenderness of meat. During ageing, proteolytic enzymes degrade the proteins and loosen up the structure of the meat. This can be seen by an increased Myofibrillar Fragmenting Index or increased degradation of specific proteins such as the cytoskeletal protein desmin (Kristensen *et al.*, 2003, 2006; Therkildsen *et al.*, 2002a). The effect of ageing depends on the time and temperature during ageing, and also on the growth rate of the animal up to slaughter, as a fast growth-rate increases the activity of proteolytic enzymes in the meat (Kristensen *et al.*, 2006; Therkildsen, 1999; Therkildsen *et al.*, 2002a,b).

Also intramuscular fat (IMF) is an important factor in tenderness (Aaslyng *et al.*, 2004b; Brewer *et al.*, 2001; Fortin *et al.*, 2005). The increase in tenderness with increasing IMF in pork is not linear but can more be seen as a limit at between 1.5–3% (depending on the country), below which the tenderness is regarded as unacceptable (Fortin *et al.*, 2005). In beef, a ‘window of acceptability’ has been defined as at least 3% being necessary to gain acceptable palatability, at least 5% to get a medium palatability, while an IMF content of at least 7% is necessary for a high palatability (Savell *et al.*, 1988).

The sarcomere length can vary depending on the chilling regime and on the suspension of the hot carcass. A short sarcomere length induces tough meat. In pork, a linear relationship between sarcomere length and tenderness exists for sarcomeres below 2 μm , independent of muscle. Above 2 μm sarcomere length, the meat is always regarded as tender (Wheeler *et al.*, 2000).

A popular way to increase tenderness is to marinate the meat, using a solution with NaCl or CaCl₂, perhaps including spices, sugars or other ingredients. Injecting this into the meat increases tenderness, juiciness and flavour. This is especially popular in USA, but has also become more widespread in Europe. In beef, a linear relationship has been demonstrated, with decreasing shear force (corresponding to increasing tenderness) as salt concentration is increased (Baublits *et al.*, 2006); and marinating of a potentially non-tender muscle such as knuckle (M. rectus femoris) can increase the tenderness up to the level of a non-marinated loin (Rosenvold *et al.*, 2006). For a meat producer, it is important to ensure high tenderness to obtain meat of high acceptability. Which is the best way to gain tenderness (IMF, ageing, chilling) must be chosen from a knowledge of the individual production systems.

1.2.3 Flavour

Flavour is a very important eating quality attribute in all meats. Not only are the preferred flavours important – it is just as, or even more, important to avoid off-flavours.

Fried flavour is generated during heat treatment at high temperatures. The aroma compounds are formed by several pathways, e.g. lipid oxidation and Maillard reaction between reducing sugars and amino acids; and the reactions between compounds from these two systems results in a large number of volatile compounds (Mottram, 1994). Thermal degradation of other compounds, such as thiamin, also contributes to the flavour of meat (Tai *et al.*, 1999). Non-volatile compounds, such as inosine monophosphate (IMP) and its degradation products, free amino acids, smaller peptides and lactic acid and ions, also have an impact on flavour (Fuke *et al.*, 1991; Maga, 1994; Nishimura *et al.*, 1988; Tikk *et al.*, 2006). Even though flavour is important for preference (Aaslyng *et al.*, 2007b), the consumers willingness to pay for improved flavour has not been investigated as thoroughly as for the tenderness.

Increasing IMF, to a degree, increases the fried flavour of both pork and beef (Brewer *et al.*, 2001; Killinger *et al.*, 2004). The main contributors to meaty volatiles are the phospholipids (Mottram *et al.*, 1983) and the content of this fraction is constant, as they are the structural lipids present in the membrane of muscle cells. The effect of IMF on flavour could therefore be due not only to increased flavour development but also to improved flavour release. This agrees with findings showing only minor effects of fatty acid composition on the flavour of pork and beef (Elmore *et al.*, 1999; Tikk *et al.*, 2007; Wood *et al.*, 2003) even though some fatty acids, especially omega-3-fatty acids, can induce off-flavours if present at too high concentrations (Aaslyng *et al.*, 2007c; Elmore *et al.*, 1999).

As the Maillard reaction is essential for developing fried flavour during heat treatment, the content of carbohydrates and amino acids seems to be crucial. Meat has a high protein content and amino acids should be present in large amounts. Carbohydrates are therefore more likely to be the limiting factor. Investigations have therefore focused more and more on the content of carbohydrates in the meat (Aliani *et al.*, 2005; Koutsidis *et al.*, 2007; Meinert *et al.*, 2006, 2007).

Several off-flavours have been reported. In pork, the boar taint is crucial as also is piggy flavour (Hansen *et al.*, 2006; Meinert *et al.*, 2006), sow flavour (Sindelar *et al.*, 2003a,b) and sometimes simply off flavours/abnormal flavours (Wood *et al.*, 1995, 2004). In beef and veal especially, livery flavours are described (Yancey *et al.*, 2006), but also metallic, cowy, milky, grassy, painty and sour flavours are mentioned (Calkins *et al.*, 2007).

1.2.4 Juiciness

Juiciness is said to be an important factor in the eating quality of meat. How important it is depends, to a large degree, on the meal composition, as a steak needs to be juicier than small slices in a stew. However, to get a tasty piece of meat requires some meat juice.

The main factor determining the juiciness of meat is the end-point temperature. The higher the end-point temperature, the higher is the cooking loss and the lower the juiciness (Aaslyng *et al.*, 2003). The connection between cooking loss and juiciness is not simple. In pork, meat of low pH had a higher cooking loss than meat of normal pH but this difference was not reflected in the juiciness, as they were equally juicy (Aaslyng *et al.*, 2003).

Increased amounts of intramuscular fat also increased juiciness, but only when the meat was fried to a high end-point temperature. At lower end-point temperatures, at which the meat was juicier, intramuscular fat had no effect on juiciness (Aaslyng *et al.*, 2004b).

To increase juiciness of meat, the most important factor must be to educate consumers not to over-cook the meat. This can be done by advising consumers on cooking times (<http://www.danishmeat.dk>) or teaching them to use a frying thermometer (Edwards *et al.*, 2005) to ensure a safe, well tasting meal.

1.3 Eating meat for nutrition

Meat is an important nutritional source in the diet of most people. It contains a high amount of protein, including essential amino acids, and other beneficial nutrients such as iron and vitamins. But it has some negative effects. Meat has a high content of saturated fatty acids, of which we need to reduce the intake to minimize the adverse effects of cardiovascular disease and the risk of cancer (NNF, 2004).

1.3.1 Protein

Eating meat means eating a huge amount of protein. Protein is an important nutrient, but in the western world lack of protein is not a problem. Investigations have shown that a diet high in protein can induce weight loss, perhaps because it is more satisfying compared with a carbohydrate-rich diet, so the intake of energy is less (Richelsen, 2006). In a world with increasing problems with obesity, this effect could be important. A EU-project – DIOGENES (Diet, Obesity and Genes) – is currently investigating these relationships (<http://www.diogenes-eu.org/>).

1.3.2 Fat content and fatty acid composition

We are advised to reduce the amount of fat we eat, especially saturated fat (NNF, 2004). Fat from animal sources (milk, meat, egg) is very saturated and the authorities in many countries therefore advise the population to reduce the intake of animal fat. This has been taken in by consumers, as most now prefer meat without visible fat.

Meat contains variable amounts of fat, depending on the source (chicken, beef, and pork) and the cutting (Danish Meat Association, 2007). A large amount of fat is visible, either between muscles (intermuscular fat) or as the fat layer (subcutaneous fat), and can be removed by the industry before sale or by the consumer

Table 1.1 Fatty acid composition (%) in pork, beef and chicken. (Fødevaredatabanken version 6, Danmarks Fødevareforskning).

	Pork	Beef	Chicken
SFA*	39.8%	45–49%	22.0%
MUFA	46.0%	43–50%	42.9%
PUFA	12.2%	2–10%	33.5%
n-6 % of fat	11.2%	1.8–8.6%	27.2%
n-3 % of fat	1.0%	0.4–1.4%	6.3%
n-6/n-3	11	Appr. 6	4

* This percentage is for both lean and fat meat cuts even though a lean piece of meat will contain a higher percent phospholipids and thereby less saturated fat compared with a fattier piece of meat.

before or after cooking. Most meat types are actually very lean and the amount of intramuscular fat should not be reduced too much as this will reduce the eating quality (Aaslyng *et al.*, 2004b). Many consumers regard chicken as the most lean meat type (Andersen, Pers. Comm., 2007), but this is not strictly true. Pork and veal can be just as lean after removing the visible fat.

In minced meat, it is more difficult for the consumer to remove the fat, as it is mixed into the meat. The industry has therefore a responsibility to offer consumers lean minced meat as an option in the shops.

Not only the amount of fat, but also the fat quality is important with regard to health. The fatty acid composition of meat depends on the feed of the animal. Pork and chicken (monogastric animals) easily reflect the fatty acid composition of the feed in the fat (Affentranger *et al.*, 1996; Gatlin *et al.*, 2002; Nürnberg *et al.*, 1999; Tikk *et al.*, 2007), but also the fatty acid composition of beef can vary with varying feed (Elmore *et al.*, 1999, 2004). Table 1.1 shows the general fatty acid composition of pork, beef and chicken.

The fatty acids of beef are more saturated than the fatty acids of pork and chicken. In comparison, the relationship between omega-3 and omega-6 fatty acids are better in pork and chicken as the proportions are below 4, as is often recommended (NNF, 2004). The proportion between omega-3 and omega-6 fatty acids in pork depends to a large extent on the feed, and ratios as low as 1.4 have been reported (Nuernberg *et al.*, 2005).

It is possible to increase the content of omega-3 fatty acids by feeding pigs with, e.g. linseed oil (Nürnberg *et al.*, 1999), and in France and Canada pork and pork products have been marketed with a health claim for a high content of omega-3 fatty acids. This is one way of meeting consumer demand for healthy meat products.

1.3.3 Minerals and vitamins

Iron is a main mineral in our body as it is essential in the transportation of oxygen

around the body. Iron deficiency is well known, especially in young women (Samuelson *et al.*, 2003). Meat is an excellent source of iron as it contains high amounts of the easily absorbable haem iron. At the same time a 'meat factor' is present as meat seems to enhance the absorption of non-haem iron (Bæch *et al.*, 2003a,b). Even small amounts – ≥ 50 g a day – of meat enhance the iron uptake from food in general (Bæch *et al.*, 2003a).

Selenium is a micronutrient necessary for some antioxidative enzymes. Pork has been shown to be an excellent source of selenium (Skibsted, 1997). The reported content of selenium in meat varies between countries, perhaps due to variation in the method of analysis (Strong, 2006).

The intake of D-vitamin is too low in many countries, and even though it can be synthesized by exposure to the sun, a higher intake through the diet is desirable. Meat contributes about 26% of the D-vitamin intake in a normal diet (Lyhne *et al.*, 2005). D-vitamin is present in the fatty part of the meat and also in the rind of pork (Fødevaredatabasen, 2007). It is not known if exposure to sun of the live animal enhances the concentration of D-vitamin in the rind.

Sodium chloride is a salt often used in meat products for its taste and functionality, and for its effect of prolonging the shelf-life of a product. However, it is recommended that we reduce our intake of sodium-ion substantially (Loria *et al.*, 2001; NNF, 2004). Meat products are responsible for a relatively high amount of our intake of sodium (NNF, 2004), and decreased use of sodium by the meat industry is therefore desirable. Countries such as Finland and UK have already worked for some time on mapping routes for reducing sodium chloride without reducing eating quality or shelf-life, and other parts of Europe and North America have now also started focusing on reducing sodium.

1.4 Variability in meat and meat products

Meat is a biological source, and natural biological variations between animals will always exist. How can a consumer be certain to get what he wants? Is there a need for improved quality?

A steak or a chop needs to be fried for a certain time depending on how thick it is. To increase the success rate for the consumer when frying the meat, the slices in a package needs to be similar in size. This sounds easy, but requires good workmanship from the industry.

Tenderness is, as stated in the introduction, an important factor for consumer satisfaction and possibilities abound to increase tenderness. Marbling is an important factor in both beef and pork, but again a large variation exists in the degree of marbling both between animals and within the same muscle (Aaslyng, 2002). Also, other factors can influence tenderness, such as the age of the animal (especially beef) and the chilling regime. The effect of these factors can not be seen on the raw meat and branding is therefore necessary to help the consumer to choose tender meat. The branding could be built upon a combination of sorting the carcasses to get marbled meat, of having a code of practice to ensure optimal

chilling or ageing of the meat to increase tenderness. This aspect is discussed in more depth in Chapter 3 of this book.

To produce healthy products, the variation in the nutritional quality of the meat must not be too high. Feeding is known to alter the fatty acid content and distribution in the meat and restriction of the fat in the feed can therefore be relevant if the focus is on healthy products. This aspect is discussed in depth in Chapters 7 and 15 of this book.

Also, the technological quality has a natural variation. In pork, the pH variation in a survey of five Belgian slaughterhouses was between 5.56 ± 0.03 and 5.76 ± 0.04 (Lammens *et al.*, 2007). This is reflected in a variation in drip loss and colour. Gentle handling of the pigs up to slaughter (Støier *et al.*, 2001) combined with rapid chilling (Maribo *et al.*, 1998), can improve the technological quality and reduce the natural variation. In beef, technological quality is reflected in the pH. The pH is measured the day after slaughter and is used as a quality attribute to screen out carcasses of low quality.

When producing meat products, other factors can be of relevance to obtain a high quality compared with those measured in fresh meat. To produce dry-cured ham products from pork, proteolytic enzyme activity is very important. This can, however, vary between animals depending on the growth rate up to slaughter (Kristensen *et al.*, 2003; Therkildsen *et al.*, 2002a,b). To produce bacon, the distribution between fat and meat influences the quality of the product, and variations are also seen here. To overcome this, sorting on fatness is a possibility. In the production of sausages, raw meat quality variation has an influence, especially the fatty acid composition. Too high a degree of unsaturation might be nutritionally beneficial, but the quality of the sausages can be reduced. The fatty acid composition depends on the feeding and also on the location of the fat in the carcass. The problem can be overcome either by restrictive feeding of the animals or by combining fat sources with different degrees of saturation.

The natural variation between animals is thus a challenge for the industry, requiring a large degree of sorting or combination of raw materials to gain the optimum quality. On the other hand, it is also a possibility to take advantage of the variation to increase the quality by choosing the optimum for each product and to meet market demand.

1.5 Future trends

The meat industry has a challenge in the future to meet consumers' demand for healthy products with high eating quality. How can the consumers and the industry meet? First of all the consumers demands must be known. Not just the demands of today, but also the expected demands in the future.

When buying meat, the consumer must either guess about the eating quality or trust the supplier. Quality branding for assured high eating quality is a way to make it easier for the consumer to choose between products when buying. Documentation of the history of the meat back to a farm or even to the specific animal is

another way of convincing the consumer that the meat industry has control of the production and thereby the quality of the meat.

To meet the demand for healthy meats, development of low-fat products with optimal fatty acid composition is one of the future challenges for the industry. A further focus on developing meat products with low concentrations of sodium is also one of the major challenges for the industry.

Convenience is another future challenge for the meat industry, not only in producing ready meals or meal components, but also in ensuring a high degree of convenience of the fresh meat. It must be easy for the consumer to go home and prepare the meat.

Retailers demand a long shelf-life for the meat and meat products, even though the consumers perhaps prefer freshness. This is another challenge for the meat industry. How do we produce fresh meat with a superior quality and a long shelf-life? Case ready production can be a way to achieve this.

To meet the challenges of the future, co-operation between the industry and scientists within the fields of meat quality, sensory science and microbiology is therefore of importance to ensure that research and product development gain from each other.

1.6 Acknowledgement

I would like to thank Susanne Støier, Niels T. Madsen, Ina Clausen, Christian Vestergaard from Danish Meat Research Institute and Grethe Andersen from Danish Meat for valuable inspiration, discussions and help during the writing of this chapter. I would also like to thank Sisse Fagt from the Technical University of Denmark, the National Food Institute, for letting me use her results even before they are published.

1.7 References

- Aaslyng, M D (2002), 'Quality indicators for raw meat', in Kerry, J Kerry, J and Ledward D, *Meat Processing. Improving quality*, 1st edn, Cambridge, Woodhead Publishing Limited, 157–174.
- Aaslyng, M D, Bejerholm, C, Erthbjerg, P, Bertram, H C and Andersen, H (2003), Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food Quality and Preferences*, 14, 277–288.
- Aaslyng, M D and Hviid, M (2004a), The secret behind tender pork. *Fleischwirtschaft International*, 2, 52–54.
- Aaslyng, M D and Støier, S (2004b), 'The effect of intramuscular fat on eating quality of pork depending on end point temperature,' Helsinki, Finland, 548–550.
- Aaslyng, M D and Meinert, L (2007a), 'We do not eat pork, we eat meals. But how is the meal with pork?', 7th. *Pangborn Sensory Science Symposium*, 12–16 August, Minneapolis, Minnesota.
- Aaslyng, M D, Oksama, M, Olsen, E V, Bejerholm, C, Baltzer, M, Andersen, G, Bredie, W L P, Byrne, D V and Gabrielsen, G (2007b), The impact of the sensory quality of pork on consumer preference. *Meat Science*, 76, 61–73.

- Aaslyng, M D and Schäfer, A (2007c), The effect of free fatty acids on the odour of pork investigated by sensory profiling and GC-O-MS. *Eur. J. Food Technol.*, Accepted.
- Affentranger, P, Gerwig, C, Seewer, G J F, Schwörer, D and Künzi, N (1996), Growth and carcass characteristics as well as meat and fat quality of three types of pigs under different feeding regimens. *Livestock Production Science*, 45, 187–196.
- Aliani, M and Farmer, L J (2005), Precursors of chicken flavor. II. Identification of key flavor precursors using sensory methods, *Journal of Agricultural and Food Chemistry*, 53 (16), 6455–6462.
- Andersen, G (2007), Personal Communication, ga@danishmeat.dk
- Anon. (2007), *Mad og Drikke i Middelalderen* Europæisk Middelalderfestival, Horsens kommune, Kultur og fritidsforvaltningen, Horsens, Denmark.
- Bæch, S B, Hansen, M, Bukhave, K, Jensen, M, Kristensen, L, Purslow, P P, Skibsted, L H and Sandström, B (2003a), Nonheme iron absorption from a phytate rich meal is enhanced by addition of small amounts of pork meat. *Am. J. Clin. Nutr.*, 77, 173–179.
- Bæch, S B, Hansen, M, Bukhave, K, Kristensen, L, Jensen, M, Sørensen, S S and Sandström, B (2003b), Increasing the cooking temperature of meat does not affect nonheme iron absorption from a phytate-rich meal in women. *Human Nutria. and Metabolism Research Comm.*, 133, 94–97.
- Baublits, R T, Pohlman, F W, Brown, A H, Yancey, E J and Johnson, Z B (2006), Impact of muscle type and sodium chloride concentration on the quality, sensory and instrumental color characteristics of solution enhanced whole-muscle beef. *Meat Science*, 72(4), 704–712.
- Boleman, S J, Boleman, S L, Miller, R K, Taylor, J F, Cross, H R, Wheeler, T L, Koohmaraie, M, Shackelford, S D, Miller, M F, West, R L, Johnson, D D and Savell, J W (1997), Consumer evaluation of beef of known categories of tenderness. *J. Anim. Sci.*, 75, 1521–1524.
- Breadsworth, A and Bryman, A (2004), Meat consumption and meat avoidance among young people. An 11-year longitudinal study. *British Food Journal*, 106 (4), 313–327.
- Brewer, M S, Zhu, L G and McKeith, F K (2001), Marbeling effects on quality characteristics of pork loin chops: Consumer purchase intent, visual and sensory characteristics. *Meat Science*, 59, 153–163.
- Bryhni, E A, Byrne, D V, Rødbotten, M, Møller, S, Claudi-Magnussen, C, Karlsson, A, Agerhem, H, Johansson, M and Martens, M (2003), Consumer and sensory investigations in relation to physical/chemical aspects of cooked pork in Scandinavia. *Meat Science*, 65, 737–747.
- Calkins, C R and Hodgen, J M (2007), A fresh look at meat flavor. *Meat Science*, 77, 63–80.
- Cosgrove, M, Flynn, A, Kiely, M (2005), Consumption of red meat, white meat and processed meat in Irish adults in relation to dietary quality. *British Journal of Nutrition*, 93, 933–942.
- Danish Meat Association (2007): http://www.danishmeat.dk/smcms/forside/undervisning/Materialer_svin/mad_mat/Index.htm?ID=13206
- Edwards, Z M, Takeuchi, M T, Hillers, V N, McCurdy, S M and Edlefsen, M (2005), Use of behavioral change theories in development of educational materials to promote food thermometer use. *Food Protection Trends*, 26 (2), 82–88.
- Elmore, J S, Mottram, D S, Enser, M and Wood, J D (1999), Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *J. Agric. Food Chem.*, 47, 1619–1625.
- Elmore, J S, Warren, H E, Mottram, D S, Scollan, N D, Enser, M, Richardson, R I and Wood, J D (2004), A comparison of the volatiles and fatty acid compositions of grilled beef muscles from Aberdeen Angus and Holstein-Friesian steers fed diets based on silage or concentrates. *Meat Science*, 68, 27–33.
- Fagt, S. (2007), Personal Communication, sfa@food.dtu.dk

- Fagt, S and Groth, M V (1992), *Udviklingen i danskernes fødevarerforbrug 1955–1990. Beskrivelse af den danske kost på grundlag af fødevarerstatistikker og næringsberegnete data*. Levnedsmiddelstyrelsen, Søborg, Denmark.
- Fagt, S, Matthiesen, J, Trolle, E, Lyhne, N, Christensen, T, Hinsch, H-J, Hartkopp, H B, Biltoft-Jensen, A, Møller, A and Daae, A-S (2002), 2. *Danskernes kostvaner 2000–2001. Udvikling i danskernes kost – forbrug, indkøb og vaner*, 1st. edn, Fødevaredirektoratet, Denmark, Søborg.
- Fødevaredatabasen (2007) (version 6, Danmarks Fødevareforskning). www.foodcomp.dk
- Fortin, A, Robertson, W and Tong, A K W (2005), The eating quality of Canadian pork and its relationship with intramuscular fat. *Meat Science*, 69, 297–305.
- Fuke, S and Konosu, S (1991), Taste-active components on some foods: A review of Japanese research. *Physiology and Behavior*, 49, 863–868.
- Garemo, M, Arvidsson Lenner, R, Nilsson, E K, Borres, M P and Strandvik, B (2007), Food choice, socio-economic characteristics and health in 4-year olds in a well-educated urban Swedish community. *Clinical Nutrition*, 26 (1), 133–140.
- Gatlin, L A, See, M T, Hansen, J A, Sutton, D and Odle, J (2002), The effect of dietary fat sources, levels, and feeding intervals on pork fatty acid composition. *J. Anim. Sci.*, 80, 1606–1615.
- Groth, M V and Fagt, S (2003), *Danskernes kostvaner. Måltidsvaner, holdninger, sociale forskelle og sammenhæng med anden livsstil*, 9th edn, Fødevaredirektoratet, Denmark, Søborg.
- Guenther, P M, Jensen, H J, Batres-Marquez, S P and Chen, C-F (2005), Sociodemographic, knowledge and attitudinal factors related to meat consumption in the United States. *J. American Dietetic Association*, 105 (8), 1266–1274.
- Hansen, L L, Claudi-Magnussen, C, Jensen, S K and Andersen, H J (2006), Effect of organic pig production systems on performance and meat quality. *Meat Science*, 74, 605–615.
- Holm, L and Møhl, M (2000), The role of meat in everyday food culture: An analysis of an interview study in Copenhagen. *Appetite*, 34, 277–283.
- Horowitz, R (2006), *Putting Meat on the American Table. Taste, Technology, Transformation*. The Johns Hopkins University Press, Baltimore, Maryland.
- Huffman, K L, Miller, M F, Hoover, L C, Wu, C K, Brittin, H C and Ramsey, C B (1996), Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. *J. Anim. Sci.*, 74, 91–97.
- Jeremiah, L E, Tong, A K W, Jones, S D M and McDonell, C (1992), Consumer acceptance of beef with different levels of marbling. *J. Consumer Studies and Home Economics*, 16, 375–387.
- Killinger, K M, Calkins, C R, Umberger, W J, Feuz, D M and Eskridge, K M (2004), Consumer sensory acceptance and value for beef steaks of similar tenderness, but differing in marbling level. *J. Anim. Sci.*, 82, 3294–3301.
- Koizumi, S, Jussaume, R A, Kobayashi, S, Pan, I N, Takadu, S, Nishino, M, Saito, H, Baba, M and Nagano, M (2001), Study on consumer behaviour for meat consumption in the U.S. *Animal Science*, 72 (4), 329–343.
- Koutsidis, G, Elmore, J S, Oruna-Concha, M J, Campo, M M, Wood, J D and Mottram, D S (2007), Water-soluble precursors of beef flavour: I. Effect of diet and breed. *Meat Science*, doi:10.1016/j.meatsci.2007.08.008. 2007.
- Kristensen, L, Therkildsen, M, Aaslyng, M D, Oksbjerg, N and Ertbjerg, P (2006), Compensatory growth improves meat tenderness in gilts but not in barrows. *J. Anim. Sci.*, 82, 3617–3624.
- Kristensen, L, Therkildsen, M, Riis, B, Sørensen, M T, Oksbjerg, N, Purslow, P P and Ertbjerg, P (2003), Dietary-induced changes of muscle growth rate in pigs: Effects on in vivo and post mortem muscle proteolysis and meat quality. *J. Anim. Sci.*, 80, 2862–2871.
- Kubberød, E, Dingstad, G I, Ueland, O and Risvik, E (2006), The effect of animality on disgust response at the prospect of meat preparation: An experimental approach from Norway, *Food Quality and Preference*, 17 (3–4) 199–208.

- Kubberød, E, Ueland, Ø, Rødbotten, M, Westad, F and Risvik, E (2002a), Gender specific preferences and attributes towards meat. *Food Quality and Preferences*, 13, 285–294.
- Kubberød, E, Ueland, Ø, Tronstad, Å and Risvik, E (2002b), Attitudes towards meat and meat-eating among adolescents in Norway: A qualitative study. *Appetite*, 38, 53–62.
- Lammens, V, Peeters, E, De Maere, H, De Mey, E, Paelinck, H, Leyten, J and Geers, R (2007), A survey of pork quality in relation to pre-slaughter conditions, slaughterhouse facilities and quality assurance. *Meat Science*, 75, 381–387.
- Loria, C M, Obarzanek, E and Ernst, N D (2001), Choose and prepare foods with less salt: Dietary advice for all Americans. *J. Nutr.*, 131, 536–551.
- Lyhne, N, Christensen, T, Groth, M V, Fagt, S, Biloft-Jensen, A, Hartkopp, H B, Hinsch, H-J, Matthiesen, J, Møller, A, Saxholt, E and Trolle, E (2005), *Danskernes kostvaner 2000–2002. Hovedresultater*. Dansk Fødevareforskning, Denmark, Søborg.
- Madsen, N T (2007), Personal Communication, ntm@danishmeat.dk
- Maga, J A (1994), 'Umami flavour of meat', in F. Shahidi, *Flavor of Meat and Meat Products*, New York, Chapman and Hall, 98–115.
- Maribo, H, Olsen, E V, Barton-Gade, P, Møller, A J and Karlsson, A (1998), Effect of early post mortem cooling on temperature, pH fall and meat quality in pigs. *Meat Science*, 50 (1), 115–129.
- Marques-Vidal, P, Ravasco, P, Dias, C and Camilo, M (2006), Trends of food intake in Portugal, 1987–1999: Results from the national health surveys. *Eur. J. Clinical Nutrition*, 60, 1415–1422.
- Meinert, L, Andersen, L T, Bredie, W L P, Bjerregaard, C and Aaslyng, M D (2006), Chemical and sensory characterisation of pan-fried pork flavour: Interactions between raw meat quality, ageing and frying temperature. *Meat Science*, 75, 229–242.
- Meinert, L, Christiansen, S, Kristensen, L, Bjerregaard, C and Aaslyng, M D (2007), Eating quality of pork from pure breed pigs compared with DLY studied by focus group research and meat quality analyses. *Food Quality and Preference*. Submitted.
- Miller, M F, Carr, M A, Ramsey, C B, Crockett, K L and Hoover, L C (2001), Consumer thresholds for establishing the value of beef tenderness. *J. Anim. Sci.*, 79, 3062–3068.
- Mottram, D S (1994), 'Flavor compounds formed during the Maillard reaction', in T H Parliment, M J Morello and R J McGorin, *Thermally Generated Flavors. Maillard, Microwave and Extrusion Processes*, 543, Washington, D.C., Am. Chem. Soc., 104–125.
- Mottram, D S and Edwards, R A (1983), The role of triglycerides and phospholipids in the aroma of cooked beef. *J. Sci. Food Agric.*, 34, 517–522.
- Naska, A, Fouskakis, D, Oikonomou, E, Almeida, M, Berg, M, Gedrich, K, Moreias, O, Nelson, M, Trygg, K, Turrini, A, Remaut, A, Volatier, J, Trichopoulou, A and DAFNE participants. (2006), Dietary patterns and their socio-demographic determinants in 10 European countries: Data from the DAFNE databank. *Eur. J. Clinical Nutrition*, 60, 181–190.
- Ngapo, T M, Martin, J F and Dransfield, E (2007), International preferences for pork appearance: 1. Consumer choices. *Food Quality and Preference*, 18, 26–36.
- Nishimura, T, Rhue, M R, Okitani, A and Kato, H (1988), Components contributing to the improvement of meat taste during storage. *Agric. Biol. Chem.*, 52 (9), 2323–2330.
- NNF (2004), Nordic Nutrition Recommendation 2004, 4th edn Integration nutrition and physical activity. Nordic Council of Ministers, Copenhagen, Denmark
- Nuernberg, K, Fischer, K, Nuernberg, G, Kuechenmeister, U, Klosowska, D, Eliminowska-Wenda, G, Fielder, I and Ender, K (2005), Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. *Meat Science*, 70, 63–74.
- Nürnberg, K, Küchenmeister, U, Nürnberg, G, Ender, K and Hackl, W (1999), Influence of exogenous application of *n*-3 fatty acids on meat quality, lipid composition and oxidative stability in pigs. *Arch. Anim. Nutr.*, 52, 53–56.

- Richelsen B. (2006), Giver proteinrige slankekur bedre langtidseffekter med henblik på vægttabet? *Motions og ernæringsrådets nyhedsbrev*, 4, 5–6.
- Rosenvold, K, Clausen, I and Madsen, N T (2006), 'Mechanical tenderisation and enhancement improve eating quality of beef from dairy cows', *International Congress of Meat Science and Technology 2006*, Dublin, Ireland, 611–612.
- Samuelson, G, Lönnnerdal, G, Kempe, B, Elverby, J-E and Bratteby, L-E (2003), Serum ferritin and transferrin receptor concentrations during the transition from adolescence to adulthood in a healthy Swedish population. *Acta Paediatr.*, 92, 5–11.
- Savell, J W and Cross, H R (1988), 'The role of fat in the palatability of beef, pork and lamb', in National Research Council *Designing Foods: Animal Product Options in the Marketplace*, Washington D.C., National Academy Press, 345–355.
- Sindelar, J J, Prochaska, F, Britt, J, Smith, G L, Miller, R K, Templeman, R and Osburn, W (2003a), Strategies to eliminate atypical flavours and aromas in sow loins. I. Optimization of sodium tripolyphosphate, sodium bicarbonate, and injection level. *Meat Science*, 65, 1211–1222.
- Sindelar, J J, Prochaska, F, Britt, J, Smith, G L, and Osburn, W (2003b), Strategies to eliminate atypical aromas and flavors in sow loins – part II: consumer acceptance of loins marinated with sodium tripolyphosphate and sodium bicarbonate. *Meat Science*, 65, 1223–1230.
- Sivertsen, H, Kubberød, E and Hildrum, K I (2002), Consumer preferences of beef tenderness and mechanical measurements. *J. Sensory Studies*, 17, 365–378.
- Skibsted, L (1997), Dietary treatments and oxidative stability of muscle and meat products: Nutritive value, sensory quality and safety. Final synthesis report, 195.
- Sloan, A E (2003), What, When and Where Americans eat: 2003. *Food Technology*, 57 (8), 48–66.
- Støier, S, Aaslyng, M D, Olsen, E V and Henckel, P (2001), The effect of stress during lairage and stunning on muscle metabolism and drip loss in Danish pork. *Meat Science*, 59, 127–131.
- Strong, M (2006), 'Red meat in the diet', *International Congress of Meat Science and Technology*, August, Dublin, 43–54.
- Tai, C-Y, Yang, J and Ho, C-T (1999), 'Effect of thiamin oxidation on thermal formation of meaty aroma compounds', in Y L Xiong, *Quality Attributes of Muscle Foods*, Kluwer Academic/Plenum Publishers, 173.
- Therkildsen, M (1999), *Biological factors affecting beef tenderness with emphasis on growth rate and muscle protein degradation*, Ph.D. thesis, The Royal Veterinary and Agricultural University, Copenhagen, Denmark.
- Therkildsen, M, Riis, B, Karlsson, A, Kristensen, L, Ertbjerg, P, Purslow, P, Aaslyng, M D and Oksbjerg, N (2002a), Dietary induced changes in growth rate affect muscle proteolytic potential and meat texture. Effects of duration of the compensatory growth. *Animal Science*, 80, 2862–2871.
- Therkildsen, M, Riis, B, Karlsson, A, Kristensen, L, Purslow, P P, Aaslyng, M D and Oksbjerg, N (2002b), Compensatory growth response in pigs, muscle protein turn-over and meat texture: Effects of restriction/realimentation period. *Animal Science*, 75, 367–377.
- Tikk, K, Tikk, M, Aaslyng, M D, Karlsson, A H, Lundström, K and Andersen, H J (2007), The significance of fat supplemented diets on pork quality – connections between specific fatty acids and sensory attributes of pork. *Meat Science*, 77, 275–286.
- Tikk, M, Tikk, K, Tørngren, M A, Meinert, L, Aaslyng, M D, Karlsson, A H and Andersen, H J (2006), Development of inosine monophosphate and its degradation products during ageing of pork of different qualities in relation to basic taste and retronasal flavor perception of the meat. *J. Agric. Food Chem.*, 54, 7769–7777.
- Wheeler, T L, Schackelford, S D and Koohmaraie, M (2000), Variation in proteolysis, sarcomere length, collagen content and tenderness among pork muscles. *J. Anim. Sci.*, 78, 958–965.

- Wood, J D, Nute, G R, Fursey, G A J and Cuthbertson, A (1995), The effect of cooking conditions on the eating quality of pork. *Meat Science*, 40, 127–135.
- Wood, J D, Nute, G R, Richardson, R I, Whittington, F M, Southwood, O, Plastow, G, Mansbridge, R, Costa, N D and Chang, K C (2004), Effect of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science*, 67, 651–667.
- Wood, J D, Richardson, R I, Nute, G R, Fisher, A V, Campo, M M, Kasapidou, E, Sheard, P R and Enser, M (2003), Effects of fatty acids on meat quality: A review. *Meat Science*, 66, 21–32.
- Yancey, E J, Grobbel, J P, Dikeman, M E, Smith, J S, Hachmeister, K A, Chambers, E I, Gadgil, P, Milliken, G A and Dressler, E A (2006), Effects of total iron, myoglobin, hemoglobin and lipid oxidation of uncooked muscles on livery flavor development and volatiles of cooked beef steaks. *Meat Science*, 73, 680–686.

2

Biology and regulation of carcass composition

P. L. Greenwood, NSW Department of Primary Industries, Australia, and F. R. Dunshea, University of Melbourne, Australia

Abstract: This chapter provides an overview of the generally accepted understanding of the biology and regulation of carcass composition. More specifically, patterns of growth are briefly described and an overview of the biology of carcass tissue growth and development is provided. Regulation of compositional characteristics by nutrition, genotype and metabolic modifiers is also reviewed. Finally, future trends in research relating to the regulation of carcass composition and meat production are discussed.

Key words: carcass composition, nutrition, genetics, metabolic modifiers, meat production trends.

2.1 Introduction

The composition of carcasses and meat are important for consumer acceptance and health, and for meat processors due to the economics associated with meeting specific market requirements, maximising yield of saleable meat, and minimising wastage due to excessive fatness and bone content. Carcass and meat composition are important to producers of meat due to their importance for growth and the efficiency of utilisation of nutrients, and in meeting market specifications to maximise returns.

Defining carcass composition depends on the market segment for which the meat is destined. In a more traditional sense, carcass composition refers to the absolute or relative amounts of muscle, fat and bone, or of protein, lipid, ash and water. From a processor perspective, this definition can be further refined to

encompass quantities of retail meat, fat trim and bone and, within the wholesale component of meat, into primal cuts that may be prepared with or without bone. However, the complexity of the modern marketplace has resulted in a redefining of carcass composition to include other factors such as those listed below and detailed elsewhere in this book.

Consumer requirements for meat vary widely depending upon socio-economic factors. In developing countries, there is an increasing demand for protein of which meat is an important and growing contributor. In developed countries, there is an increasingly segmented marketplace that demands meat products based on factors, detailed elsewhere within this book, that include for example: calorific, fat and protein content; specific dietary components such as omega-3 and other fatty acids, iron, zinc, antioxidants and other macro and micronutrients; intramuscular fat content or marbling; tender, juicy and flavourful meat; retail attractiveness including colour; residue free meat; organically produced meat; animal welfare considerations; environmental impact; and low cost meat.

For the meat producer, the primary objective is maximum efficiency of nutrient utilisation to achieve specific market requirements that maximise income. To meet this goal it is necessary to utilise a genotype capable of meeting market specifications, and to provide a suitable environment that includes enough nutrients of a quality appropriate for the various stages of growth and development of that genotype. The profitability of processing animals for meat depends upon a supply of product that is within specification, maximising the yield of saleable meat of an appropriate specification, and minimising the amount of fat that has to be trimmed from a carcass and the amount of bone relative to saleable meat.

In this chapter, we provide an overview of the generally accepted understanding of the biology and regulation of carcass composition. More specifically, we briefly describe patterns of growth, provide an overview of the biology of carcass tissue growth and development, and review regulation of compositional characteristics by nutrition, genetics and metabolic modifiers. Finally, we discuss future trends in research relating to carcass composition and its regulation.

2.2 Patterns of growth of carcass tissues

The carcass tissues follow well-defined patterns of growth, referred to as allometric or relative growth due to their differing rates of accretion during development, growth and maturation (Hammond, 1932; Palsson, 1955). Overall, relative growth of bone precedes that of muscle which precedes that of fat. Hence, at younger ages and lighter body weights bone is present at greater proportions than muscle and fat, respectively, than at older ages and heavier weights. Carcass lipid increases at an increasing rate compared to carcass protein and hence contributes to carcass energy content at an increasing rate during postnatal growth (Fig. 2.1). This relationship is more evident when assessed on a bodyweight- or carcass weight-specific basis (Fig. 2.2). The relative proportions of the carcass tissues will differ at a given age or weight due to genotype or gender, depending

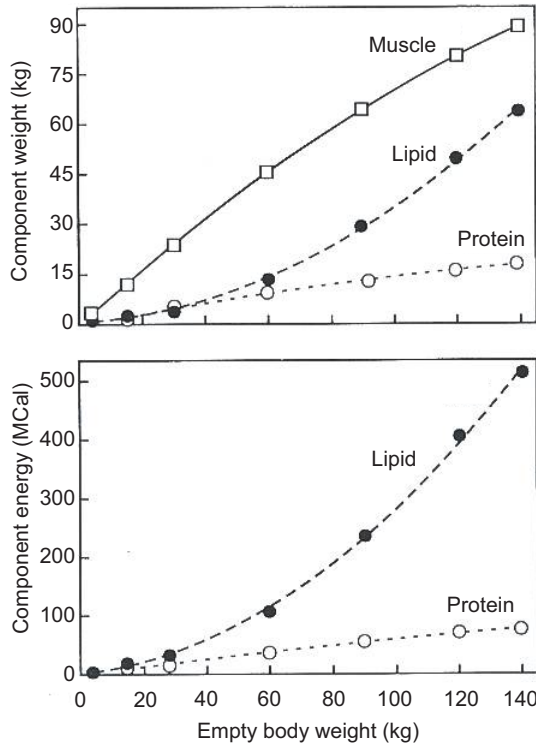


Fig. 2.1 Pattern of growth of carcass components and the contribution of protein and fat to energy stored in the body of the pig. From Etherton and Walton (1986) in Mitchell *et al.* (2001). © *J Anim Sci*.

upon the maturity pattern and mature size, and on the supply of nutrients and growth rate. For example, later maturing, higher mature weight animals will have a greater proportion of lean tissues (muscle and bone) at the same age or weight than earlier maturing, lower mature weight animals, which will be fatter. Similarly, animals of the same genotype nourished to grow rapidly to a given age or weight will generally have a lower proportion of lean tissues and a higher proportion of fat than those nourished to grow more slowly (Fig. 2.3). This is due to the priority of use of available nutrients favouring bone over muscle over fat accretion when nutrients are limiting (Hammond, 1944; Pálsson, 1955). Conversely, as the supply of nutrients increases, adipose tissue accretion increases by more, relative to muscle and bone.

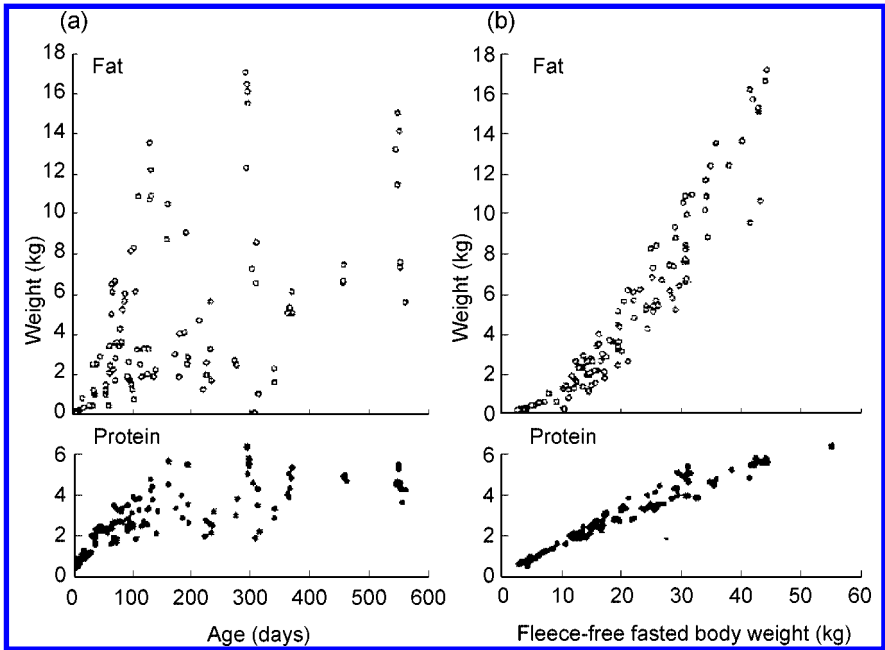


Fig. 2.2 The relationship between weight of protein and fat in lambs and (a) age and (b) fleece-free fasted body weight (FFBW). Black (1983) in Oddy and Sainz (2002). Reprinted by permission of CSIRO Publishing and CABI, Wallingford, UK.

2.3 Biology of carcass tissue development and growth

2.3.1 Embryonic and fetal development

General

Most fetal growth occurs during the final third or so of pregnancy in cattle (Winters *et al.*, 1942) and sheep (Wallace, 1948a,b; Joubert, 1956; Everitt, 1968), and from about day 70 onwards in pigs (Marrable and Ashdown, 1967), although genesis and growth of vital organs and body tissues commences during early gestation. Development of the vital organs generally precedes that of the carcass tissues (Palsson, 1955; Black, 1983). However, development of muscle is evident by about day 30 of gestation in sheep (Wilson *et al.*, 1992), and of bone by about day 35 of gestation (Altschul, 1987) in sheep.

Bone

Growth of the skeleton, particularly of long bones, is the primary determinant of mature size, and is an important regulator of skeletal muscle growth due to stretch induced hypertrophy of muscle.

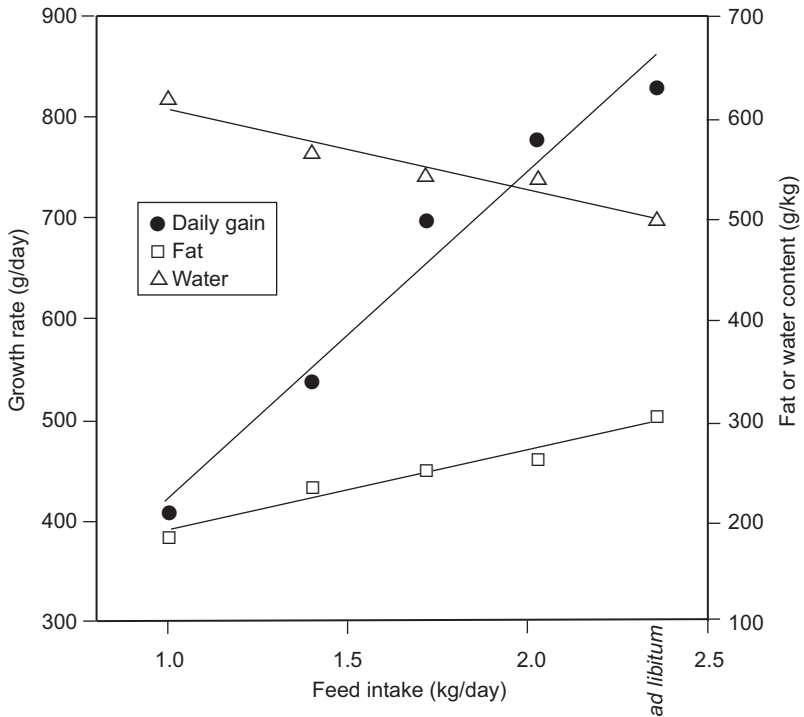


Fig. 2.3 Relationship between feed intake and growth of pigs from 20 to 45 kg and body composition at 45 kg. From Mitchell *et al.* (2001) using data from Campbell *et al.* (1983). Reprinted from *Biology of the Domestic Pig*, edited by Wilson G. Pond and Harry J. Mersmann. Copyright © 2001 by Cornell University. Used by permission of the publisher, Cornell University Press.

During early fetal life, bone commences developing on non-ossified, cartilaginous rods, plates and disks that act as templates for subsequent ossification and bone formation. In sheep, ossification commences from about day 35 for the jaw bones (Altschul, 1987), followed by commencement of mineralisation of long bones of the fore- and hind-limbs, in that order, and of other bones of the skull and thorax by day 41 of gestation (Wenham, 1981; Altschul, 1987). Ossification of the long bones commences in the first trimester in pigs (Hodges, 1953; Connolly *et al.*, 2004) and cattle (Gjesdal, 1969; Sterba, 2004).

The three major cell types that contribute to formation of the skeleton are the chondrocytes, which are of mesenchymal origin and form cartilage, osteoblasts, which also originate from the mesenchyme and produce bone matrix, and the osteoclasts, which resorb bone and are of haematopoietic origin (Erlebacher *et al.*, 1995).

Long bone development is characterised by endochondral ossification, with development of a solid, central primary core of ossification, the diaphysis, which develops towards the ends of the still growing cartilaginous model. Ossification

also occurs within secondary sites, the epiphyses, which develop into a shell encompassing plates that become the epiphyseal growth plates between the diaphysis and the epiphyses at the ends of the long bone. This process is accompanied by continual remodeling as a result of resorption and apposition (anabolism) of the growing bone and development of a central core. This core contains blood vessels, reticuloendothelial cells, and connective tissue including adipocytes that form bone marrow.

Growth of bones occurs due to continued proliferation of cells known as chondrocytes that arise from progenitor cells within the growth plate, and which secrete the chondroid matrix. Existing cells become more distant from the proliferative zone of the growth plate as proliferation and bone growth continues, and subsequently hypertrophy and extrude calcium which mineralizes the chondroid or cartilage matrix. In turn, the mineralized chondroid matrix is invaded by blood vessels and osteoblasts which secrete osteoid and form bone. Concurrently, osteocytes remove residual chondroid matrix from the newly formed spongy bone, which is replaced by dense bone.

Compared to soft tissues, the skeletal size of the fetus is relatively resistant to the growth-limiting effects of restricted nutrient supply due to maternal nutritional and/or placental limitations. Although some between-fetus variation in bone development and skeletal size can occur within the first third of pregnancy (Hafez and Rajakoski, 1964), most of the increase in between-animal variation in size of the fetal skeleton occurs during the final third of gestation (McDonald *et al.*, 1977; Greenwood *et al.*, 2002b).

Bone represents a much higher proportion of body weight in newborn livestock than muscle, adipose, and other soft tissues. Bone growth continues during postnatal life until growth plate closure, which generally occurs shortly after puberty. At this time, progenitor cells cease proliferation and are consumed into mineralised tissue, and the growth plate is replaced by bone. Apart from gross differences in size and structure of bones between the livestock species, finer-scale structural differences also exist at birth and during postnatal growth (Mori *et al.*, 2005).

Muscle

Muscle fibres form as a result of the alignment and fusion of myoblasts, which arise from mesodermal somites, to form primary myotubes in the first instance (Hauschka, 1994). Subsequently, myoblasts align and fuse on the surface of established myotubes and/or myofibres to form secondary and/or tertiary myofibres. These latter phases account for most of the increase in the number of fibres within a muscle.

The timing of stages of myogenesis in livestock (Table 2.1) was previously reviewed by Picard *et al.* (2002) and Brameld *et al.* (2003). In sheep, primary myogenesis commences at about day 32 of gestation, secondary myogenesis by about day 38, and myogenesis is concluded by about day 110 (Wilson *et al.*, 1992; Maeir *et al.*, 1992; Greenwood *et al.*, 1999), at which time the number of muscle fibres is established and hypertrophy of myofibres commences (Greenwood *et al.*,

Table 2.1 Timing (days of gestation) of muscle development in livestock (after Brameld *et al.*, 2003)

	Myogenesis		Muscle hypertrophy	Gestation length	References
	1°	2°/3°			
Pig	35–60	54–90 (3°: ~ 35pn*)	70 →	~ 114	Wigmore and Stickland (1983); Lefaucheur <i>et al.</i> (1995); McPherson <i>et al.</i> (2004)
Sheep	32–38	38–110	110 →	~ 147	Wilson <i>et al.</i> (1992); Maeir <i>et al.</i> (1992); Greenwood <i>et al.</i> (1999)
Cattle	<47–90	90–200	200 →	~ 280	Russell and Oteruelo (1981); Robelin <i>et al.</i> (1991); Picard <i>et al.</i> (2002)

* days postnatal

1999). In cattle, primary myotubes appear prior to day 47 of gestation (Russell and Oteruelo, 1981) and secondary myotubes are present from around day 90 (Russell and Oteruelo, 1981; Robelin *et al.*, 1991), with myogenesis complete by about day 200 (Picard *et al.*, 2002). In the pig, primary myogenesis is evident by day 35 of gestation, secondary myogenesis by day 55, and myogenesis is believed to be completed by day 90 (Wigmore and Stickland, 1983; reviewed by Novakofski and McCusker, 2001), although there is some evidence of a wave of tertiary myogenesis during early postnatal life in pigs (Lefaucheur *et al.*, 1995).

While it is believed that the number of primary myotubes influences the total number of myofibres formed within a muscle, the number of secondary and/or tertiary myofibres formed appears more susceptible to environmental influences. Evidence exists that the number of myofibres can be altered at least to some extent by maternal nutritional deprivation during early to mid pregnancy in sheep (Everitt, 1968; Zhu *et al.*, 2004) and pigs (Wigmore and Stickland, 1983), but less so in cattle (Greenwood and Cafe, 2007). In pigs, improved nutrition (Dwyer *et al.*, 1993; Gatford *et al.*, 2003) during early to mid pregnancy may increase myofibre number, although this is not always the case (Nissen *et al.*, 2003). Likewise, somatotropin treatment of sows during early to mid gestation increased fetal muscle fibre numbers in some studies (Rehfeldt *et al.*, 1993, 2001) but not others (Gatford *et al.*, 2003) (see reviews by Rehfeldt *et al.*, 2004 and Rehfeldt and Kuhn, 2006). However, the extent to which these findings may also reflect differences in the growth of intrafascicularly terminating myofibres during early life is unclear (see Swatland and Cassens, 1973), and may account for differences in findings between apparently similar studies. Hence, an assessment of prenatal effects on myofibre number may be more reliable if undertaken later in life (for example, Greenwood and Cafe, 2007), when radial growth rather than elongation of myofibres is most responsible for myofibre hypertrophy (Swatland, 1994),

provided that accurate measurement of muscle cross-sectional area can be made. By comparison, with studies on environmental or exogenous effects during early to mid pregnancy, in sheep severely growth-retarded during later fetal life due to a placental restriction on growth (Greenwood *et al.*, 2000b), the number of myofibres formed was unaffected in various anatomical muscles relative to normally grown fetuses (Greenwood *et al.*, 1999, 2000a; McCoard *et al.*, 2000). However, runt pigs have fewer myofibres than their larger counterparts, demonstrating the severe influences on development that can occur during myogenesis (Hegarty and Allen, 1978; Rehfeldt and Kuhn, 2006).

Following completion of myogenesis, muscle satellite cell cycle activity is believed to be the sole source of new myonuclei in support of myofibre hypertrophy (MacConnachie *et al.*, 1964; Shafiq *et al.*, 1968) which continues, albeit at declining rates (Allbrook *et al.*, 1971; Greenwood *et al.*, 1999), until some time approaching mature muscle mass. By this time, the number of nuclei in muscle fibres, as indicated by the amount of DNA present, is maximal (Trenkel *et al.*, 1978; Solomon *et al.*, 1986; Di Marco *et al.*, 1987; Novakofski and McCusker, 2001), while the ratio of protein to DNA which is indicative of cell size in syncytial tissues such as muscle, continues to increase until mature muscle mass is attained (LaFlamme *et al.*, 1973; Trenkel *et al.*, 1978; Solomon *et al.*, 1986; Novakofski and McCusker, 2001). Hence, factors that regulate muscle growth, including age and liveweight (Allbrook *et al.*, 1971; Greenwood *et al.*, 1999), nutrition (Greenwood *et al.*, 1999) and growth promotants (Mulvaney *et al.*, 1988), also regulate muscle satellite cell activity (Greenwood *et al.*, 1999) and accumulation of myonuclei (Swatland, 1977; Trenkle *et al.*, 1978; Greenwood *et al.*, 2000a) either directly or indirectly, due to influences on muscle protein accretion rates (Greenwood *et al.*, 1999, 2000a).

It has been proposed that the extent to which commencement of myofibre hypertrophy during late gestation is delayed, as indicated by the ratio of protein to DNA or of sarcoplasm to myonuclei, may determine the extent to which capacity for postnatal muscle growth is compromised by severe fetal growth retardation (Figs 2.4 and 2.5; Greenwood *et al.*, 1999, 2000a). This may help explain why consequences of altered fetal growth and development during early gestation are usually more transient, irrespective of apparent effects on myofibre number during fetal life, than those that result from more severe, chronic effects on growth and size of fetuses during pregnancy (Rehfeldt *et al.*, 2004; Greenwood and Thompson, 2007). Irrespective of the direct effects of fetal nutrition on muscle development, the importance of prenatal effects on postnatal capacity for growth of the skeleton must also be considered in determining the consequences of fetal development on postnatal muscle growth.

Adipose tissue

The ontogeny and metabolism of brown adipose tissue in ruminant species has been reviewed by Smith and Carstens (2005), and development of white adipose tissue and lipid metabolism by Mersmann and Smith (2005). By contrast with ruminants, which have predominantly brown fat at birth (Alexander, 1978),

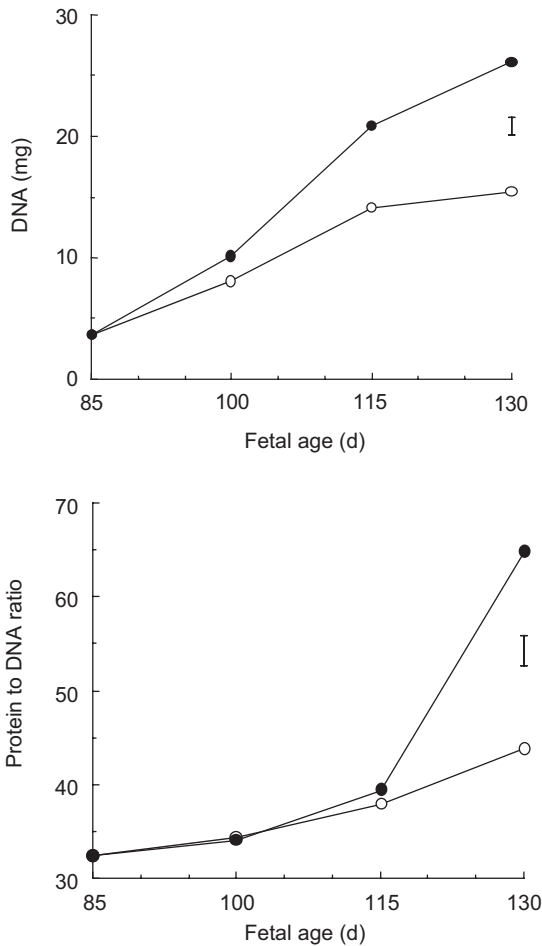


Fig. 2.4 Accretion of DNA and ratio of protein to DNA in normal (solid circles) and growth-retarded (open circles) fetal sheep *semimembranosus* muscle during growth from 85 to 130 days of pregnancy, demonstrating the commencement of muscle hypertrophy in normal lambs at ~115 days of age (from Greenwood *et al.*, 1999).

newborn pigs have little if any brown fat at birth (Herpin *et al.*, 2005). Ruminants (Alexander, 1978) and pigs (Herpin *et al.*, 2005) have only small amounts of white adipose tissue at birth.

In fetal sheep and cattle, non-subcutaneous adipose tissue is composed of brown fat, whereas subcutaneous adipose tissue, which regresses during late gestation, is composed of white fat (Alexander *et al.*, 1975; Alexander, 1978). Ruminant fetal adipose tissue depots comprising brown fat have high thermogenic potential, which aids survival of the newborn.

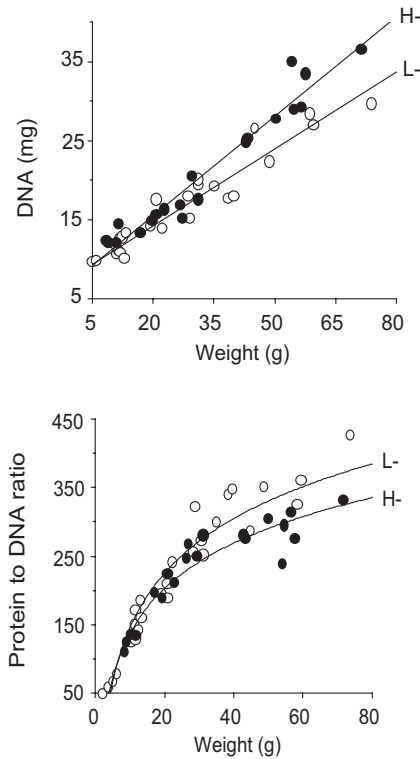


Fig. 2.5 Accretion of DNA and ratio of protein to DNA in normal (H-, solid circles, 4.8 kg) and low (L-, open circles, 2.2 kg) birth weight sheep *semitendinosus* muscle during postnatal growth to 20 kg live weight, demonstrating reduced muscle cellularity in the severely growth-retarded newborns (from Greenwood *et al.*, 2000a). Results are presented relative to weight of *M. semitendinosus*.

Accumulation of extractable lipid commences prior to day 50 of pregnancy in fetal sheep (Alexander, 1978). Lipid accumulation is observed in adipocyte cells from about day 70 in subcutaneous and perirenal adipose depots (Gemmell and Alexander, 1978), dissectible perirenal-abdominal adipose tissue is present from about day 70 of gestation and dissectible subcutaneous adipose tissue is present from about day 89 (Alexander, 1978). While pre-adipose cells of both depots have similar morphology, between days 89 and 90 of gestation perirenal fat develops into mitochondria-rich brown adipose tissue cells, whereas in subcutaneous depots white adipose cells with fewer mitochondria with less cristae develop during the same period (Gemmell and Alexander, 1978).

The amount of perirenal adipose tissue in sheep increases by about one-third during the last 3 weeks of gestation (Alexander, 1978) and adipocyte volume by about 40% during the final 4 weeks of gestation (Vernon *et al.*, 1981), suggesting that hypertrophy rather than hyperplasia of adipocytes is responsible for the

increase in mass during this period. Concurrently, subcutaneous fat involutes between about day 115 and term, by which time little if any is present (Alexander, 1978). In the fetal pig, whole-body lipid content increased from about 0.25% to 0.75% from 30 days of gestation until term (Mitchell *et al.*, 2001), which compares with 2% or so in newborn lambs (Alexander, 1974; Greenwood *et al.*, 1998).

Brown fat transforms into white fat during the first weeks of postnatal life in ruminants (Gemmell *et al.*, 1972; Alexander *et al.*, 1975; Clarke *et al.*, 1997) and to a large extent adipocyte hyperplasia is a postnatal phenomenon (Allen, 1976) as discussed later.

2.3.2 Postnatal development and growth

Birth and early postnatal development is characterised by maturation of organ tissue and metabolic systems essential for survival and subsequent growth, provision of immunoglobulins in colostrum, and a massive increase in the supply of nutrients. Birth is also characterised by a pronounced shift in the composition of nutrients available to the newborn from the dam, most notably a large increase in the supply of lipids in milk, and of nutrients subsequently available as solid feed.

Although bone and muscle continue to grow in an essentially linear manner during postnatal growth to mature size, adipose tissue grows at an increasing rate (Fig. 2.1). Hence, during postnatal growth, the percentage of bone and muscle in the body declines while that of adipose tissue increases (Black, 1983; Mitchell *et al.*, 2001).

Growth of bone continues until closure of the growth plates post-puberty, at about which time mature skeletal size is attained. Muscle growth continues as a result of hypertrophic accretion of muscle protein coupled with the incorporation of nuclei which result from cell cycle activity and mitosis in satellite cells (Novakofski and McCusker, 2001). Cell cycle activity of satellite cells continually slows until it essentially ceases when the number of myonuclei in muscle reaches a maximum, after which time protein accretion continues for a period until the maximum ratio of sarcoplasm to myonuclei or of protein to DNA is reached. At this time mature lean body mass is attained. Growth of fatty tissues during postnatal life is supported by postnatal hyperplasia, particularly at younger ages (Allen, 1976), and by hypertrophy of adipocytes until a maximal size is reached, beyond which they do not grow (Mersmann and Smith, 2005). Adipose depots continue to grow as long as the supply of energy in excess of requirements for other bodily functions is exceeded, and involves both hypertrophy and hyperplasia (Mersmann and Smith, 2005).

2.4 Consequences of prenatal and postnatal growth and development for carcass composition and meat quality

In this section we briefly review the consequences at market weights of altered growth and development during embryonic, fetal, pre-weaning, backgrounding or grow-out, and finishing phases.

The extent to which prenatal and postnatal growth and development of the carcass tissues is influenced by the external environment differs due to the moderating influence of the dam on the fetus and neonate which ceases at weaning, and due to changes in the amount and types of nutrients available to, and utilised by, the offspring. These changes are associated with a shift from autocrine and paracrine regulation of tissue development, towards endocrine regulation as the animal ages and is increasingly subjected to effects of the external environment. Consequently, the extent to which the marketable carcass is influenced by environmental effects and alterations in growth and development at different stages of life also varies.

Postnatal nutritional effects on body composition are complex due to factors including the partition of metabolisable energy between the requirements of maintenance, protein synthesis, lipogenesis, and heat loss associated with nutrient utilisation (Black, 1983; Burrin, 2001; Oddy and Sainz, 2002). Factors that influence the partition of absorbed nutrients and hence affect body composition include: maturity of the animal; level of feeding relative to maintenance; protein absorption relative to requirements; balance of protein to energy in the diet and available for absorption by the animal; prior nutrition that may result in compensatory growth and affect the composition of gain; and climatic conditions that may limit growth rate and fat synthesis. A detailed account of influences of these factors on the composition of livestock and their carcasses at market weights is beyond the scope of this chapter; hence the reader is referred to the aforementioned reviews and to Part III (Chapters 14 to 19) of this book.

2.4.1 Prenatal growth and development (including weight and composition at birth)

The embryonic and early fetal periods, encompassing early to mid gestation, represent phases of maximal development as genesis of most organs and tissues commences and is completed during this time, as detailed in Section 2.3. Generally speaking, this is a hyperplastic period of tissue development when increasing cell number is the major contributor. This contrasts with later fetal, neonatal and postnatal development when maturation and, increasingly, hypertrophy of tissues and organs occurs. Hence, it is the embryonic and early fetal periods of development that are considered to hold promise to enhance subsequent productive characteristics. To date, most research relating to this period in livestock has focused on postnatal consequences of altered maternal nutrition prior to conception and/or during early to mid gestation (reviewed, in sheep, by Greenwood and Thompson, 2007). Beyond ensuring maternal nutrition is adequate during late gestation and lactation to support adequate fetal and neonatal growth, little of consequence to establish reliable treatments or management practices to enhance carcass tissue development has ensued from this research thus far. However, a recent study has reported that increasing gestation feeding allowance had little effect on pig growth performance to slaughter but did reduce carcass fatness (Lawlor *et al.*, 2007).

Table 2.2 Consequences of growth *in utero* for carcass and yield characteristics of beef cattle at 30 months of age (Greenwood *et al.*, 2006a; Greenwood and Cafe, 2007)

Variable	Prenatal growth/Birth weight		Significance of difference (<i>P</i>)
	Low (<i>n</i> = 120)	High (<i>n</i> = 120)	
Birth weight (kg)	28.6	38.8	<0.001
<i>At equivalent age (30 months):</i>			
Carcass weight (kg)	364	396	<0.001
Retail yield (kg)	239	257	<0.001
<i>At equivalent carcass weight (380 kg):</i>			
Eye muscle area (cm ²)	90.4	88.9	0.25
P8 fat depth (mm)	21.3	19.6	0.048
Rib fat depth (mm)	10.9	10.5	0.35
Aus-Meat marble score	1.83	1.86	0.56
USDA marble score	447	444	0.98
<i>Longissimus</i> IMF%	6.8	7.0	0.62
Ossification score	206	195	0.009
Retail yield (kg)	249	247	0.20
Bone (kg)	66.9	67.6	0.10
Fat trim (kg)	54.6	56.0	0.58

Note: Values are predicted means from REML analyses including effects of birth weight, pre-weaning growth, sex/year cohort, sire-genotype and their interactions, with carcass weight as a covariate (linear and, where significant, quadratic) to predict means at equivalent carcass weight. IMF = intramuscular fat.

Severe, chronic fetal growth retardation, resulting in low birth weight, may limit skeletal and muscle growth potential and result in a predisposition towards reduced mature lean body mass and increased fatness at any given postnatal live weight in pigs (Powell and Aberle, 1980; Collins *et al.*, 2007a) and sheep (Villette and Theriez, 1981; Greenwood *et al.*, 1998; Louey *et al.*, 2005). Fetal growth retardation may also limit postnatal growth capacity in cattle although it had little effect on *bovine* carcass composition in the longer term (Table 2.2; Tudor *et al.*, 1980; Greenwood *et al.*, 2006a; Greenwood and Cafe, 2007), despite an increase in ossification of bone (Greenwood *et al.*, 2006a) suggesting a potential affect on mature size. Hence, it appears that effects of prenatal growth on postnatal carcass composition are of consequence only if more prolonged and severe fetal growth retardation, resulting in very low birth weight, occurs.

A direct influence of prenatal growth and nutrition on postnatal adiposity of livestock is uncertain. In severely growth-retarded newborn lambs, differences in fatness compared to normal size newborn lambs were not evident at birth but occurred due to rapid accretion of fat during the early post-partum period, and this difference persisted during subsequent growth to weaning (Greenwood *et al.*, 1998; de Blasio *et al.*, 2007). Rather than being a specific cause of increased postnatal adiposity, restricted prenatal growth may predispose the growth-retarded

newborn to increased adiposity during early postnatal life due to more fetal-like metabolic characteristics at birth, low maintenance energy requirements and limited capacity for lean tissue growth, coupled with high early postnatal relative feed intake (Greenwood *et al.*, 1998, 2000a, 2002a; Greenwood and Bell, 2003). As discussed earlier, adipose tissue in fetal ruminants is mainly brown fat, which has high thermogenic potential for the newborn and transforms into white fat during the early postpartum period, and adipocyte hyperplasia continues during postnatal life. In this regard, the newborn pig also has very low levels of white adipose tissue at birth (<2%), which accretes very rapidly post-partum, as reviewed by Herpin *et al.* (2005).

Increasingly, studies are being conducted that seek to limit intrauterine growth retardation and variability in growth between fetuses within *porcine* litters and/or enhance fetal growth and development in livestock species. This research has focused more on early to mid pregnancy, and aims to alter the uterine environment in which the embryo resides and to increase placental blood flow and/or the amount and quality of nutrients supplied to the fetus. These studies, which have been reviewed by Wu *et al.* (2006) and Rehfeldt and Kuhn (2006), have included: maternal supplementation with arginine and other factors that increase placental blood flow and fetal growth; supplementation with selenium due to its antioxidant properties; and administration of somatotropin and other growth promotants or repartitioning agents to the pregnant dam, with a view to enhancing embryonic, placental and fetal growth and muscle development. Results of studies on growth promotants and repartitioning agents have been variable, and while supplementation with factors that increase placental blood flow have had positive effects *in utero*, postnatal consequences have not been reported. Hence, the challenge still remains to develop reliable management practices during fetal development to enhance postnatal growth and carcass characteristics.

Altered maternal nutrition and/or restricted growth during prenatal life has also had little effect at typical market weights on meat quality characteristics, including ultimate pH, objective textural measurements, intramuscular fat percentage, water-holding capacity and meat colour, in sheep (Greenwood and Thompson, 2007), cattle (Greenwood *et al.*, 2006a; Greenwood and Cafe, 2007) or pigs (Nissen *et al.*, 2003). However, at least in pigs, there is evidence that differences in carcass composition and muscle fibres between light and heavy birth weight pigs may impact on meat quality, with drip loss higher in meat from light birth weight animals (Rehfeldt and Kuhn, 2006). Also, low birth weight pigs have fewer but larger muscle fibres at slaughter (Gondret *et al.*, 2006). While Gondret *et al.* (2006) found no difference in glycolytic potential at slaughter, postmortem pH decline or drip loss of meat of light and heavy birth weight pigs, a trained sensory panel found the loin from the light birth weight pigs to be less tender.

Irrespective of the outcomes of studies conducted to date on postnatal consequences of altered maternal environments and fetal growth and development, the extent to which variation in prenatal development contributes to unexplained variation in postnatal growth and commercially important carcass and meat quality characteristics remains unclear and warrants further investigation (Bell, 2006).

This is particularly so, given recent advances in the understanding of epigenetic mechanisms and of technologies capable of elucidating these mechanisms (see [Wu et al.](#), 2006).

2.4.2 Pre-weaning growth and development (including weight and composition at weaning)

The pre-weaning period is characterised by an increase in the supply of nutrients, and a shift in the quality of nutrients away from primarily glucose and amino acids towards a diet higher in fat. While growth of the fetus during late pregnancy is constrained (Bell *et al.*, 2005), far more rapid growth is achievable from birth to weaning when lactational performance and nutrient intake is not seriously compromised.

Animals that are substantially smaller at weaning generally remain smaller during subsequent growth (Berge, 1991; King, 2003; Pluske *et al.*, 2005; Greenwood *et al.*, 2006a; Cafe *et al.*, 2006b). Prior to weaning, it is possible to alter the composition of the young animal by altering the total supply of nutrients (Berge, 1991; Cafe *et al.*, 2006a). Both increased total nutrient supply and more rapid growth to weaning generally result in increased fatness at weaning (Hodge, 1974; Greenwood *et al.*, 1998; Cafe *et al.*, 2006a), which can contribute to a somewhat fatter carcass at heavier market weights (Table 2.3, Greenwood *et al.*, 2006a; Greenwood and Cafe, 2007), although not consistently in ruminants (Berge, 1991) or pigs (Wolter *et al.*, 2002a; Klindt, 2003).

Despite potential to affect gross carcass composition by altering nutrition and growth to weaning, meat quality characteristics, including ultimate pH, objective measures of texture, water-holding capacity, colour, and marbling score and intramuscular fat percentage, are little affected in the longer term by nutrition during this period in cattle (Greenwood *et al.*, 2006a; Greenwood and Cafe, 2007) and likely other species.

2.4.3 Early weaning

Early weaning has attracted interest as a means of enhancing the reproductive performance of the dam by conserving maternal body energy reserves prior to mating.

In recent large-scale studies, early weaning of lambs did not affect time to reach a market live weight of 45 kg provided that post-weaning nutrition was not compromised, due to more rapid growth post-weaning than in early- rather than later-weaned lambs (Hopkins *et al.*, 2007a). There was little effect of early, compared to later, weaning on lean, fat or mineral ash (bone) percentage of carcasses, although when combined with restricted growth post-weaning, early weaning resulted in heavier carcasses with larger *longissimus* cross-sectional areas at 45 kg live weight (Hopkins *et al.*, 2007a). Weight at weaning had little effect on meat quality attributes (Hopkins *et al.*, 2007b).

Evidence from studies of cattle suggests that consequences of early weaning or

Table 2.3 Consequences of growth from birth to weaning for carcass characteristics of beef cattle at 30 months of age (Greenwood *et al.*, 2006a; Greenwood and Cafe, 2007)

Variable	Pre-weaning growth		Significance of difference (<i>P</i>)
	Slow (<i>n</i> = 119)	Rapid (<i>n</i> = 121)	
Pre-weaning ADG (g)	554	875	<0.001
Weaning weight (kg)	151	221	<0.001
<i>At equivalent age (30 months)</i>			
Carcass weight (kg)	368	393	<0.001
Retail yield (kg)	242	254	<0.001
<i>At equivalent carcass weight (380 kg)</i>			
Eye muscle area (cm ²)	90.1	89.2	0.55
P8 fat depth (mm)	20.1	20.8	0.41
Rib fat depth (mm)	10.4	11.0	0.33
Aus-Meat marble score	1.92	1.77	0.15
USDA marble score	450	441	0.49
<i>Longissimus</i> IMF%	6.88	6.98	0.80
Ossification score	202	199	0.53
Retail yield (kg)	251	246	<0.001
Bone (kg)	67.8	66.7	0.053
Fat trim (kg)	52.8	57.8	<0.001

Note: Values are predicted means from REML analyses including effects of birth weight, pre-weaning growth, sex/year cohort, sire-genotype and their interactions, with carcass weight as a covariate (linear and, where significant, quadratic) to predict means at equivalent carcass weight. IMF = intramuscular fat.

weaning at light weights on subsequent carcass and beef quality characteristics are influenced by supplements fed prior to weaning and the diet onto which the calves are weaned (Tudor *et al.*, 1980; Wertz *et al.*, 2001). Early weaning may (Myers *et al.*, 1999a,b; Fluharty *et al.*, 2000; Schoonmaker *et al.*, 2004) or may not (Myers *et al.*, 1999c; Barker-Neef *et al.*, 2001; Schoonmaker *et al.*, 2001, 2003; Arthington *et al.*, 2005) result in altered carcass grades, fatness or intramuscular fat content, or affect other meat quality characteristics in cattle (Myers *et al.*, 1999c; Schoonmaker *et al.*, 2001; Meyer *et al.*, 2005).

Hohenshell *et al.* (2000) did not detect any differences in carcass yield, percentage of lean tissue or loin muscle depths between early (10 days of age) and conventionally weaned (30 days of age) pigs raised under the same environment. However, in another study, Dunshea *et al.* (2003) found that pigs weaned at 14 days of age had higher P2 back fat at 23 weeks of age compared to those weaned at 28 days of age (13.1 v 10.9 mm), while in another investigation Dritz *et al.* (1996) observed that pigs weaned at 9 days of age and fed a low complexity diet post-weaning had a higher carcass lipid content than pigs weaned onto the same diet at 19 days of age. More recently, Collins *et al.* (2005) found that early weaned

(17 days of age) pigs were leaner than those weaned later (23 days of age). There are few data on the effect of weaning age on meat quality attributes of pigs.

2.4.4 Post-weaning growth and development

Results of studies on the influence of post-weaning nutrition and growth rates on carcass composition of lambs vary, as discussed by Black (1983) and Lewis *et al.* (2006), due to factors described previously. In this regard, it has been proposed that 'management systems capable of attaining specific growth rates tailored for appropriate genotypes offer the best options to meet market specifications in terms of muscle development, fatness, including marbling, and consumer assessed meat quality' (Oddy and Sainz, 2002). Generally consistent with results from pigs (Fig. 2.2), these authors concluded that in lambs of similar age and genotype, more rapid compared to slower growth results in fatter carcasses with smaller muscles and more intramuscular fat at the same live weight.

Short-term severe nutritional restriction post-weaning may increase the time taken to reach market weight but appears to have little effect on carcass characteristics. In sheep held at their weaning weight or fed *ad libitum* for 55 days following weaning at 20 or 30 kg live weight, little or no difference in the percentages of carcass fat, lean and bone mineral content at 45 kg live weight were observed (Hopkins *et al.*, 2007a). Similarly, in cattle grown on pasture following severe nutritional restriction for 120 days that resulted in a 15% live weight loss during the immediate post-weaning period, differences in compositional characteristics at approximately 500 kg live weight including dressing percentage, subcutaneous fat depth, eye muscle area, marbling score, intramuscular fat content, and bone mineral content, were not evident (Tomkins *et al.*, 2006). In this latter study, objective and subjective (taste panel) measures of beef quality did not differ due to nutrition immediately post-weaning (Tomkins *et al.*, 2006). In contrast, lambs fed a restricted diet post-weaning tended to have less acceptable meat quality, although this effect was less evident in lambs of sires with high breeding values for fatness (Hopkins *et al.*, 2007b).

Compensatory growth responses after periods of induced dietary restriction have been shown to occur in pigs (McMeekan, 1940; Elsley, 1963; Campbell and Biden, 1978; Chiba 1994, 1995; Wolter *et al.*, 2002a,b,c; 2003a,b; Whang *et al.*, 2003; Collins *et al.*, 2007b) although the growth and carcass composition responses have been variable, possibly related to the length, duration, type (energy v protein) and severity of the restriction.

More rapid growth of cattle at pasture over the entire post-weaning period to the same market weight or to feedlot entry can increase carcass fatness and intramuscular fat percentage at slaughter following finishing (Robinson *et al.*, 2001; McKiernan and Wilkins, 2007; Wilkins *et al.*, 2009; Table 2.4). Despite this, retail beef yield and visual marbling scores did not differ at the same carcass weight following differing backgrounding growth rates (Wilkins *et al.*, 2009; Table 2.4). Prolonged, post-weaning growth at more rapid rates may also improve beef-eating quality (Perry and Thompson, 2005; McKiernan *et al.*, 2007), although

Table 2.4 Consequences of growth from weaning (backgrounding) to feedlot entry at the same live weight (approximately 380 kg) on carcass characteristics of beef steers following 100 day feedlot finishing (adapted from Wilkins *et al.*, 2008)

Variable	Backgrounding growth		Significance of difference (<i>P</i>)
	Slow (<i>n</i> = 260)	Rapid (<i>n</i> = 299)	
Backgrounding ADG (g)	467	747	<0.001
HSCW (kg)	363	357	0.002
Dressing %	55.8	56.2	0.106
P8 fat depth (mm)	16.9	17.7	0.118
Rib fat depth (mm)	9.7	11.2	<0.001
AUS marble score	1.36	1.39	0.224
USDA marble score	347	347	0.540
<i>Longissimus</i> IMF%	3.61	4.15	0.001
Eye muscle area (cm ²)	81.3	82.9	0.008
VIAscan retail yield %	68.1	68.0	0.307
Ossification score	184	181	0.006
Meat colour	2.49	2.32	0.002
Fat colour	0.84	0.79	0.280

Note: Values are predicted means from analyses including effects of backgrounding growth, cohort, sire-genotype and their interactions, with carcass weight as a covariate to predict means at equivalent carcass weight where significant. IMF = intramuscular fat.

improvements were not consistent across breed types, geographic locations, and muscles (Perry and Thompson, 2005). In this regard, while beef-eating quality characteristics decline with age (Perry and Thompson, 2005), serious adverse effects of growth on beef tenderness are evident only if retarded growth during backgrounding results in animals being substantially older (for example, by about 9 months) when they reach the same market weight (Purchas *et al.*, 2002).

In recent studies of lambs at pasture, more rapid compared to slower growth for their entire postnatal life resulted in increased carcass fatness at equivalent carcass weight when assessed by CT-scan, but not when determined chemically (Cake *et al.*, 2006; Hegarty *et al.*, 2006). However, prolonged slower growth post-weaning increased toughness (Hopkins *et al.*, 2005) and ossification score (Cake *et al.*, 2006).

2.4.5 Finishing

More rapid growth of cattle during finishing or use of feedlot compared to pasture finishing generally increases carcass fatness and intramuscular fat content (Robinson *et al.*, 2001; Troy *et al.*, 2002; Reverter *et al.*, 2003), and may (Johnston *et al.*, 2003; Perry and Thompson, 2005) or may not (Troy *et al.*, 2002) improve meat tenderness, depending upon factors such as prior nutrition and the duration of concentrate feeding.

2.5 Influences of metabolic modifiers on carcass characteristics

There are a number of technologies that impact on carcass characteristics and meat quality and these have been reviewed recently (Beermann and Dunshea, 1995; Dunshea *et al.*, 2005; Dikeman, 2007). The major metabolic modifiers used in the livestock industries are the hormone growth implants (HGP), β -agonists and somatotropin.

2.5.1 Hormone growth implants

Estrogenic and androgenic growth-promoting agents have been widely used in the beef cattle industry for almost fifty years to increase growth performance and profitability. A number of individual compounds are approved for use either alone or in combination and these include naturally occurring steroids such as 17-oestradiol, progesterone and testosterone, and their synthetic counterparts zeranol, melengestrol acetate and trenbolone acetate. Doses of individual compounds vary among the several approved combination implants. Estrogenic products are effective in steers, androgenic products are effective in heifers, and combination products are also effective. Detailed descriptions of the chemistry and mechanisms of action of estrogenic and androgenic compounds have been published (Hancock *et al.*, 1991; Sillence, 2004). These anabolic agents increase rates of muscle protein synthesis and deposition and/or decrease protein degradation, and also decrease the amount of fat at a particular live weight. Although implants increase feed intake by 5 to 10%, they decrease the amount of energy required for maintenance, increasing the amount available for growth, thereby improving feed efficiency by 5 to 15%. Daily gain is improved by up to 25% when aggressive implant strategies are used in cattle fed high-concentrate diets (Bartle *et al.*, 1992; Johnson *et al.*, 1996; Perry *et al.*, 1991). Comprehensive summaries of the effects of implant strategies using various combinations of commercial products indicate that increasing the anabolic implant dose, up to a point, increases the weight at which animals reach a common body composition or lean-to-fat ratio (Bartle *et al.*, 1992; Guioy *et al.*, 2002). Unlike for ruminant animals, anabolic implants appear to have little effect upon growth performance and carcass quality in pigs and so are not used in pork production.

The effects of estrogenic and androgenic agents on meat quality have been the subject of much debate. A review of possible effects of implant strategies on beef quality concluded that current anabolic implants have only subtle, if any, effects on tenderness measured either objectively or subjectively (Nichols *et al.*, 2002). For example, in one large study with 2748 steers, Barham *et al.* (2003) reported that there was no significant effect of moderate implant strategies on shear force in 21-day aged steaks, although differences in tenderness were detected by a trained sensory panel but not by an untrained consumer panel. For steaks that were aged for less than 21 days, HGP treatment resulted in increased shear values. Another comprehensive study conducted at five ranches, which assessed implanting at up to five stages through growth, found that implanting at one or more stages had negative effects on meat palatability, marbling scores and shear force (Platter *et al.*,

2003). The Meat Standards Australia (MSA) beef grading system, which predicts palatability of individual muscles from a range of production and processing inputs, has recently incorporated a negative effect of anabolic implants into its prediction model. The effect is dependent upon the muscle and can be moderated by ageing of the meat and tenderstretch hanging. A number of studies contributed to the MSA decision to incorporate effect of anabolic implants. Firstly, targeted taste panel tests of beef cuts were undertaken using untrained consumer studies. It was concluded that heifers or steers implanted with Revalor-H and -S, and slaughtered within the implant payout period, resulted in tougher meat. The greatest response was in those muscles with the highest ageing rates *post-mortem*. This was consistent with a mechanism for increased lean deposition being due, in part, to reduced protein degradation, possibly as a result of increased calpastatin activity in the live animal, which results in lower ageing rates and less tender meat *post-mortem*. This effect was most evident in those muscles with the fastest ageing rates, as presumably these muscles would respond most to increased calpastatin activity (Thompson *et al.*, 2008). Those authors also found effects on compressive toughness, consistent with changes in connective tissue structure accompanying changes in calpastatin activity, but having longer half-lives. In a second study, a number of different hormone growth promotant (HGP) implant strategies resulted in a decrease in sensory scores, particularly tenderness (Watson *et al.*, 2008). Whilst all HGP implant strategies resulted in an increase in ossification scores and a decrease in marbling scores relative to controls, the differences in sensory scores were still apparent after adjustment to the same ossification and marbling scores. Finally, Watson (2008) undertook a meta-analysis on 32 studies with 22 treatment-control comparisons for taste panel tenderness evaluations; and on 18 studies, with 24 treatment-control comparisons for shear-force measurements. When all these data were considered, it was concluded there was a small but highly significant increase in shear force of 0.3 kg and a 5% decrease in taste-panel tenderness score (Watson, 2008). Consistent with earlier analyses, however, more aggressive growth promotion strategies led to greater effects on tenderness.

2.5.2 β -agonists

The β -agonist ractopamine has recently been approved for use in many countries as an in-feed ingredient to increase lean tissue growth and improve production efficiency in pigs. Treatment of pigs with β -agonists, particularly ractopamine, generally has resulted in dose-dependent improvements in average daily gain, feed conversion ratio and carcass lean content. Feed intake is typically unchanged (Adeola *et al.*, 1990; Gu *et al.*, 1991; Yen *et al.*, 1991) or decreased slightly (Adeola *et al.*, 1990; Watkins *et al.*, 1990; Mitchell *et al.*, 1991) during β -agonist treatment. While there is general agreement that protein deposition is increased during β -agonist treatment, effects on fat deposition have been equivocal. For example, while ractopamine increased protein deposition in boars, gilts and barrows, there was little effect on fat deposition (Dunshea *et al.*, 1993a). A review of the literature suggests that ractopamine does not decrease backfat measured

along the midline (Aalhus *et al.*, 1990; Dunshea *et al.*, 1993a,b, 1998a) whereas backfat depths measured off the midline have been either decreased (Aalhus *et al.*, 1990; Adeola *et al.*, 1990; Dunshea *et al.*, 1993a) or unchanged (Mitchell *et al.*, 1991; Dunshea *et al.*, 1993b, 1998a; Sainz *et al.*, 1993). Because ractopamine has been approved in the USA and elsewhere, additional data on its effects have become available. A summary of proprietary information from 20 studies conducted in the late 1980s to early 1990s indicated a reduction in backfat at the 10th rib in relatively fat animals (Schinckel *et al.*, 2001). In a more recent experiment with leaner pigs, there was a reduction in 10th rib backfat due to ractopamine feeding, although the response was not as great (Schinckel *et al.*, 2001). However, a summary of a number of Australian studies with ractopamine using a variety of treatment regimens in different classes of pigs concluded that although lean meat yield was increased, there were no significant effects of ractopamine on backfat measured at either the P2 site or over the leg (Smits and Cadogan, 2003).

The β -agonists act directly through β -adrenergic receptors on adipocytes and influence cellular metabolism via signalling cascades. In overview, ractopamine or other β -agonists indirectly lead to decreased *lipogenesis* (fat synthesis and storage) and increased *lipolysis* (fat mobilisation and hydrolysis) (Liu and Mills, 1989; Dunshea, 1993; Mersmann, 1998). The rate of fat accumulation in adipocytes or growth of the adipose tissue mass slows, particularly in ruminants, resulting in a leaner animal. The magnitude of these changes is influenced by dose and duration of treatment with the β -agonist, the type of β -agonist, and the animal species (Beermann, 1993; Mersmann, 1998). For example, ractopamine and other β -agonists do not appear to decrease fat deposition in pigs because of a combination of rapid down-regulation of adipocyte β -adrenergic receptors (Dunshea, 1993; Dunshea and King, 1995; Dunshea *et al.*, 1998b) and a relative insensitivity of *porcine* adipocytes to β -agonists (Dunshea and D'Souza, 2003). On the other hand β -agonists have pronounced effects on fat deposition in ruminants (Dunshea *et al.*, 2005).

Skeletal myocytes also express β -adrenergic receptors, which transduce signal from β -agonist to muscle metabolic enzymes in a dose-dependent manner (Byrem *et al.*, 1996). While there is uncertainty as to whether the increased protein deposition is due to changes in synthesis and/or degradation, the majority of the data support increased synthesis, at least in pigs (Beermann, 2002). In ruminants, it appears that a variety of different β -agonists increase the activity of the major skeletal muscle protease inhibitor calpastatin (Kretchmar *et al.*, 1990; Koohmaraie *et al.*, 1991; Wheeler and Koohmaraie, 1992; McDonagh *et al.*, 1999) suggesting that β -agonists decrease protein degradation. Some of the discrepancies between studies probably relate to differences in the β -agonist studied as well as differences between species.

Effects of β -agonists on meat quality are equivocal, particularly in pigs. At least some of the confusion must arise from the fact that all β -agonists are not equally potent and also that cells, tissues and individual animals vary greatly in expression of β -adrenergic receptors and the metabolic pathways linked to them. In order to

reconcile many of the, at times, subtle effects of β -agonists on meat quality, the readily available literature on meat quality was collated for a number of muscles from a variety of genotypes treated with a range of doses of various β -agonists (salbutamol, cimaterol, clenbuterol, ractopamine, zilpaterol and L644,969) for ruminants and pigs (Dunshea *et al.* 2005). While there were insufficient data obtained from ruminants to conduct a meta-analysis, it seems reasonable to conclude that most β -agonists decrease intramuscular fat and increase shear force, drip loss and ultimate pH. There are much more data for pigs, and from the meta-analysis it appears that there are differences in the responses to the various β -agonists, with cimaterol having the most pronounced effects on reducing intramuscular fat and increasing shear force and drip loss. On the other hand, ractopamine and salbutamol had no effect on intramuscular fat content and had either no effect (ractopamine) or decreased drip loss (salbutamol). Ractopamine and salbutamol both increased shear force by approximately 0.5 kg. In general, β -agonist treatment caused a slight (+0.02) increase in ultimate pH and decreased the a^* and b^* colour values of pork loins, indicating a decrease in redness and yellowness of the meat. The limited data on consumer preferences would suggest that ractopamine causes a decrease in tenderness (–6%), a negligible decrease in flavor (–1%), and has no effect on juiciness. In keeping with the lack of effect of ractopamine on intramuscular fat, there is relatively little effect of ractopamine on the fatty acid composition of intramuscular or subcutaneous fat. It should be borne in mind when summarising these effects that most of the data on pork quality from pigs fed ractopamine were obtained in studies where 20 ppm of ractopamine were fed. Under most commercial situations, the levels of ractopamine fed are between 5 and 10 ppm and so effects on meat quality may be less than suggested by the meta-analysis.

2.5.3 Somatotropin

Somatotropin (ST) is a naturally occurring protein hormone produced by the anterior pituitary gland and secreted into the circulation. Somatotropin has several important roles in the regulation of development and growth of skeletal muscle, bone, adipose tissue, and the liver in growing animals and plays an integral role in the coordination of lipid, protein, and mineral metabolism in mammalian species. Elevation of plasma ST redirects nutrients toward increased muscle and bone growth and decreased adipose tissue growth in meat animals (Etherton and Bauman, 1998). In 1994, the US Food and Drug Administration approved a prolonged release *bST* formulation for use in lactating dairy cows but currently no ST products are approved for beef or lamb production. On the other hand, *porcine* ST is approved for use in many countries (Dunshea *et al.*, 2002), although not in the USA or the EU. As a result of these commercial applications and approvals, most of the data discussed will be from pigs treated with *pST*, although some ruminant data exists, particularly with respect to mechanisms of action.

Exogenous *pST* treatment consistently improves average daily gain, feed conversion efficiency and protein deposition and reduces fat deposition. Its

efficacy in improving growth performance is unquestioned (Etherton *et al.*, 1987; Campbell *et al.*, 1988, 1990a,b, 1991; King *et al.*, 2000). Under commercial conditions, *p*ST is delivered as either a daily, bidaily or thrice weekly injection (ca. 5 mg/day), administered using a propane-powered applicator (Dunshea, 2002; Dunshea *et al.*, 2002). Dose-dependent increases in protein deposition and reductions in feed intake, fat deposition and carcass fat have been observed (Etherton *et al.*, 1987; Krick *et al.*, 1992). Qualitatively similar responses occur in ruminants although the responses may not be as marked because the supply of nutrients may be limiting (National Research Council, 1994).

The increased protein deposition is not restricted to skeletal muscle as ST increases protein deposition in all tissues including skin (Caperna *et al.*, 1994; Robertson *et al.*, 1997; Suster *et al.*, 2004) and visceral organs (Caperna *et al.*, 1994). Indeed, the proportional increases in protein deposition in skin and viscera may be greater than in skeletal muscle, contributing to a reduction in dressing rate (ca. -1% in pigs). Also, ST can increase bone deposition (Suster *et al.*, 2004), impacting on boneless meat yield percentage. Therefore, the increases in growth rate observed with ST treatment need to be offset against the decreases in dressing and boneless meat yield percentage.

Slight or no effect of *p*ST on objective and subjective measures of meat tenderness, appearance and shelf-life have been reported in some studies (Wander *et al.*, 1993; Aalhus *et al.*, 1997). However, others reported that pork from *p*ST treated pigs had lower consumer preference scores for tenderness, juiciness and overall acceptability (D'Souza and Mullan, 2002). In order to reconcile many of the, at times, subtle effects of *p*ST on meat quality, the readily available literature on meat quality were collated for a number of muscles from a variety of genotypes treated with a range of doses of *p*ST, and the data obtained for the loin muscles were subjected to a meta-analysis (Dunshea *et al.*, 2005). From this meta-analysis it appeared that *p*ST decreases intramuscular fat (-12%), increases shear force (+9%) and reduces drip loss (-6%). The limited data on consumer preferences suggested that there is a decrease in tenderness (ca. -9%), although there is no effect upon juiciness or flavor. Importantly, there may be some interactions between muscle types, processing, gender and genotype (Aalhus *et al.*, 1997; D'Souza and Mullan, 2002), although with this limited data set, this type of analysis was unable to determine these effects. In general, it appears that *p*ST does cause a small increase in shear force and sensory perceptions of toughness in pork but it is uncertain whether this would be consistently detected by consumers as there is no difference in perceptions of flavor or juiciness. Certainly, greater rates of deposition of collagen and other connective tissue proteins into the perimysial connective tissue would be expected to increase sensory toughness and perhaps also shear force (Harper, 1999). There are far fewer studies in ruminants, but those that have been conducted suggest similar findings as for pigs, at least with respect to intramuscular fat and shear force. In this context, *b*ST treatment was found to decrease intramuscular fat in goats (Kouakou *et al.*, 2005) and Friesian heifers (Vestergaard *et al.*, 1995), and marbling score in steers (Dalke *et al.*, 1992). While there were no significant effects on shear force, the magnitude of the increases

were similar in the former two studies to that observed in pork from pigs treated with *pST*.

The majority of studies have reported no effect of *pST* on muscle fibre type distribution (Beermann *et al.*, 1990; Oksbjerg *et al.*, 1995; Ono *et al.*, 1995; Aalhus *et al.*, 1997). However, some authors have reported an increase in the proportion of fast oxidative–glycolytic myofibres and a decrease in proportion of fast glycolytic myofibres in the *longissimus* muscle (Solomon *et al.*, 1990, 1991). In contrast, a decrease in the proportion of fast oxidative–glycolytic fibres and an increase in the proportion of fast glycolytic fibres were evident in another study (Whipple *et al.*, 1992). Solomon *et al.* (1990) reported that *pST* administration increased muscle fibre size and subsequent shear force, an objective measure of tenderness, of fresh pork. The use of *pST* has also been reported to reduce calcium-activated proteolysis in the *longissimus* muscle, thereby preventing improvements in tenderness during the ageing process (Weikard *et al.*, 1992).

2.6 Genotypic influences on carcass composition

Domesticated ruminants and pigs have been selected for traits including survival and reproductive capacity, growth rate, muscularity, yield of meat, and eating quality characteristics including intramuscular fat content. In this section we provide a brief overview of effects of breed, quantitative selection, single genes with large effects, and of gene markers, on carcass characteristics in livestock. We also refer to recent research in sheep that has used estimated breeding values for growth, muscling and fatness to assess genotypic effects within differing production environments. More specific details of genotypic effects are provided in Part II (Chapters 9 to 13) of this book, and the reader is also referred to earlier reviews of the genetics of carcass and quality traits of pork (Sellier, 1998; Rohrer, 2001), sheep meat (Banks, 1997; Thompson and Ball, 1997) and beef (Marshall, 1999; Cundiff, 2006).

Highly selected livestock, such as European cattle breeds, display productive advantages in more temperate climates within highly managed systems, whereas tropically adapted animals such as *Bos indicus* cattle and various sheep and goat breeds have the capacity to survive and produce in hotter and/or more humid environments in which other types perform poorly. For example, *Bos indicus* breeds survive well in the tropics and produce a high proportion of lean beef from their carcass, although they do produce somewhat tougher meat than *Bos taurus* cattle (Thompson, 2002).

Substantial genotypic advantage conferred by using specific breeds of livestock may be captured by the use of pure-bred herds or flocks (for example, Dorset v Suffolk sheep: Beermann *et al.*, 1995), terminal sires (for example, Piedmontese v Wagyu-sired cattle: Greenwood *et al.*, 2006a), or within composite herds comprising animals with defined proportions of genetic material from specific breeds or genotypes. Industry-based breeding and selection programs within and across improved breeds of livestock utilise quantitative selection techniques and

progeny testing to improve traits of interest such as growth, muscling, fatness, marbling, survival and reproduction. Increasingly, these programs are looking to incorporate effects of gene markers into their data bases (Nicholas, 2006).

Examples of breeds or genotypes of livestock with more extreme carcass characteristics include:

- European breeds of cattle that are late-maturing and display high degrees of muscling, in some cases associated with mutations resulting in non-functional myostatin and extreme levels of muscling, for example, Belgian Blue and Piedmontese cattle (Bellinge *et al.*, 2004);
- Wagyu and Hanwoo cattle from Japan and Korea, respectively, that produce highly marbled beef (Pethick *et al.*, 2004);
- Texel sheep which have a mutation in the myostatin gene that causes translational inhibition of the mRNA into myostatin, resulting in high muscling (Laville *et al.*, 2004; Clop *et al.*, 2006);
- Callipyge sheep, which have a mutation which, when present in heterozygous offspring that inherit the mutation from their sire, produces extreme muscling, particularly of the hindquarters, but which is associated with extremely tough meat (Freking *et al.*, 2004);
- Pietran pigs that have high levels of muscling (Sellier, 1998).

The most extreme effects of specific genes on carcass characteristics result in *double muscled* cattle and the *Callipyge* sheep phenotypes. Although mutations for these genes result in increased muscularity, their specific causes and effects differ substantially, as summarised in [Table 2.5](#).

Recent studies have assessed the use of sires with a range of Australian sheep breeding values (ASBVs) for muscling (eye muscle depth), fatness (subcutaneous fat depth) and growth (post-weaning weight) on a broad range of commercial, cellular, and biochemical measures ([Table 2.6](#)). Furthermore, associations of ASBVs with factors including age, live weight, carcass weight, gender of offspring, and nutrition have also been assessed (Hegarty *et al.*, 2006; Warner *et al.*, 2007). This approach to understanding influences of quantitative selection for traits differs from previous single-trait selection line studies by assessing effects across a continuum of breeding values for a trait (for example, muscling), while accounting for effects of breeding values for other associated traits (for example, growth and fatness). Among the numerous experimental, single-trait selection lines are those for growth rate or weaning weight (Thompson *et al.*, 1985a,b,c; Parnell *et al.*, 1986; Oddy and Sainz, 2002), fatness (Abdullah *et al.*, 1998), muscularity (Cafe *et al.*, 2006c) and net feed efficiency (Arthur *et al.*, 2004).

Use of sires with greater ASBVs for muscling (eye muscle depth) increased slaughter weight and proportion of lean while reducing subcutaneous fat depth and the proportion of bone in the carcass ([Table 2.6](#)). This results in an increase in carcass conformation score and the muscle-to-bone ratio (Cafe *et al.*, 2006), which is consistent across ages ranging from 4 to 22 months (Warner *et al.*, 2007). This advantage of high muscling breeding value and the growth advantages due to high sire ASBV for post-weaning weight were maintained within low and high

Table 2.5 Comparison of characteristics of *Callipyge* sheep and double-muscled cattle

	<i>Callipyge</i> sheep	Double-muscled cattle
Specific cause	Uncertain (DLK-1 involved)	Myostatin (GDF8) mutation (non-functional myostatin)
Location of single nucleotide polymorphism(s)	<i>Ovine</i> Chromosome 18	<i>Bovine</i> chromosome 2 (<i>Ovine</i> chromosome 2) ¹
Genotype resulting in mutant phenotype	Heterozygote with mutant allele inherited from sire (polar overdominance)	Homozygous for mutant allele (heterozygote has intermediate phenotype)
Phenotypic expression	Primarily postnatal	Prenatal and postnatal
Location of muscle hypertrophy	Hindquarter and loin	More generalised
Myofibres of affected muscles (cf. normal)	No hyperplasia More type 2X Far less type 2A Less type 1 in some affected muscles Type 2 hypertrophy Hyperplasia	Hyperplasia More type 2X Less type 2A May have type 2 hypertrophy depending on mutation and genetic background of cattle
Predominant mechanism in enhanced muscle growth	More glycolytic Reduced protein degradation (more calpastatin)	More glycolytic Increased protein synthesis
Meat quality (cf. normal)	Much tougher, pale	Similar or more tender, pale

¹ Mutation in Texel breed of sheep which affects translation into myostatin protein.

nutritional systems. In contrast to increased muscularity at any given weight that is generally associated with large mature size within breeds and strains of sheep (Black, 1983; Beermann *et al.*, 1995), offspring of sires selected using ASBVs for eye muscle depth had reduced mature weight, which was particularly associated with the skeleton (Warner *et al.*, 2007).

Increasing sire breeding values for muscling also increased the percentage of type 2X (fast glycolytic) myofibres (Greenwood *et al.*, 2006b) and connective tissue seam thickness (Allingham *et al.*, 2006), and reduced the percentage of type 2A (fast oxidative–glycolytic) myofibres (Greenwood *et al.*, 2006b). Furthermore, the intramuscular fat content (Hopkins *et al.*, 2005) declined, and there was a reduction in consumer acceptance of meat (Hopkins *et al.*, 2005) with increased breeding value for muscularity. These latter findings are consistent with those for pigs genetically selected for greater eye muscle area, which also has adverse consequences for eating quality associated with an increasing proportion of fast glycolytic myofibres (Rehfeldt *et al.*, 2000; Fiedler *et al.*, 2004). They suggest that continued genetic selection for muscling should incorporate eating quality characteristics and may also need to include myofibre characteristics within index-based genetic selection programs if acceptable eating quality is to be maintained while increasing meat yield. It was also evident that site-specific genetic selection

Table 2.6 Effects of increasing the estimated breeding values of sires for post-weaning eye-muscle depth (PEMD) on major growth and carcass characteristics of lambs (adapted from Hegarty *et al.*, 2006c). Regression coefficients are provided for each trait significantly affected ($P < 0.05$) by PEMD within multifactor analyses

Unaffected	Positively affected	Negatively affected
Post-weaning LWG ^A	Slaughter weight (0.59 kg) ^A Carcass EMD (0.61 mm) ^A Conformation score (0.03–0.85) ^{AB} Carcass protein (0.037 kg) ^B Four hindquarter muscles (0.021 kg) ^B	Carcass C-fat depth (–0.136 mm) ^A
Radius, ulna lengths ^C	Proportion of lean in carcass (0.015) ^C	Proportion of bone in carcass (–0.022) ^C
Bone mid-shaft width ^C	Carcass muscle to bone ratio (0.20) ^C RNA:DNA in muscle (0.351) ^D Protein:DNA in muscle (24.9) ^D Total protein in muscle (1.29 g, <i>semimembranosus</i>) (3.17 g, <i>longissimus</i>) ^D % type 2X myofibres (0 to 2.14) ^E	DNA concentration in muscle (–10.2 µg/g) ^D % type 2A myofibres (0 to –1.29) ^E
Cooking loss	Connective tissue seam thickness (219 µm) ^F	Muscle fascicle width (–0.27 mm) ^F
<i>Longissimus</i> shear force ^G		<i>Longissimus</i> IMF% (–0.11) ^G
Meat colour (<i>L,a,b</i>) ^G		Overall consumer liking (–1.32) ^G

^AHegarty *et al.* (2006a); ^BHegarty *et al.* (2006b); ^CCake *et al.* (2006); ^DGreenwood *et al.* (2006c);

^EGreenwood *et al.* (2006b), range indicates muscle × EBV interaction; ^FAllingham *et al.* (2006);

^GHopkins *et al.* (2005). ~ Colour assessed approximately 24h after slaughter and 30–40 min after cutting.

for increased muscling has differing affects across different muscles (Greenwood *et al.*, 2006b,c), with implications for compositional and eating quality characteristics (Hegarty *et al.*, 2006).

Marker-assisted selection has been made possible through the development of molecular technologies that allow identification of specific gene markers or single nucleotide polymorphisms (SNPs) for commercially important traits. A detailed history of developments in the search for DNA markers is provided by Nicholas (2006). The search for SNPs in ruminants was enhanced by development of the *bovine* gene map, which was made possible through the identification of quantita-

tive trait loci (QTL) using microsatellite markers on specific chromosomal regions, the development of linkage and radiation-hybrid maps covering a large proportion of the genome coupled with loci physically mapped using *in situ* hybridisation, and the use of Location Database (LDB) to integrate this information. Identification of markers in or near specific genes has been possible using fine-mapping of QTL, although identification of the causative quantitative trait nucleotide (QTN) has occurred infrequently due to difficulties in their identification, as detailed by Nicholas (2006).

DNA sequencing of the *bovine* genome and the advent of large-scale, high throughput detection and SNP genotyping now allows for genome-wide studies to determine the association of SNPs with commercially important traits (Hawkin *et al.*, 2004). Differential expression of genes using, in particular, microarrays containing many thousands of genes, is also used as a means of identifying important genes (Lehnert *et al.*, 2006a,b, 2007) and gene networks (Reverter *et al.*, 2006) that regulate development of carcass tissue traits and their response to the environment. More recently, these developments have allowed for expression QTL (eQTL) studies to be undertaken in which microarray profiling is integrated with high throughput SNP genotyping to allow for a detailed molecular phenotype to be rapidly linked to a genome region responsible for a specific trait (Lehnert *et al.*, 2006b).

2.7 Future perspectives

In the future, there will be continual tension between the desire to produce and consume value-added products with social credence (e.g. animal welfare friendly, environmentally sound, residue-free livestock products), health values in developed countries, and the need to provide animal protein to people in the rapidly developing nations. There is no doubt that as the standard of living increases, so too does the aspiration to consume animal protein and move up the food chain. For example, the growth in consumption of animal protein in China has been staggering over the last few decades compared to the steady rise in the USA, for example, and this will continue. To satisfy the demand for animal protein there needs to be enormous advances in the efficiency of conversion of carbon and nitrogen into meat, milk and wool, while maintaining or increasing the credence value of these products.

The desire to convert grain and forage into animal protein will coincide with the need to reduce reliance on fossil fuels and the increased use of plant biomass, including grain, for ethanol production. At the very least, this is going to increase the cost of grain (Lyons, 2007) but more likely will result in regional shortages of grain for livestock feed. In an often drought-stricken nation such as Australia that has a heavy reliance on fossil fuels, we have some serious challenges ahead. Coupled with this is the uncertainty that global warming and climate change brings (http://www.ipcc.ch/15_wmo_congress_pdf/metz_cg15.pdf).

Another tension is that between the need to produce meat and animal products

as efficiently as possible and the credence values around 'hormone-free animal products' in some communities or sectors. The classic example of this is the ban on hormonal growth promotants (HGP) in the European Union (EU) that applies to both products for domestic consumption and import. As mentioned previously, there is no doubt about the improvements in efficiency of milk and meat production, and reduced effluent production in cattle and pigs treated with HGP, somatotropin or β -agonists (Beermann and Dunshea, 2004; Dunshea *et al.*, 2005). The EU banned importation of beef from cattle administered HGP in 1989. The EU action was affirmed and reaffirmed through risk assessment documents released in 1999 and 2002, principally in response to public concern about involuntary hormone exposure. This action was not supported at the time by either the EU's own scientific committee (the Lamming Committee) or the World Health Organization's joint FAO/WHO Expert Committee on Food Additives, and it may be suggested that the original EU ban was a political risk assessment reflecting the credence values of the EU (Beermann and Dunshea, 2005). When data from the literature are collated and subject to meta-analyses, it appears that conservative use of each of these technologies will result in a 5–10% (0.3–0.5 kg) increase in shear force with a similar reduction in perception of tenderness (Dunshea *et al.*, 2005). However, it should be borne in mind that the magnitudes of these increases are similar to those observed with similar increases in carcass leanness obtained through other means (e.g. nutritional, genetic selection) and may be an inherent consequence of the production of leaner meat. To counter this, there are some other metabolic factors and dietary additives that offer some potential to improve meat quality and it is possible that these can be used on their own or in conjunction with somatotropin, approved β -agonists and anabolic implants to maintain or improve meat quality (Dunshea *et al.*, 2005). If we are to meet the increased demand for animal protein as a result of people in the developing world wishing to move up the food chain, then we really do need to embrace these technologies. In addition, we should continue to look for dietary means of manipulating metabolism or somatotropin secretion, such as with dietary cysteamine (Dunshea, 2007). Similar concerns and tensions exist for the use of antibiotics and ionophores in ruminant diets and their potential for greenhouse gas mitigation.

2.8 Sources of further information and advice

Pond WG and Bell AW (editors), 'Encyclopedia of Animal Science', New York, Taylor & Francis Group. <http://www.dekker.com/sdek/linking~db=enc~content=t713172958>
Livestock Library, <http://www.livestocklibrary.com.au/>

2.9 References

- Aalhus JL, Jones SDM, Schaefer AL, Tong AKW, Robertson WM, Merrill JK and Murray AC (1990), 'The effect of ractopamine on performance, carcass composition and meat quality of finishing pigs', *Can. J. Anim. Sci.*, 70, 943–952.
Aalhus J L, Best D R, Costello F and Schaefer A L (1997), 'The effects of porcine

- somatotropin on muscle fibre morphology and meat quality of pigs of known stress susceptibility', *Meat Sci.*, 45, 283–295.
- Abdullah A Y, Purchas R W and Davies A S (1998), 'Patterns of growth for muscularity and other composition characteristics of Southdown lambs selected for high and low backfat depth', *NZ J. Agric. Res.*, 41, 367–376.
- Adeola O, Darko E A, He P and Young L G (1990), 'Manipulation of porcine carcass composition by ractopamine', *J. Anim. Sci.*, 68, 3633–3641.
- Alexander G (1974), 'Birth weight of lambs: Influences and consequences'. In *Size at Birth*. Ciba Foundation Symposium 27 (new series). Amsterdam, ASP, 215–239.
- Alexander G (1978), 'Quantitative development of adipose tissue in foetal sheep', *Aust. J. Biol. Sci.*, 31, 489–503.
- Alexander G, Bennett J W and Gemmell R T (1975), 'Brown adipose tissue in the newborn calf', *J. Physiol.*, 244, 223–234.
- Allbrook D B, Han M F and Hellmuth A E (1971), 'Regulation of muscle satellite cells in relation to age and mitotic activity', *Pathology*, 3, 233–243.
- Allen C E (1976), 'Cellularity of adipose tissue in meat animals', *Fed. Proc.*, 35, 2302–2307.
- Allingham P G, Gardner G E, Taylor M, Hegarty R S and Harper G S (2006), 'Effects of sire genotype and plane of nutrition on fascicular structure of *M. longissimus thoracis et lumborum* and its effects on eating quality', *Aust. J. Exp. Agric.*, 57, 641–650.
- Altschul M (1987), 'Early ossification of the fetal ovine skeleton', MSc Thesis, Ithaca, Cornell University.
- Arthington J D, Spears J W and Miller D C (2005), 'The effect of early weaning on feedlot performance and measures of stress in beef calves', *J. Anim. Sci.*, 83, 933–939.
- Arthur P F, Archer J A and Herd R M (2004), 'Feed intake and efficiency in beef cattle: Overview of recent Australian research and challenges for the future', *Aust. J. Exp. Agric.*, 44, 361–369.
- Banks R G (1997), 'Genetics of lamb and meat production', in Piper L and Ruvinsky A, *The Genetics of Sheep*, Wallingford, CAB International, 505–522.
- Barham B L, Brooks J C, Blanton Jr J R, Herring A D, Carr M A, Kerth C R and Miller M F (2003), 'Effects of growth implants on consumer perceptions of meat tenderness in beef steers', *J. Anim. Sci.*, 81, 3052–3056.
- Barker-Neef J M, Buskirk D D, Black J R, Doumit M E and Rust S R (2001), 'Biological and economic performance of early-weaned Angus steers', *J. Anim. Sci.*, 79, 2762–2769.
- Bartle S J, Preston R L, Brown R E and Grant R J (1992), 'Trenbolone acetate/estradiol combinations in feedlot steers: Dose-response and implant carrier effects', *J. Anim. Sci.*, 70, 1326–1332.
- Beermann D H (1993), 'Beta adrenergic agonists and growth', in Schreibman M P, Scanes C G and Pang P K T (eds) *The Endocrinology of Growth, Development and Metabolism in Vertebrates*, San Diego, CA, Academic Press, 345–366.
- Beermann D H (2002), 'β-adrenergic agonist receptor modulation of skeletal muscle growth', *J. Anim. Sci.*, 80 (E. Suppl. 1), 18–23.
- Beermann D H and Dunshea F R (2005), *Animal Agriculture's Future through Biotechnology Part 3. Metabolic Modifiers for Use in Animal Production*. Issue paper 30. Council for Agricultural Science and Technology, Iowa.
- Beermann D H, Fishell V K, Roneker K, Boyd R D, Armbruster G and Souza L (1990), 'Dose-response relationships between porcine somatotropin, muscle composition, muscle fiber characteristics and pork quality', *J. Anim. Sci.*, 68, 2690–2697.
- Beermann D H, Robinson T F and Hogue D E (1995), 'Impact of composition manipulation on lean lamb production in the United States', *J. Anim. Sci.*, 73, 2493–2502.
- Bell A W (2006), 'Prenatal programming of postnatal productivity and health of livestock: A brief review', *Aust. J. Exp. Agric.*, 46, 725–732.
- Bell A W, Greenwood P L and Ehrhardt R A (2005), 'Regulation of metabolism and growth during prenatal life', in Burrin D G and Mersmann H J, *Biology and Metabolism of Growing Animals*, Amsterdam, Elsevier Science BV, 3–34.

- Bellinge R H S, Liberles D A, Iaschi S P A, O'Brien P A and Kay G K (2004), 'Myostatin and its implications on animal breeding: A review', *Anim. Genetics*, 36, 1–6.
- Berge P (1991), 'Long-term effects of feeding during calfhood on subsequent performance in beef cattle (a review)', *Livest. Prod. Sci.*, 28, 179–201.
- Black J L (1983), 'Growth and development of lambs', in Haresign W, *Sheep Production*, London, Butterworths, 21–58.
- Brameld J M, Fahey A J, Langley-Evans S C and Buttery P J (2003), 'Nutritional and hormonal control of muscle growth and fat deposition', *Arch Tierz Dummerstorf*, Special Issue, 143–156.
- Burrin D G (2001), 'Nutrient requirements and metabolism', in Pond W G and Mersmann H J, *Biology of the Domestic Pig*, Ithaca, Cornell University Press, 309–389.
- Byrem T M, Beermann D H and Robinson T F (1996), 'Characterization of dose-dependent metabolic responses to close arterial infusion of cimaterol in the hindlimb of steers', *J. Anim. Sci.*, 74, 2907–2916.
- Cafe L, Hennessy D W, Hearnshaw H, Morris S G and Greenwood P L (2006a), 'Influences of nutrition during pregnancy and lactation on birth weights and growth to weaning of calves sired by Piedmontese or Wagyu bulls', *Aust J. Exp. Agric.*, 46, 245–255.
- Cafe L M, Hearnshaw H, Hennessy D W and Greenwood P L (2006b), 'Growth and carcass characteristics at heavy market weights of Wagyu-sired steers following slow or rapid growth to weaning', *Aust. J. Exp. Agric.*, 46, 951–955.
- Cafe L M, O'Rourke B, McKiernan W and Greenwood P L (2006c), 'Carcass and yield characteristics of steers from Angus muscling selection lines with normal and mutant myostatin alleles', *Aust Soc Anim Prod 26th Biennial Conf.*, Short Communication 13.
- Cake M A, Gardner G E, Hegarty R S, Boyce M D and Pethick D W (2006), 'Effect of nutritional restriction and sire genotype on forelimb bone growth and carcass composition in crossbred lambs', *Aust. J. Agric. Res.*, 57, 605–616.
- Campbell R G and Biden R S (1978), 'The effect of protein nutrition between 5.5 and 20 kg live weight on the subsequent performance and carcass quality of pigs', *Anim. Prod.*, 27, 223–228.
- Campbell R G, Taverner M R and Curic D M (1983), 'The influence of feeding level from 20 to 45 kg live weight on the performance and body composition of female and entire male pigs', *Anim. Prod.*, 36, 193–199.
- Campbell R G, Steele N C, Caperna T J, McMurtry J P, Solomon M B and Mitchell A D (1988), 'Interrelationships between energy intake and endogenous porcine growth hormone administration on the performance, body composition and protein and energy metabolism of growing pigs weighing 25 to 55 kg liveweight', *J. Anim. Sci.*, 66, 1643–1655.
- Campbell R G, Johnson R J, King R H and Taverner M R (1990a), 'Effects of gender and genotype on the response of growing pigs to exogenous administration of porcine growth hormone', *J. Anim. Sci.*, 68, 2674–2681.
- Campbell R G, Johnson R J, King R H, Taverner M R and Meisinger D J (1990b), 'Interaction of dietary protein content and exogenous porcine growth hormone administration on protein and lipid accretion rates in growing pigs', *J. Anim. Sci.*, 68, 3217–3225.
- Campbell R G, Johnson R J, Taverner M R and King R H (1991), 'Interrelationships between exogenous porcine somatotropin (PST) administration and dietary protein and energy intake on protein deposition capacity and energy metabolism of pigs', *J. Anim. Sci.*, 69, 1522–1531.
- Caperna T J, Gavelek D and Vossoughi J (1994), 'Somatotropin alters collagen metabolism in growing pigs', *J. Nutr.*, 124, 770–778.
- Chiba L I (1994), 'Effects of dietary amino acid content between 20 and 50 kg and 50 and 100 kg live weight on the subsequent and overall performance of pigs', *Livest. Prod. Sci.*, 39, 213–221.

- Chiba L I (1995), 'Effects of nutritional history on the subsequent and overall growth performance and carcass traits of pigs', *Livest. Prod. Sci.*, 41, 151–161.
- Clarke L, Buss D S, Juniper D T, Lomax M A and Symonds M E (1997), 'Adipose tissue development during early postnatal life in ewe-reared lambs', *Exp. Physiol.*, 82, 1015–1027.
- Clop A, Marcq F, Takeda H, Pirrottin D, Toroir X, Bibe B, Bouix J, Caiment F, Elsen J-M, Eychenne F, Larzul C, Laville E, Meisch F, Milenkovic D, Tobin J, Charlier C and Georges M (2006), 'A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep', *Nature Genetics*, 38, 813–818.
- Collins C L, Henman D J and Dunshea F R (2007a), 'Reduced protein intake during the weaner period has variable effects on subsequent growth and carcass composition of pigs', *Aust. J. Exp. Agric.*, 47, 1333–1340.
- Collins C L, Leury B J and Dunshea F R (2007b), 'Light and heavy birth weight pigs respond differently to a period of protein restriction during early growth', in Paterson J, *Manipulating Pig Production*, Werribee, Australasian Pig Science Association, 55.
- Collins C L, Leury B J, Tatham B G and Dunshea F R (2005), 'Early weaning affects pig growth performance and carcass characteristics', in Paterson J, *Manipulating Pig Production*, Werribee, Australasian Pig Science Association, 117.
- Connolly S A, Jaramillo D, Hong J K and Shapiro F (2004), 'Skeletal development in fetal pig specimens: MR imaging of femur with histologic comparison', *Radiology*, 233, 505–514.
- Cundiff L V (2006), 'The impact of quantitative genetics on productive, reproductive and adaptive traits in beef cattle', in *Australian Beef – the Leader! The Impact of Science on the Beef Industry*, Armidale, Cooperative Research Centre for Beef Genetic Technologies, 29–46.
- Dalke B S, Roeder R A, Kasser T R, Veenhuizen J J, Hunt C W, Hinman D D and Schelling G T (1992), 'Dose-response effects of recombinant bovine somatotropin implants on feedlot performance in steers', *J. Anim. Sci.*, 70, 2130–2137.
- De Blasio M J, Gatford K L, Robinson J S and Owens J A (2007), 'Placental restriction of fetal growth reduces size at birth and alters postnatal growth, feeding activity, and adiposity in the young lamb', *Am. J. Physiol.*, 292, R875–R886.
- Dikeman M E (2007), 'Effects of metabolic modifiers on carcass traits and meat quality', *Meat Sci.*, 77, 121–135.
- Di Marco O N, Baldwin R L and Calvert C C (1987), 'Relative contributions of hyperplasia and hypertrophy to growth in cattle', *J. Anim. Sci.*, 65, 150–157.
- Dritz S S, Owen K Q, Nelssen J L, Goodband R D and Tokach M D (1996), 'Influence of weaning age and nursery diet complexity on growth performance and carcass characteristics and composition of high-health status pigs from weaning to 109 kilograms', *J. Anim. Sci.*, 74, 2975–2984.
- D'Souza D N and Mullan B P (2002), 'The effect of genotype, sex and management strategy on the eating quality of pork' *Meat Sci.*, 60, 95–101.
- Dunshea F R (1993), 'Effect of metabolism modifiers on lipid metabolism in the pig', *J. Anim. Sci.*, 71, 1966–1977.
- Dunshea F R (2002), 'Metabolic and production responses to different porcine somatotropin injection regimes in pigs', *Aust. J. Agric. Res.*, 53, 785–793.
- Dunshea F R (2005), 'Sex and porcine somatotropin impact on variation in growth performance and back fat thickness', *Aust. J. Exp. Agric.*, 45, 677–682.
- Dunshea, F R (2007), 'Porcine somatotropin and cysteamine hydrochloride improve growth performance and reduce back fat in finisher gilts', *Aust. J. Exp. Agric.*, 47, 796–800.
- Dunshea F R and D'Souza D N (2003), 'Fat deposition in the pig', in Paterson J A, *Manipulating Pig Production Volume IX*, Werribee, Australasian Pig Science Association, 127–150.
- Dunshea F R and Gannon N J (1995), 'Nutritional and other factors affecting efficacy of β -agonists in pigs', *Rec. Adv. Anim. Nutr. Aust.*, 10, 46–52.

- Dunshea F R and King R H (1995), 'Responses to homeostatic signals in ractopamine-treated pigs', *Br. J. Nutr.*, 73, 809–818.
- Dunshea F R, Harris D M, Bauman D E, Boyd R D and Bell A W (1992), 'Effect of porcine somatotropin on in vivo glucose kinetics and lipogenesis in growing pigs', *J. Anim. Sci.*, 70, 141–151.
- Dunshea F R, King R H and Campbell R G (1993a), 'Interrelationships between dietary protein and ractopamine on protein and lipid deposition in finishing gilts', *J. Anim. Sci.*, 71, 2931–2941.
- Dunshea F R, King R H, Campbell R G, Sainz R D and Kim Y S (1993b), 'Interrelationships between sex and ractopamine on protein and lipid deposition in rapidly growing pigs', *J. Anim. Sci.*, 71, 2919–2930.
- Dunshea F R, King R H, Eason P J and Campbell R G (1998a), 'Interrelationships between dietary ractopamine, energy intake, and sex in pigs', *Aust. J. Agric. Res.*, 49, 565–574.
- Dunshea F R, Leury B J and King R H (1998b), 'Lipolytic responses to catecholamines in ractopamine-treated pigs', *Aust. J. Agric. Res.*, 49, 875–881.
- Dunshea F R, Colantoni C, Howard K, McCauley I, Jackson P, Long K A, Lopaticki S, Nugent E A, Simons J A, Walker J and Hennessy D P (2001), 'Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance', *J. Anim. Sci.*, 79, 2524–2535.
- Dunshea F R, Cox M L, Borg M R, Sillence M N and Harris D R (2002), 'Porcine somatotropin (pST) administered using a commercial delivery system improves growth performance of rapidly-growing, group-housed finisher pigs', *Aust. J. Agric. Res.*, 53, 287–293.
- Dunshea F R, Kerton D K, Cranwell P D, Campbell R G, Mullan B P, King R H, Power G N and Pluske J R (2003), 'Lifetime and post-weaning determinants of performance indices of pigs', *Aust. J. Agric. Res.*, 54, 364–370.
- Dunshea F R, D'Souza D N, Pethick D W, Harper G S and Warner R D (2005), 'Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat', *Meat Sci.*, 71, 8–38.
- Dwyer C M, Stickland N C and Fletcher J M (1993), 'The influence of maternal nutrition on muscle fibre number development in the porcine fetus and on subsequent postnatal growth', *J. Anim. Sci.*, 72, 911–917.
- Elsley F W H (1963), 'Studies of growth and development in the young pig Part II. A comparison of the performance to 200lb of pigs reared along different growth curves to 56 days of age', *J. Anim. Sci.*, 61, 243–251.
- Erlebacher A, Filvaroff E H, Gitelman S E and Derynck R (1995), 'Towards a molecular understanding of skeletal development', *Cell*, 80, 371–378.
- Etherton T D and Walton P E (1986), 'Hormonal and metabolic regulation of lipid metabolism in domestic livestock', *J. Anim. Sci.*, 63, 76–88.
- Etherton T D and Bauman D E (1998) 'Biology of somatotropin in growth and lactation of domestic animals', *Physiol. Rev.*, 78, 745–761.
- Etherton T D, Wiggins J P, Evock C M, Chung C S, Rebhun J F, Walton P E and Steele N C (1987), 'Stimulation of pig growth performance by porcine growth hormone: Determination of the dose–response relationship', *J. Anim. Sci.*, 64, 433–443.
- Everitt G C (1968), 'Prenatal development of uniparous animals with particular reference to the influence of maternal nutrition in sheep', in Lodge G A and Lamming G E, *Growth and Development of Mammals*, Proceedings of the 14th Nottingham Easter School, London, Butterworths, 131–157.
- Fiedler I, Dietl G, Rehfeldt C, Wegner J and Ender K (2004), 'Muscle fibre traits as additional selection criteria for muscle growth and meat quality in pigs – results of a simulated selection', *J. Anim. Breed. Genetics*, 121, 331–344.
- Fluharty F L, Loerch S C, Turner T B, Moeller S J and Lowe G D (2000), 'Effect of weaning age and diet on growth and carcass characteristics in steers', *J. Anim. Sci.*, 78, 1759–1767.

- Freking B A, Smith T P L and Leymaster K A (2004), 'The *Callipyge* mutation for sheep muscular hypertrophy – genetics, physiology and meat quality', in te Pas MFW, Everts ME and Haagsman HP, *Muscle Development in Livestock Animals*, Wallingford, CAB International, 317–342.
- Gatford K L, Ekert J E, Blackmore K, De Blasio M J, Boyce J M, Owens J A, Campbell R G and Owens P C (2003), 'Variable maternal nutrition and growth hormone treatment in the second quarter of pregnancy in pigs alter semitendinosus muscle in adolescent progeny', *Br. J. Nutr.*, 90, 283–293.
- Gatford K L, Boyce J M, Blackmore K, Smits R J, Campbell R G and Owens P C (2004), 'Long-term, but not short-term, treatment with somatotropin during pregnancy in under-fed pigs increases the body size of progeny at birth', *J. Anim. Sci.*, 82, 93–101.
- Gemmell R T and Alexander G (1978), 'Ultrastructural development of adipose tissue in foetal sheep', *Aust. J. Biol. Sci.*, 31, 505–515.
- Gemmell R T, Bell A W and Alexander G (1972), 'Morphology of adipose tissues in lambs at birth and during subsequent transition of brown to white adipose tissue in cold and warm conditions', *Am. J. Anat.*, 133, 143–164.
- Gjesdal F (1969), 'Age determination of bovine fetuses', *Acta Vet. Scand.*, 10, 197–218.
- Gondret F, Lefaucheur L, Juin H, Louveau I and Lebret B (2006), 'Low birth weight is associated with enlarged muscle fiber area and impaired meat tenderness of the *longissimus* muscle in pigs', *J. Anim. Sci.*, 84, 93–103.
- Greenwood P L, Hunt A S, Hermanson J W and Bell A W (1998), 'Effects of birth weight and postnatal nutrition on neonatal sheep: I. Body growth and composition, and some aspects of energetic efficiency', *J. Anim. Sci.*, 76, 2354–2367.
- Greenwood P L, Slepatis R M, Hermanson J W and Bell A W (1999), 'Intrauterine growth retardation is associated with reduced cell cycle activity, but not myofibre number, in ovine fetal muscle', *Reprod. Fertil. Dev.*, 11, 281–291.
- Greenwood P L, Hunt A S, Hermanson J W and Bell A W (2000a), 'Effects of birth weight and postnatal nutrition on neonatal sheep: II. Skeletal muscle growth and development', *J. Anim. Sci.*, 78, 50–61.
- Greenwood P L, Slepatis R M and Bell A W (2000b), 'Influences on fetal and placental weights during mid to late gestation in prolific ewes well-nourished throughout pregnancy', *Reprod. Fertil. Dev.*, 12, 149–156.
- Greenwood P L, Hunt A S, Slepatis R M, Finnerty K D, Alston C, Bermann D H and Bell A W (2002a), 'Effects of birth weight and postnatal nutrition on neonatal sheep: III. Regulation of energy metabolism', *J. Anim. Sci.*, 80, 2850–2861.
- Greenwood P L, Slepatis R M, McPhee M J and Bell A W (2002b), 'Prediction of stage of pregnancy in prolific sheep using ultrasound measurement of fetal bones', *Reprod. Fertil. Dev.*, 14, 7–13.
- Greenwood P L and Bell A W (2003), 'Consequences of intrauterine growth retardation for postnatal growth, metabolism and pathophysiology', *Reprod. Suppl.*, 61, 195–206.
- Greenwood P L and Thompson A N (2007), 'Consequences of maternal nutrition during pregnancy and of fetal growth for productivity in sheep', *Rec. Adv. Anim. Nutr. Aust.*, 16, 169–180.
- Greenwood P L, Cafe L M, Hearnshaw H, Hennessy D W, Thompson J M and Morris S G (2006a), 'Long-term consequences of birth weight and growth to weaning for carcass, yield and beef quality characteristics of Piedmontese- and Wagyu-sired cattle', *Aust. J. Exp. Agric.*, 46, 257–269.
- Greenwood P L, Gardner G E and Hegarty R S (2006b), 'Lamb myofibre characteristics are influenced by sire estimated breeding values and pastoral nutritional system', *Aust. J. Exp. Agric.*, 57, 627–639.
- Greenwood P L, Gardner G E and Hegarty R S (2006c), 'Indices of cellular development in muscles of lambs are influenced by sire estimated breeding values and pastoral nutritional system', *Aust. J. Exp. Agric.*, 57, 651–659.

- Greenwood P L and Cafe L M (2007), 'Prenatal and pre-weaning growth and nutrition of cattle: Long-term consequences for beef production', *Animal*, 1, 1297–1313.
- Gu Y, Schinckel A P, Forrest J C, Kuel C H and Watkins L E (1991), 'Effects of ractopamine, genotype, and growth phase on finishing performance and carcass value in swine: II. Estimation of lean growth rate and lean feed efficiency', *J. Anim. Sci.*, 69, 2694–2702.
- Guiroy P J, Tedeschi L O, Fox D G and Hutcheson J P (2002), 'The effects of implant strategy on finished body weight of beef cattle', *J. Anim. Sci.*, 80, 1791–1800.
- Hafez E S E and Rajakoski E (1964), 'Placental and fetal development during multiple bovine pregnancy. Anatomical and physiological studies', *Anat. Rec.*, 150, 303–316.
- Hammond J (1932), *Growth and the Development of Mutton Qualities in the Sheep*, Edinburgh, Oliver and Boyd.
- Hammond J (1944), 'Physiological factors affecting birth weight', *Proc. Nutr. Soc.*, 2, 8–14.
- Hancock D L, Wagner J F and Anderson D B (1991), 'Effects of estrogens and androgens on animal growth', in Pearson A M and Dutson T R, *Growth Regulation in Farm Animals*, Essex, Elsevier Applied Science, 255–297.
- Harper G S (1999), 'Trends in skeletal muscle biology and the understanding of toughness in beef', *Aust. J. Agric. Res.*, 50, 1105–1129.
- Hauschka S D (1994), 'The embryonic origin of muscle', in Engel A G and Franzini-Armstrong C, *Myology. Basic and Clinical. 2nd edition*, New York, McGraw-Hill, 3–73.
- Hawkin R J, Barris W C, McWilliam S M and Dalrymple B P (2004), 'An interactive bovine in silico SNP database (IBISS)', *Mammalian Genome*, 15, 819–827.
- Hegarty P V J and Allen C E (1978), 'Effect of pre-natal runtting on the postnatal development of skeletal muscles in swine and rats', *J. Anim. Sci.*, 46, 1634–1640.
- Hegarty R S, Shands C, Marchant R, Hopkins D L, Ball A J and Harden S (2006a), 'Effects of available nutrition and sire breeding values for growth and muscling on development of crossbred lambs. 1: Growth and carcass characteristics', *Aust. J. Agric. Res.*, 57, 593–603.
- Hegarty R S, Hopkins D L, Farrell T C, Banks R and Harden S (2006b), 'Effects of available nutrition and sire breeding values for growth and muscling on development of crossbred lambs. 2: Composition and commercial yield', *Aust. J. Agric. Res.*, 57, 617–626.
- Hegarty R S, Warner R D and Pethick D W (2006c), 'Genetic and nutritional regulation of lamb growth and muscle characteristics', *Aust. J. Agric. Res.*, 57, 721–730.
- Herpin P, Louveau I, Damon M and Le Dividich J (2005), 'Environmental and hormonal regulation of energy metabolism in early development in the pig', in Burrin D G and Mersmann H J, *Biology and Metabolism of Growing Animals*, Amsterdam, Elsevier Science BV, 353–374.
- Hodge R W (1974), 'Efficiency of food conversion and body composition of the preruminant lamb and young pig', *Br. J. Nutr.*, 32, 113–126.
- Hodges Jr P C (1953), 'Ossification in the fetal pig; A radiographic study', *Anat. Rec.*, 116, 315–325.
- Hohenshell L M, Cunnick J E, Ford S P, Kattesh H G, Zimmerman D R, Wilson M E, Matteri R L, Carroll J A and Lay Jr. D C (2000), 'Few differences found between early- and late-weaned pigs raised in the same environment', *J. Anim. Sci.*, 78, 38–49.
- Hopkins D L, Hegarty R S and Farrell T C (2005), 'Relationship between sire EBVs and the meat and eating quality of meat from their progeny grown on two planes of nutrition', *Aust. J. Exp. Agric.*, 45, 525–533.
- Hopkins D L, Stanley D F, Martin L C, Ponnampalam E N and van de Ven R (2007a), 'Sire and growth path effects on sheep meat production 1. Growth and carcass characteristics', *Aust. J. Exp. Agric.*, 47, 1208–1218.
- Hopkins D L, Stanley D F, Toohey E S, Gardner G E, Pethick D W and van de Ven R (2007b), 'Sire and growth path effects on sheep meat production 2. Meat and eating quality', *Aust. J. Exp. Agric.*, 47, 1219–1228.
- Johnson B J, Anderson P T, Meiske J C and Dayton W R (1996), 'Effect of a combined

- trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics, and carcass composition of feedlot steers', *J. Anim. Sci.*, 74, 363–371.
- Johnston D J, Reverter A, Ferguson D M, Thompson J M and Burrow H M (2003), 'Genetic and phenotypic characterisation of animal, carcass, and meat quality traits from temperate and tropically adapted beef breeds. 3: Meat quality traits', *Aust. J. Agric. Res.*, 54, 135–147.
- Joubert D M (1956) 'A study of prenatal growth and development in the sheep', *J. Agric. Sci., Camb.*, 47, 382–427.
- King R H (2003), 'Feeding the sow to increase piglet weaning weight', *Rec. Adv. Anim. Nutr. Aust.*, 14, 87–91.
- King R H, Campbell R G, Smits R J, Morley W C, Ronnfeldt K, Butler K and Dunshea F R (2000), 'Interrelationships between dietary lysine, sex, and porcine somatotropin administration on growth performance and protein deposition in pigs between 80 and 120 kg live weight', *J. Anim. Sci.*, 78, 2639–2651.
- Klindt J (2003), 'Influence of litter size and creep feeding on preweaning gain and influence of preweaning growth on growth to slaughter in barrows', *J. Anim. Sci.*, 81, 2434–2439.
- Koohmaraie M, Shackelford S D, Muggli-Cockett N E and Stone R T (1991), 'Effect of the β -adrenergic agonist L644,969 on muscle growth, endogenous proteinase activities, and postmortem proteolysis in wether lambs', *J. Anim. Sci.*, 69, 4823–4835.
- Kouakou B, Gelaye S, Kannan G, Pringle T D and Amoah E A (2005), 'Blood metabolites, meat quality and muscle calpain-calpastatin activities in goats treated with low doses of recombinant bovine somatotropin', *Small Rumin. Res.*, 57, 203–212.
- Kretchmar D H, Hathaway M R, Epley R J and Dayton W R (1990), 'Alterations in postmortem degradation of myofibrillar proteins in muscle of lambs fed a β -adrenergic agonist', *J. Anim. Sci.*, 68, 1760–1772.
- Krick B J, Roneker K R, Boyd R D, Beermann D H, David P J and Meisinger D J (1992), 'Influence of genotype and sex on the response of growing pigs to recombinant porcine somatotropin', *J. Anim. Sci.*, 70, 3024–3034.
- LaFlamme L F, Trenkle A and Topel D G (1973), 'Effect of castration or breed type on growth of the *longissimus* muscle in male cattle', *J. Anim. Sci.*, 37, 249–256.
- Laville E, Bouix J, Sayd T, Bibe B, Elsen J M, Larzul C, Eychenne F, Marcq F and Georges M (2004), 'Effects of a quantitative trait locus for muscle hypertrophy from Belgian Texel sheep on carcass conformation and muscularity', *J. Anim. Sci.*, 82, 3128–3137.
- Lawlor P G, Lynch P B, O'Connell M K, McNamara L, Reid P and Stickland N C (2007), 'The influence of over feeding sows during gestation on reproductive performance and pig growth to slaughter', *Archiv. fur Tierzucht.*, 50, 82–91.
- Lefaucheur L, Edom F, Ecolan P and Butler-Browne G S (1995), 'Pattern of muscle fiber type formation in the pig', *Dev. Dynamics*, 203, 27–41.
- Lehnert S A, Byrne K A, Reverter A, Nattrass G S, Greenwood P L, Wang Y H, Hudson N J and Harper G S (2006a), 'Gene expression profiling of bovine skeletal muscle in response to and during recovery from chronic and severe undernutrition', *J. Anim. Sci.*, 84, 3239–3250.
- Lehnert S A, Wang Y H, Tan S H and Reverter A (2006b), 'Gene expression-based approaches to beef quality research', *Aust. J. Exp. Agric.*, 46, 165–172.
- Lehnert S A, Reverter A, Byrne K A, Wang Y, Nattrass G S, Hudson N J and Greenwood P L (2007), 'Gene expression studies of developing bovine *longissimus* muscle from two different beef cattle breeds', *BMC Dev. Biol.*, 7, 95.
- Lewis R M, Emmans G C and Simm G (2006), 'Describing effects of genetic selection, nutrition, and their interplay in prime lambs using growth and efficiency functions', *Aust. J. Agric. Res.*, 57, 707–719.
- Liu C Y and Mills S E (1989), 'Determination of the affinity of ractopamine and clenbuterol for the beta-adrenoreceptor of the porcine adipocyte', *J. Anim. Sci.*, 67, 2937–2942.
- Louey S, Cock M L and Harding R (2005), 'Long-term consequences of low birthweight on

- postnatal growth, adiposity and brain weight at maturity in sheep', *J. Reprod. Dev.*, 51, 59–68.
- Lyons T P (2007), 'The new energy crisis: Food, feed, or fuel? Will ethanol displace gasoline or simply take food off our plates and feed from our animals? How can new technologies help?', in Lyons T P, Jaques K A and Hower J M, *Nutritional Biotechnology in the Feed and Food Industries*, Nottingham, Nottingham University Press, 1–10.
- MacConnachie H F, Enesco M and Leblond C P (1964), 'The mode of increase in the number of skeletal muscle nuclei in the postnatal rat', *Am. J. Anat.*, 114, 245–253.
- Maeir A, McEwan J C, Dodds K G, Fischman D A, Fitzsimmons R B and Harris A J (1992), 'Myosin heavy chain composition of single fibres and their origins and distribution in developing fascicles of sheep tibialis cranialis muscles', *J. Muscle Res. Cell. Motil.*, 13, 551–572.
- Marrable A W and Ashdown R R (1967), 'Quantitative observations on pig embryos of known ages', *J. Anim. Sci.*, 69, 443–447.
- Marshall D M (1999), 'Genetics of meat quality', in Fries R and Ruvinsky A, *The Genetics of Cattle*, Wallingford, CAB International, 605–636.
- McCoard S A, McNabb W C, Peterson S W, McCutcheon S N and Harris P M (2000), 'Muscle growth, cell number, type and morphometry in single and twin fetal lambs during mid to late gestation', *Reprod. Fertil. Dev.*, 12, 319–327.
- McDonagh M B, Fernandez C and Oddy V H (1999), 'Hind-limb protein metabolism and calpain system activity influence post-mortem change in meat quality in lamb' *Meat Sci.*, 52, 9–18.
- McDonald I, Wenham G and Robinson J J (1977), 'Studies on reproduction in prolific ewes 3. The development in size and shape of the foetal skeleton', *J. Agric. Sci., Camb.*, 89, 373–391.
- McKiernan W and Wilkins J (2007), 'The effect of genetic potential and pre feedlot growth path on beef eating quality', in Jones A, *Agribusiness Livestock Updates 2007*, Perth, Western Australian Department of Agriculture, 124–125.
- McMeekan C P (1940), 'Growth and development in the pig, with special references to carcass quality characters. III. Effects of plane of nutrition on the form and composition of the bacon pig', *J. Agric. Sci., Camb.*, 30, 511–569.
- McPherson R L, Ji F, Wu G, Blanton Jr. J R and Kim S W (2004), 'Growth and compositional changes of fetal tissues in pigs', *J. Anim. Sci.*, 82, 2534–2540.
- Meer D L, Kerley M S, Walker E L, Keisler D H, Pierce V L, Schmidt T B, Stahl C A, Linville M L and Berg E P (2005), 'Growth rate, body composition, and meat tenderness in early vs. traditionally weaned beef calves', *J. Anim. Sci.*, 83, 2752–2761.
- Mersmann H J (1998), 'Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action', *J. Anim. Sci.*, 76, 160–172.
- Mersmann H J and Smith S B (2005), 'Development of white adipose tissue lipid metabolism', in Burrin D G and Mersmann H J, *Biology and Metabolism of Growing Animals*, Amsterdam, Elsevier Science BV, 275–302.
- Meyer D L, Kerley M S, Walker E L, Keisler D H, Pierce V L, Schmidt T B, Stahl C A, Linville M L and Berg E P (2005), 'Growth rate, body composition, and meat tenderness in early vs. traditionally weaned beef calves', *J. Anim. Sci.*, 83, 2752–2761.
- Mitchell A D, Solomon M B and Steele N C (1991), 'Influence of level of dietary protein or energy on effects of ractopamine in finishing swine', *J. Anim. Sci.*, 69, 4487–4495.
- Mitchell A D, Scholz A M and Mersmann H J (2001), 'Growth and body composition', in Pond W G and Mersmann H J, *Biology of the Domestic Pig*, Ithaca, Cornell University Press, 225–308.
- Mori R, Kodaka T, Soeta S, Sato J, Kakino J, Hamato S, Takaki H and Yaito Y (2005), 'Preliminary study of histological comparison on the growth patterns of long-bone cortex in young calf, pig and sheep', *J. Vet. Med. Sci.*, 67, 1223–1229.
- Mulvaney D R, Marple D N and Merkel R A (1988), 'Proliferation of skeletal muscle satellite

- cells after castration and administration of testosterone propionate', *Proc. Soc. Exp. Biol. Med.*, 188, 40–45.
- Myers S E, Faulkner D B, Ireland F A, Berger L L and Parrett D F (1999a), 'Production systems comparing early weaning to normal weaning with or without creep feeding for beef steers', *J. Anim. Sci.*, 77, 300–310.
- Myers S E, Faulkner D B, Ireland F A and Parrett D F (1999b), 'Comparison of three weaning ages on cow-calf performance and steer carcass traits', *J. Anim. Sci.*, 77, 323–329.
- Myers S E, Faulkner D B, Nash T G, Berger L L, Parrett D F and McKeith F K (1999c), 'Performance and carcass traits of early-weaned steers receiving either a pasture growing period or a finishing diet at weaning', *J. Anim. Sci.*, 77, 311–322.
- National Research Council (1994), *Metabolic Modifiers: Effects on the Nutrient Requirements of Food-Producing Animals*. Subcommittee on Effects of Metabolic Modifiers on the Nutrient Requirements of Food-Producing Animals. Committee on Animal Nutrition Board on Agriculture. National Research Council. National Academy Press. Washington, D.C.
- Nicholas F W (2006), 'Discovery, validation and delivery of DNA markers', *Aust. J. Exp. Agric.*, 46, 155–158.
- Nichols W T, Galyean M L, Thomson D U and Hutcheson J P (2002), 'Effects of steroid implants on the tenderness of beef', *Professional Anim. Scientist*, 18, 202–210.
- Nissen P M, Danielsen V O, Jorgensen P F and Oksbjerg N (2003), 'Increased maternal nutrition of sows has no beneficial effects on muscle fiber number or postnatal growth and has no impact on the meat quality of the offspring', *J. Anim. Sci.*, 81, 3018–3027.
- Novakofski J E and McCusker R H (2001), 'Skeletal and muscular systems', in Pond W G and Mersmann H J, *Biology of the Domestic Pig*, Ithaca, Cornell University Press, 454–501.
- Oddy V H and Sainz R D (2002), 'Nutrition for sheep-meat production', in Dove H and Freer M, *Sheep Nutrition*, Collinwood, CSIRO Publishing, 237–262.
- Oksbjerg N, Petersen J S, Sorensen M T, Henckel P, Agergaard N, Bejerholm C and Erlandsen E (1995), 'The influence of porcine growth hormone on muscle fibre characteristics, metabolic potential and meat quality', *Meat Sci.*, 39, 375–385.
- Ono Y, Solomon M B, Evock-Clover C M, Steele N C and Maruyama K (1995), 'Effects of porcine somatotropin administration on porcine muscles located within different regions of the body', *J. Anim. Sci.*, 73, 2282–2288.
- Palsson H (1955), 'Conformation and body composition', in Hammond J, *Progress in the Physiology of Farm Animals*, London, Butterworths, 430–542.
- Parnell P F, Barlow R and Tier B (1986), 'Realised responses to divergent selection for yearling growth rate in Angus cattle', in Dickerson G E and Johnson R K, *Proceedings of the 3rd World Congress of Genetics Applied to Livestock Production XI*, Lincoln, University of Nebraska, 330–334.
- Perry D and Thompson J M (2005), 'The effect of growth rate during backgrounding and finishing on meat quality traits in beef cattle', *Meat Sci.*, 69, 691–702.
- Perry T C, Fox D G and Beermann D H (1991), 'Effect of an implant of trenbolone acetate and estradiol on growth, feed efficiency, and carcass composition of Holstein and beef steers', *J. Anim. Sci.*, 69, 4696–4702.
- Pethick D W, Harper G S and Oddy V H (2004), 'Growth, development and nutritional manipulation of marbling in cattle: A review', *Aust. J. Exp. Agric.*, 44, 705–715.
- Picard B, Lefaucheur L, Berri C and Duclos M J (2002), 'Muscle fibre ontogenesis in farm animal species', *Reprod. Nutr. Dev.*, 42, 415–431.
- Platter W J, Tatum J D, Belk K E, Scanga J A and Smith G C (2003), 'Effects of repetitive use of hormonal implants on beef carcass quality, tenderness, and consumer ratings of beef palatability', *J. Anim. Sci.*, 81, 984–996.
- Pluske J R, Payne H G, Williams I H and Mullen B P (2005), 'Early feeding for lifetime performance of pigs', *Rec. Adv. Anim. Nutr. Aust.*, 15, 171–181

- Powell S E and Aberle E D (1980), 'Effects of birth weight on growth and carcass composition of swine', *J. Anim. Sci.*, 50, 860–868.
- Purchas R W, Burnham D L and Morris S T (2002), 'Effects of growth potential and growth path on tenderness of beef *longissimus* muscle from bulls and steers', *J. Anim. Sci.*, 80, 3211–3221.
- Rehfeldt C and Kuhn G (2006), 'Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis', *J. Anim. Sci.*, 84 Suppl, E113–E123.
- Rehfeldt C, Fiedler I, Weikard R, Kanitz E and Ender K (1993), 'It is possible to increase skeletal muscle fibre number in utero', *Biosci. Rep.*, 13, 213–220.
- Rehfeldt C, Fiedler I, Dietl G and Ender K (2000), 'Myogenesis and postnatal muscle cell growth as influenced by selection', *Livest. Prod. Sci.*, 66, 177–188.
- Rehfeldt C, Kuhn G, Vanselow J, Furbass R, Fiedler I, Nurnberg G, Clelland A K, Stickland N C and Ender K (2001), 'Maternal treatment with somatotropin during early gestation affects basic events of myogenesis in pigs', *Cell. Tissue Res.*, 306, 429–440.
- Rehfeldt C, Nissen P M, Kuhn G, Vestergard M, Ender K and Oksbjerg N (2004), 'Effects of maternal nutrition and porcine growth hormone (pGH) treatment during gestation on endocrine and metabolic factors in sows, fetuses and pigs, skeletal muscle development, and postnatal growth', *Domest. Anim. Endocrinol.*, 27, 267–285.
- Reverter A, Johnston D J, Perry D, Goddard M E and Burrow H M (2003), 'Genetic and phenotypic characterisation of animal, carcass, and meat quality traits from temperate and tropically adapted beef breeds. 2: Abattoir carcass traits', *Aust. J. Agric. Res.*, 54, 119–134.
- Reverter A, Hudson N J, Wang Y, Tan S-H, Barris W, Byrne K A, McWilliam S M, Bottema C D, Kister A, Greenwood P L, Harper G S, Lehnert S A and Dalrymple B P (2006), 'A gene coexpression network for bovine skeletal muscle inferred from microarray data', *Physiol. Genomics*, 28, 76–83.
- Robelin J, Lacourt A, Bechet D, Ferrara M, Briand Y and Geay Y (1991), 'Muscle differentiation in the bovine fetus: A histological and histochemical approach', *Growth Dev. Aging*, 55, 151–161.
- Robertson J G, Walton P E, Dunshea F R, Dunaiski V, Ballard F J and Belford D A (1997), 'Growth hormone but not insulin-like growth factor-I improves wound strength in pigs', *Wound Repair and Regeneration*, 5, 168–174.
- Robinson D L, Oddy V H, Dicker R W and McPhee M J (2001), 'Post-weaning growth of cattle in northern New South Wales 3. Carry-over effects on finishing, carcass characteristics and intramuscular fat', *Aust. J. Exp. Agric.*, 41, 1041–1049.
- Rohrer G A (2001), 'Genetics', in Pond W G and Mersmann H J, *Biology of the Domestic Pig*, Ithaca, Cornell University Press, 122–149.
- Russell R G and Oteruelo F T (1981), 'An ultrastructural study of the differentiation of skeletal muscle in the bovine fetus', *Anat. Embryol.*, 162, 403–417.
- Sainz R D, Kim Y S, Dunshea F R and Campbell R G (1993), 'Temporal changes in growth enhancement by ractopamine in pigs: Performance aspects', *Aust. J. Agric. Res.*, 44, 1449–1455.
- Schinckel A P, Richert B T, Herr C T, Einstein M E and Kendall D C (2001), 'Effects of ractopamine on swine growth, carcass composition and quality', in *Second International Virtual Conference on Pork Quality*. www.conferencia.uncnet.br/pork/programa.en.html
- Schoonmaker J P, Fluharty F L, Loerch S C, Turner T B, Moeller S J and Wulf D M (2001), 'Effect of weaning status and implant regimen on growth, performance, and carcass characteristics of steers', *J. Anim. Sci.*, 79, 1074–1084.
- Schoonmaker J P, Cecava M J, Faulkner D B, Fluharty F L, Zerby H N and Loerch S C (2003), 'Effect of source of energy and rate of growth on performance, carcass characteristics, ruminal fermentation, and serum glucose and insulin of early-weaned steers', *J. Anim. Sci.*, 81, 843–855.
- Schoonmaker J P, Cecava M J, Fluharty F L, Zerby H N and Loerch S C (2004), 'Effect of

- source and amount of energy and rate of growth in the growing phase on performance and carcass characteristics of early- and normal-weaned steers', *J. Anim. Sci.*, 82, 273–282.
- Sellier P (1998), 'Genetics of meat and carcass traits', in Rothschild M F and Ruvinsky A, *The Genetics of the Pig*, Wallingford, CAB International, 463–510.
- Shafiq S A, Gorycki M A and Mauro A (1968), 'Mitosis during postnatal growth in skeletal and cardiac muscle of the rat', *J. Anat.*, 103, 135–141.
- Sillence M N (2004), 'Technologies for the control of fat and lean deposition in livestock', *Vet J.*, 167, 242–257.
- Smith S B and Carstens G E (2005), 'Ontogeny and metabolism of brown adipose tissue in livestock species', in Burrin D G and Mersmann H J, *Biology and Metabolism of Growing Animals*, Amsterdam, Elsevier Science BV, 303–322.
- Smits R J and Cadogan D J (2003), 'The use of ractopamine as the commercial product, Paylean®, for the Australian pig industry', *Rec. Adv. Anim. Nutr. Aust.*, 14, 143–150.
- Solomon M B, West R L and Henges Jr J F (1986), 'Growth and muscle development characteristics of purebred Angus and Brahman bulls', *Growth*, 50, 51–67.
- Solomon M B, Campbell R G and Steele N C (1990), 'Effect of sex and exogenous porcine somatotropin on *longissimus* muscle fiber characteristics of growing pigs', *J. Anim. Sci.*, 68, 1176–1181.
- Solomon M B, Campbell R G, Steele N C and Caperna T J (1991), 'Effects of exogenous porcine somatotropin administration between 30 and 60 kilograms on *longissimus* muscle fiber morphology and meat tenderness of pigs grown to 90 kilograms', *J. Anim. Sci.*, 69, 641–645.
- Storba O (2004), 'Prenatal development of metacarpus and metatarsus of cattle', *Acta Vet. Brno.*, 73, 405–412.
- Suster D, Leury B J, King R H, Mottram M and Dunshea F R (2004), 'Interrelationships between porcine somatotropin (pST), betaine, and energy level on body composition and tissue distribution of finisher boars', *Aust. J. Agric. Res.*, 55, 983–990.
- Swatland H J (1977), 'Accumulation of myofiber nuclei in pigs with normal and arrested development', *J. Anim. Sci.*, 44, 759–764.
- Swatland H J (1994), *Structure and Development of Meat Animals and Poultry*, Lancaster, Technomic Publishing Company.
- Swatland H J and Cassens R G (1973), 'Inhibition of muscle growth in foetal sheep', *J. Agric. Sci., Camb.*, 80, 503–509.
- Thompson J (2002), 'Managing meat tenderness', *Meat Sci.*, 62, 295–308.
- Thompson J M and Ball A J (1997), 'Genetics of meat quality', in Piper L and Ruvinsky A, *The Genetics of Sheep*, Wallingford, CAB International, 523–538.
- Thompson, J M, Parks J R and Perry D (1985a), 'Food intake, growth and body composition in Australian Merino sheep selected for high and low weaning weight. 1. Food intake, feed efficiency and growth', *Anim. Prod.*, 40, 55–70.
- Thompson J M, Butterfield R M and Perry D (1985b), 'Food intake, growth and body composition in Australian Merino sheep selected for high and low weaning weight. 2. Chemical and dissectible body composition', *Anim. Prod.*, 40, 71–84.
- Thompson, J M and Parks J R (1985), 'Food intake, growth and body composition in Australian Merino sheep selected for high and low weaning weight. 3. Energy balance', *Anim. Prod.*, 40, 85–91.
- Thompson J M, McIntyre B M, Tudor G D, Pethick D W, Polkinghorne R and Watson R (2008), 'Effects of hormonal growth promotants (HGP) on growth, carcass characteristics, the palatability of different muscles in the beef carcass and their interaction with aging', *Aust. J. Exp. Agric.*, 48, 1405–1414.
- Tomkins N W, Harper G S, Bruce H L and Hunter R A (2006), 'Effect of different post-weaning growth paths on long-term weight gain, carcass characteristics, and eating quality of beef cattle', *Aust. J. Exp. Agric.*, 46, 1571–1578.
- Trenkel A, DeWitt D L and Topel D G (1978), 'Influence of age, nutrition and genotype on

- carcass traits and cellular development of the *M. longissimus* of cattle', *J. Anim. Sci.*, 46, 1597–1603.
- Troy D, Murray B, O'Sullivan A, Mooney T, Moloney A and Kerry J (2002), *Influence of Feeding Systems on the Eating Quality of Beef*, Research Report No. 58, Dublin, Teagasc.
- Tudor G D, Utting D W and O'Rourke P K (1980), 'The effect of pre- and post-natal nutrition on the growth of beef cattle. III. The effect of severe restriction in early postnatal life on the development of body components and chemical composition', *Aust. J. Agric. Res.*, 31, 194–201.
- Vernon R G, Robertson J P, Clegg R A and Flint D J (1981), 'Aspects of adipose-tissue metabolism in foetal lambs', *Biochem. J.*, 196, 819–824.
- Vestergaard M, Purup S, Henckel P, Tonner E, Flint D J, Jensen L R and Sejrsen K (1995), 'Effects of growth hormone and ovariectomy on performance, serum hormones, insulin-like growth factor-binding proteins, and muscle fiber properties of prepubertal Friesian heifers', *J. Anim. Sci.*, 73, 3574–3584.
- Villette Y and Theriez M (1981), 'Influence of birth weight on lamb performances. II. – Carcass chemical composition of lambs slaughtered at the same weight.' *Ann. Zootech.*, 30, 169–182.
- Wallace L R (1948a), 'The growth of lambs before and after birth in relation to level of nutrition. Part II', *J. Agric. Sci., Camb.*, 38, 243–302.
- Wallace L R (1948b), 'The growth of lambs before and after birth in relation to level of nutrition. Part III', *J. Agric. Sci., Camb.*, 38, 367–401.
- Wander R C, Clark S L, Hu C Y, Holmes Z A and Schrupf E (1993), 'Interaction of porcine somatotropin administration to growing pigs and frozen storage of carcass on lipids and quality characteristics of roasts', *J. Food Composition Anal.*, 6, 62–74.
- Warner R D, Pethick D W, Greenwood P L, Ponnampalam E N, Banks R G and Hopkins D L (2007), 'Unravelling the complex interactions between genetics, animal age and nutrition as they impact on tissue deposition, muscle characteristics and quality of Australian sheep meat', *Aust. J. Exp. Agric.*, 47, 1339–1238.
- Watkins L E, Jones D J, Mowrey D H, Anderson D B and Veenhuizen E L (1990), 'The effect of various levels of ractopamine hydrochloride on the performance and carcass characteristics of finishing swine', *J. Anim. Sci.*, 68, 3588–3595.
- Watson R (2008), 'Meta-analysis of the published effects of HGP use on beef palatability in steers as measured by objective and sensory testing', *Aust. J. Exp. Agric.*, 48, (in press).
- Watson R, Polkinghorne R, Gee A, Porter M, Thompson J M, Ferguson D, Pethick D W and McIntyre B M (2008), 'Effect of hormonal growth promotants on palatability and carcass traits of various muscles from steer and heifer carcasses from *Bos indicus*/*Bos taurus* composite cross', *Aust. J. Exp. Agric.*, 48, 1415–1424.
- Weikard R, Rehfeldt C and Ender K (1992), 'Changes in muscle structure and protein metabolism of pigs in response to porcine somatotrophin (pST)', *Archiv. Fur Tierzucht.*, 35, 273–284.
- Wenham G (1981), 'A radiographic study of early skeletal development in foetal sheep', *J. Agric. Sci., Camb.*, 96, 39–44.
- Wertz E, Berger L L, Walker P M, Faulkner D B, McKeith F K and Rodriguez-Zas S (2001), 'Early weaning and postweaning nutritional management affect feedlot performance of Angus × Simmental heifers and the relationship of 12th rib fat and marbling score to feed efficiency', *J. Anim. Sci.*, 79, 1660–1669.
- Wang K Y, Kim S W, Donovan S M, McKeith F K and Easter R A (2003), 'Effects of protein deprivation on subsequent growth performance, gain of body components, and protein requirements in growing pigs', *J. Anim. Sci.*, 81, 705–716.
- Wheeler T L and Koohmaraie M (1992), 'Effects of the beta-adrenergic agonist L644,969 on muscle protein turnover, endogenous proteinase activities, and meat tenderness in steers', *J. Anim. Sci.*, 70, 3035–3043.
- Whipple G, Hunt M C, Klemm R D, Kropf D H, Goodband R D and Schrick B R (1992),

- 'Effects of porcine somatotropin and supplemental lysine on porcine muscle histochemistry', *J. Muscle Foods*, 3, 217–227.
- Wigmore P M C and Stickland N C (1983), 'Muscle development in large and small pig fetuses', *J. Anat.*, 137, 235–245.
- Wilkins J F, McKiernan W A, Irwin J, Orchard B and Barwick S A (2009), 'Beef production carcass traits and meat quality of steer progeny of sires differing in genetic potential for fatness and meat yield following growth at divergent rates', *Anim. Prod. Sci.*, (submitted).
- Wilson S J, McEwan J C, Sheard P W, Harris A J (1992), 'Early stages of myogenesis in a large mammal: Formation of successive generations of myotubes in sheep tibialis cranialis muscle', *J. Muscle Res. Cell. Motil.*, 13, 534–550.
- Winters L M, Green W W and Comstock R E (1942), 'Pre-natal development of the bovine', *Tech. Bulletin No. 151*, Univ. of Minnesota Agric. Experiment Station.
- Wolter B F, Ellis M, Corrigan B P and DeDecker J M (2002a), 'The effect of birth weight and feeding of supplemental milk replacer to piglets during lactation on preweaning and postweaning growth performance and carcass characteristics', *J. Anim. Sci.*, 80, 301–308.
- Wolter B F, Ellis M, DeDecker J M, Curtis S E, Hollis G R, Shanks R D, Parr E N and Webel D M (2002b), 'Effects of double stocking and weighing frequency on pig performance in wean-to-finish production systems', *J. Anim. Sci.*, 80, 1442–1450.
- Wolter B F, Ellis M, Curtis S E, Parr E N and Webel D M (2002c), 'Effects of feeder-trough space and variation in body weight within a pen of pigs on performance in a wean-to-finish production system', *J. Anim. Sci.*, 80, 2241–2246.
- Wolter B F, Ellis M, Corrigan B P, DeDecker J M, Curtis S E, Parr E N and Webel D M (2003a), 'Impact of early postweaning growth rate as affected by diet complexity and space allocation on subsequent growth performance of pigs in a wean-to-finish production system', *J. Anim. Sci.*, 81, 353–359.
- Wolter B F, Ellis M, Corrigan B P, DeDecker J M, Curtis S E, Parr E N and Webel D M (2003b), 'Effect of restricted postweaning growth resulting from reduced floor and feeder-trough space on pig growth performance to slaughter weight in a wean-to-finish production system', *J. Anim. Sci.*, 81, 836–842.
- Wu G, Bazer F W, Wallace J M and Spencer T E (2006) 'Board Invited Review: Intrauterine growth retardation: Implications for the animal sciences' *J. Anim. Sci.*, 84, 2316–2337.
- Yen J T, Nienaber J A, Klindt J and Crouse J D (1991), 'Effect of ractopamine on growth, carcass traits, and fasting heat production of U.S. contemporary crossbred and Chinese Meishan', *J. Anim. Sci.*, 69, 4810–4822.
- Zhu M-J, Ford S P, Nathanielz P W and Du M (2004), 'Effect of maternal nutrient restriction in sheep on the development of fetal skeletal muscle', *Biol. Reprod.*, 71, 1968–1973.

3

Fresh meat texture and tenderness

D. A. King, T. L. Wheeler, S. D. Shackelford and M. Koohmaraie,
US Meat Animal Research Center, USA

Abstract: Strategies to consistently produce tender meat products should help maintain and build consumer confidence. Numerous antemortem and postmortem factors can impact upon tenderness, both positively and negatively. Generally, these effects are mediated through alteration of sarcomere length, postmortem proteolysis, or connective tissue integrity. The interaction of these component traits is complex and muscle dependent. Therefore, antemortem and postmortem management decisions must be made carefully, possibly on a muscle-by-muscle basis. This chapter discusses antemortem and postmortem management strategies that can be used to influence meat tenderness attributes. Additionally, tenderness assessment and prediction are addressed.

Key words: genetic markers, management, meat tenderness, prediction.

3.1 Introduction

Consumers have certain expectations regarding the quality of the meat they purchase. Eating satisfaction is determined by the perceived value delivered by three palatability traits: tenderness, juiciness, and flavor. Each of these traits is important and deficiency in any one of them could result in consumer dissatisfaction. However, the majority of meat palatability research has emphasized tenderness because of its importance in consumer perceptions of muscle foods. The importance of tenderness is evidenced by consumers' ability to discern differences in tenderness and willingness to pay premiums for guaranteed tender products (Boleman *et al.*, 1997; Lusk *et al.*, 2001; Shackelford *et al.*, 2001). Additional evidence comes from consumer survey results indicating that, of the three palatability traits, tenderness is the most important contributor to their eating satisfaction (Miller *et al.*, 1995). Certain cuts of beef have been reported to be inconsistent with regard to tenderness and need improvement relative to consumer

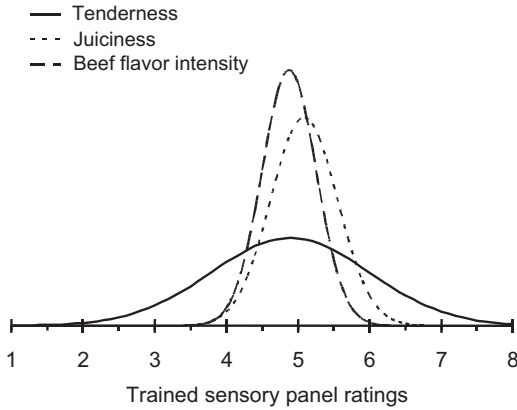


Fig. 3.1 Variation in tenderness, flavor, and juiciness ratings for beef *longissimus* steaks from young, grain-fed steers. Adapted from Wheeler *et al.* (2001a).

expectations (Neely *et al.*, 1998; Savell *et al.*, 1999; Brooks *et al.*, 2000; Behrends *et al.*, 2005).

Tenderness is the most variable of the three palatability traits (Fig. 3.1). In large studies comparing animals of diverse genetic backgrounds reared in a calf-fed, corn–corn silage based production system, animal-to-animal variation in tenderness traits is more than double the variation observed in flavor intensity and juiciness (Wheeler *et al.*, 1996a, 2001a). Furthermore, tenderness differences across muscles within carcasses have been reported to be larger than differences in flavor or juiciness (Carmack *et al.*, 1995; Shackelford *et al.*, 1995; Rhee *et al.*, 2004). Additionally, Rhee *et al.* (2004) and Searls *et al.* (2005) reported substantial differences in Warner–Bratzler shear force values across locations within some muscles.

3.2 Muscle constituents and structure contributing to tenderness variation

Physical and chemical properties of muscle interact to determine the tenderness of the resulting meat products. Therefore, it is important to understand the muscle constituents and structures that contribute to the variation in tenderness. For a review of the macroscopic and microscopic structure of muscle, muscle contraction, and the conversion of muscle to meat, the reader is referred to Aberle *et al.* (2001). Numerous sources contribute to the variation in tenderness of meat; however, these effects ultimately are asserted by altering one or more of three component traits: connective tissue amount and quality, sarcomere length, and the rate and extent of postmortem proteolysis of key structural proteins are considered to be the primary component traits contributing to explainable tenderness variation.

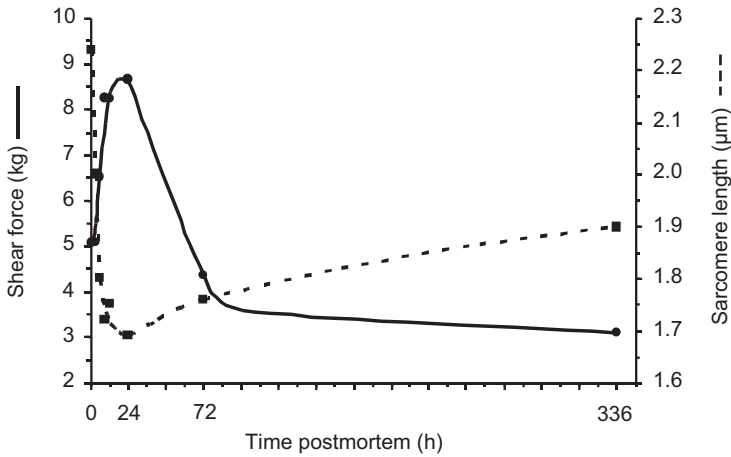


Fig. 3.2 Changes in Warner–Bratzler shear force and sarcomere length in lamb *longissimus* muscle during chilling and postmortem storage. Adapted from Wheeler and Koohmaraie (1994).

The connective tissue fraction of meat, predominantly comprised of collagen, provides structural support to muscles and transfers force generated during contraction to the skeleton. The contribution of collagen to meat tenderness is determined by a number of factors, such as total collagen concentration, types of collagen present, and cross-linking of the collagen matrix. The effects of connective tissue on tenderness is often referred to as ‘background toughness’ (Marsh, 1977) and are thought to be a minor source of animal-to-animal variation in *longissimus* tenderness of young animals. However, as animals mature, inter-molecular cross-linking stabilizes the collagen matrix (Avery *et al.*, 1996). The stabilized collagen is more resistant to solubilization by heating, which increases meat toughness (Goll *et al.*, 1964; Shorthose and Harris, 1990).

Total collagen content varies between muscles depending on their skeletal location and function in the live animal, and partially explains tenderness differences between muscles (McKeith *et al.*, 1985; Wheeler *et al.*, 2000b; Rhee *et al.*, 2004). For example, muscles located in the limbs of the animal, which are used for locomotion, generally have greater collagen concentrations than support muscles located in the epaxial regions.

The contractile state of muscle is a key contributor to the myofibrillar tenderness of meat. The increased overlap of the thick and thin filaments associated with contracted muscle (i.e. shorter sarcomeres) is associated with greater toughness (Locker and Hagyard, 1963; Marsh and Leet, 1966). Wheeler and Koohmaraie (1994) measured the effects of rigor and early postmortem storage on sarcomere length of lamb *longissimus* and the consequential effects on tenderness (Fig. 3.2). Sarcomere length was longest at death and was reduced to a minimum at 24 h.

Warner–Bratzler shear force increased to a maximum at 24 h and improved dramatically during 14 d of ageing. When sarcomere shortening during rigor was prevented, the increase in Warner–Bratzler shear force during the first hours postmortem was not observed (Koohmaraie *et al.*, 1996a). However, tenderization still occurred. Collectively, these results indicate that lamb *longissimus* is intermediate in tenderness at death and rigor shortening causes a reduction in tenderness during the first 24 h. At some point after death, degradation of structural proteins is initiated and results in tenderization. Veiseth *et al.* (2004) reported that calpain degradation of key structural proteins began within a few hours postmortem. Therefore, in muscles with low to intermediate collagen concentrations, the ultimate tenderness of the muscle is dictated by the extent of sarcomere shortening that is allowed to occur and how much postmortem protein degradation occurs.

After death, tenderization begins and will continue at varying rates and for varying periods of time postmortem. For detailed reviews of the tenderization process, the reader is referred to Koohmaraie (1992a, 1995, 1996). Postmortem changes that are observed during refrigerated storage include loss of Z-disk and sarcomere integrity resulting from proteolytic degradation of numerous cytoskeletal proteins such as troponin-T, titin, nebulin, desmin, vinculin, filamin, synemin, and dystrophin (Taylor *et al.*, 1995; Koohmaraie, 1992a, 1996). The proteins observed to be degraded in postmortem meat function to provide muscle integrity through involvement in inter- (e.g. desmin and vinculin) and intra-myofibril (e.g. titin, nebulin, and possibly troponin-T) linkages and the attachment of muscle cells to the basal lamina (e.g. laminin, and fibronectin) (Price, 1991; Robson *et al.*, 1997). Therefore, it is evident that degrading these proteins results in a weakening of the myofibril structure, resulting in tenderization. However, it is notable that the major contractile proteins, actin and myosin, are unaffected during postmortem storage.

Numerous investigations have been conducted to deduce the proteolytic system responsible for these changes. Lysosomal proteases (e.g. cathepsins; Goll *et al.*, 1983; Ouali and Valin, 1980; Zeece *et al.*, 1992), multicatalytic proteinase complex (Goll, 1991; Koohmaraie, 1992c, 1994) and the calpain system (Ouali and Talmant, 1990; Koohmaraie *et al.*, 1992b, 1995, 1996) have all been examined as potentially causing the tenderization observed during postmortem storage. Of these, only the calpain system has been found to produce the changes normally seen in postmortem muscle (Koohmaraie, 1996). Calpain mediated proteolysis of cytoskeletal proteins begins soon after death (Veiseth *et al.*, 2004) and continues for some period of time, depending on species.

Collectively, connective tissue content, sarcomere length, and the extent of postmortem proteolysis of myofibrillar proteins can explain the majority of the variations in tenderness. However, their interaction is complex and is muscle dependent. For example, psoas major is very tender due to long sarcomere lengths and low collagen content, despite a very low amount of postmortem proteolysis. Biceps femoris is relatively tough due to high collagen concentration and medium sarcomere length, despite having as much or more proteolysis as all other muscles (Rhee *et al.*, 2004).

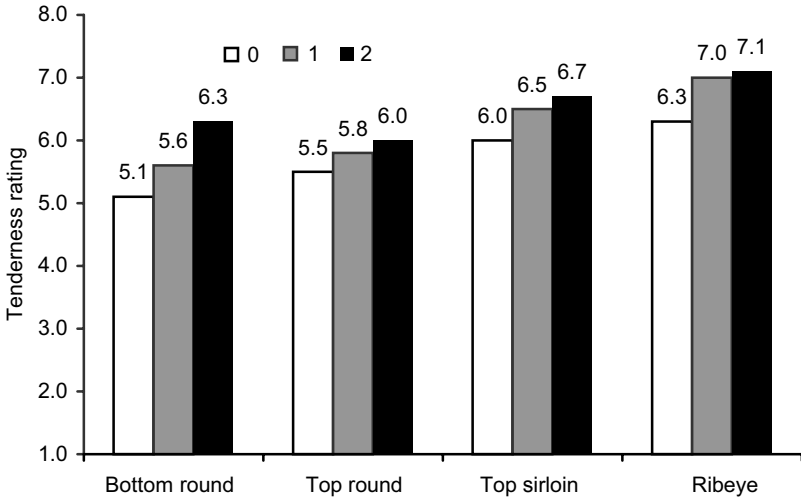


Fig. 3.3 Trained sensory panel tenderness ratings for four beef muscles from Piedmontese cross cattle with 0, 1, or 2 copies of the inactive myostatin allele. Adapted from Wheeler *et al.* (2001b).

3.3 Antemortem factors affecting meat tenderness

3.3.1 Genetic conditions affecting tenderness

Meat tenderness is affected by complex interactions of multiple antemortem and postmortem factors. Genetics determine an animal's potential for producing tender meat, and the interaction of genetics with ante- and postmortem environment and management will determine the ultimate tenderness of the meat from an animal. A mutation in the myostatin gene has been associated with the condition in cattle known as 'double muscling' (Grobet *et al.*, 1997; Kambadur *et al.*, 1997; McPherron and Lee, 1997; Smith *et al.*, 1997). This syndrome is characterized by embryonic hyperplasia caused by inactive myostatin, which normally inhibits cell-proliferation. Carcasses of double-muscled cattle yield a greater percentage of retail product than carcasses of normal cattle (Arthur, 1995; Wheeler *et al.*, 1997a). Additionally, meat from these animals is more tender, primarily due to reduced collagen concentration (Wheeler, unpublished data). Animals with one or two inactive myostatin alleles produced ribeye, top sirloin, bottom round, and top round steaks that received greater trained sensory panel tenderness ratings than animals with two normal alleles at the myostatin locus (Fig. 3.3; Wheeler *et al.*, 2001b). In addition, bottom round steaks from animals with two inactive myostatin allele received higher tenderness ratings than steaks from animals with one inactive allele. In all muscles evaluated, increasing the number of inactive myostatin

alleles decreased collagen concentration (Wheeler, unpublished data). This trend was more pronounced in the bottom round, which had the highest collagen content of the four muscles evaluated.

Cattle with *Bos indicus* inheritance are commonly used in tropical and subtropical environments. The heat tolerance and insect resistance possessed by these breeds, coupled with their maternal characteristics, have made them a valuable part of beef production in these regions. However, *Bos indicus* cattle, especially Brahman, have been repeatedly reported to produce tougher meat than *Bos taurus* cattle (Koch *et al.*, 1982; Peacock *et al.*, 1982; Crouse *et al.*, 1989; Wheeler *et al.*, 1990a,b, 1996a, 2001a). Increased toughness of meat produced by *Bos indicus* cattle has been demonstrated to be due to increased calpastatin levels (Wheeler *et al.*, 1990b; Whipple *et al.*, 1990b; Pringle *et al.*, 1997), resulting in less proteolytic degradation (Whipple *et al.*, 1990b) and slower improvements in tenderness with ageing (Wheeler *et al.*, 1990a,b; O'Connor *et al.*, 1997) in meat from *Bos indicus*-influenced carcasses. Breeding programs utilizing *Bos taurus* × *Bos indicus* matings are commonly used to capitalize on heterosis and the positive traits possessed by *Bos indicus* breeds. Crouse *et al.* (1989) reported that increasing the proportion of Brahman inheritance from 0 to 100% resulted in progressive decreases in trained sensory panel tenderness ratings. This effect was even more pronounced as percentage of Sahiwal inheritance increased from 0 to 100%. Additionally, the variation in tenderness within a breed group increased as the percentage of *Bos indicus* inheritance increased. In partial agreement, Johnson *et al.* (1990) reported that increasing Brahman influence reduced tenderness scores and inhibited improvements in Warner–Bratzler shear force after 5 or 10 d of ageing. However, Johnson *et al.* (1990) reported no difference in tenderness or ageing response between Angus and ¼ Brahman steers. The use of composite breeds comprised of 3/8 *Bos indicus* inheritance and 5/8 *Bos taurus* inheritance is commonly used by beef producers to incorporate the positive attributes of *Bos indicus* cattle. Bidner *et al.* (2002) reported that calves sired by bulls of Brahman derivative breeds (3/8 Brahman) were less tender after 10 d of ageing than calves sired by Angus bulls. This is in agreement with the results of O'Connor *et al.* (1997) which found that *longissimus* steaks from 3/8 *Bos indicus* steers produced higher shear force values when compared to *Bos taurus* steers after ageing up to 35 d postmortem, regardless of the source of the *Bos taurus* germplasm.

A muscle hypertrophy condition in lamb that causes dramatic toughening of the resulting meat is called callipyge (Cockett *et al.*, 1994, 2005; Koohmaraie *et al.*, 1995; Carpenter *et al.*, 1996; Freking *et al.*, 1998). The hypertrophy is caused by decreased protein degradation, and is associated with increased calpastatin activity in the hypertrophied muscles (Koohmaraie *et al.*, 1995; Lorenzen *et al.*, 2000). The response of various muscles to the callipyge condition is proportional to the increase in calpastatin activity within the muscle (Koohmaraie *et al.*, 1995). The meat from callipyge lamb is less tender than normal lamb because the higher calpastatin activity inhibits the rate and extent of postmortem proteolysis by μ -calpain (Koohmaraie *et al.*, 1995).

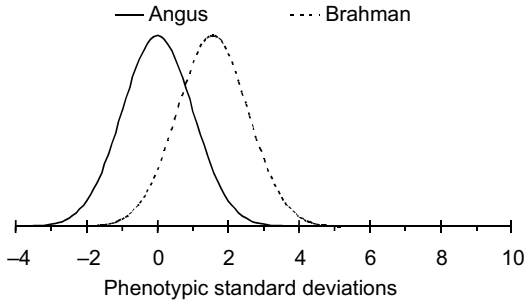


Fig. 3.4 Phenotypic variation in *longissimus* tenderness of Angus- (most tender breed represented) and Brahman- (toughest breed represented) sired progeny. Adapted from Wheeler *et al.* (2001a).

3.3.2 Genomic approaches to meat tenderness

Measures of tenderness have been reported to be moderately heritable, with estimates ranging from 0.30 to 0.53 (Shackelford *et al.*, 1994; Wheeler *et al.*, 1996a, 2001a, 2004a, 2005; Dikeman *et al.*, 2005). Smith *et al.* (2003) estimated that 46% of the variation in tenderness is genetic and 54% is environmental. This indicates that improving tenderness via genetic selection is possible. Palatability trait differences have been characterized among cattle breeds (Koch *et al.*, 1982; Wheeler *et al.*, 1996a, 2001a, 2004a, 2005) and are considered in cross-breeding programs. However, substantial variation in tenderness exists within breeds and the opportunity for improving tenderness by selecting seedstock within a breed may be as great, or greater, than by changing breeds (Fig. 3.4; Wheeler, *et al.*, 1996a, 2001a).

Several quantitative trait loci (QTL) associated with tenderness have been identified (Smith *et al.*, 2003). Of these, two have been found that correspond to the genes responsible for μ -calpain (CAPN1) and its inhibitor calpastatin (CAST). Two SNP markers with significant utility for marker-assisted selection have been identified for the CAPN1 gene (Page *et al.*, 2002; White *et al.*, 2005). The allele associated with improved tenderness in one of these markers (CAPN1 316) is present at low to intermediate frequencies in *Bos taurus* populations (Table 3.1; Page *et al.*, 2004), but segregate less appreciably in *Bos indicus* populations (Casas *et al.*, 2005). A second marker (CAPN1 4751) was reported by White *et al.* (2005) to segregate in both *Bos taurus* and *Bos indicus* populations. These authors suggested that simultaneous analysis of the CAPN1 316 and 4751 markers would provide optimal information in marker assisted selection for tenderness at the μ -calpain locus. Several markers flanking the CAST gene have been demonstrated to be associated with tenderness (Casas *et al.*, 2006; Schenkel *et al.*, 2006). The favorable allele of one of these markers has been reported to have a frequency of approximately 60% in commercial cattle (Table 3.1.; Schenkel *et al.*, 2006). Casas *et al.* (2006) reported that the effects of the CAPN1 and CAST loci appear to be

Table 3.1 Frequencies of favorable alleles and effects of unfavorable alleles of SNP markers for Warner–Bratzler shear force values measured at 14 d postmortem

Population	Favorable allele	Frequency (%)	Effect of unfavorable allele 14 d WBSF (kg)		Source
			Heterozygous unfavorable	Homozygous unfavorable	
CAST					
<i>Bos taurus</i>	T	79	0.29	0.31	Casas <i>et al.</i> (2006)
<i>Bos indicus</i>	T	72	0.28	0.48	Casas <i>et al.</i> (2006)
<i>Bos taurus</i> × <i>Bos indicus</i>	T	83	0.05	0.47	Casas <i>et al.</i> (2006)
CAST _{RsaI}					
<i>Bos taurus</i>	C	63	0.16	0.21	Schenkel <i>et al.</i> (2006)
CAPN1 ₃₁₆					
<i>Bos taurus</i>	C	32	0.16	0.29	Page <i>et al.</i> (2004)
<i>Bos indicus</i>	C	1	–	–	Casas <i>et al.</i> (2005)
<i>Bos taurus</i> × <i>Bos indicus</i>	C	41	0.18	0.55	White <i>et al.</i> (2005)
CAPN1 ₄₇₅₁					
<i>Bos taurus</i>	C	58	–0.014	0.27	White <i>et al.</i> (2005)
<i>Bos indicus</i>	C	10	0.40	–	White <i>et al.</i> (2005)
<i>Bos taurus</i> × <i>Bos indicus</i>	C	64	0.28	0.44	White <i>et al.</i> (2005)

additive and do not interact. Therefore, it appears that markers for both of these genes can be used simultaneously in breeding programs to improve tenderness. Markers for both genes are available in commercial tests. The effects of these markers on tenderness are relatively small (Table 3.1), but indicate significant opportunity to improve the tenderness of the beef population.

Continuing efforts are needed to validate the effectiveness of these markers on independent resource populations as well as samples of commercial cattle. Additionally, it is likely that additional markers will be identified and developed as new populations become available. Furthermore, understanding how the genes identified by these markers interact with one another and with environmental factors to affect tenderness is needed. Knowledge of those interactions would allow the development of expected progeny differences (EPDs) that are adjusted based on genetic marker information (Smith *et al.*, 2003). Finally, research on the interaction between genotype and management strategies will allow a whole-system approach to improving tenderness.

3.3.3 Grain feeding effects on tenderness

In the U.S. and other countries, market animals are commonly placed in feed lots and given *ad libitum* access to concentrate-based rations to produce rapid, efficient

growth and to ensure that animals reach slaughter weights at young ages. This practice has been reported to produce heavier, fatter, and more muscular carcasses compared to forage feeding (Bowling *et al.*, 1977; Aberle *et al.*, 1981). Concentrate-fed animals also produced steaks that were more tender than forage-fed animals. Increased mass and fat thickness in grain-fed carcasses slows chilling and, consequently, reduces sarcomere shortening during the onset of rigor (Tatum, 1981). Bowling *et al.* (1977) compared the tenderness of meat from grain-fed steer carcasses and slowly or conventionally chilled forage-fed carcasses. Sarcomere lengths were longest in grain-fed carcasses, shortest in conventionally chilled, forage-fed carcasses, and intermediate in slowly chilled, forage-fed carcasses. Trained sensory panel tenderness ratings were highest for meat from grain-fed carcasses and did not differ across chilling rates.

These data suggest that increased tenderness associated with grain feeding is not solely due to reduced sarcomere shortening. The improved tenderness of grain-fed animals is likely attributable to increased growth rate, which has been associated with increased protein turnover (Koochmaraie *et al.*, 2002), postmortem proteolysis (Aberle *et al.*, 1981; Purchas *et al.*, 2002), and collagen solubility (Aberle *et al.*, 1981). Aberle *et al.* (1981) reported that increasing time on a high-energy diet to 70 d increased tenderness ratings, myofibril fragmentation index (a measure of proteolytic degradation), and collagen solubility. These authors attributed these differences to rapid growth in grain-fed animals. These findings are consistent with those of May *et al.* (1992), who found that sensory panel tenderness ratings were optimized at 84 d on feed. Tatum *et al.* (1980) found no improvements in tenderness associated with feeding steers longer than 100 d. Short *et al.* (1999) reported that tenderness increased with increasing time on feed, but steers placed on feed at 18 mo of age showed no further improvement after 90 d on feed while steers placed on feed at 6 mo of age displayed slight improvements through 270 d on feed. These findings suggest that grain feeding cattle for 90 to 100 d should optimize tenderness.

3.3.4 Effects of growth promotants on meat tenderness

Improving the rate and efficiency of growth in market animals is an important economic consideration to livestock producers. Therefore, the administration of agents that partition nutrients towards muscle deposition is a common practice. Though numerous metabolic modifiers have been utilized in meat production, the most commonly used include anabolic steroids and beta adrenergic agonists (BAA). Despite substantial evidence demonstrating the efficacy and safety of using growth promotants, their use is prohibited in some countries, particularly the European Union.

The vast majority of cattle fed in the U.S. receive anabolic steroid implants, which can be broadly classified according to the chemical nature of their active ingredients (estrogens, progestins, androgens, and combination) and the concentration of each (mild or strong; Montgomery *et al.*, 2001). Of single ingredient implants, estrogenic implants are considered most effective in steers while andro-

genic implants are considered most effective in heifers (Dikeman, 2003). Combination implants containing both estrogens and androgens generally provide greater growth responses than single ingredient implants and are, therefore, considered to be most 'aggressive' (Montgomery *et al.*, 2001; Dikeman, 2003). Animals may be implanted at multiple stages of production and may receive multiple implants during the finishing phase. Endless combinations of compounds, dosages, and timing that might be utilized in an implant strategy make discerning the effects of implants extremely complex. However, anabolic implants are generally reported to have neutral or negative effects on meat tenderness compared to non-implanted controls (Morgan, 1997; Montgomery *et al.*, 2001; Dikeman, 2003). In a review of available literature, Morgan (1997) estimated that meat from implanted animals has a Warner–Bratzler shear force value 0.5 kg higher than meat from non-implanted animals. These effects are largely dependent on the implanting strategy used. As implanting strategies increase in aggressiveness (use of androgenic, combination and/or multiple implants), the negative effects on tenderness are amplified (Morgan, 1997; Platter *et al.*, 2003). Schneider *et al.* (2007) reported that Warner–Bratzler shear force values increased linearly as the cumulative dose of estradiol 17- β plus trenbolone acetate (1:10 ratio) increased in two sequential implants. Samber *et al.* (1996) studied several implant protocols and reported that using a mild implanting strategy at the beginning of the feeding period was less detrimental to tenderness than strategies using aggressive implants at the beginning of the feeding period. Platter *et al.* (2003) studied the effects of various lifetime implanting protocols (from approximately 2 mo of age through finishing) and found that Warner–Bratzler shear force values increased as the number of lifetime implants increased from zero to two and from two to three. Steers implanted four or five times had shear force values intermediate to the shear force values of steers implanted two or three times. Dikeman (2003) concluded that cattle should not be implanted within 70 d of slaughter.

Interest in the use of BAA (e.g., Cimaterol, L_{644,969}, ractopamine, zilpaterol) in meat animals has increased because of the dramatic increases in lean growth associated with their use. Numerous reports indicate that BAA negatively affects meat tenderness (Dikeman, 2003). The toughening effect is thought to be due to reduced proteolysis resulting from increased calpastatin activity (Koochmaraie *et al.*, 1991, 1996b). In the U.S., BAA have recently been approved for use in swine (ractopamine) and beef cattle (ractopamine and zilpaterol). The compounds also are approved for cattle in some other countries. Feeding pigs either 10 or 20 ppm ractopamine resulted in higher Warner–Bratzler shear force values, but did not affect tenderness scores compared to non-supplemented pigs (Carr *et al.*, 2005). This is in agreement with the findings of Uttaro *et al.* (1993), who reported that 20 ppm ractopamine increased Warner–Bratzler shear force values and decreased myofibril fragmentation in loin chops of supplemented pigs compared with those of non-supplemented pigs. Gruber *et al.* (2007) reported that cattle fed 200 mg/steer/d ractopamine produced steaks that were less tender than non-supplemented control through 21 d of postmortem ageing. In their study, the effect of ractopamine on tenderness was larger in Brahman crossbred steers than in

Continental or British crossbred cattle (0.5, 0.3, 0.2 kg Warner–Bratzler shear force, respectively). Avendaño-Reyes *et al.* (2006) compared cattle fed ractopamine and zilpaterol to non-supplemented controls and found that both compounds increased Warner–Bratzler shear force values of *longissimus* steaks by 10 and 16%, respectively. Strydom and Nel (1999) fed zilpaterol (0.15 mg/kg live weight/d) to steers for the final 30 or 50 days on feed and reported that both zilpaterol treatments reduced sensory panel tenderness ratings by 0.8 units (8 point scale) compared to controls. In comparison, Casey *et al.* (1997) reported even greater increases in Warner–Bratzler shear force due to zilpaterol fed to steers that had been either implanted with 24 mg estradiol and 120 mg trenbolone acetate (1.5 kg) or not implanted (2 kg). Available information indicates that feeding currently available BAA would have detrimental effects on tenderness.

3.4 Postmortem technologies affecting meat tenderness

Once an animal has been harvested, postmortem management strategies can be employed to optimize the tenderness of the resulting meat. These strategies either manipulate the inherent biochemical processes that are active in postmortem muscle, or physically or chemically alter the myofibrillar or connective tissue structures of the muscle. When applied appropriately, postmortem technologies can improve the overall palatability and consistency of meat being produced.

3.4.1 Postmortem ageing

Soon after slaughter, the calpain enzyme system begins degrading cytoskeletal proteins (Koohmaraie, 1996; Veiseth *et al.*, 2004). The rate and extent of the ageing response is influenced by numerous factors such as the calpastatin activity in the muscle, muscle pH, and storage temperature. Though tenderization with ageing is well established, identifying optimum ageing time is difficult. Koohmaraie (1996) recommended that beef, lamb, and pork *longissimus* be stored for 10–14 d, 7–10 d, and 5 d, respectively, to ensure adequate tenderization. It is important to note the results of muscle profiling studies (e.g. Rhee *et al.*, 2004; Von Seggern *et al.*, 2005) suggest that the contribution of component traits driving tenderness differences are muscle dependent. Therefore, the ageing time needed to optimize tenderness is likely to differ across muscles. Gruber *et al.* (2006) reported that 17 muscles differed in ageing response (2 to 28 d of storage) with the decrease in Warner–Bratzler shear force ranging from 0.5 kg in US Choice biceps femoris to 2.5 kg in US Select *longissimus lumborum*. Mies *et al.* (1999) reviewed literature pertaining to different subprimal cuts of beef and recommended ageing times of 12, 11, 11–15, 14, 21, 12, and 15 d for chuck roll (*longissimus thoracis* and complexus), shoulder clod (*triceps brachii* and *infraspinatus*), ribeye roll (*longissimus thoracis*), strip loin (*longissimus lumborum*), top sirloin (*gluteus medius*), bottom round (*biceps femoris*), and top round (*semimembranosus* and *adductor*)

subprimals, respectively. The identification of optimum ageing times for various muscles is further complicated by differing reports on the magnitude of tenderness improvements due to ageing. These inconsistencies in the literature may be partially due to meat that is inherently tender requiring less ageing time to reach a point where no further increases are detectable. Additionally, in some studies, ageing treatments may be confounded with intramuscular differences in tenderness (Rhee *et al.*, 2004) which may potentially mask the effects of ageing on tenderness. King *et al.* (2007) attempted to mitigate the confounding effects of location within the muscle in an investigation of ageing effects on gluteus medius and triceps brachii tenderness by evaluating 50 muscles of each at six ageing times (7 to 42 d postmortem) and sampling a single location in each muscle for tenderness evaluations. In that experiment, slice shear force values decreased by 24 and 21% in gluteus medius and triceps brachii, respectively. However, most of the improvement in slice shear force values (18%) had been achieved by 28 d postmortem in both muscles.

3.4.2 Electrical stimulation

Electrical stimulation applied to pre-rigor carcasses causes muscle contraction and, consequently, rapid depletion of glycogen stores in muscle before chilling. Electrical stimulation has been reported to enhance the tenderness of beef (Cross *et al.*, 1979, 1984; Savell *et al.*, 1981). The improvement in tenderness is generally attributed to the prevention of cold-induced toughening (Cross *et al.*, 1984), activation of endogenous proteases (Bowling *et al.*, 1978; Cross, 1979), and physical disruption of muscle fibers (Savell *et al.*, 1978). Hopkinson *et al.*, (1985) reported that Warner–Bratzler shear force values of *longissimus* steaks from electrically stimulated steer carcasses at 2 d postmortem were similar to *longissimus* steaks from non-stimulated carcasses aged for 14 d (6.41 and 6.35, respectively). This is in agreement with the results of Savell *et al.* (1981), who reported that the improvement in Warner–Bratzler shear force values due to electrical stimulation at 1 d postmortem (25.8%) was similar to the improvement due to 8 d of postmortem ageing (25.1%). Savell *et al.* (1981) also reported that the improvement in tenderness due to electrical stimulation was greater in carcasses that were initially tough than in those carcasses that were inherently tender. Electrical stimulation effects on tenderness are highly dependent on the parameters used in electrical stimulation application. Generally, high voltage is required to elicit a significant tenderization response. The extensive literature regarding electrical stimulation should be consulted for additional information.

3.4.3 Chilling effects on tenderness

During the first 24 h postmortem, anaerobic glycolysis depletes muscle glycogen causing the accumulation of lactate. As a result, muscle pH declines from 7.4 to approximately 5.5. As ATP stores are depleted, actomyosin bonds are fixed. During this process, sarcomere shortening occurs, causing toughening (Wheeler

and Koohmaraie, 1994). At lower temperatures, the sarcoplasmic reticulum's ability to sequester calcium is reduced (Whiting, 1980). As a result, low temperatures (below 10 °C) in early postmortem muscle possessing adequate ATP stores to cause contraction result in sarcomere shortening (Locker and Hagyard, 1963), which causes toughening (Marsh and Leet, 1966; Marsh *et al.*, 1968). This condition is termed 'cold shortening'. Marsh and Leet (1966) suggested that cold shortening could be minimized by not allowing muscle temperatures to fall below 10 °C until muscle pH had declined below 6.2.

Additionally, the rate of temperature decline can have profound effects on proteolytic degradation of muscle proteins. Extremely rapid chilling of longissimus and triceps brachii muscles resulted in less desmin degradation (10 percentage points) during the first 24 h postmortem (King *et al.*, 2003). Rates of desmin degradation were similar in rapidly chilled and conventionally chilled muscles during the next 13 d of ageing. In contrast, Whipple *et al.* (1990a) compared conventional chilling to holding carcasses at ambient temperatures for 6 h before chilling. Delayed chilling provided a more favorable environment for proteolysis, resulting in higher myofibril fragmentation index values at 3, 7, and 14 d postmortem and lower shear force values at 1 d postmortem in carcasses subjected to delayed chilling. However, Warner–Bratzler shear force values were not affected by delayed chilling at 14 d postmortem. Though high-temperature conditioning is effective in improving tenderness, this practice is not recommended due to food safety concerns.

3.4.4 Pre-rigor alterations in carcass position

The extent of sarcomere shortening during the onset of rigor mortis in an intact carcass is limited by skeletal restraint. Herring *et al.* (1965a,b) and Hostetler *et al.* (1970) found that hanging carcasses by the achilles tendon stretches some muscles while allowing others to contract, which impacted tenderness. Several investigators have examined the use of alternative methods of hanging or altering carcasses to increase the tension on valuable muscles. In a method called 'tenderstretch', Hostetler *et al.* (1970 and 1972) hung carcasses by the obturator foramen to stretch the *longissimus* and other valuable muscles. This method is used by some processors in Europe. Carcasses in Australia can be tenderstretched to qualify for a higher quality grade. This strategy is not currently used in the U.S., primarily because tenderstretched carcasses require more space in the chilling cooler, and the shape of muscles from tenderstretched carcasses are not conducive to traditional fabrication methods. However, with the advent of marketing individual muscles, this strategy might merit reconsideration by U.S. processors. Another procedure termed 'tender cut' involves severing the bones and connective tissue at the 12th thoracic vertebrae and in the pelvic girdle to stretch the *longissimus* and round muscles (Wang *et al.*, 1994, 1996; Ludwig *et al.*, 1997). This process places the weight of the carcass on the muscle rather than the skeleton, thereby lengthening sarcomeres and improving tenderness in economically important muscles.

3.4.5 Enhancement strategies to improve tenderness

Processing strategies can be applied to post-rigor muscle to improve palatability. Blade tenderization is commonly applied to cuts destined for food service establishments. In this process, thin, needle-like blades are used to disrupt muscle fibers and connective tissue. George-Evins *et al.* (2004) reported that blade tenderization improved the tenderness of gluteus medius steaks without negative effects on other palatability traits. Savell *et al.* (1977) evaluated the use of blade tenderization on four beef muscles and noted that a single pass through a blade tenderizer decreased shear force values of gluteus medius, semimembranosus, and longissimus steaks, but did not affect biceps femoris steaks relative to non-tenderized controls. A second application of blade tenderization further reduced Warner–Bratzler shear force values of semimembranosus and also improved biceps femoris steaks. Additionally, after a single application of blade tenderization, longissimus steaks received higher sensory panel ratings for overall and myofibrillar tenderness, and perceivable connective tissue (less connective tissue), but were also rated as less juicy. Another approach to muscle enhancement is to use injection of marinades containing non-meat ingredients to increase palatability traits (Miller, 1998). Marinades commonly contain salts (e.g. sodium chloride), water-binders (e.g. sodium phosphates), exogenous enzymes (e.g. papain, bromelain, and ficin), and/or antioxidants (e.g. rosemary). The effects of these solutions on tenderness are mediated through the dilution of myofibrillar proteins, the degradation of myofibrillar or connective tissue proteins by exogenous enzymes or the activation of endogenous enzymes by calcium chloride (Koohmaraie *et al.*, 1989; Wheeler *et al.*, 1992, 1993). Vote *et al.* (2000) found that injecting beef strip loins with distilled water or 0.25% sodium tripolyphosphate solution decreased tenderness relative to untreated controls, but injecting with 0.25% sodium tripolyphosphate, 2.5% sodium lactate, 0.5% sodium chloride solution improved tenderness. Additionally, Vote *et al.* (2000) reported that increasing the level of injection from 7.5 to 15% of green weight provided incremental increases in tenderness. Mueller *et al.* (2006) reported that beef round muscles injected (12% pickup) with a 5% sodium chloride and 2.95% sodium tripolyphosphate solution received higher consumer ratings for tenderness, juiciness, and flavor than blade tenderized or non-injected muscles. Calcium chloride has been used to activate the calpain system and increase tenderization in beef longissimus, semimembranosus, and triceps brachii compared to non-injected or water-injected controls, regardless of injection level, calcium chloride concentration, or time of injection (Wheeler *et al.*, 1993; 1997b); however, no commercial implementation of calcium chloride marinades has occurred to our knowledge.

3.5 Laboratory tenderness assessment

The evaluation of strategies intended to improve tenderness requires that tenderness assessments be accurate and repeatable. AMSA (1995) provides a set of guidelines for tenderness assessment by trained sensory panels and shear force

measurements. Regardless of the method used, care must be exercised to collect accurate and repeatable tenderness data.

3.5.1 Trained sensory panel

The gold standard for tenderness assessment is a trained sensory panel comprised of highly trained individuals. There are numerous ways to use a trained sensory panel, depending on the question to be addressed. One of the most common is a trained descriptive attribute panel for detecting differences in traits such as tenderness, juiciness, and flavor intensity. Some considerations for the selection and training of panel members include availability, motivation, skill, and consistency (Wheeler *et al.*, 1997d). Panelists should be identified, screened to assure their ability to discern differences, and trained as described by Cross *et al.* (1978) and AMSA (1995). Samples presented to the panel must be of sufficient quantity to allow all panel members to evaluate the sample adequately and representative of the inferences to be made. Constant evaluation of the panel and periodic refresher training of the panelists is necessary to prevent drift and to maintain accuracy and precision. In addition, a well trained sensory panel is expensive for a laboratory to maintain.

3.5.2 Untrained/consumer panel

Consumer evaluations of meat products provide excellent information regarding acceptability of products. In-home tests provide important information about consumer acceptance and preferences when actually using the product (e.g. Neely *et al.*, 1998; Savell *et al.*, 1999). However, these studies are complicated by lack of control over sample preparation, consumers failing to complete the study, and the potential for incomplete or incorrect recording of data. Central location consumer panels provide the opportunity to collect consumer impressions while controlling sample presentation and provide a data collection environment that may improve accuracy (e.g. Hoover *et al.*, 1995; Carr *et al.*, 2004). Wheeler *et al.* (2004b) evaluated untrained laboratory consumer panels in relation to slice shear force and noted that consumer panels were able to detect differences in tenderness categories with as few as four panelists, although the correlation of mean panel ratings to slice shear force values increased as the number of panelists increased to 16 ($r = -0.68$ and -0.92 for panels consisting of 4 and 16 panelists, respectively). The repeatability of mean panel scores of duplicate samples was 0.80, although accuracy and repeatability varied widely among individual panelists. These data suggest that, depending on the samples of interest, untrained laboratory consumer panels may be a valuable tool in evaluating meat tenderness.

3.5.3 Warner–Bratzler shear force

Sensory panel data collection is a slow, cumbersome process. The need to collect data on a large number of samples in a narrow time frame has led to the widespread

use of instrumental measures of tenderness. The most common instrumental objective tenderness measurement is Warner–Bratzler shear force. Warner–Bratzler shear force involves shearing 1.27 cm-diameter cores (removed from cooked steaks parallel to the muscle fiber orientation) with a V-notch blade (1.016 mm-thick with a half-round bevel) attached to a load cell on a Warner–Bratzler shear machine or a universal testing machine. Though collection of shear force data is viewed as routine, differences in accuracy and repeatability exist between individual laboratories conducting tests (Wheeler *et al.*, 1997c). Obtaining accurate and repeatable Warner–Bratzler shear force data requires investigators to be vigilant with regard to numerous factors such as cooking methodology, core removal, and shearing of cores (Wheeler *et al.*, 1996b, 1998). For detailed discussions of standardizing protocols, the reader is referred to Savell *et al.* (1994) and Wheeler *et al.* (1997d).

3.5.4 Slice shear force

Efforts to develop a rapid tenderness measurement to be used in on-line tenderness classification programs resulted in the advent of slice shear force (Shackelford *et al.*, 1999). Slice shear force involves the shearing of a 1 cm thick \times 5 cm long slice removed parallel to the muscle fiber orientation with a flat, blunt-ended blade. The thickness and bevel of the slice shear force blade is the same as those used for the Warner–Bratzler V-notch blade. Slice shear force values are highly repeatable ($r = 0.91$) and more strongly related to trained sensory panel ratings than Warner–Bratzler shear force values ($r = -0.82$ and -0.77 , respectively; Shackelford *et al.*, 1999). Additionally, removing ‘good’ slices for slice shear force is easier than acquiring ‘good’ cores for Warner–Bratzler shear force. Finally, since slice shear force can be measured on hot steaks and is measured on one slice rather than 6 or more cores, lab throughput is increased relative to Warner–Bratzler shear force.

Other instrumental measures of tenderness have been used, depending on the type of information desired by the investigator. These include compression tests, Allo–Kramer shear, the MIRINZ tenderometer, as well as various modifications of shear tests. Regardless of the type of shear force measured, investigators should be cognizant of the limitations of shear force data. It is common to attempt to identify thresholds for consumer acceptance. However, the inherent differences between labs and varying consumer acuity and perceptions for tenderness make these attempts dubious (Wheeler *et al.*, 1997c,d). A level of tenderness that is perfectly acceptable to one consumer may be completely unacceptable to another. In general, inferences made using shear force data should be limited to relative treatment differences within an experiment. Finally, Warner–Bratzler and slice shear force values do not accurately reflect differences in tenderness between muscles (Harris and Shorthose, 1988). Shackelford *et al.* (1995) reported that Warner–Bratzler shear force values identified few differences among 10 beef muscles, but trained sensory panel ratings stratified these muscles into four tenderness groups. This is likely because shear force measures do not accurately represent the contribution of connective tissue to tenderness differences between

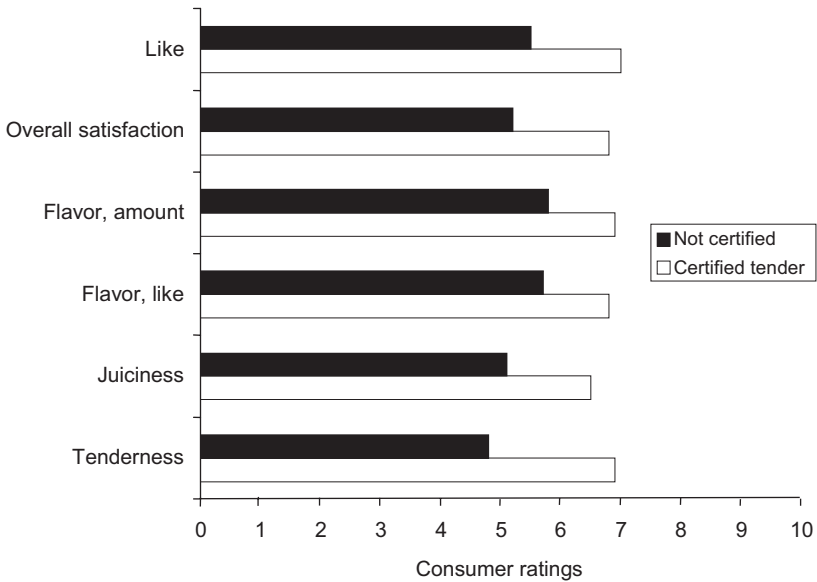


Fig. 3.5 Consumer ratings of sensory traits of steaks certified tender or not-certified by a slice shear force tenderness classification system. Adapted from Shackelford *et al.* (2001).

muscles (Bouton *et al.*, 1978). Those conclusions were confirmed by the results of Rhee *et al.* (2004).

3.6 On-line tenderness prediction

Many investigators have attempted to apply technologies such as Tendertec (George *et al.*, 1997; Belk *et al.*, 2001), connective tissue probe (Swatland, 1995; Swatland and Findlay, 1997; Swatland *et al.*, 1998), elastography (Berg *et al.*, 1999), near-infrared spectroscopy (Hildrum *et al.*, 1994; Park *et al.*, 1998), ultrasound (Park and Whittaker, 1991; Park *et al.*, 1994), image analysis (Li *et al.*, 1999, 2001), lean color attributes (Wulf *et al.*, 1997), image analysis traits using prototype Beef Cam modules (Belk *et al.*, 2000), and a combination of colorimetric, marbling, and hump height traits (Wulf and Page, 2000), for the purpose of tenderness classification of meat, with little success. In the early 1990s, scientists at USMARC concluded that a direct measure of tenderness was required to obtain an accurate enough measurement to create a useful tenderness classification system, if the tenderness assessment could be made at normal processing speeds. To this end, slice shear force was developed and incorporated into a rapid process for cooking and obtaining a slice shear force value of the longissimus muscle as carcasses were presented for grading (Shackelford *et al.* 1998, 1999). Consumers gave higher ratings for all sensory traits to U.S. Select beef classified as tender by slice shear force compared to beef not classified as tender (Fig. 3.5; Shackelford *et al.*, 2001).

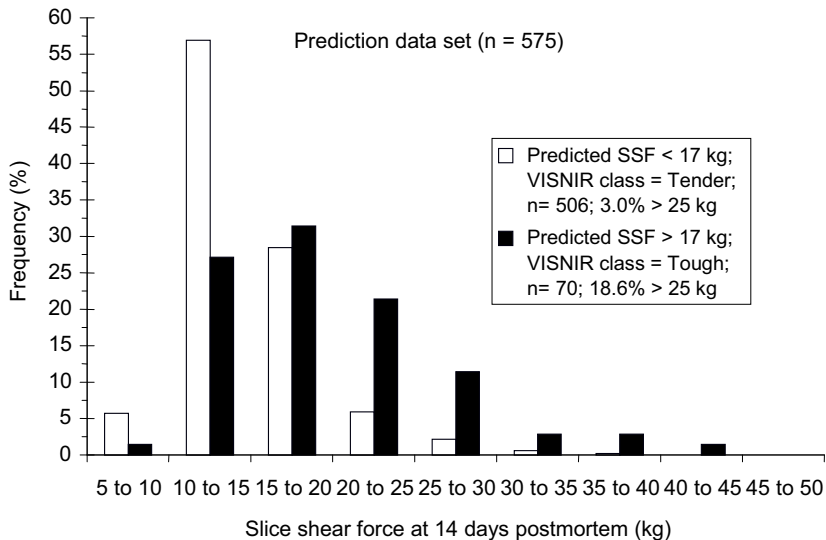


Fig. 3.6 Distribution of slice shear force values of *longissimus* steaks predicted as tough or tender by VISNIR spectroscopy. Adapted from Shackelford *et al.* (2005).

To date, one large beef processor is using the slice shear force technology in cooperation with a large retailer to produce a guaranteed-tender product line. Anticipating that the majority of the industry would deem it to be too invasive and costly for implementation, simultaneous efforts were focused on developing a non-invasive approach. Visible and near-infrared-reflectance (VISNIR) spectroscopy technology appeared to have the most potential to classify beef carcasses according to tenderness (Shackelford *et al.*, 2005). Early application of VISNIR tenderness classification was focused on US Select carcasses because these carcasses are discounted relative to US Choice carcasses, even though the vast majority of US Select carcasses are acceptably tender. Shackelford *et al.*, (2005) reported that VISNIR spectroscopy was effective in identifying carcasses that produced tender meat after ageing. In the validation data set reported in that study, carcasses predicted to be tender had higher sensory panel tenderness ratings compared to those not predicted to be tender (5.6 versus 5.1, respectively). Furthermore, only 5.5 percent of carcasses predicted to be tender had slice shear force values greater than 25 kg compared to 30.1% of those not predicted to be tender (Fig. 3.6). Future research objectives related to tenderness prediction include the application of this technology to carcasses of all quality grades, muscles other than the *longissimus*, and other species.

3.7 Conclusions

Tenderness is critical to the consumer acceptance of meat products. Numerous

antemortem and postmortem factors can impact tenderness, both positively and negatively. Therefore, antemortem and postmortem management decisions must be made carefully. Recent advances in the development of genetic markers for use in selection decisions are encouraging. Meanwhile, antemortem management should strive to optimize efficiency and palatability traits. Tenderness prediction technology can be used to segregate carcasses into product lines. Additionally, postmortem strategies can be used singularly or in concert to further ensure the eating quality of muscle foods.

3.8 Sources of further information and advice

Muscle structure, contraction, and conversion of muscle to meat

Aberle, E.D., Forrest, J.C., Gerrard, D.E., and Mills, E.W. (2001), *Principles of Meat Science*, Dubuque, IA, USA, Kendall/Hunt Publishing

Postmortem tenderization

Koohmaraie, M. (1994), Muscle proteinases and meat ageing. *Meat Sci.*, **36**, 93–104.

Koohmaraie, M. (1995), The biological basis of meat tenderness and potential genetic approaches for its control and prediction. *Proc. Recip. Meat Conf.*, **48**, 69–75.

Koohmaraie, M. (1996), Biochemical factors regulating the toughening and tenderization process of meat. *Meat Sci.*, **43**, S193–S201

Growth promotants

Dikeman, M.E. (2003), Metabolic modifiers and genetics: Effects on carcass traits and meat quality. *Intl. Cong. Meat Sci. Technol.*, **49**, 1–38.

Montgomery, T.H., Dew, P.F. and Brown, M.S. (2001), Optimizing carcass value and the use of anabolic implants in beef cattle. *J. Anim. Sci.*, **79** (E-Suppl), E296–306.

Morgan, J.B., (1997), Implant programs effects on USDA beef carcass quality grade traits and meat tenderness. *Proc. Impact of implants on performance and carcass value of beef cattle*, Okla. Agric. Exp. Statn., Stillwater, P-957:147–154.

Tenderness measurement

AMSA (1995), *Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat*. Savoy, IL, American Meat Sci. Assoc.

Cross, H.R., Moen R. and Stanfield, M.S. (1978), Training and testing of judges for sensory analysis of meat quality. *Food Technol.*, **37**, 48.

Savell, J. W., Miller, R. K., Wheeler, T., Koohmaraie, M., Shackelford, S., Morgan, B., Calkins, C., Miller, M., Dikeman, M., McKeith, F., Dolezal, G., Henning, B., Busboom, J., West, R., Parrish, F., and Williams, S. (1994), Standardized Warner–Bratzler shear force procedures for genetic evaluation. Available: <http://meat.tamu.edu/shear.pdf> Accessed: 30 May, 2007.

Wheeler, T.L., Shackelford, S.D. and Koohmaraie, M. (1997d), Standardizing collection and interpretation of Warner–Bratzler shear force and sensory tenderness data. *Proc. Recip. Meat Conf.*, **50**, 68–77.

3.9 References

Aberle, E.D., Forrest, J.C., Gerrard, D.E., and Mills, E.W. (2001), *Principles of Meat Science*, Dubuque, IA, Kendall/Hunt Publishing.

- Aberle, E.D., Reeves, E.S., Judge, M.D., Hunsley, R.E., and Perry, T.W. (1981), Palatability and muscle characteristics of cattle with controlled weight gain: Time on a high energy diet. *J. Anim. Sci.*, 52, 757–763.
- AMSA (1995), Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat. Savoy, IL, Am. Meat Sci. Assoc..
- Arthur, P.F. (1995), Double muscling in cattle: A review. *Aust. J. Agric. Res.*, 46, 1493–1515.
- Avendano-Reyes, L., Torres-Rodriguez, V., Meraz-Murillo, F.J., Perez-Linares, C., Figueroa-Saavedra, F., and Robinson, P.H. (2006), Effects of two {beta}-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. *J. Anim. Sci.*, 84, 3259–3265.
- Avery, N.C., Sims, T.J., Warkup, C., and Bailey, A.J. (1996), Collagen cross-linking in porcine m. longissimus lumborum: Absence of a relationship with variation in texture at pork weight. *Meat Sci.*, 42 (3), 355–369.
- Behrends, J.M., Goodson, K.J., Koohmaraie, M., Shackelford, S.D., Wheeler, T.L., Morgan, W.W., Reagan, J.O., Gwartney, B.L., Wise, J.W., and Savell, J.W. (2005), Beef customer satisfaction: Factors affecting consumer evaluations of calcium chloride-injected top sirloin steaks when given instructions for preparation. *J. Anim. Sci.*, 83 (12), 2869–2875.
- Belk, K.E., Scanga, J.A., Wyle, A.M., Wulf, D.M., Tatum, J.D., Reagan, J.O., and Smith, G.C. (2000), The use of video image analysis and instrumentation to predict beef palatability. *Proc. Recip. Meat Conf.*, 53, 10–15.
- Belk, K.E., George, M.H., Tatum, J.D., Hilton, G.G., Miller, R.K., Koohmaraie, M., Reagan, J.O., and Smith, G.C. (2001), Evaluation of the Tendertec beef grading instrument to predict the tenderness of steaks from beef carcasses. *J. Anim. Sci.*, 79 (3), 688–697.
- Berg, E.P., Kallel, F., Hussain, F., Miller, R.K., Ophir, J., and Kehtarnavaz, N. (1999), The use of elastography to measure quality characteristics of pork semimembranosus muscle. *Meat Sci.*, 53 (1), 31–35.
- Bidner, T.D., Wyatt, W.E., Humes, P.E., Franke, D.E., and Blouin, D.C. (2002), Influence of Brahman-derivative breeds and Angus on carcass traits, physical composition, and palatability. *J. Anim. Sci.*, 80, 2126–2133.
- Boleman, S.J., Boleman, S.L., Miller, R.K., Taylor, J.F., Cross, H.R., Wheeler, T.L., Koohmaraie, M., Shackelford, S.D., Miller, M.F., West, R.L., Johnson, D.D., and Savell, J.W. (1997), Consumer evaluation of beef of known categories of tenderness. *J. Anim. Sci.*, 75, 1521–1524.
- Bouton, P.E., Ford, A.L., Harris, P.V., Shorthose, W.R., Ratcliff, D., and Morgan, J.H.L. (1978), Influence of animal age on the tenderness of beef: Muscle differences. *Meat Sci.*, 2, 301–311.
- Bowling, R.A., Smith, G.C., Carpenter, Z.L., Dutson, T.R., and Oliver, W.M. (1977), Comparison of forage-finished and grain-finished beef carcasses. *J. Anim. Sci.*, 45, 209–215.
- Bowling, R.A., Smith, G.C., Dutson, T.R., and Carpenter, Z.L. (1978), Effect of prerigor conditioning treatments on lamb muscle shortening, pH, and ATP. *J. Food Sci.*, 43, 502–514.
- Brooks, J.C., Belew, J.B., Griffin, D.B., Gwartney, B.L., Hale, D.S., Henning, W.R., Johnson, D.D., Morgan, J.B., Parrish, F.C. Jr., Reagan, J.O., and Savell, J.W. (2000), National Beef Tenderness Survey – 1998. *J. Anim. Sci.*, 78, 1852–1860.
- Carmack, C.F., Kastner, C.L., Dikeman, M.E., Schwenke, J.R., and Garcia Zepeda, C.M. (1995), Sensory evaluation of beef-flavor intensity, tenderness, and juiciness among major beef muscles. *Meat Sci.*, 39, 143–147.
- Carpenter, C.E., Rice, O.D., Cockett, N.E., and Snowden, G.D. (1996), Histology and composition of muscles from normal and callipyge lambs. *J. Anim. Sci.*, 74 (2), 388–393.
- Carr, M.A., Crockett, K.L., Ramsey, C.B., and Miller, M.F. (2004), Consumer acceptance of calcium chloride-marinated top loin steaks. *J. Anim. Sci.*, 82, 1471–1474.
- Carr, S.N., Ivers, D.J., Anderson, D.B., Jones, D.J., Mowrey, D.H., England, M.B., Killefer,

- J., Rincker, P.J., and McKeith, F.K. (2005), The effects of ractopamine hydrochloride on lean carcass yields and pork quality characteristics. *J. Anim. Sci.*, 83, 2886–2893.
- Casas, E., White, S.N., Riley, D.G., Smith, T.P.L., Brenneman, R.A., Olson, T.A., Johnson, D.D., Coleman, S.W., Bennett, G.L., and Chase, C.C. Jr. (2005), Assessment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in *Bos indicus* cattle. *J. Anim. Sci.*, 83 (1), 13–19.
- Casas, E., White, S.N., Wheeler, T.L., Shackelford, S.D., Koohmaraie, M., Riley, D.G., Chase, C.C. Jr., Johnson, D.D., and Smith, T.P.L. (2006), Effects of calpastatin and {micro}-calpain markers in beef cattle on tenderness traits. *J. Anim. Sci.*, 84 (3), 520–525.
- Casey, N.H., Montgomery, T.H., Scheltens, M.L. (1997), The effect of zilpaterol on feedlot performance, carcass quality, USDA carcass grades and meat quality. *Intl. Cong. Meat Sci. and Technol.*, 43, 262–263.
- Cockett, N.E., Jackson, S.P., Shay, T.D., Nielsen, D., Moore, S.S., Steele, M.R., Barendse, W., Green, R.D., and Georges, M. (1994), Chromosomal localization of the callipyge gene in sheep (*Ovis aries*) using bovine DNA markers. *Proc. Natl. Acad. Sci., USA*, 91, 3019–3023.
- Cockett, N.E., Smit, M.A., Bidwell, C.A., Segers, K., Hadfield, T.L., Snowden, G.D., Georges, M., and Charlier, C. (2005), The callipyge mutation and other genes that affect muscle hypertrophy in sheep. *Genet. Sel. Evol.*, 37(Suppl. 1), S65–S81.
- Cross, H.R. (1979), Effects of electrical stimulation on meat tissue and muscle properties – A review. *J. Food Sci.*, 44, 509–523.
- Cross, H.R., Crouse, J.D., and MacNeill, M.D. (1984), Influence of breed, sex, age, and electrical stimulation on carcass and palatability traits of three bovine muscles. *J. Anim. Sci.*, 58, 1358–1365.
- Cross, H.R., Moen R., and Stanfield, M.S. (1978), Training and testing of judges for sensory analysis of meat quality. *Food Technol.*, 37, 48
- Crouse, J.D., Cundiff, L.V., Koch, R.M., Koohmaraie, M., and Seideman, S.C. (1989), Comparison of *Bos indicus* and *Bos taurus* inheritance for carcass beef characteristics and meat palatability. *J. Anim. Sci.*, 67, 2661–2668.
- Dikeman, M.E. (2003), Metabolic modifiers and genetics: Effects on carcass traits and meat quality. *Intl. Cong. Meat Sci. Technol.*, 49, 1–38.
- Dikeman, M.E., Pollak, E.J., Zhang, Z., Moser, D.W., Gill, C.A., and Dressler, E.A. (2005), Phenotypic ranges and relationships among carcass and meat palatability traits for fourteen cattle breeds, and heritabilities and expected progeny differences for Warner–Bratzler shear force in three beef cattle breeds. *J. Anim. Sci.*, 83 (10), 2461–2467.
- Freking, B.A., Keele, J.W., Nielsen, M.K., and Leymaster, K.A. (1998), Evaluation of the ovine callipyge locus: II. Genotypic effects on growth, slaughter, and carcass traits. *J. Anim. Sci.*, 76 (10), 2549–2559.
- George, M.H., Tatum, J.D., Dolezal, H.G., Morgan, J.B., Wise, J.W., Calkins, C.R., Gordon, T., Reagan, J.O., and Smith, G.C. (1997), Comparison of USDA quality grade with Tendertec for the assessment of beef palatability. *J. Anim. Sci.*, 75 (6), 1538–1546.
- George-Evins, C.D., Unruh, J.A., Waylan, A.T., and Marsden, J.L. (2004), Influence of quality classification, aging period, blade tenderization, and endpoint cooking temperature on cooking characteristics and tenderness of beef gluteus medius steaks. *J. Anim. Sci.*, 82, 1863–1867.
- Goll, D.E. (1991), Role of proteinases and protein turnover in muscle growth and meat quality. *Proc. Recip. Meat Conf.*, 44, 25–36.
- Goll, D.E., Bray, R.W., and Hoekstra, W.G. (1964), Age-associated changes in bovine muscle connective tissue. 3. Rate of solubilization at 100 °C. *J. Food Sci.*, 29, 622–628.
- Goll, D.E., Otsuka, Y., Nagainis, P.A., Shannon, J.D., Sathe, S.K., and Muguruma, M. (1983), Role of muscle proteinases in maintenance of muscle integrity and mass. *J. Food Biochem.*, 7, 137–177.
- Grobet, L., Poncelet, D., Royo, L.J., Brouwers, B., Pirottin, D., Michaux, C., Menissier, F., Zanottie, M., Dunner, S., and Georges, M. (1997), Molecular definition of an allelic series

- of mutations disrupting the myostatin function and causing double-muscling in cattle. *Mamm. Genome*, 9, 210–213.
- Gruber, S.L., Tatum, J.D., Scanga, J.A., Chapman, P.L., Smith, G.C., and Belk, K.E. (2006), Effects of postmortem aging and USDA quality grade on Warner–Bratzler shear force values of seventeen individual beef muscles. *J. Anim. Sci.*, 84, 3387–3396.
- Gruber, S.L., Tatum, J.D., Engle, T.E., Prusa, K.J., Laudert, S.B., Schroeder, A.L., and Platter, W.J. (2007), Effects of ractopamine supplementation and postmortem aging on longissimus muscle palatability of beef steers differing in biological type. *J. Anim. Sci.* 2008, 86, 205–210.
- Harris, P.V. and Shorthose, W.R. (1988), ‘Meat texture’, in Lawrie, R.A., *Developments in Meat Science – 4*. London, Elsevier Applied Science Publishers, 245–286.
- Herring, H.K., Cassens, R.G. and Briskey, E.J. (1965a), Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length, and fiber diameter. *J. Food Sci.*, 30, 1049–1054.
- Herring, H.K., Cassens, R.G., and Briskey, E.J. (1965b), Sarcomere length of free and restrained bovine muscles at low temperatures as related to tenderness. *J. Sci. Food Agric.*, 16, 378–384.
- Hildrum, K.I., Nilsen, B.N., Mielnik, M., and Naes, T. (1994), Prediction of sensory characteristics of beef by near-infrared spectroscopy. *Meat Sci.*, 38, 67–80.
- Hoover, L.C., Cook, K.D., Miller, M.F., Huffman, K.L., Wu, C.K., Lansdell, J.L., and Ramsey, C.B. (1995), Restaurant consumer acceptance of beef loin strip steaks tenderized with calcium chloride. *J. Anim. Sci.*, 73, 3633–3638.
- Hopkinson, S.F., Ringkob, T.P., and Bailey, C.M. (1985), Cutability and the effect of electrical stimulation and aging on tenderness of beef from young intact males and castrates. *J. Anim. Sci.*, 60, 675–681.
- Hostetler, R.L., Landmann, W.A., Link, B.A., and Fitzhugh, H.A. Jr. (1970), Influence of carcass position during rigor mortis on tenderness of beef muscles: Comparison of two treatments. *J. Anim. Sci.*, 73, 1064–1073.
- Hostetler, R.L., Link, B.A., Landmann, W.A., and Fitzhugh, H.A. (1972), Effect of carcass suspension on sarcomere length and shear force of some major bovine muscles. *J. Food Sci.*, 37, 132–135.
- Johnson, D.D., Huffman, R.D., Williams, S.E., and Hargrove, D.D. (1990), Effects of percentage Brahman and Angus breeding, age-season of feeding, and slaughter end point on meat palatability and carcass characteristics. *J. Anim. Sci.*, 68, 1980–1986.
- Kambadur, R., Sharma, M., Smith, T.P.L., and Bass, J.J. (1997), Mutations in myostatin (GDF8) in double-muscling Belgian Blue and Piedmontese cattle. *Genome Res.*, 7, 910–915.
- King, D.A., Dikeman, M.E., Wheeler, T.L., Kastner, C.L., and Koohmaraie, M. (2003), Chilling and cooking rate effects on some myofibrillar determinants of tenderness of beef. *J. Anim. Sci.*, 81, 1473–1481.
- King, D.A., Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. (2007), Comparison of palatability characteristics of beef gluteus medius and triceps brachii muscles. *J. Anim. Sci.*, (in press).
- Koch, R.M., Dikeman, M.E., and Crouse, J.D. (1982), Characterization of biological types of cattle (Cycle III). I. Carcass composition, quality and palatability. *J. Anim. Sci.*, 54, 35–45.
- Koohmaraie, M. (1992a), The role of Ca(2+)-dependent proteases (calpains) in post mortem proteolysis and meat tenderness. *Biochimie*, 74, 239–45.
- Koohmaraie, M. (1992b), Effect of pH, temperature, and inhibitors on autolysis and catalytic activity of bovine skeletal muscle mu-calpain. *J. Anim. Sci.*, 70, 3071–3080.
- Koohmaraie, M. (1992c), Ovine skeletal muscle multicatalytic proteinase complex (proteasome): Purification, characterization, and comparison of its effects on myofibrils with mu-calpains. *J. Anim. Sci.*, 70, 3697–3708.
- Koohmaraie, M. (1995), The biological basis of meat tenderness and potential

- genetic approaches for its control and prediction. *Proc. Recip. Meat Conf.*, 48, 69–75.
- Koohmaraie, M. (1996), Biochemical factors regulating the toughening and tenderization process of meat. *Meat Sci.*, 43, S193–S201.
- Koohmaraie, M., Crouse, J.D., and Mersmann, H.J. (1989), Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of concentration and ionic strength. *J. Anim. Sci.*, 67, 934–942.
- Koohmaraie, M., Doumit, M.E., and Wheeler, T.L. (1996a), Meat toughening does not occur when rigor shortening is prevented. *J. Anim. Sci.*, 74, 2935–2942.
- Koohmaraie, M., Kent, M.P., Shackelford, S.D., Veiseth, E., and Wheeler, T.L. (2002), Meat tenderness and muscle growth: Is there any relationship? *Meat Sci.*, 62, 345–352.
- Koohmaraie, M., Shackelford, S.D., Muggli-Cockett, N.E., and Stone, R.T. (1991), Effect of the beta-adrenergic agonist L644,969 on muscle growth, endogenous proteinase activities, and postmortem proteolysis in wether lambs. *J. Anim. Sci.*, 69, 4823–4835.
- Koohmaraie, M., Shackelford, S.D., and Wheeler, T.L. (1996b), Effects of a beta-adrenergic agonist (L-644,969) and male sex condition on muscle growth and meat quality of callipyge lambs. *J. Anim. Sci.*, 74, 70–79.
- Koohmaraie, M., Shackelford, S.D., Wheeler, T.L., Lonergan, S.M., and Doumit, M.E. (1995), A muscle hypertrophy condition in lamb (Callipyge): Characterization of effects on muscle growth and meat quality traits. *J. Anim. Sci.*, 73, 3596–3607.
- Li, J., Tan, J., Martz, F.A., and Heymann, H. (1999), Image texture features as indicators of beef tenderness. *Meat Sci.*, 53, 17–22.
- Li, J., Tan, J., and Shatadal, P. (2001), Classification of tough and tender beef by image texture analysis. *Meat Sci.*, 57, 341–346.
- Locker, R.H. and Hagyard, C.J. (1963), A cold shortening effect in beef muscles. *J. Food Sci. Agric.*, 14, 787–793.
- Lorenzen, C.L., Koohmaraie, M., Shackelford, S.D., Jahoor, F., Freetly, H.C., Wheeler, T.L., Savell, J.W., and Fiorotto, M.L. (2000), Protein kinetics in callipyge lambs. *J. Anim. Sci.*, 78, 78–87.
- Ludwig, C.J., Claus, J.R., Marriott, N.G., Johnson, J., and Wang, H. (1997), Skeletal alteration to improve beef longissimus muscle tenderness. *J. Anim. Sci.*, 75, 2404–2410.
- Lusk, J.L., Fox, J.A., Schroeder, T.C., Mintert, J., and Koohmaraie, M. (2001), In-store valuation of steak tenderness. *Am. J. Agric. Econ.*, 83, 539–550.
- Marsh, B.B. (1977), The nature of tenderness. *Proc. Recip. Meat Conf.*, 30, 69–74.
- Marsh, B.B. and Leet, N.G. (1966), Studies in meat tenderness. III. The effects of cold shortening on tenderness. *J. Food Sci.*, 31, 450–459.
- Marsh, B.B., Woodhams, P.R., and Leet, N.G. (1968), Studies in meat tenderness. 5. The effects on tenderness of carcass cooling and freezing before the completion of rigor mortis. *J. Food Sci.*, 33, 12–18.
- May, S.G., Dolezal, H.G., Gill, D.R., Ray, F.K., and Buchanan, D.S. (1992), Effect of days fed, carcass grade traits, and subcutaneous fat removal on postmortem muscle characteristics and beef palatability. *J. Anim. Sci.*, 70, 444–453.
- McKeith, F.K., De Vol, D.L., Miles, R.S., Bechtel, P.J., and Carr, T.R. (1985), Chemical and sensory properties of thirteen major beef muscles. *J. Food Sci.*, 50, 869–872.
- McPherron, A.C. and Lee, S.J. (1997), Double muscling in cattle due to mutations in the myostatin gene. *Proc. Natl. Acad. Sci., USA*, 94, 12457–12561.
- Mies, P.D., Belk, K.E., Tatum, J.D., and Smith, G. C. (1999), Effects of postmortem aging on beef tenderness and aging guidelines to maximize tenderness of different beef subprimal cuts. Available: http://ansci.colostate.edu/files/meat_science/mies.pdf Accessed: May 31, 2007.
- Miller, M.F., Huffman, K.L., Gilbert, S.Y., Hamman, L.L., and Ramsey, C.B. (1995), Retail consumer acceptance of beef tenderized with calcium chloride. *J. Anim. Sci.*, 73, 2308–2314.
- Miller, R. K. (1998), Functionality of non-meat ingredients used in enhanced pork. Available: <http://www.meatscience.org/Pubs/porkfact.asp> Accessed April 5, 2007.

- Montgomery, T.H., Dew, P.F., and Brown, M.S. (2001), Optimizing carcass value and the use of anabolic implants in beef cattle. *J. Anim. Sci.*, 79 (E-Suppl), E296–306.
- Morgan, J.B. (1997), Implant programs effects on USDA beef carcass quality grade traits and meat tenderness. Proc. Impact of implants on performance and carcass value of beef cattle, Okla. Agric. Exp. Statn., Stillwater, P-957, 147–154.
- Mueller, S.L., King, D.A., Baird, B.E., McKenna, D.R., Osburn, W.N., and Savell, J.W. (2006), In-home consumer evaluations of individual muscles from beef rounds subjected to tenderization treatments. *Meat Sci.*, 74 (2), 272–280.
- Neely, T.R., Lorenzen, C.L., Miller, R.K., Tatum, J.D., Wise, J.W., Taylor, J.F., Buyck, M.J., Reagan, J.O., and Savell, J.W. (1998), Beef customer satisfaction: Role of cut, USDA quality grade, and city on in-home consumer ratings. *J. Anim. Sci.*, 76, 1027–1033.
- O'Connor, S.F., Tatum, J.D., Wulf, D.M., Green, R.D., and Smith, G.C. (1997), Genetic effects on beef tenderness in *Bos indicus* composite and *Bos taurus* cattle. *J. Anim. Sci.*, 75, 1822–1830.
- Ouali, A. and Talmant A. (1990), Calpains and calpastatin distribution in bovine, porcine, and ovine skeletal muscles. *Meat Sci.*, 28, 331–348.
- Ouali, A. and Valin, C. (1980), Effect of muscle lysosomal enzymes and calcium activated neutral proteinase on myofibrillar ATPase activity: Relationship with ageing changes. *Meat Sci.*, 5, 233–245.
- Page, B.T., Casas, E., Heaton, M.P., Cullen, N.G., Hyndman, D.L., Morris, C.A., Crawford, A.M., Wheeler, T.L., Koohmaraie, M., Keele, J.W. and Smith, T.P.L. (2002), Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *J. Anim. Sci.*, 80, 3077–3085.
- Page, B.T., Casas, E., Quaas, R.L., Thallman, R.M., Wheeler, T.L., Shackelford, S.D., Koohmaraie, M., White, S.N., Bennett, G.L., Keele, J.W., Dikeman, M.E. and Smith, T.P.L. (2004), Association of markers in the bovine CAPN1 gene with meat tenderness in large crossbred populations that sample influential industry sires. *J. Anim. Sci.*, 82, 3474–3481.
- Park, B., Chen, Y.R., Hruschka, W.R., Shackelford, S.D., and Koohmaraie, M. (1998), Near-infrared reflectance analysis for predicting beef longissimus tenderness. *J. Anim. Sci.*, 76, 2115–2120.
- Park, B. and Whittaker, A.D. (1991), Non-intrusive measurement of meat tenderness. *ASAE (Am. Soc. Ag. Eng.)* St. Joseph, MO. pp 1–13.
- Park, B., Whittaker, A.D., Miller, R.K., and Hale, D.S. (1994), Ultrasonic spectral analysis for beef sensory analysis. *J. Food Sci.*, 59, 697–724.
- Peacock, F.M., Koger, M., Palmer, A.Z., Carpenter, J.W., and Olson, T.A. (1982), Additive breed and heterosis effects for individual and maternal influences on feedlot gain and carcass traits of Angus, Brahman, Charolais, and crossbred steers. *J. Anim. Sci.*, 55, 797–803.
- Platter, W.J., Tatum, J.D., Belk, K.E., Scanga, J.A., and Smith, G.C. (2003), Effects of repetitive use of hormonal implants on beef carcass quality, tenderness, and consumer ratings of beef palatability. *J. Anim. Sci.*, 81, 984–996.
- Price, M.G. (1991), Striated muscle endosarcomeric and exosarcomeric lattices. *Advances in Structural Biology*, 1, 175–207.
- Pringle, T.D., Williams, S.E., Lamb, B.S., Johnson, D.D., and West, R.L. (1997), Carcass characteristics, the calpain proteinase system, and aged tenderness of Angus and Brahman crossbred steers. *J. Anim. Sci.*, 75, 2955–2961.
- Purchas, R.W., Burnham, D.L., and Morris, S.T. (2002), Effects of growth potential and growth path on tenderness of beef longissimus muscle from bulls and steers. *J. Anim. Sci.*, 80, 3211–3221.
- Rhee, M.S., Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. (2004), Variation in palatability and biochemical traits within and among eleven beef muscles. *J. Anim. Sci.*, 82, 534–550.
- Robson, R. M., Huff-Lonerger, E., Parrish, F. C. Jr., Ho, C.-Y., Stromer, M. H., Huiatt, T.

- W., Bellin, R. M., and Sernett, S. W. (1997), Postmortem changes in myofibrillar and other cytoskeletal proteins in muscle. *Proc. Recip. Meat Conf.*, 50, 43–52.
- Samber, J. A., Tatum, J. D., Wray, M. I., Nichols, W. T., Morgan, J. B., and Smith, G. C. (1996), Implant program effects on performance and carcass quality of steer calves finished for 212 days. *J. Anim. Sci.*, 74, 1470–1476.
- Savell, J. W., Dutson, T. R., Smith, G. C., and Carpenter, Z. L. (1978), A research note: Structural changes in electrically stimulated beef muscle. *J. Food Sci.*, 43, 1606–1609.
- Savell, J. W., Lorenzen, C. L., Neely, T. R., Miller, R. K., Tatum, J. D., Wise, J. W., Taylor, J. F., Buyck, M. J., and Reagan, J. O. (1999), Beef customer satisfaction: Cooking method and degree of doneness effects on the top sirloin steak. *J. Anim. Sci.*, 77, 645–652.
- Savell, J. W., Miller, R. K., Wheeler, T., Koohmaraie, M., Shackelford, S., Morgan, B., Calkins, C., Miller, M., Dikeman, M., McKeith, F., Dolezal, G., Henning, B., Busboom, J., West, R., Parrish, F., and Williams, S. (1994), Standardized Warner–Bratzler shear force procedures for genetic evaluation. Available: <http://meat.tamu.edu/shear.pdf> Accessed: May 30, 2007.
- Savell, J. W., McKeith, F. K., and Smith, G. C. (1981), Reducing postmortem aging time of beef with electrical stimulation. *J. Food Sci.*, 46, 1777–1781.
- Savell, J. W., Smith, G. C., and Carpenter, Z. L. (1977), Blade tenderization of four muscles from three weight-grade groups of beef. *J. Food Sci.*, 42, 866–870.
- Schenkel, F. S., Miller, S. P., Jiang, Z., Mandell, I. B., Ye, X., Li, H., and Wilton, J. W. (2006), Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *J. Anim. Sci.*, 84, 291–299.
- Schneider, B. A., Tatum, J. D., Engle, T. E., and Bryant, T. C. (2007), Effects of heifer finishing implants on beef carcass traits and longissimus tenderness. *J. Anim. Sci.*, 85, 2019–2030.
- Searls, G. A., Maddock, R. J., and Wulf, D. M. (2005), Intramuscular tenderness variation within four muscles of the beef chuck. *J. Anim. Sci.*, 83, 2835–2842.
- Shackelford, S. D., Koohmaraie, M., Cundiff, L. V., Gregory, K. E., Rohrer, G. A., and Savell, J. W. (1994), Heritabilities and phenotypic and genetic correlations for bovine postrigor calpastatin activity, intramuscular fat content, Warner–Bratzler shear force, retail product yield, and growth rate. *J. Anim. Sci.*, 72, 857–863.
- Shackelford, S. D., Wheeler, T. L., and Koohmaraie, M. (1995), Relationship between shear force and trained sensory panel tenderness ratings of 10 major muscles from *Bos indicus* and *Bos taurus* cattle. *J. Anim. Sci.*, 73, 3333–3340.
- Shackelford, S. D., Wheeler, T. L., and Koohmaraie, M. (1998), Coupling of image analysis and tenderness classification to simultaneously evaluate carcass cutability, longissimus area, subprimal cut weights, and tenderness of beef. *J. Anim. Sci.*, 76, 2631–2640.
- Shackelford, S. D., Wheeler, T. L., and Koohmaraie, M. (1999), Evaluation of slice shear force as an objective method of assessing beef longissimus tenderness. *J. Anim. Sci.*, 77, 2693–2699.
- Shackelford, S. D., Wheeler, T. L., and Koohmaraie, M. (2005), On-line classification of US Select beef carcasses for longissimus tenderness using visible and near-infrared reflectance spectroscopy. *Meat Sci.*, 69, 409–415.
- Shackelford, S. D., Wheeler, T. L., Meade, M. K., Reagan, J. O., Byrnes, B. L., and Koohmaraie, M. (2001), Consumer impressions of Tender Select beef. *J. Anim. Sci.*, 79, 2605–2614.
- Short, R. E., Grings, E. E., MacNeil, M. D., Heitschmidt, R. K., Williams, C. B., and Bennett, G. L. (1999), Effects of sire growth potential, growing–finishing strategy, and time on feed on performance, composition, and efficiency of steers. *J. Anim. Sci.*, 77, 2406–2417.
- Shorthose, W. R. and Harris, P. V. (1990), Effect of animal age on the tenderness of selected beef muscles. *J. Food Sci.*, 55, 1–8, 14.
- Smith, T. P. L., Lopez-Corrales, N. L., Kappes, S. M., and Sonstegard, T. S. (1997), Myostatin maps to the interval containing the bovin mh locus. *Mamm. Genome*, 8, 742–744.

- Smith, T.P.L., Thallman R.M., Casas, E., Shackelford, S.D., Wheeler, T.L., and Koohmaraie, M. (2003), Theory and application of genome-based approaches to improve the quality and value of beef. *Outlook on Agriculture*, 32, 253–265.
- Strydom, P.E. and Nel, E. (1999), The effect of supplementation period of beta-agonist (zilpaterol), electrical stimulation and ageing period on meat quality characteristics. *Intl. Cong. Meat Sci. Technol.*, 45, 474–475.
- Swatland, H.J. (1995), *On-line Evaluation of Meat*. pp. 229–258. Technomic Publishing Co, Inc.
- Swatland, H.J., Brooks, J.C., and Miller, M.F. (1998), Possibilities for predicting taste and tenderness of broiled beef steaks using an optical–electromechanical probe. *Meat Sci.*, 50, 1–12.
- Swatland, H.J. and Findlay, C.J. (1997), On-line probe prediction of beef toughness, correlating sensory evaluation with fluorescence detection of connective tissue and dynamic analysis of overall toughness. *Food Qual. Pref.*, 8, 233–239.
- Tatum, J.D. (1981), Is tenderness nutritionally controlled? *Proc. Recip. Meat Conf.*, 34, 65–67.
- Tatum, J.D., Smith, G.C., Berry, B.W., Murphey, C.E., Williams, F.L., and Carpenter, Z.L. (1980), Carcass characteristics, time on feed and cooked beef palatability attributes. *J. Anim. Sci.*, 50, 833–840.
- Taylor, R.C., Geesink, G.H., Thompson, V.F., Koohmaraie, M., and Goll, D.E. (1995), Is Z-disk degradation responsible for postmortem tenderization? *J. Anim. Sci.*, 73, 1351–1367.
- Uttaro, B.E., Ball, R.O., Dick, P., Rae, W., Vessie, G., and Jeremiah, L.E. (1993), Effect of ractopamine and sex on growth, carcass characteristics, processing yield, and meat quality characteristics of crossbred swine. *J. Anim. Sci.*, 71, 2439–2449.
- Veiseth, E., Shackelford, S.D., Wheeler, T.L., and Koohmaraie, M. (2004), Indicators of tenderization are detectable by 12 h postmortem in ovine longissimus. *J. Anim. Sci.*, 82, 1428–1436.
- Von Seggern, D.D., Calkins, C.R., Johnson, D.D., Brickler, J.E., and Gwartney, B.L. (2005), Muscle profiling: Characterizing the muscles of the beef chuck and round: 51st International Congress of Meat Science and Technology (ICoMST). *Meat Sci.*, 71, 39–51.
- Vote, D.J., Platter, W.J., Tatum, J.D., Schmidt, G.R., Belk, K.E., Smith, G.C., and Speer, N.C. (2000), Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. *J. Anim. Sci.*, 78, 952–957.
- Wang, H., Claus, J.R., and Marriot, N.G. (1994), Selected skeletal alterations to improve tenderness of beef round muscles. *J. Musc. Foods*, 5, 137–147.
- Wang, H., Claus, J.R. and Marriot, N.G. (1996), Prerigor treatment and endpoint temperature effects on U.S. Choice beef tenderness. *J. Musc. Foods*, 7, 45–54.
- Wheeler, T.L., Crouse, J.D. and Koohmaraie, M. (1992), The effect of postmortem time of injection and freezing on the effectiveness of calcium chloride for improving beef tenderness. *J. Anim. Sci.*, 70, 3451–3457.
- Wheeler, T.L., Cundiff, L.V., Koch, R.M., and Crouse, J.D. (1996a), Characterization of biological types of cattle (Cycle IV): Carcass traits and longissimus palatability. *J. Anim. Sci.*, 74, 1023–1035.
- Wheeler, T.L., Cundiff, L.V., Koch, R.M., Dikeman, M.E. and Crouse, J.D. (1997a), Characterization of different biological types of steers (Cycle IV): Wholesale, subprimal, and retail product yields. *J. Anim. Sci.*, 75, 2389–2403.
- Wheeler, T.L., Cundiff, L.V., Shackelford, S.D., and Koohmaraie, M. (2001a), Characterization of biological types of cattle (Cycle V): Carcass traits and longissimus palatability. *J. Anim. Sci.*, 79, 1209–1222.
- Wheeler, T.L., Cundiff, L.V., Shackelford, S.D., and Koohmaraie, M. (2004a), Characterization of biological types of cattle (Cycle VI): Carcass, yield, and longissimus palatability traits. *J. Anim. Sci.*, 82, 1177–1189.
- Wheeler, T.L., Cundiff, L.V., Shackelford, S.D., and Koohmaraie, M. (2005), Characteri-

- zation of biological types of cattle (Cycle VII): Carcass, yield, and longissimus palatability traits. *J. Anim. Sci.*, 83, 196–207.
- Wheeler, T.L. and Koohmaraie, M. (1994), Prerigor and postrigor changes in tenderness of ovine longissimus muscle. *J. Anim. Sci.*, 72, 1232–1238.
- Wheeler, T.L., Koohmaraie, M., Lansdell, J.L., Siragusa, G.R., and Miller, M.F. (1993), Effects of postmortem injection time, injection level, and concentration of calcium chloride on beef quality traits. *J. Anim. Sci.*, 71, 2965–2974.
- Wheeler, T.L., Koohmaraie, M., and Shackelford, S.D. (1997b), Effect of postmortem injection time and postinjection aging time on the calcium-activated tenderization process in beef. *J. Anim. Sci.*, 75, 2652–2660.
- Wheeler, T.L., Savell, J.W., Cross, H.R., Lunt, D.K., and Smith, S.B. (1990a), Effect of postmortem treatments on the tenderness of meat from Hereford, Brahman, and Brahman cross beef cattle. *J. Anim. Sci.*, 68, 3677–3686.
- Wheeler, T.L., Savell, J.W., Cross, H.R., Lunt, D.K., and Smith, S.B. (1990b), Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. *J. Anim. Sci.*, 68, 4206–4220.
- Wheeler, T.L., Shackelford, S.D., Casas, E., Cundiff, L.V., and Koohmaraie, M. (2001b), The effects of Piedmontese inheritance and myostatin genotype on the palatability of longissimus thoracis, gluteus medius, semimembranosus, and biceps femoris. *J. Anim. Sci.*, 79, 3069–3074.
- Wheeler, T.L., Shackelford, S.D., Johnson, L.P., Miller, M.F., Miller, R.K., and Koohmaraie, M. (1997c), A comparison of Warner–Bratzler shear force assessment within and among institutions. *J. Anim. Sci.*, 75, 2423–2432.
- Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. (1996b), Sampling, cooking, and coring effects on Warner–Bratzler shear force values in beef. *J. Anim. Sci.*, 74, 1553–1562.
- Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. (1997d), Standardizing collection and interpretation of Warner–Bratzler shear force and sensory tenderness data. *Proc. Recip. Meat Conf.*, 50, 68–77.
- Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. (2000a), Relationship of beef longissimus tenderness classes to tenderness of gluteus medius, semimembranosus, and biceps femoris. *J. Anim. Sci.*, 78, 2856–2861.
- Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. (2000b), Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. *J. Anim. Sci.*, 78, 958–965.
- Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. (2004b), The accuracy and repeatability of untrained laboratory consumer panelists in detecting differences in beef longissimus tenderness. *J. Anim. Sci.*, 82, 557–562.
- Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. (1998), Cooking and palatability traits of beef longissimus steaks cooked with a belt grill or an open hearth electric broiler. *J. Anim. Sci.*, 76, 2805–2810.
- Whipple, G., Koohmaraie, M., Dikeman, M.E., and Crouse, J.D. (1990a), Effects of high-temperature conditioning on enzymatic activity and tenderness of *Bos indicus* longissimus muscle. *J. Anim. Sci.*, 68, 3654–3662.
- Whipple, G., Koohmaraie, M., Dikeman, M.E., Crouse, J.D., Hunt, M.C., and Kemn, R.D. (1990b), Evaluation of attributes that affect longissimus muscle tenderness in *Bos taurus* and *Bos indicus* cattle. *J. Anim. Sci.*, 65, 597–607.
- White, S.N., Casas, E., Wheeler, T.L., Shackelford, S.D., Koohmaraie, M., Riley, D.G., Chase, C.C. Jr., Johnson, D.D., Keele, J.W., and Smith, T.P.L. (2005), A new single nucleotide polymorphism in CAPN1 extends the current tenderness marker test to include cattle of *Bos indicus*, *Bos taurus*, and crossbred descent. *J. Anim. Sci.*, 83, 2001–2008.
- Whiting, R.C. (1980), Calcium uptake by bovine muscle mitochondria and sarcoplasmic reticulum. *J. Food Sci.*, 45, 288–292.
- Wulf, D.M., O'Connor, S.F., Tatum, J.D., and Smith, G.C. (1997), Using objective measures of muscle color to predict beef longissimus tenderness. *J. Anim. Sci.*, 75, 684–692.

- Wulf, D.M. and Page, J.K. (2000), Using measurements of muscle color, pH, and electrical impedance to augment the current USDA beef quality grading standards and improve the accuracy and precision of sorting carcasses into palatability groups. *J. Anim. Sci.*, 78, 2595–2607.
- Zeece, M.G., Woods, T.L., Keen, M.A., and Reville, W.J. (1992), Role of proteinases and inhibitors in postmortem muscle protein degradation. In *Proceedings of the Recip. Meat Conf.*, 45, 51–61.

4

Meat color

R. A. Mancini, University of Connecticut, USA

Abstract: The main theme of this chapter is an overview of the factors that influence meat color and myoglobin chemistry. Meat color is primarily determined by several factors interacting in concert rather than one single trait. These factors can occur both antemortem and postmortem. Several sections are included, such as established factors influencing color, typical procedures used to evaluate color, and new developments in myoglobin chemistry in relation to our fundamental knowledge of meat color. Potential areas of research include cutting edge experiments in myoglobin chemistry, particularly via proteomics and mutants. In addition, development of color measurement methodology that is able to quantify carboxymyoglobin content is needed. Understanding myoglobin chemistry and the factors influencing meat color are critical for improving shelf-life. Future research that is directed at both fundamental myoglobin chemistry and applied technologies should be valuable to the meat industry.

Key words: color, myoglobin, packaging, mitochondria, hemoglobin, beef.

4.1 Introduction

Color influences meat purchasing decisions more than any other quality factor because consumers use discoloration as an indicator of product spoilage and wholesomeness. As a result, nearly 15% of retail beef is discounted in price due to surface discoloration, which corresponds to annual revenue losses of \$1 billion (Smith *et al.*, 2000). Recuperation of lost profit associated with improvements in product color life is dependent on our fundamental knowledge of myoglobin redox chemistry.

4.2 Myoglobin chemistry

Myoglobin is the sarcoplasmic protein that is responsible for meat color. Centrally located within this globular single-chain protein is heme iron, which plays a vital role in the visible color transformations that occur on the surface of meat products. Myoglobin contains 8 α -helices, often referred to as segments A through H, joined by short nonhelical strands. Although myoglobin contains 153 residues, two histidines (64 and 93) are essential to myoglobin structure and function, as well as to muscle-food color stability.

Myoglobin's prosthetic group is an iron-containing protoporphyrin (heme) located within a hydrophobic pocket of the protein. Iron within the heme ring has the ability to form six bonds. Four of iron's available bonds are with pyrrole nitrogens, connecting iron to the protoporphyrin. The 5th possible bond coordinates with the proximal histidine (H93, F8), which, in addition to hydrophobic interactions, links the prosthetic group to the apoprotein. A 6th bond or coordination site is available for ligand binding. This site can reversibly, as well as preferentially, bind ligands, two concepts that influence color development and stability. The distal histidine (H64, E7) also influences meat color by contributing to steric effects and acting as a swinging gate that partially regulates ligand access within the hydrophobic heme pocket. The ligand present and the redox state of iron result in one of four chemical forms of myoglobin that are primarily responsible for meat color (Fig. 4.1). Further information regarding the role of myoglobin chemistry in meat color is available in: Baron and Andersen, 2002; Cornforth, 1994; Faustman and Cassens, 1990a; Giddings, 1977; Mancini and Hunt, 2005; Pegg and Shahidi, 1997; and Seideman *et al.*, 1984.

4.2.1 Deoxymyoglobin

Deoxymyoglobin results from a combination of an unoccupied (no ligand present) 6th coordination site and ferrous heme iron (Fe^{2+}). This derivative is purplish-red or purplish-pink and, as the name implies, is associated with meat products that are not exposed to oxygen (deoxy); for example, vacuum packaged product and the interior of steaks packaged in PVC. However, a thorough vacuum is necessary as very low oxygen tension (<1.4 mm Hg; Brooks, 1935) is required to maintain myoglobin in a deoxygenated state.

4.2.2 Oxymyoglobin

Oxygenation occurs when deoxymyoglobin is exposed to oxygen, a process that results in the development of oxymyoglobin and a bright cherry-red color. This color change from purple to red is often referred to as bloom. No change in iron's valence occurs during oxygenation and the protein remains in the ferrous redox state. However, oxymyoglobin's 6th coordination site on iron is occupied by diatomic oxygen. A hydrogen bond between the distal histidine and the second oxygen atom stabilizes O_2 bound to the 6th coordination site of iron. This

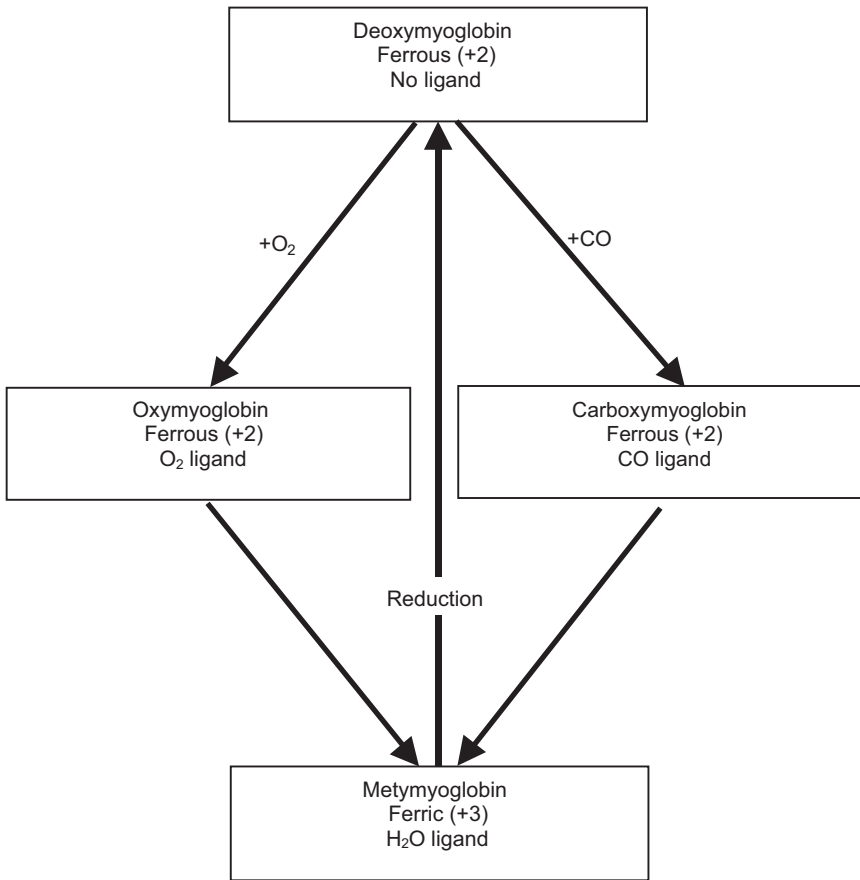


Fig. 4.1 Color changes and myoglobin redox states on the surface of postmortem muscle.

interaction between histidine and bound oxygen brings the iron more in line with the protoporphyrin plane and makes oxymyoglobin slightly more compact compared with deoxymyoglobin.

From a muscle appearance standpoint, competition for oxygen between mitochondria and myoglobin is a key element in the formation of bright cherry-red oxymyoglobin. In particular, increased oxygen consumption by processes other than myoglobin results in a darker, deoxygenated meat color due to limited oxygen availability to myoglobin. Within the mitochondria, cytochrome c oxidase is responsible for essentially all oxygen uptake from myoglobin (Wittenberg *et al.*, 2003). Postmortem competition for oxygen is evident in dark-cutting beef, which results from numerous factors, the most prominent being long-term stress of the animal prior to slaughter. Muscle glycogen is continuously depleted during stress, and, as a result, little substrate is available for anaerobic glycolysis at the time of

slaughter. Limited postmortem glycolysis causes minimal pH decline and results in an elevated ultimate pH, a factor that promotes mitochondrial respiration and competition for oxygen in meat. Low oxygen partial pressure resulting from increased mitochondrial activity helps to maintain pigments in a deoxygenated state. In this situation, meat fails to bloom (oxygenate) and does not form the characteristic bright cherry-red color associated with fresh beef.

As time of exposure to the atmosphere increases, oxygen diffuses into the interior of a meat product and oxymyoglobin penetrates deeper beneath the meat's surface. Depth of oxygen penetration and the resulting thickness of the oxymyoglobin layer are influenced by muscle temperature and pH, oxygen partial pressure, and competition for oxygen by other respiratory processes.

4.2.3 Metmyoglobin

Oxidation of heme iron within either deoxy- or oxymyoglobin results in metmyoglobin. Brown discoloration associated with consumer dissatisfaction is due to both the amount of surface area covered by metmyoglobin as well as subsurface myoglobin. As atmospheric oxygen diffuses into meat products, it initially leaves a layer of bright-red oxymyoglobin on the surface. However, the partial pressure of oxygen decreases as depth beneath the surface increases, to a point at which the interior of a steak or roast may remain deoxygenated and purple in color. Between the surface where oxygen levels are great enough to form oxymyoglobin and the interior where anaerobic oxygen levels maintain deoxymyoglobin is a layer of metmyoglobin. This layer of brown results from low oxygen partial pressure (low meaning not sufficient for oxygenation or deoxygenation), which promotes myoglobin oxidation. Metmyoglobin beneath the surface (located between superficial oxymyoglobin and interior deoxymyoglobin) will eventually shift towards the surface. Rate of discoloration depends on several factors, including oxygen partial pressure, temperature, pH, postmortem age, reducing activity, and microbial growth.

4.2.4 Metmyoglobin reduction

Oxidation of ferrous deoxy- and oxymyoglobin to ferric metmyoglobin decreases shelf-life, whereas maintaining myoglobin in a ferrous state promotes desirable meat color life. Because myoglobin autoxidation in meat products is inevitable, chemical reduction of metmyoglobin's ferric iron plays an important role in meat color life. Unfortunately, this process is not straightforward and is influenced by oxygen scavenging enzymes, reducing enzyme systems, and the NADH pool, all of which are continually depleted as time postmortem progresses. Although critical to myoglobin redox stability, postmortem replenishment of the NADH pool has received little attention in the published meat science literature.

Metmyoglobin reduction involves the transfer of an electron from a donating source to the ferric heme of myoglobin (Taylor *et al.*, 1942). Early work noted that *in vivo* pigment reduction needed pyridine nucleotides and electron transferring

compounds (Huennekens *et al.*, 1957). It is now widely accepted that the pyridine nucleotide that donates electrons to metmyoglobin is NADH, which is the limiting factor in non-enzymatic metmyoglobin reduction (Bekhit *et al.*, 2005; Brown *et al.*, 1969). In addition to direct reduction of metmyoglobin by NADH, electron transfer and mediation in muscle involving a reductase enzyme system has been demonstrated. Livingston *et al.* (1985) suggested that metmyoglobin reductase is an NADH:cytochrome b5 complex. Metmyoglobin reduction in meat is dependent on numerous factors including NADH stores, protein oxidation, and loss of enzyme activity (Faustman and Cassens, 1990a,b).

4.2.5 Deoxygenation and subsequent reoxygenation

Conversion of oxymyoglobin to deoxymyoglobin on the surface of fresh meat is a two-step process of particular importance to packaging bloomed product in either vacuum or ultra-low-oxygen atmospheres. In this case, oxymyoglobin is not converted directly to deoxymyoglobin, but first is oxidized by low-oxygen partial pressures. Continued oxygen depletion via endogenous oxygen consumption is then necessary to result in oxygen partial pressures low enough to produce deoxymyoglobin. Therefore, the meat must be capable of further oxygen consumption coupled with reduction of ferric to ferrous iron to regenerate deoxymyoglobin. From a meat packaging standpoint, this two-step process is often troublesome because deoxymyoglobin formation and subsequent rebloom depend on both reducing capacity and reduction in oxygen tension. To counteract some of these problems, low levels of carbon monoxide can be added to meat packages.

4.2.6 Carboxymyoglobin

Carbon monoxide binds strongly to myoglobin to form a bright cherry-red pigment, carboxymyoglobin. The unoccupied 6th coordinate of heme iron in deoxymyoglobin likely favors reaction with carbon monoxide. Thus, at the levels of carbon monoxide accepted for use in meat packaging (<0.4%), deoxymyoglobin is likely to be more readily converted to carboxymyoglobin than are oxymyoglobin or metmyoglobin, from a fundamental molecular standpoint. Nevertheless, the reaction characteristics of carbon monoxide with myoglobin, and the interconversion(s) between carboxy- and other myoglobin forms are poorly understood.

4.2.7 Hemoglobin

The appearance of meat products is influenced not only by muscle color, but also by bone marrow and fat discoloration. There is an abundance of peer-reviewed literature focused on the mechanism of muscle discoloration as well as factors and methodologies that maintain desirable product appearance. However, there is a

lack of published research evaluating the discoloration of bone marrow and fat. This is somewhat surprising because industry has suggested that marrow and fat color affect consumer acceptance.

Like muscle color, marrow discoloration is probably due to heme pigment oxidation within marrow. However, unlike muscle, bone marrow is abundant in hemoglobin rather than myoglobin. As a result, the redox state of hemoglobin likely dictates marrow color changes from red to brown or gray, and eventually black. Cutting bone exposes the deoxygenated pigments in marrow to oxygen, resulting in a bright-red pigment (oxyhemoglobin) that can be oxidized to an undesirable methemoglobin. Similarly, the color of subcutaneous fat is likely due to either the redox state of residual hemoglobin (capillaries and/or hemorrhage) or carotenoid level (Irie, 2001). Texture (soft versus hard fat) determines oxygen permeability and thus affects overall fat color and hemoglobin redox state.

4.3 Antemortem factors affecting meat color

Antemortem factors that indirectly affect meat color include animal diet and stress. The primary mechanisms by which these two factors influence color are likely via changes in muscle pH and/or myoglobin redox chemistry. Antemortem factors can influence two aspects of meat color: (i) overall muscle lightness/darkness and (ii) color stability. In general, increased muscle pH results in a darker, yet more stable color.

4.3.1 Diet

Diet affects several traits associated with meat color, including metabolism, glycogen storage and utilization, pH, chilling rate, and antioxidant accumulation. For example, forage-based diets might promote oxidative metabolism rather than anaerobic muscle metabolism and glycogen storage (Vestergaard *et al.*, 2000; Baublits *et al.*, 2004). As a result, bulls fed forage-based diets have less muscle glycogen, higher pH, and thus, darker muscle color than bulls fed *ad libitum* concentrates. In addition to overall muscle lightness, decreasing residual glycogen increases redness and decreases yellowness (Immonen *et al.*, 2000).

Swine diets low in digestible carbohydrates, often referred to as strategic finishing, can improve pork color by reducing muscle glycogen stores and increasing *longissimus* darkness (Rosenvold *et al.*, 2001a,b). Glycolytic potential is often used to quantify muscular capacity for anaerobic metabolism, which is directly related to pork lightness. For example, *longissimus* quality is inversely related to glycolytic potential, with L* values increasing 0.99 and 1.32 units for every one standard deviation increase in ante- and postmortem glycolytic potential, respectively (Hamilton *et al.*, 2003). Positive correlations between loin glycolytic potential and L* ($r = 0.33$ Moeller *et al.*, 2003; $r = 0.40$ Meadus *et al.*, 2000) suggests that increases in glycolytic potential promote acidity and paleness.

Substrates found in muscle that can be converted to lactic acid play a role in

determination of glycolytic potential, as ultimate lactate level and L^* are linearly related, with increased paleness resulting from higher lactate levels (Juncher *et al.*, 2001). In addition, free glucose content is related to muscle darkening ($r = 0.52$). Most data indicate that reducing glycolytic potential and free glucose may improve pork muscle color.

Diet also indirectly affects muscle lightness via alterations in fat thickness and subsequent chilling rate. This is readily observed when the overall lightness of meat from grass- versus grain-fed cattle is compared. For example, pastured steers have less subcutaneous fat than grain-finished steers, which results in faster carcass chilling for pasture-fed animals. Warmer muscle temperatures shortly after harvest promote glycolytic enzyme activity and pH decline, increasing protein denaturation and muscle lightness in grain-finished animals relative to pasture-finished animals (Bruce *et al.*, 2004). This idea is supported by a significant correlation between subcutaneous fat depth and muscle L^* values (decreased L^* corresponds to increased darkening, $r = 0.63$), as well as fat thickness and pH ($r = -0.82$; Bruce *et al.*, 2004).

Changes in color stability resulting from grass-based diets are primarily attributed to the relationship between lipid and pigment oxidation, particularly antioxidant accumulation and the instability of polyunsaturated fatty acids, rather than chilling rate. For example, color stability of *longissimus* steaks from cattle fed on high-herbage diets was greater than that for steaks from cattle fed *ad libitum* concentrate (O'Sullivan *et al.*, 2003). Increased lipid stability, possibly resulting from increased lipid-soluble antioxidants and decreased 18:3 fatty acids, could be responsible for diet-based improvements in color life. Diet can also influence fat color, likely due to β -carotene accumulation within lipid tissue. For example, grazing was related to subcutaneous fat color (r -value between dietary concentrate and yellowness $= -0.52$; French *et al.*, 2000). Yet, no dietary effects on *longissimus* yellowness were noted. Others have reported that dietary β -carotene supplementation (7500 mg/day for 28 days prior to harvest) improved *semimembranosus* and *longissimus* color life without effecting b^* values (Muramoto *et al.*, 2003).

4.3.2 Housing

Husbandry practices, such as housing environment, play a role in beef color by influencing animal activity. Similar to diet, the underlying mechanism is likely to be via changes in muscle fiber type and metabolism. For example, increased physical activity associated with loose-housed bulls increased muscle pigmentation and darkness compared with bulls housed in tie-stalls (Vestergaard *et al.*, 2000). Conversely, the less active tie-stall housing decreased slow-contracting fiber amount, vascularization, and oxidative metabolic capacity. It is possible that increased oxidative muscle capacity could decrease lactate production while also increasing pyruvate oxidation within mitochondria, β -oxidation, and time to muscle exhaustion. Changes in metabolism were likely due to alterations in muscle fiber type resulting from increased physical activity. Alterations in fiber type also have been associated with improvements in pork color. For example, vitamin D_3

supplementation may activate the calcineurin pathway in pork muscle, promoting both slow-twitch fiber expression and oxidative muscle metabolism (Wilborn *et al.*, 2004)

4.3.3 Genetics

Genetics play a significant role in pork color, primarily due to alterations in stress susceptibility and postmortem muscle metabolism. Genotypes commonly associated with pork color defects include halothane, ryanodine, and napole. For example, pork *longissimus* color is detrimentally affected by the presence of the n halothane allele. Typically, the halothane genotype influences initial, 45-minute, and 24-hour postmortem muscle pH ($nn < Nn < NN$), resulting in a lower incidence of PSE (8%) in negative pigs (NN) compared with nn genotypes (100% PSE; Channon *et al.*, 2000; Fisher *et al.*, 2000; Eggert *et al.*, 2002; Fernandez *et al.*, 2002; Moelich *et al.*, 2003). Susceptibility to malignant hyperthermia resulting from mutations affecting alleles associated with ryanodine receptors also detrimentally affect lightness (Kuchenmeister *et al.*, 2000; Moeller *et al.*, 2003; Bertram *et al.*, 2000; Piedrafitra *et al.*, 2001; Van Oeckel *et al.*, 2001; Velarde *et al.*, 2001).

Genetic effects on pork color have traditionally been associated with alterations in lightness (incidence of PSE). However, the role of genetics in muscle redness has recently been reported. For example, although meat from pigs containing either the halothane or the RN- gene is paler, these genes tend to improve *longissimus* redness (Bertram *et al.*, 2000; Lindahl *et al.*, 2004; Fabrega *et al.*, 2002). Possible mechanisms for increased redness include increased pigment content and oxymyoglobin concentration (Bertram *et al.*, 2000; Fabrega *et al.*, 2002). It is also possible that genotypic improvements in redness are due to temperature- and pH-induced effects on oxygen consumption (Lindahl *et al.*, 2004). More specifically, redness and degree of bloom can be enhanced by stress-induced increases in *longissimus* temperature. Warm muscle temperatures will promote inactivation of proteins involved in oxygen consumption, resulting in greater surface oxygenation (greater degree of bloom) due to less competition for oxygen by enzymes (Rosenvold *et al.*, 2003). However, stress-related elevation of postmortem muscle temperature also can inactivate metmyoglobin reductase and therefore decrease color-stability during display. Another recent development in the role of swine genetics in color is the identification of quantitative trait loci positions for L*, a*, and pigment content (de Koning *et al.*, 2001; Ovilo *et al.*, 2002).

4.4 Laboratory analysis of meat color

There are several ways to measure meat color, and selecting the most suitable and efficient method is often not straightforward. In general, researchers have two primary options for measuring color: visual and instrumental evaluation. Within

each of these categories are two subcategories: consumer and trained panels for visual methodology, and surface reflectance and extraction for instrumental techniques.

4.4.1 Visual color evaluation

Data obtained from hedonic scales is the true standard upon which consumer satisfaction should be based. Consumer panels are often costly and sometimes difficult to conduct, as 50 or more panelists are necessary to obtain a useful indication of purchasing decisions. Although consumer panels are not suitable for describing meat color, they are ideal for evaluating acceptance and satisfaction. On the other hand, properly trained panelists can describe color attributes and stability with a high degree of repeatability and minimal personal bias or preference. However, trained panelists are not practical indicators of meat product acceptability and consumer preference. Descriptive panels can be composed of four to eight panelists, each of whom should be screened for color blindness and for their ability to determine color differences using the Farnsworth–Munsell 100-Hue Test (Farnsworth, 1957). Lighting is often overlooked when conducting visual panels; however, type and intensity can drastically influence panelist evaluations. Recommended lighting is 1614 lux of fluorescent lighting with a color temperature of 3000 to 3500K.

One obstacle encountered when evaluating meat color is that discoloration often does not uniformly cover the whole meat surface. Rather, browning may occur only in ‘spots’ or localized areas on the surface. For example, a beef steak might be essentially bright cherry-red, yet have a ‘dime-sized’ area of metmyoglobin. Thus, trained panelists can simultaneously assign both an ‘overall surface’ (average color score) and ‘worst point’ score (area at least 1cm² in diameter) to the same sample. Similarly, ‘two-toning’ can result in distinct dark and light regions, and thus, may be a variable of interest. Change in color from that observed during initial evaluations, referred to as color stability, is another commonly measured trait. Evaluation of cooked color, cured color, and iridescence are also of interest to researchers.

4.4.2 Instrumental color evaluation

The disadvantages associated with conducting visual panels have caused researchers to rely on instrumental measures of color and color stability. Reflectance is more commonly used due to its rapid and nondestructive nature, as well as its ability to characterize surface color, which more accurately indicates what a consumer may see. Conversely, extraction techniques are more time consuming, often render the sample useless for repeated measures, and primarily characterize interior rather than surface pigments. Nevertheless, both reflection and extraction techniques are useful for pigment quantification. Currently, differentiating between oxymyoglobin and carboxymyoglobin in meat samples is difficult. The most commonly used reflectance variables are:

L^* = indicates lightness, 100 = white, 0 = black, increased values are lighter

a^* = measures redness, positive values are red, increases in a^* are more red

b^* = measures yellowness, increases in b^* are more yellow

Hue angle = $\tan^{-1} b^*/a^*$, larger angles are more yellow and more discolored

Saturation index = $(a^2 + b^2)^{1/2}$, larger values are more intense color

$K/S\ 474\text{ nm} \div K/S\ 525$ = estimates surface deoxymyoglobin content

$K/S\ 610\text{ nm} \div K/S\ 525$ = estimates surface oxymyoglobin content

$K/S\ 572\text{ nm} \div K/S\ 525$ = estimates surface metmyoglobin content

Several methods are available to estimate the amounts of each myoglobin redox form on the surface of meat (AMSA, 1991). Since color research often requires non-invasive, repeatable, and rapid estimation of surface myoglobin redox state, most quantification methodology is based on reflectance spectrophotometry and isobestic wavelengths (wavelength at which reflectance is equal for two or more redox forms). In order to account for variation due to light scatter, reflectance data often are transformed into K/S values using the Kubelka–Munk equation $[(1-R)^2/2R]$.

Using reflectance to estimate myoglobin redox forms on the surface of meat relies on formulae that require reference values for 0 and 100% of deoxymyoglobin, oxymyoglobin, and metmyoglobin (AMSA, 1991). Although these 100% reference values are available in the literature, researchers should determine standards that are specific to each individual project rather than rely on published ratios. Researchers can characterize surface discoloration with either increases in metmyoglobin content (based on reflectance at 572 nm) or decreases in oxymyoglobin content (based on reflectance at 610 nm).

Determining the redox state of myoglobin samples *in vitro* is accomplished using absorbance at 503, 557, and 582 nm, representative of metmyoglobin, deoxymyoglobin, and oxymyoglobin, respectively (Tang *et al.*, 2004). In addition, absorbance at 525 nm is used to determine myoglobin concentration. However, traditional methodology does not incorporate wavelength maxima for carboxymyoglobin. Rather, A_{503}/A_{581} can be used to assess oxidation of carboxymyoglobin solutions *in vitro*. In addition, A_{543}/A_{581} may be useful for differentiating between 100% carboxymyoglobin and 100% oxymyoglobin. Further research should be designed to better assess carboxymyoglobin oxidation on the surface of meat samples.

4.4.3 Assessing metmyoglobin reducing activity

Two other traits essential to meat color stability are metmyoglobin reducing activity and oxygen consumption. Similar to measuring color, several techniques exist for assessing these two variables and researchers are currently trying to find the most efficient and reliable methodology. Comparison of procedures to assess muscle reducing capacity has been performed (Sammel *et al.*, 2002; Claus *et al.*, 2005; McKenna *et al.*, 2005). Commonly used assays for determining reducing activity involve a two-phase procedure in which (i) myoglobin is oxidized via packaging in 1% O_2 or submerging samples in nitrite to induce metmyoglobin

formation and (ii) an assay step promotes metmyoglobin reduction. Changes in redox state during the reduction step are used to calculate absolute (simple subtraction) and relative (ratio) differences between initial metmyoglobin (pre-reduction) and postreduction metmyoglobin content. Values indicate the muscle's ability to reduce oxidized pigment. Other researchers have proposed using pre-reduction metmyoglobin values as an indicator of resistance to induced metmyoglobin formation and muscle reducing capacity (McKenna *et al.*, 2005; Mancini *et al.*, 2007)

4.4.4 Assessing oxygen consumption rate

Determining postmortem oxygen consumption also can be difficult for intact meat samples. One method involves placing meat samples in either a pouch or a polypropylene bottle, which is flushed with oxygen and sealed prior to measuring initial oxygen and carbon dioxide concentrations using a headspace analyzer (McKenna *et al.*, 2005; Sammel *et al.*, 2002). Samples are incubated, and oxygen and carbon dioxide concentrations are again determined after incubation. Oxygen consumption rate is then determined using the difference between pre- and post-incubation gas composition. Another method is based on surface pigment reduction (Madhavi *et al.*, 1993). Meat samples are allowed to bloom and surface oxymyoglobin content is determined. Samples are then vacuum packaged, and incubated to induce pigment deoxygenation and reduction. Differences in pre- and post-incubation oxymyoglobin values are used to quantify oxygen consumption. Development of a procedure to reliably measure oxygen consumption in postmortem muscle is necessary.

4.5 Postmortem factors affecting meat color

4.5.1 Cold chain management: temperature effects on raw color

One of the key concepts in food preservation is proper cold chain management. Thus, refrigerated temperatures during both storage and display must be maintained in order to maximize meat product color and shelf-life. Increased temperature accelerates myoglobin oxidation, lipid oxidation, and microbial growth, all of which will limit shelf-life. In addition, lighting intensity within a display case will influence rate of discoloration.

4.5.2 Packaging effects on raw color

Packaging has a significant effect on meat color stability. Common types of packaging include traditional polyvinyl chloride overwrap, vacuum, and modified atmosphere. Traditional packaging (Styrofoam trays and PVC overwrap) exposes meat to the atmosphere, which allows for the development of bright, cherry-red oxymyoglobin on the product surface. Vacuum packaging can improve color life compared with tradition PVC overwrap; however, purplish-deoxymyoglobin can

negatively affect consumer purchasing. Recently, an increased demand for case-ready meat products has led processors to implement modified atmosphere packaging including high-oxygen, ultra-low-oxygen, and carbon monoxide packaging.

High-oxygen atmospheres typically contain 80% O₂, which promotes both myoglobin oxygenation and depth of oxymyoglobin penetration. This prolongs the amount of time before metmyoglobin migrates to the muscle surface. Although high-oxygen atmospheres stabilize color during storage, rancidity often develops while color remains desirable (Jayasingh *et al.*, 2002). Conversely, ultra-low-oxygen atmospheres minimize lipid oxidation and aerobic microorganism growth. However, levels of residual oxygen within ultra-low oxygen packages must remain less than 1% for pork and less than 0.05% for beef, in order to limit metmyoglobin formation at low partial pressures. Because inadequate pigment reducing capacity and limited post-storage surface oxygenation decrease the efficacy of ultra-low-oxygen atmospheres, carbon monoxide has been added to packages because of its high affinity for myoglobin and its ability to form a bright cherry-red carboxymyoglobin (Hunt *et al.*, 2004; Jayasingh *et al.*, 2001; Luno *et al.*, 2000; Sørheim *et al.*, 1999). Hunt *et al.* (2004) concluded that the use of 0.4% carbon monoxide improved beef color during storage without masking spoilage. Others have reported the effectiveness of 0.5% CO for improving color stability (Jayasingh *et al.*, 2001; Luno *et al.*, 2000). Similar to oxygen penetration, depth of carbon monoxide penetration beneath the meat surface (thickness of the carboxymyoglobin layer) increases as exposure time increases (Krause *et al.*, 2003). Although dependent on concentration and time, it is likely that carbon monoxide will completely penetrate meat samples and convert interior pigments to carboxymyoglobin.

Carboxymyoglobin is rarely included in 'meat color triangles', which usually consider only interconversions between deoxy-, oxy-, and metmyoglobin. However, the use of carbon monoxide packaging makes carboxymyoglobin increasingly relevant to meat color research. Several fundamental principles of carboxymyoglobin chemistry are unclear from a meat-science standpoint. For example, most meat researchers base post-packaging carboxymyoglobin stability on deoxymyoglobin's strong affinity for carbon monoxide. Yet, the stability of carboxymyoglobin resulting from 0.4% CO packaging once the protein is challenged with an oxygen-containing atmosphere is not straightforward, but important, as meat stored in 0.4% carbon monoxide can be exposed to the atmosphere during retail merchandizing. For example, meat color (likely a combination of carboxy- and oxymyoglobin) deteriorates during display upon removal of product from carbon monoxide packaging (Hunt *et al.*, 2004). Similarly, removing beef samples from a carbon monoxide atmosphere results in a gradual decrease in the depth of carboxymyoglobin penetration (Jayasingh *et al.*, 2001).

The idea of ligand dissociation and replacement, in particular myoglobin's tendency to release carbon monoxide and subsequently bind oxygen once carboxymyoglobin is exposed to the atmosphere is a fundamental mechanism that

should be reviewed. In addition, the likelihood of replacing an oxygen ligand with carbon monoxide (conversion of oxy- to carboxymyoglobin) should receive some future attention. This is particularly important when packaging previously bloomed steaks in carbon monoxide. It is likely that relative partial pressures of oxygen and carbon monoxide will dictate which myoglobin derivative prevails. The idea of interconversions between oxy- and carboxymyoglobin on the surface of meat does not contradict myoglobin's binding preference for carbon monoxide, but rather suggests that myoglobin may prefer to hold onto oxygen rather than carbon monoxide.

4.5.3 Packaging effects on cooked color

The effects of modified atmosphere packaging on raw meat pigment chemistry can translate into premature browning and persistent pink cooked color concerns. Cooking causes myoglobin denaturation, which is responsible for the characteristic dull brown color of cooked meat products. Consumers generally assume that a brown color in the center of beef indicates that the product has reached a temperature sufficient to inactivate foodborne pathogens. Unfortunately, the relative brown color of cooked product interiors is not necessarily a reliable indicator that beef has been pasteurized (Bigner-George *et al.*, 2000).

Premature browning is a condition in cooked beef in which myoglobin denaturation occurs at a temperature lower than that necessary to inactivate pathogens, and thus, falsely conveys that thermal pasteurization has been achieved. For example, premature browning has been noted at 55 °C, a temperature at which some pink color is normally expected (Lavelle *et al.*, 1995). Yet, meat should be cooked to an internal temperature of 71 °C in order to ensure that *E. coli* O157:H7 is destroyed (USDA, 1997). Killinger *et al.* (2000) reported that the incidence of premature browning averaged 47% in ground beef purchased from local retail stores. It is well documented that several intrinsic (myoglobin redox state, muscle source, and antioxidants) and extrinsic (packaging, storage, and cooking from a frozen state) factors influence the susceptibility of beef to premature browning.

Incidence of premature browning is related to the redox state of myoglobin in raw beef prior to cooking (Hague *et al.*, 1994). For example, raw beef containing relatively more metmyoglobin will prematurely brown at a lower temperature than raw beef with predominantly ferrous myoglobin (Warren *et al.*, 1996). Maintenance of beef pigments in a deoxygenated state with vacuum packaging will decrease the occurrence of premature browning (Lavelle *et al.*, 1995). Machlik (1965) demonstrated that the temperature at which myoglobin denatures is dependent on the protein's redox status. Specifically, relative resistance of myoglobin to heat-induced denaturation is as follows: deoxy > oxy > metmyoglobin. Thus, Hunt *et al.* (1999) suggested that cooked color can reliably predict doneness only when deoxymyoglobin is the predominant pigment in raw beef prior to cooking.

The effect of modified atmosphere packaging on cooked meat color has been

explored in recent years. The tendency of beef to undergo premature browning is often greater when stored in high-oxygen packaging (containing 80% oxygen) than in PVC overwrap and vacuum packaging (Suman *et al.*, 2005; Seyfert *et al.*, 2004a,b). This is attributed to the fact that high-oxygen atmospheres result in deeper oxygen penetration; thus, promoting a meat pigment beneath the surface that is less heat stable compared with traditional PVC overwrap and/or vacuum packaging. In contrast, packaging containing 0.4% CO will minimize the occurrence of premature browning (John *et al.*, 2004 and 2005).

4.5.4 Packaging effects on bone marrow discoloration

High-oxygen packaging increases bone marrow discoloration, which has a detrimental effect on beef and pork shelf-life (Mancini *et al.*, 2005a; Grobbel *et al.*, 2006; Raines *et al.*, 2006). Although the exact mechanism of discoloration is unknown, it is likely that the aqueous phase of bone marrow, in particular hemoglobin redox state, is the principal determinant of marrow color (Mancini *et al.*, 2004 and 2005a). Red blood cell disruption via product fabrication releases hemoglobin, which oxidizes to methemoglobin, resulting in marrow darkening (Gill, 1996). Thus, maintenance of ferrous hemoglobin on the surface of cut bones is critical to improving beef shelf-life. Water-soluble reducing agents, such as ascorbic acid and sodium erythorbate, or modified atmosphere packaging containing 0.4% carbon monoxide, can improve rib and vertebrae color stability.

4.6 Product enhancement

Recently, an increased demand for central packaging of case-ready meat products has led processors to implement numerous injection-enhancement technologies that add value and lengthen product shelf-life. Basic muscle enhancement involves adding water, salt, and phosphate to meat, via injection of whole muscle cuts. The enhanced product is subsequently fabricated into steaks and/or roasts, packaged in modified-atmosphere packs, and shipped to retailers, who are able to display prepackaged-enhanced product without the additional labor associated with cutting and packaging. Unfortunately, pro-oxidants, packaging atmospheres containing high levels of oxygen, and potential microbial spoilage can limit shelf-life. To counteract these processes, as well as improve tenderness and juiciness, industry utilizes a variety of functional ingredients. Particularly relevant to product color life is lactate, which has been described as a 'color stabilizer' in both raw and cooked meat products (Kim *et al.*, 2006; Mancini *et al.*, 2005b; Lawrence *et al.*, 2003; Prestat *et al.*, 2002; Maca *et al.*, 1997 and 1999; Papadopoulos *et al.*, 1991). Lactate's role in improved color stability has been attributed to changes in postmortem muscle biochemistry, particularly replenishing the NADH pool and increasing metmyoglobin reduction. Enhancing beef loins with potassium lactate increases lactate dehydrogenase activity, NADH concentration, and metmyoglobin-reducing activity, all of which are involved in color stability. It is likely that

lactate-injection promotes NADH production via increased lactate dehydrogenase activity (Kim *et al.*, 2006).

4.6.1 Iridescence

Iridescence is a physical phenomenon resulting in a shiny, rainbow-like appearance that can be misinterpreted by consumers as an indication of chemical additives and/or microbial spoilage. The most common colors associated with iridescence on the surface of cooked meat products are green, red, orange, and yellow. Other than producing background colors that can be mixed to result in a kaleidoscope-like appearance, myoglobin likely has a negligible role in iridescence. In contrast, the mechanism of iridescence is primarily associated with product surface microstructure and light diffraction (Swatland, 1984).

Iridescence is commonly associated with both raw and cooked *semitendinosus* muscles, likely due to fiber orientation during slicing (Wang, 1991; Lawrence *et al.*, 2002; Kukowski *et al.*, 2004). For example, *semitendinosus* fibers are often more perpendicular to the blade during slicing than the fibers of other muscles (Lawrence *et al.*, 2002). Conversely, *semitendinosus* muscle cut longitudinally to fiber orientation does not produce iridescence. Both fiber type (red v white) and diameter (small v large) can cause muscle-to-muscle variation in iridescence.

Structural uniformity on the surface of a muscle food generates light diffraction patterns that are conducive to iridescence whereas disruption of surface microstructure limits iridescence (Wang, 1991). The practical implication of this is that dull blades cause light to be reflected in a more irregular pattern due to meat product surface roughening. Conversely, the shearing mechanism of sharp blades results in a smooth surface that increases the occurrence of iridescence.

4.7 New developments and new areas of research

Recent developments in meat color research are focused on fundamental myoglobin chemistry. For example, proteomic techniques have been used to better understand the interrelation between lipid and pigment oxidation. Lipid oxidation generates secondary products such as aldehydes, which are responsible for off-flavors and off-odors associated with muscle food rancidity. In addition, secondary lipid oxidation products accelerate heme protein oxidation, resulting in a positive correlation between metmyoglobin formation and lipid oxidation (Faustman *et al.*, 1999). More specifically, covalent modification of amino acids by adduction has been suggested as the reason for aldehyde-induced myoglobin oxidation (Faustman *et al.*, 1999). One particular aldehyde, 4-hydroxy-nonenal, is currently being used as a model for investigating lipid oxidation-induced meat discoloration. Mass spectrometry based proteomic analyses revealed that 4-hydroxy-nonenal adduction occurs exclusively at histidine residues (Lee *et al.*, 2003; Alderton *et al.*, 2003; Suman *et al.*, 2006 and 2007). Tandem mass spectrometry and proteomic data-mining confirmed six different nucleophilic histidine residues (HIS 24, 64,

93, 116, 119, and 152) readily adducted by 4-hydroxy-nonenal via Michael addition. Adducted residues include the proximal and distal histidine, which helps to explain the detrimental effect of 4-hydroxy-nonenal on heme redox stability.

Other fundamental research has evaluated the role of myoglobin in lipid oxidation with the use of protein mutants (Grunwald and Richards, 2006a,b). Amino acid substitutions were used to elucidate the role of released heme and released iron in lipid oxidation. In addition, peroxynitrite has received some recent attention due to its ability to induce rapid oxymyoglobin oxidation (Connolly *et al.*, 2002). The mechanism of increased pigment oxidation was attributed to a peroxynitrite-induced oxidation of iron, whereas limited pigment reduction resulted from small changes in myoglobin's structure. Thus, peroxynitrite may be useful for a better understanding of fundamental myoglobin chemistry and should be further researched.

4.8 Future directions

To improve our knowledge of meat color, both applied and fundamental research is needed. Researchers should 'think outside the box' when attempting to better understand myoglobin redox chemistry. For example, can biomedical technologies be used to improve our basic understanding of myoglobin and cytochrome chemistry? Novel approaches to solving established meat color problems have the potential to improve shelf-life. There is also a need for development of methodology that adequately quantifies metmyoglobin reducing activity, oxygen consumption, and carboxymyoglobin content.

4.9 Conclusion

Meat color is not dictated solely by one factor, but by several factors that work in concert to influence color life. Understanding both ante- and postmortem factors affecting color is necessary to maximize consumer satisfaction. A solid understanding of myoglobin chemistry will promote research efforts to improve postmortem muscle quality.

4.10 Sources of further information and advice

AMSA Color Guidelines (1991)
Faustman and Phillips (2001)
Mancini and Hunt (2005)

4.11 References

Alderton, A. L., Faustman, C., Liebler, D. C. and Hill, D. W. (2003), Induction of redox instability of bovine myoglobin by adduction with 4-hydroxy-2-nonenal. *Biochemistry*, 42 (15), 4398–4405.

- AMSA (1991), Guidelines for meat color evaluation. *Reciprocal Meat Conference Proceedings*, 44, 1–17.
- Baron, C. P. and Andersen, H. J. (2002), Myoglobin-induced lipid oxidation. A review. *Journal of Agricultural and Food Chemistry*, 50 (14), 3887–3897.
- Baublits, R. T., Brown, A. H., Jr, Pohlman, F. W., Johnson, Z. B., Onks, D. O. and Loveday, H. D. (2004), Brown carcass and beef color characteristics of three biological types of cattle grazing cool-season forages supplemented with soyhulls. *Meat Science*, 68 (2), 297–303.
- Bekhit, A. E. D. and Faustman, C. (2005), Metmyoglobin reducing activity. *Meat Science*, 71 (3), 407–439.
- Bertram, H. C., Petersen, J. S. and Andersen, H. J. (2000), Relationship between RN-genotype and drip loss in meat from Danish pigs. *Meat Science*, 56 (1), 49–55.
- Bigner-George, M. E. and Berry, B. W. (2000), Thawing prior to cooking affects sensory, shear force, and cooking properties of beef patties. *Journal of Food Science*, 65 (1), 2–8.
- Brooks, J. (1935), The oxidation of haemoglobin to methaemoglobin by oxygen. II. The relation between the rate of oxidation and the partial pressure of oxygen. *Proceedings of the Royal Society*, London, Series. B, 118, 560–577.
- Brown W. D. and Snyder H. E. (1969), Nonenzymatic reduction and oxidation of myoglobin and hemoglobin by nicotinamide adenine dinucleotides and flavins. *Journal of Biological Chemistry*, 244, 6702–6706.
- Bruce, H. L., Stark, J. L. and Beilken, S. L. (2004), The effects of finishing diet and postmortem ageing on the eating quality of the M. longissimus thoracis of electrically stimulated Brahman steer carcasses. *Meat Science*, 67 (2), 261–268.
- Channon, H. A., Payne, A. M. and Warner, R. D. (2000), Halothane genotype, pre-slaughter handling and stunning method all influence pork quality. *Meat Science*, 56 (3), 291–299.
- Claus, J., Mohanan, S. and Russell, R. (2005), Biochemical and physical properties of ten different beef muscles in relation to meat color. In *Proceedings of the 51st International Congress of Meat Science and Technology* (Abstract 11611), 7–12 August 2005, Baltimore, Maryland.
- Connolly, B. J., Brannan, R. G. and Decker, E. A. (2002), Potential of peroxyxynitrite to alter the color of myoglobin in muscle foods. *Journal of Agricultural and Food Chemistry*, 50 (18), 5220–5223.
- Cornforth, D. (1994), Color – its basis and importance. In: A.M. Pearson and T.R. Dutson, Editors, *Advances in meat research Vol. 9*, Blackie Academic and Professional, London (1994), pp. 34–78.
- de Koning, D. J., Harlizius, B., Rattink, A. P., Groenen, M. A. M., Brascamp, E. W. and van Arendonk, J. A. M. (2001), Detection and characterization of quantitative trait loci for meat quality traits in pigs. *Journal of Animal Science*, 79 (11), 2812–2819.
- Eggert, J. M., Depreux, F. F., Schinckel, A. P., Grant, A. L. and Gerrard, D. E. (2002), Myosin heavy chain isoforms account for variation in pork quality. *Meat Science*, 61 (2), 117–126.
- Fabrega, E., Manteca, X., Font, J., Gisbert, M., Carrion, D. and Velarde, A. (2002), Effects of halothane gene and pre-slaughter treatment on meat quality and welfare from two pig crosses. *Meat Science*, 62 (4), 463–475.
- Farnsworth, D. (1957), *The Farnsworth-Munsell 100-Hue test for the examination of color discrimination*. Macbeth, Division of Kollmorgen Instruments Corporation, Newburgh, New York.
- Faustman, C. and Cassens, R. G. (1990a), The biochemical basis for meat discolouration in fresh meat: A review. *Journal of Muscle Foods*, 1, 217–243.
- Faustman, C. and Cassens, R. G. (1990b), Influence of aerobic metmyoglobin reducing capacity on color stability of beef. *Journal of Food Science*, 55 (5), 1278–1279, 1283.
- Faustman, C., Liebler, D. C., McClure, T. D. and Sun, Q. (1999), Alpha, beta-unsaturated aldehydes accelerate oxymyoglobin oxidation. *Journal of Agricultural and Food Chemistry*, 47 (8), 3140–3144.

- Faustman, C. and Phillips, A. (2001), Measurement of discoloration in fresh meat. In *Current Protocols in Food Analytical Chemistry*, F3.3.1–F3.3.13.
- Fernandez, X., Neyraud, E., Astruc, T. and Sante, V. (2002), Effects of halothane genotype and pre-slaughter treatment on pig meat quality. Part 1. Postmortem metabolism, meat quality indicators and sensory traits of *m. longissimus lumborum*. *Meat Science*, 62 (4), 429–437.
- Fisher, P., Mellett, F. D. and Hoffman, L. C. (2000), Halothane genotype and pork quality. 1. Carcass and meat quality characteristics of three halothane genotypes. *Meat Science*, 54 (1), 97–105.
- French, P., Stanton, C., Rawless, F., O’Riordan, E. G., Monahan, F. J. and Caffery, P. J. (2000), Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *Journal of Animal Science*, 55 (11), 2849–2855.
- Giddings, G. G. (1977), The basis of color in muscle foods. *CRC Critical Reviews in Food Science and Nutrition*, 9, 81–114.
- Gill, C. O. (1996), Extending the storage life of raw chilled meats. *Meat Science*, 43, S99–S109.
- Grobbe, J. P., Dikeman, M. E., Smith, J. S., Kropf, D. H. and Milliken, G. A. (2006), Effects of polyvinyl chloride overwrap film, high oxygen modified atmosphere packaging, or ultra-low modified atmosphere packaging on bone marrow discoloration in beef humerus, rib, thoracic vertebrae, and scapula. *Journal of Animal Science*, 84, 694–701.
- Grunwald, E. W. and Richards, M. P. (2006a), Mechanisms of heme protein-mediated lipid oxidation using hemoglobin and myoglobin variants in raw and heated washed muscle *Journal of Agricultural and Food Chemistry*, 54 (21), 8271–8280.
- Grunwald, E. W. and Richards, M. P. (2006b), Studies with myoglobin variants indicate that released heme is the primary promoter of lipid oxidation in washed fish muscle *Journal of Agricultural and Food Chemistry*, 54 (12), 4452–4460.
- Hague, W. A., Warren, K. E., Hunt, M. C., Kropf, D. H., Kastner, C. L., Stroda, S. L. and Johnson, D. E. (1994), Endpoint-temperature, internal cooked color, and expressible juice color relationships in ground beef patties. *Journal of Food Science*, 59 (3), 465–470.
- Hamilton, D. N., Miller, K. D., Ellis, M., McKeith, F. K. and Wilson, E. R. (2003), Relationships between longissimus glycolytic potential and swine growth performance, carcass traits, and pork quality. *Journal of Animal Science*, 81 (9), 2206–2212.
- Huennekens F. M., Caffrey R. W., Basford R. E. and Gabrio B.W. (1957), Erythrocyte metabolism. IV. Isolation and properties of methemoglobin reductase. *Journal of Biological Chemistry*, 227, 261–272.
- Hunt, M. C., Mancini, R. A., Hachmeister, K. A., Kropf, D. H., Merriman, M. and DelDuca, G. (2004), Carbon monoxide in modified atmosphere packaging affects color, shelf life, and microorganisms of beef steaks and ground beef. *Journal of Food Science*, 69 (1), C45–C52.
- Hunt, M. C., Sørheim, O. and Slinde, E. (1999), Color and heat denaturation of myoglobin forms in ground beef. *Journal of Food Science*, 64 (5), 847–851.
- Immonen, K., Ruusunen, M. and Puolanne, E. (2000), Some effects of residual glycogen concentration on the physical and sensory quality of normal pH beef. *Meat Science*, 55 (1), 33–38.
- Irie, M. (2001), Optical evaluation of factors affecting appearance of bovine fat. *Meat Science*, 57 (1), 19–22.
- Jayasingh, P., Cornforth, D. P., Brennand, C. P., Carpenter, C. E. and Whittier, D. R. (2002), Sensory evaluation of ground beef stored in high-oxygen modified atmosphere packaging. *Journal of Food Science*, 67 (9), 3493–3496.
- Jayasingh, P., Cornforth, D. P., Carpenter, C. E. and Whittier, D. (2001), Evaluation of carbon monoxide treatment in modified atmosphere packaging or vacuum packaging to increase color stability of fresh beef. *Meat Science*, 59 (3), 317–324.
- John, L., Cornforth, D. P., Carpenter, C. E., Sørheim, O., Pettee, B. C. and Whittier, D. R.

- (2004), Comparison of color and thiobarbituric acid values of cooked hamburger patties after storage of fresh beef chubs in modified atmospheres. *Journal of Food Science*, 69 (8), 608–614.
- John, L., Cornforth, D. P., Carpenter, C. E., Sørheim, O., Pettee, B. C. and Whittier, D. R. (2005), Color and thiobarbituric acid values of cooked top sirloin steaks packaged in modified atmospheres of 80% oxygen, or 0.4% carbon monoxide, or vacuum. *Meat Science*, 69 (3), 441–449.
- Juncher, D., Ronn, B., Mortensen, E. T., Henckel, P., Karlsson, A. and Skibsted, L. H. (2001), Effect of pre-slaughter physiological conditions on the oxidative stability of colour and lipid during chill storage of pork. *Meat Science*, 58 (4), 347–357.
- Killinger, K. M., Hunt, M. C., Campbell, R. E. and Kropf, D. H. (2000), Factors affecting premature browning during cooking of store purchased ground beef. *Journal of Food Science*, 65 (4), 585–587.
- Kim, Y. H., Hunt, M. C., Mancini, R. A., Seyfert, M., Loughin, T. M., Kropf, D. H. and Smith, J. S. (2006), Mechanism for lactate-color stabilization in injection-enhanced beef. *Journal of Agricultural and Food Chemistry*, 54, 7856–7862.
- Krause, T. R., Sebranek, J. G., Rust, R. E. and Honeyman, M. S. (2003), Use of carbon monoxide packaging for improving the shelf life of pork. *Journal of Food Science*, 68 (8), 2596–2603.
- Kuchenmeister, U., Kuhn, G. and Ender, E. (2000), Seasonal effects on Ca + 2 transport of sarcoplasmic reticulum and on meat quality of pigs with different malignant hyperthermia status. *Meat Science*, 55 (2), 239–245.
- Kukowski, A. C., Wulf, D. M., Shanks, B. C., Page, J. K. and Maddock, R. J. (2004), Factors associated with surface iridescence in fresh beef. *Meat Science*, 66 (4), 889–893.
- Lavelle C.L., Hunt, M.C. and Kropf, D.H. (1995), Display life and internal cooked color of ground beef from vitamin E-supplemented steers. *Journal of Food Science*, 60 (6), 1175–1178, 1196.
- Lawrence, T. E., Hunt, M. C. and Kropf, D. H. (2002), A research note: Surface roughening of precooked, cured beef round muscles reduces iridescence. *Journal of Muscle Foods*, 13 (1), 69–73.
- Lawrence, T. E., Dikeman, M. E., Hunt, M. C., Kastner, C. L. and Johnson, D. E. (2003), Effects of calcium salts on beef longissimus quality. *Meat Science*, 64, 299–308.
- Lee, S., Phillips, A. L., Liebler, D. C. and Faustman, C. (2003), Porcine oxymyoglobin and lipid oxidation in vitro. *Meat Science*, 63 (2), 241–247.
- Lindahl, G., Enfalt, A., von Seth, G., Joseli, A., Hedebo-Velander, I. and Andersen, H. J. (2004), A second mutant allele (V199I) and the PRKAG3 (RN) locus II. Effect on colour characteristics of pork loin. *Meat Science*, 66 (3), 621–627.
- Livingston, D. J., McLachlan, S. J., LaMar, G. N. and Brown, W. D. (1985), Myoglobin: Cytochrome b5 interactions and the kinetic mechanism of metmyoglobin reductase. *Journal of Biological Chemistry*, 260, 15699–15707.
- Luno, M., Roncales, P., Djenane, D. and Beltran, J. A. (2000), Beef shelf life in low O₂ and high CO₂ atmospheres containing different low CO concentrations. *Meat Science*, 55 (4), 413–419.
- Maca, J. V., Miller, R. K., Bigner, M. E., Luvia, L. M. and Acuff, G. R. (1999), Sodium lactate and storage temperature effects on shelf life of vacuum packaged beef top rounds. *Meat Science*, 53, 23–29.
- Maca, J. V., Miller, R. K., Maca, J. and Acuff, G. R. (1997), Microbiological, sensory and chemical characteristics of vacuum-packaged cooked beef top rounds treated with sodium lactate and sodium propionate. *Journal of Food Science*, 62, 586–590.
- Machlik, S. M. (1965), *The effect of heat on bovine myoglobin derivatives in model systems and in beef semitendinosus muscles*. PhD. Dissertation, Purdue University.
- Madhavi, D. L. and Carpenter, C. E. (1993), Aging and processing affect color, metmyoglobin reductase, and oxygen consumption of beef muscles. *Journal of Food Science*, 58 (5), 939–942, 94.

- Mancini, R.A. and Hunt, M.C. (2005), Current research in meat color. *Meat Science*, 71, 100–121.
- Mancini, R. A., Hunt, M. C., Hachmeister, K. A., Kropf, D. H. and Johnson, D. E. (2004), Ascorbic acid minimizes lumbar vertebrae discoloration. *Meat Science*, 68, 339–345.
- Mancini, R. A., Hunt, M. C., Hachmeister, K. A., Kropf, D. H. and Johnson, D. E. (2005a), Exclusion of oxygen from modified atmosphere packages limits beef rib and lumbar vertebrae marrow discoloration during display and storage. *Meat Science*, 69, 493–500.
- Mancini, R. A., Hunt, M. C., Hachmeister, K. A., Seyfert, M., Kropf, D. H., Johnson, D. E., Cusick, S. and Morrow, C. (2005b), The utility of lactate and rosemary in beef enhancement solutions: Effects on *longissimus* color changes during display. *Journal Muscle Foods*, 16, 27–36.
- Mancini, R. A., Seyfert, M. and Hunt, M. C. (2007), Effects of data expression, sample location, and oxygen partial pressure on initial nitric oxide metmyoglobin formation and metmyoglobin-reducing-activity measurement in beef muscle. *Meat Science*, 79 (2), June 2008, 244–251.
- McKenna, D. R., Mies, P. D., Baird, B. E., Pfeiffer, K. D., Ellebracht, J. W. and Savell, J. W. (2005), Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science*, 70 (4), 665–682.
- Meadus, W. J. and MacInnis, R. (2000), Testing for the RN- gene in retail pork chops. *Meat Science*, 54 (3), 231–237.
- Moelich, E., Hoffman, L. C. and Conradie, P. J (2003), Sensory and functional meat quality characteristics of pork derived from three halothane genotypes. *Meat Science*, 63 (3), 333–338.
- Moeller, S. J., Baas, T. J., Leeds, T. D., Emmett, R. S. and Irvin, K. M (2003), Rendement Napole gene effects and a comparison of glycolytic potential and DNA genotyping for classification of Rendement Napole status in Hampshire-sired pigs. *Journal of Animal Science*, 81 (2), 402–410.
- Muramoto, T., Nakanishi, N., Shibata, M. and Aikawa, A. (2003), Effect of dietary b-carotene supplementation on beef color stability during display of two muscles from Japanese Black steers. *Meat Science*, 63 (1), 39–42.
- O'Sullivan, A., Galvin, K., Moloney, A. P., Troy, D. J., O'Sullivan, K. and Kerry, J. P. (2003), Effect of pre-slaughter rations of forage and/ or concentrates on the composition and quality of retail packaged beef. *Meat Science*, 63 (3), 279–286.
- Ovilo, C., Clop, A., Noguera, J. L., Oliver, M. A., Barragan, C. and Rodriguez, C. (2002), Quantitative trait locus mapping for pig meat quality traits in an Iberian × Landrace F2 pig population. *Journal of Animal Science*, 80 (11), 2801–2808.
- Papadopoulos, L. S., Miller, R. K., Ringer, L. J. and Cross, H. R. (1991), Sodium lactate effect on sensory characteristics, cooked meat color and chemical composition. *Journal of Food Science*, 56, 621–626.
- Pegg, R. B. and Shahidi, F. (1997), Unraveling the chemical identity of meat pigments. *CRC Critical Reviews in Food Science and Nutrition*, 37 (6), 561–589.
- Piedrafito, J., Christian, L. L. and Lonergan, S. M. (2001), Fatty acid profiles in three stress genotypes of swine and relationships with performance, carcass and meat quality traits. *Meat Science*, 57 (1), 71–77.
- Prestat C., Jensen J., Robbins K., Ryan K., Zhu L., McKeith F. K. and Brewer M. S. (2002), Physical and sensory characteristics of precooked, reheated pork chops with enhancement solutions. *Journal of Muscle Foods*, 13, 37–51.
- Raines, C. R., Dikeman, M. E., Grobbel, J. P. and Yancey, E. J. (2006), Effects of ascorbic acid and Origanox (TM) in different packaging systems to prevent pork lumbar vertebrae discoloration. *Meat Science*, 74 (2), 267–271.
- Rosenvold, K. and Andersen, H. J (2003), The significance of preslaughter stress and diet on colour and colour stability of pork. *Meat Science*, 63 (2), 199–209.

- Rosenvold, K., Laerke, H. N., Jensen, S. K., Karlsson, A. H., Lundstrom, K. and Andersen, H. J. (2001a), Strategic finishing feeding as a tool in the control of pork quality. *Meat Science*, 59 (4), 397–406.
- Rosenvold, K., Peterson, J. S., Laerke, H. N., Jensen, S. K., Therkildsen, M. and Karlsson, A. H. (2001b), Muscle glycogen stores and meat quality as affected by strategic finishing feeding of slaughter pigs. *Journal of Animal Science*, 79 (2), 382–391.
- Sammel, L. M., Hunt, M. C., Kropf, D. H., Hachmeister, K. A. and Johnson, D. E. (2002), Comparison of assays for metmyoglobin reducing ability in beef inside and outside semimembranosus muscle. *Journal of Food Science*, 67 (3), 978–984.
- Seideman, S. C., Cross, H. R., Smith, G. C. and Durland, P. R. (1984), Factors associated with fresh meat color: A review. *Journal of Food Quality*, 6, 211–237.
- Seyfert, M., Hunt, M. C., Mancini, R. A., Kropf, D. H. and Stroda, S. L. (2004a), Internal premature browning in cooked steaks from enhanced beef round muscles packaged in high-oxygen and ultralow oxygen modified atmospheres. *Journal of Food Science*, 69 (2), 142–146.
- Seyfert, M., Mancini, R. A. and Hunt, M. C. (2004b), Internal premature browning in cooked ground beef patties from highoxygen modified-atmosphere packaging. *Journal of Food Science*, 69 (9), 721–725.
- Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J. D. and Williams, S. N (2000), Economic implications of improved color stability in beef. In E. A. Decker, C. Faustman and C. J. Lopez-Bote (Eds.), *Antioxidants in muscle foods: Nutritional strategies to improve quality* (pp. 397–426). New York: Wiley Interscience.
- Sørheim, O., Nissen, H. and Nesbakken, T. (1999), The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. *Meat Science*, 52 (2), 157–164.
- Suman, S. P., Faustman, C., Lee, S., Tang, J., Sepe, H. A., Vasudevan, P., Annamalai, T., Manojkumar, M., Marek, P. and Venkitanarayanan, K. S. (2005), Effect of erythorbate, storage and high-oxygen packaging on premature browning in ground beef. *Meat Science*, 69 (2), 363–369.
- Suman S. P., Faustman C., Stamer S. L. and Liebler, D. C. (2006), Redox instability induced by 4-hydroxy-2-nonenal in porcine and bovine myoglobins at pH 5.6 and 4 degrees C. *Journal of Agricultural and Food Chemistry*, 54, 3402–3408.
- Suman, S. P., Faustman, C., Stamer, S. L. and Liebler D. C. (2007), Proteomics of lipid oxidation-induced oxidation of porcine and bovine oxymyoglobins. *Proteomics*, 7 (4), 628–640.
- Swatland, H. J. (1984), Optical characteristics of natural iridescence in meat. *Journal of Food Science*, 49 (3), 685–686.
- Tang, J., Faustman, C. and Hoagland, T. A. (2004), Krzywicki revisited: Equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. *Journal of Food Science*, 69, C717–720.
- Taylor, J. F. and Morgan, V. E. (1942), Oxidation–reduction potentials of the metmyoglobin–myoglobin system. *Journal of Biological Chemistry*, 144, 15–20.
- USDA (1997), USDA advises consumers to use a meat thermometer when cooking hamburger. *FSIS News and Information Bulletin*. Washington, DC: FSIS, USDA.
- Van Oeckel, M. J., Warrants, N., Boucque, C. V., Delputte, P. and Depuydt, J. (2001), The preference of the consumer for pork from homozygous or heterozygous halothane negative animals. *Meat Science*, 58 (3), 247–251.
- Velarde, A., Gispert, M., Faucitano, L., Alonso, P., Manteca, X. and Diestre, A. (2001), Effects of the stunning procedure and the halothane genotype on meat quality and incidence of haemorrhages in pigs. *Meat Science*, 58 (3), 313–319.
- Vestergaard, M., Oksberg, N. and Henckel, P. (2000), Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. *Meat Science*, 54 (2), 177–185.

- Wang, H. (1991), *Causes and solutions of iridescence in precooked meat*. Ph.D. dissertation, Kansas State University, Manhattan.
- Warren, K. E., Hunt, M. C. and Kropf, D. H. (1996), Myoglobin oxidative state affects internal cooked color development in ground beef patties. *Journal of Food Science*, 61, 513–515, 519.
- Wilborn, B. S., Kerth, C. R., Owsley, W. F., Jones, W. R. and Frobish, L. T (2004), Improving pork quality by feeding supranutritional concentrations of vitamin D3. *Journal of Animal Science*, 82 (1), 218–224.
- Wittenberg, J. B. and Wittenberg, B. A. (2003), Myoglobin function reassessed. *The Journal of Experimental Biology*, 206, 2011–2020.

Flavour development in meat

J. S. Elmore and D. S. Mottram, University of Reading, UK

Abstract: Meat flavour forms during cooking, as a result of the Maillard reaction and lipid oxidation. Compositions of both Maillard precursors, i.e. sugars and amino acids, and lipids can be influenced by several factors. This chapter examines how meat flavour can be affected pre-slaughter (by the animal's diet, breed, and slaughter age, for example) as well as post-slaughter (as a result of conditioning and preservation). The characteristic aroma compounds of cooked meat from different species are discussed, as well as those compounds that cause undesirable flavours in meat, due to spoilage, contamination or diet. Methods for analysing cooked meat aroma are described and future developments in meat flavour research are discussed.

Key words: volatile compounds, lipid oxidation, Maillard reaction, diet, post-slaughter treatment, gas chromatography–mass spectrometry.

5.1 Introduction

The flavour of meat, or of any other food, is a combination of its taste and aroma, and will also be influenced by sensations such as mouthfeel and juiciness (Farmer, 1992). The contribution of flavour to the eating quality of meat is, of course, very important, and much research has been carried out on the chemistry of meat flavour, in order to determine how desirable flavour can be achieved through production and processing.

Meat flavour is thermally derived, since uncooked meat has little or no aroma, and only a blood-like taste (Mottram, 1998). During cooking, a complex series of thermally induced reactions occur between non-volatile components of lean and fatty tissues, resulting in a large number of reaction products. The volatile compounds formed in these reactions are largely responsible for the characteristic

flavours of cooked meat. Over 1000 volatile compounds have been identified in cooked meat; a much larger number has been identified in beef than in the other meats, although this is reflected in the much larger number of publications about beef flavour, compared with that of pork, sheep meat or poultry (Maarse and Visscher, 1996; Mottram, 1991). Although some authors have studied the effects of different treatments on the flavour of adipose tissue, this chapter will discuss only the effects of changes affecting muscle flavour.

5.2 Flavour formation in meat

When meat is cooked, flavour develops as a result of two main reactions. The Maillard reaction, between amino acids and reducing sugars, is responsible for typical meaty flavour and savoury, roast, and boiled character. Lipid degradation provides compounds that give fatty aromas to cooked meat, and also compounds that are responsible for some of the aroma differences between meats from different species (Wasserman and Talley, 1968; Pearson *et al.*, 1973).

5.2.1 The Maillard reaction and meatiness

Low molecular weight, water-soluble compounds have long been recognised as important precursors of the characteristic aroma of cooked meat (Macy *et al.*, 1964a and b). Amino acids, peptides and carbohydrates, in particular ribose, are important meat flavour precursors, which react together during heating in the Maillard reaction; other water-soluble compounds implicated in the formation of meat flavour include thiamine, nucleotides, and additional amino compounds, such as creatine, carnosine and creatinine (Macy *et al.*, 1964b; MacLeod, 1986).

Meat contains significant quantities of ribose, which is a pentose sugar, and its reaction with cysteine in model systems has been shown to give meat-like aromas (Morton *et al.*, 1960; Farmer *et al.*, 1989). The reaction is widely used in the preparation of reaction product flavourings with meat-like characteristics. Post slaughter, adenosine triphosphate (ATP), the ribonucleotide which is essential for muscle function in the live animal (Lawrie, 1991), is broken down enzymatically in the muscle to give inosine monophosphate (IMP). Further enzymatic breakdown of IMP may lead to hypoxanthine, ribose and ribose 5-phosphate (Fig.5.1), although most of the ribose in meat remains bound within IMP. During the cooking of meat, IMP may also provide a source of ribose.

When the free (non-protein) amino acid cysteine is heated in the presence of reducing sugars, it breaks down to acetaldehyde, hydrogen sulphide and ammonia, which can react with carbonyls produced from sugar breakdown, to give a wide range of sulphur-containing heterocyclic volatile compounds, in particular thiophenes, thiazoles and sulphur-containing furans (Farmer *et al.*, 1989). Many of these compounds have been reported in cooked meat and some, particularly furans and thiophenes substituted in the 3-position with sulphur, possess very low odour thresholds (Mottram, 1998). MacLeod (1986) compiled a list of 78 compounds

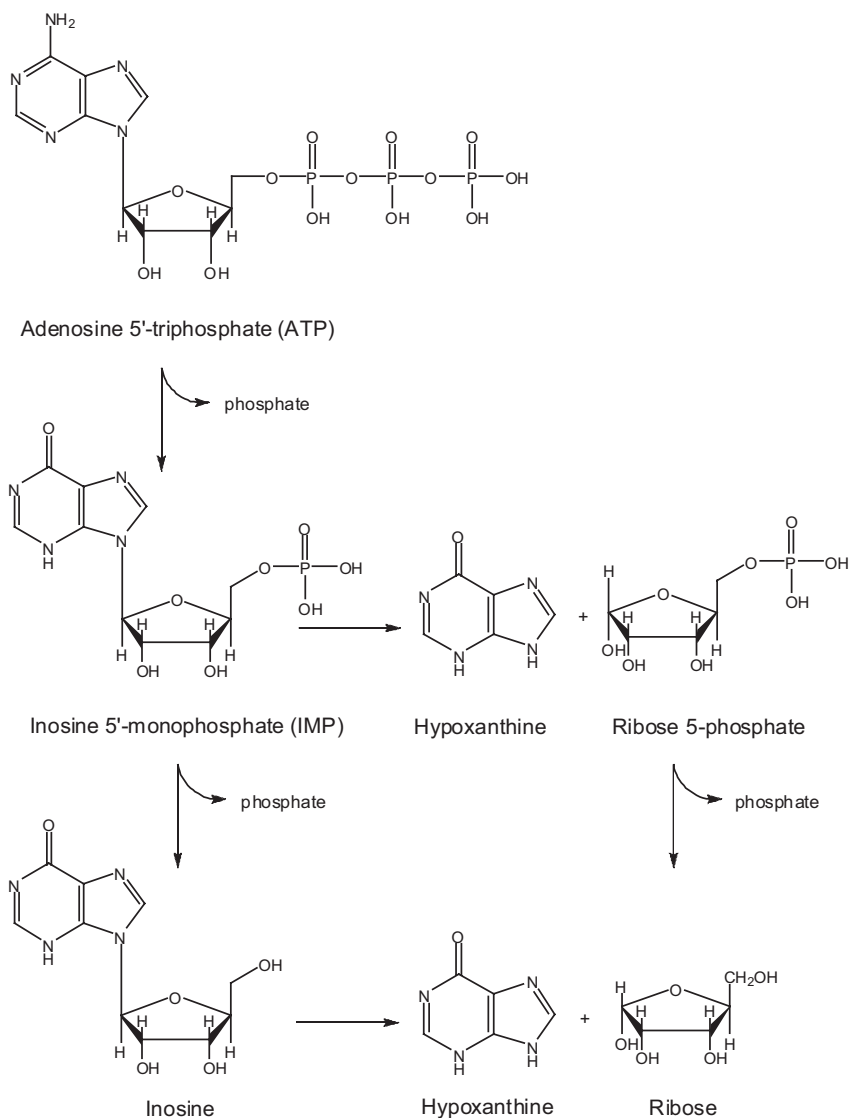


Fig. 5.1 Post-mortem decomposition of adenosine triphosphate.

that had been reported in the literature as possessing meaty aroma. Seventy-two of these compounds contained sulphur, of which 65 were heterocyclic in nature. Twenty-four of these compounds had been isolated from cooked meat at the time. At least six more of these compounds have been found in cooked meat subsequently (Mottram, 1998; Werkhoff *et al.*, 1993; Gasser and Grosch, 1990; Elmore *et al.*, 1999). [Figure 5.2](#) shows some of the sulphur-containing compounds,

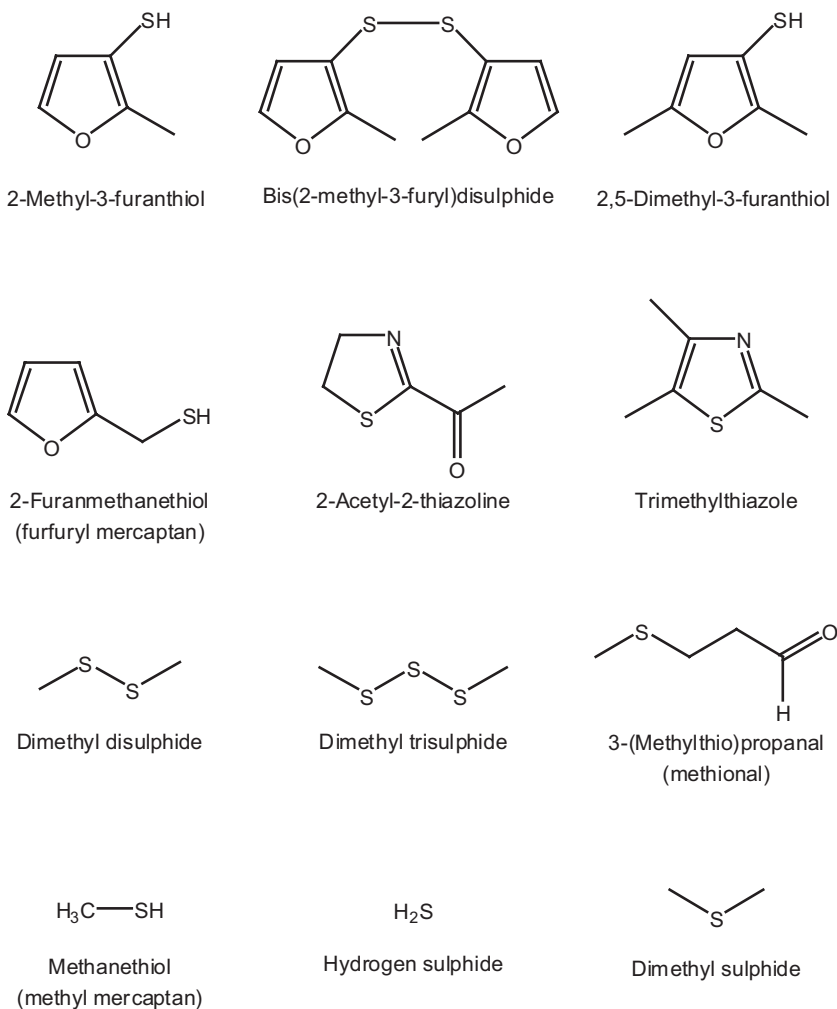


Fig. 5.2 Important sulfur-containing compounds in cooked meat aroma.

formed as a result of the Maillard reaction, which have been reported as important contributors to cooked meat aroma.

The water-soluble compounds in meat not only act as flavour precursors but also possess taste properties. MacLeod (1998) suggests that, in beef, sugars may contribute to its sweetness, while organic acids provide sour taste. Bitterness may be due to peptides and hypoxanthine, while saltiness is due to inorganic salts. Free amino acids may contribute sweetness, sourness and bitterness, while their salts may contribute to saltiness and the umami taste, a characteristic savoury quality. Important contributors to umami taste are glutamic acid and its sodium salt (MSG),

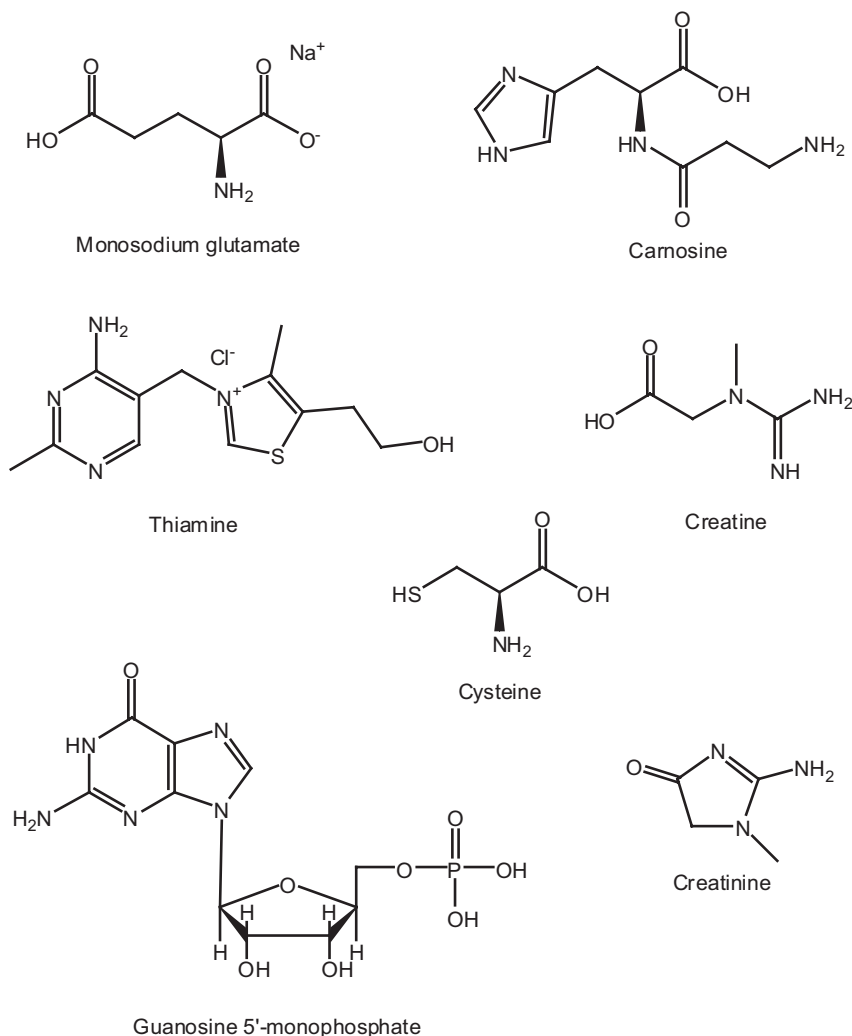


Fig. 5.3 Some water-soluble taste compounds and flavour precursors present in meat.

as well as hypoxanthine, inosine 5'-monophosphate (IMP), guanosine 5'-monophosphate and certain peptides. Figure 5.3 gives the structures of some of the water-soluble taste compounds and flavour precursors present in meat.

Aliani *et al.* (2003) measured sugars and thiamine in supermarket-purchased chicken and beef. Glucose, fructose and their phosphates were much higher in beef than chicken, whereas levels of ribose and ribose phosphate were similar in both. Levels of thiamine in both meats were thought to be too low to influence flavour formation. The same authors measured non-protein and protein amino acids in

chicken and concluded that free cysteine levels were also too low to influence cooked chicken flavour. They suggested that hydrogen sulphide, released from protein-bound cysteine, was the precursor of sulphur-containing compounds in cooked chicken. Aliani and Farmer (2005) measured nucleotides, inosine and hypoxanthine in chicken breast and leg, and found twice as much IMP in breast than leg. They also found wide variation between samples, which they suggested may be due to genetic differences, or post-slaughter conditioning. Again, these authors concluded that these differences in precursors were not sufficient to affect sensory quality.

5.2.2 Lipid oxidation and species character

Mottram and Edwards (1983) reported that lipid could contribute to cooked-meat flavour in several ways. Oxidative breakdown of lipid on cooking produces compounds that will contribute to meat aroma. These compounds may also react with compounds formed, via the Maillard reaction, to yield additional compounds. Furthermore, lipid may act as a solvent for aroma compounds, controlling their release during eating.

In meat, lipid can be broadly divided into two types: subcutaneous adipose tissue and intramuscular fat (marbling fat). Enser *et al.* (1996) measured the levels of both types of fat in shop-bought English beef, lamb and pork. They found that lamb had the highest content of both types of fat, while pork contained more adipose tissue and less intramuscular fat than beef.

On heating, the fatty acids in lipid are attacked by oxygen and decompose to form volatile aroma compounds, such as hydrocarbons, alcohols, aldehydes, ketones, alkylfurans and lactones. As the number of double bonds in the fatty acid chain increases, i.e. as its degree of unsaturation increases, its susceptibility to oxidation increases (Grosch, 1987). Although adipose tissue comprises the majority of fat in meat, its contribution to flavour formation is relatively small as it is composed almost entirely of triacylglycerols, which are relatively saturated. Polyunsaturated fatty acids (PUFA), containing two or more double bonds, are important sources of aroma volatiles in cooked meat. The PUFA composition of the triacylglycerols in beef is between 1 and 2% (Larick *et al.*, 1989, Enser *et al.*, 1996), while that of lamb adipose tissue is between 2 and 8% (Enser *et al.*, 1996; Young *et al.*, 2003). Pork adipose tissue is relatively high in linoleic acid (C18:2 *n*-6), resulting in a PUFA content of around 17% (Enser *et al.*, 1996). One group of compounds associated with adipose tissue, of great sensory importance in sheep and goat meat, are the volatile branched-chain fatty acids, which contribute to the typical aromas of the meat from these species (Ha and Lindsay, 1990; Young and Braggins, 1999). High levels of these compounds (4-methyloctanoic acid, 4-ethyloctanoic acid and 4-methylnonanoic acid) are disagreeable to many consumers. 4-Methyloctanoic and 4-ethyloctanoic acid have been shown to contribute a sweaty aroma in both sheep and goat meat, which may be responsible for the limited appeal of these meats, compared with beef, where these compounds are absent.

Phospholipids, which are essential structural components of all cells, are always present in meat. The fatty acids contained in the phospholipids are far more unsaturated than the fatty acids of the triacylglycerols (the storage lipids). The four major phospholipids of meat are phosphatidylcholine and phosphatidylethanolamine, choline plasmalogen and ethanolamine plasmalogen, with PUFA contents of 15–25%, 57–70%, 38–48% and 56–72% in beef, pork, lamb and chicken, respectively (Fogerty *et al.*, 1991). The total phospholipid contents of muscle lipids were reported by Fogerty *et al.* (1990) as approximately 2% in lamb, 6% in beef, 19% in pork, 21% in chicken, and 21% in veal, while Wood *et al.* (2003) reported values of 24–31% in four different pig breeds, and Guth and Grosch (1995) obtained values of 9–52% in cattle. Phosphatidylcholine and phosphatidylethanolamine are diacyl phosphatides, i.e. they possess two fatty acid chains, whereas choline plasmalogen and ethanolamine plasmalogen are alk-1-enyl acyl phosphatides, possessing one fatty acid and one bound fatty aldehyde. Upon cooking, the plasmalogens hydrolyse to give fatty aldehydes, principally hexadecanal, octadecanal and 9-octadecenal (Fogerty *et al.*, 1991; Dannenberger *et al.*, 2006). 12-Methyltridecanal has been identified as an important aroma compound, formed directly from the hydrolysis of plasmalogens in ruminants, particularly in beef (Guth and Grosch, 1993). The compound has been reported as having a tallowy, beef-like aroma and was one of a group of compounds that defined the characteristic aroma of stewed beef. Figure 5.4 shows the structures of the major phospholipids in meat, along with the structures of the important aldehydes formed directly from plasmalogens, and the structures of the major odour-active branched-chain amino acids found in lamb and goat adipose tissue.

Mottram and Edwards (1983) showed that, when the triacylglycerols were extracted from lean beef muscle, there was little effect on its aroma when cooked. However, when the phospholipids were also removed, meaty aroma was also removed and a roast, biscuit-like aroma appeared in its place. This effect on aroma was found to be due to a large decrease in lipid-derived aldehydes and alcohols, accompanied by an increase in the formation of compounds derived from the Maillard reaction.

Compounds formed from the decomposition of lipid during cooking include aldehydes, alcohols, ketones and alkylfurans. Lipid-derived volatiles are found at relatively high levels, compared with Maillard-derived volatiles, in lightly cooked meat, whereas the converse is true in pressure-cooked and well-done meat (Mottram, 1985; Elmore, 1999; Elmore *et al.*, 2004a,b). Figure 5.5 shows those compounds formed from lipid breakdown that have been reported as important contributors to cooked meat flavour.

5.2.3 Lipid/Maillard interactions

Many compounds formed from the interaction of lipid with the Maillard reaction (Whitfield, 1992) have been identified in meat, particularly in the pressure-cooked meat of animals that have been fed supplements high in unsaturated fats (Elmore *et al.*, 1997, 1999, 2000), and include 2-alkylthiazoles, 2-alkyl-3-thiazolines,

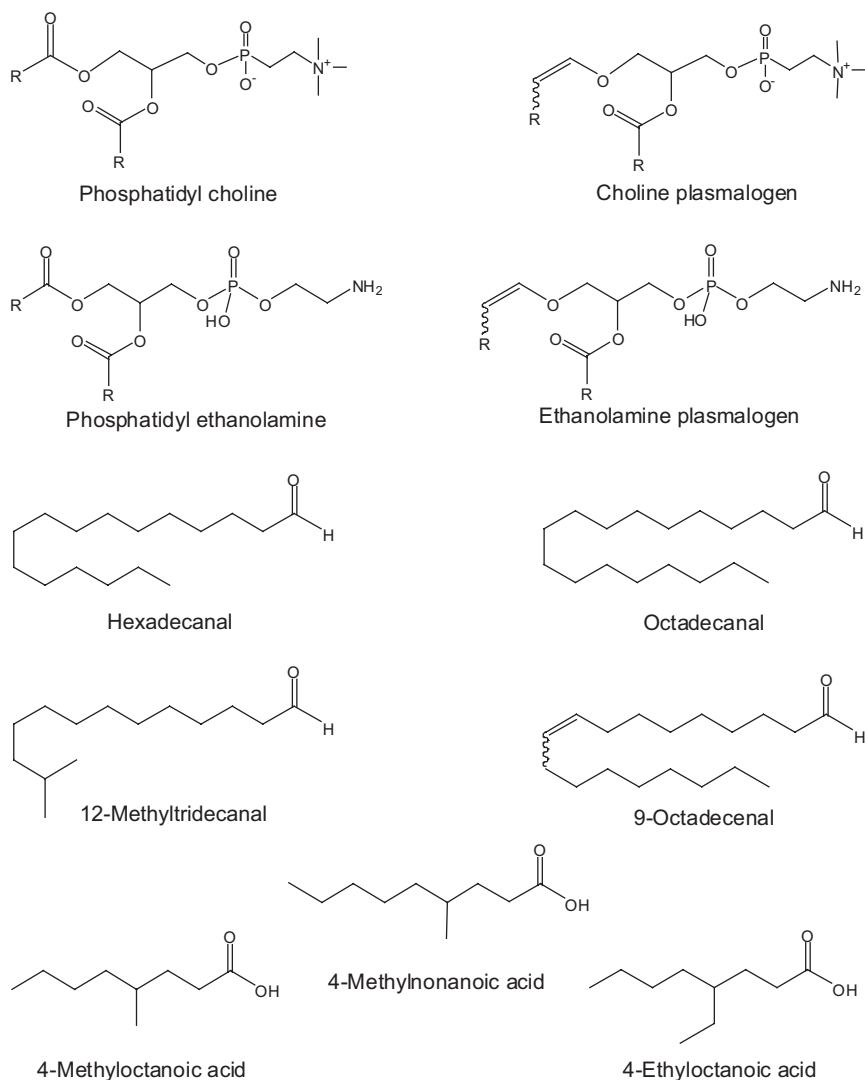


Fig. 5.4 Major phospholipids in meat, aldehydes derived from plasmalogens, and odour-potent branched-chain fatty acids found in sheep and goat meat.

2-alkylthiophenes and 2-alkyl-(2*H*)-thiapyrans (Farmer and Mottram, 1994). It is likely that concentrations of these compounds are relatively high in pressure-cooked meat because they are formed from ammonia and hydrogen sulphide, which are maintained within the closed system but would evaporate during roasting, boiling and frying (Elmore and Mottram, 1997). Tang *et al.* (1983) reported a series of 2-alkylthiazoles in fried chicken, as well as some

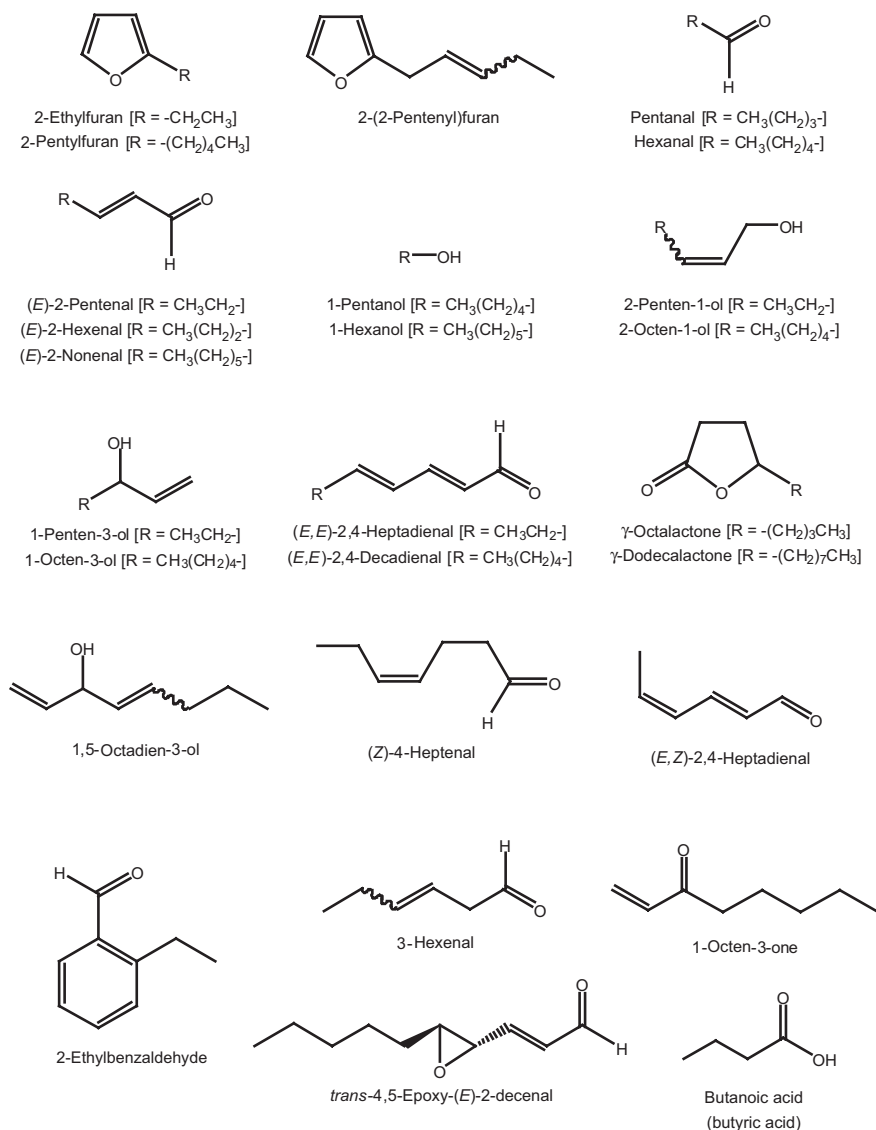


Fig. 5.5 Important compounds in cooked meat, formed from lipid oxidation.

2-alkylpyridines and 2-alkylthiophenes. Buttery (1977) also reported the presence of 2-alkylpyridines in heated lamb fat. Figure 5.6 gives the structures of some of the compounds found in cooked meat which have been formed from the reaction of products of the Maillard reaction with products of lipid oxidation.

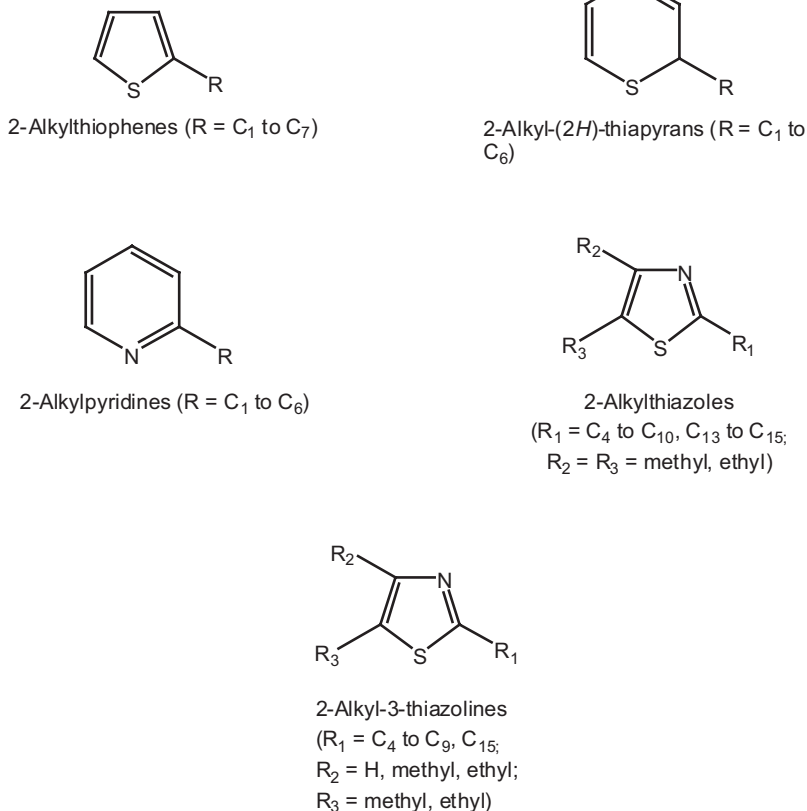


Fig. 5.6 Compounds in cooked meat, formed from the reaction between Maillard reaction and lipid oxidation products.

5.2.4 Character impact compounds

Not all of the volatile compounds found in meat contribute to its flavour and aroma. The character impact compounds are those compounds which make an important contribution to defining the typical aromas of cooked meat (Gasser and Grosch, 1988). Much of the work in this area has used the technique of aroma extract dilution analysis (AEDA), which will be described later in the chapter.

Beef was the first meat studied using this technique. Seventeen compounds in the cooked beef had high aroma values, of which 2-methyl-3-furanthiol and bis(2-methyl-3-furyl) disulphide had meat-like aromas. Both of these compounds are found in commercial meat flavourings (Ruther and Baltes, 1994). (*E*)-2-Nonenal and (*E,E*)-2,4-decadienal, which are formed from lipid breakdown, were the main contributors of fatty aromas to the cooked beef (Gasser and Grosch, 1988).

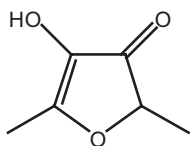
When an extract of stewed beef juice was analysed by AEDA, 4-hydroxy-2,5-

dimethyl-3(2*H*)-furanone (furanol), methanethiol and 12-methyltridecanal were found to be character impact components (Guth and Grosch, 1994). When the 15 compounds with the highest aroma values in the extract were dissolved in coconut oil, the aroma of the resulting sample bore a strong resemblance to stewed beef juice. Stewed beef was analysed by a vacuum distillation technique, whereas the cooked beef extract described above was analysed by simultaneous distillation/extraction. As the latter technique is unsuitable for the analysis of highly volatile compounds such as methanethiol, and polar compounds such as furaneol, it is likely that the compounds identified as stewed beef juice character impact compounds would be more representative than those identified in the cooked beef experiment.

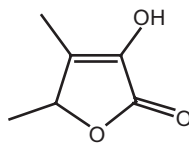
3,5-Dimethyl-2-ethylpyrazine was reported as being an important contributor to both roast beef (Cerny and Grosch, 1992) and shallow-fried beef (Specht and Baltes, 1994). Other compounds contributing to the aroma of both types of meat included the lipid-derived compounds γ -octalactone, (*E*)-2-nonenal, (*E,E*)-2,4-decadienal and 1-octen-3-one, and the Maillard-derived compounds 3-(methylthio)propanal (methional) and 2,3-diethyl-5-methylpyrazine. Furanol, 2-methoxyphenol (guaiacol) and 2-acetyl-2-thiazoline were all important contributors to roast beef, while 3,5(or 6)-dimethyl-2-vinylpyrazine and 2,5-dimethyl-3-ethylpyrazine were important in fried beef.

Less work has been carried out on the character impact compounds of pork, lamb and chicken. When cooked chicken was compared with cooked beef, 2-methyl-3-furanthiol, 2-furanmethanethiol (furfuryl mercaptan) and methional were important compounds in both meats. Bis(2-methyl-3-furyl) disulphide was much more important in beef aroma, while 2,5-dimethyl-3-furanthiol, another meaty aroma compound, and 2,4,5-trimethylthiazole, an earthy-smelling compound, were much more important in chicken aroma. (*E,E*)-2,4-Decadienal, γ -dodecalactone and nonanal were lipid-derived compounds which were also important in cooked chicken aroma (Gasser and Grosch, 1990). Furfuryl mercaptan, methional and (*E,E*)-2,4-decadienal were important in boiled chicken, along with furaneol, 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (sotolon), acetic acid, butyric acid and 2-acetyl-2-thiazoline (Kerler and Grosch, 1997).

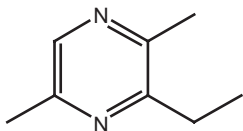
Methanethiol has been shown to be important in cooked beef, pork and chicken, while 2-methyl-3-furanthiol and furfuryl mercaptan were both important contributors to cooked beef and pork aroma (Kerscher and Grosch, 2000). Rota and Schieberle (2006) showed that furaneol was a key contributor to cooked mutton aroma but did not contribute to raw mutton aroma, whereas 4-ethyloctanoic acid and *trans*-4,5-epoxy-(*E*)-2-decenal were important in both raw and cooked mutton aroma. When comparing beef, pork and chicken, Kerscher and Grosch (2000) stated that odour differences among the three species were mainly due to different concentrations of the key odorants; only a few compounds, e.g., 12-methyltridecanal and dimethyl sulphide in beef and hydrogen sulphide in chicken, have an impact on the aroma of only one species. Figure 5.7 gives the structures of those compounds described above which have not been present in any of the previous figures.



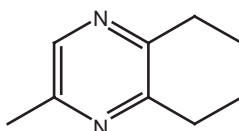
2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone
(furanol)



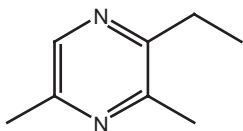
4,5-Dimethyl-3-hydroxy-2(3*H*)-furanone
(sotolon)



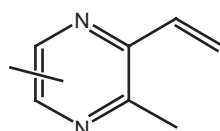
2,5-Dimethyl-3-ethylpyrazine



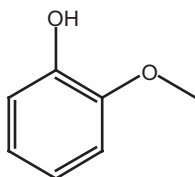
2,3-Diethyl-5-methylpyrazine



3,5-Dimethyl-2-ethylpyrazine



3,5(or 6)-Dimethyl-2-vinylpyrazine



2-Methoxyphenol
(guaiacol)

Fig. 5.7 Some character impact compounds of cooked meat.

5.3 Dietary effects on meat flavour

Pigs and poultry are monogastric animals; dietary fatty acids are absorbed from the intestine without modification and incorporated directly into tissue lipids. In mammals, linoleic acid (C18:2 *n*-6) and α -linolenic acid (C18:3 *n*-3) cannot be synthesised and tissue concentrations of these acids respond rapidly to dietary changes. Tissue concentrations of saturated and monounsaturated fatty acids are less susceptible to dietary influences as they can be biosynthesised in the animal

(Wood and Enser, 1997). The polyunsaturated fatty acid (PUFA) composition of ruminant tissue is lower than that of monogastric animals, due to hydrogenation of fatty acids by rumen bacteria. Even so, modification of the fatty acid composition of ruminant meat can be achieved through diet, and numerous authors have reviewed this topic (Melton, 1990, Scollan *et al.*, 2001; Wood *et al.*, 2003; Raes *et al.*, 2004; Vasta and Priolo, 2006).

Many workers have attempted to modify the fatty acid composition of meat, either to increase total PUFA levels in muscle or to increase the amounts of $n-3$ PUFA, in particular the long-chain PUFA C20:5 $n-3$ (eicosopentaenoic acid; EPA) and C22:6 $n-3$ (docosahexaenoic acid; DHA). Dietary intake of these PUFA has been associated with a reduction in coronary heart disease (Cooper *et al.*, 2004). EPA and DHA are naturally present at high levels in fatty fish, but consumption of fatty fish in Western societies is generally low, justifying the need to raise $n-3$ PUFA levels in meat (Raes *et al.*, 2004).

Modifying the lipid composition of animal diets will alter the lipid composition of their meat, which may affect its flavour. Lipids high in $n-3$ fatty acids will break down readily on heating to give compounds that are more unsaturated and hence more reactive than compounds formed from the breakdown of the equivalent $n-6$ fatty acids. Such compounds could affect flavour by reacting with Maillard precursors and intermediates, as well as contributing to flavour in their own right. Furthermore, they appear to catalyse the breakdown of more saturated lipids, which may also affect meat flavour (Elmore *et al.*, 1999).

5.3.1 Comparison of grass and grain diets

Cattle diets are almost always grain-based (wheat, barley, soya) or forage-based (grass, silage, hay). Grain-based, high energy diets contain relatively high amounts of linoleic acid, whereas forage-based diets are low energy diets, relatively high in α -linolenic acid. Hence, animals fed on grain, when compared with grass-fed animals of the same age, are usually heavier with a higher percentage of body fat (Muir *et al.*, 1998).

Grass- and grain-fed cattle of similar weight, reared on diets containing sugar beet pulp added to grass silage or a restricted barley/soya diet, were slaughtered at 14 and 24 months (Elmore *et al.*, 2004b). Muscle lipids showed large differences due to diet; in particular, all $n-3$ fatty acids were higher in the muscle of the grass-fed animals, while all $n-6$ fatty acids were higher in the muscle of the grain-fed animals. Linoleic acid was over two times higher in grain-fed animals, whereas α -linolenic acid was around five times higher in grass-fed animals. It is recommended that the ratio of $n-6$ to $n-3$ fatty acids in the human diet should be less than 4 (Wood *et al.*, 2003). In two separate experiments an $n-6$: $n-3$ ratio for the *longissimus dorsi* muscle in concentrates-fed cattle of 9 was measured, whereas in grass-fed animals the ratio was only 1.3 (Enser *et al.*, 1998, Elmore *et al.*, 2004).

Compounds derived from $n-6$ fatty acids, including hexanal, 1-hexanol, 1-octen-3-ol, (Z)-2-octen-1-ol and 2-pentylfuran, were higher in grilled steaks from grain-fed animals, whereas the $n-3$ derived compounds 1-penten-3-ol, (Z)-2-

penten-1-ol and 2-ethylfuran were higher in the steaks from grass-fed animals. Although the meat from the grass-fed cattle contained the more highly unsaturated fatty acids, the quantity of lipid-derived volatiles was greater in the beef from the grain-fed animals. The higher levels of the antioxidant vitamin E in the diet of grass-fed animals, relative to grain-fed animals, has been related to improved colour retention and reduced lipid oxidation in the meat of grass-fed animals (Wood *et al.*, 2003).

Nuernberg *et al.* (2005) compared the sensory properties, colour and oxidative stability of grass and concentrates-fed cattle, slaughtered at the same body weight. They confirmed that grass-fed beef was more stable, with better colour retention. However, German consumers preferred the concentrates-fed beef, scoring grass-fed beef significantly higher for fishy flavour. Sitz *et al.* (2005) confirmed a widely-held belief that the majority of American consumers preferred corn-fed beef to grass-fed, and suggested that this preference was due to the fact that corn-fed beef is far more readily available in the United States and hence consumers were more accustomed to its taste. American consumers scored corn-fed American beef higher for juiciness, tenderness, flavour and overall acceptability, when compared against barley-fed Canadian beef and grass-fed Australian beef.

Of those compounds that may be important contributors to cooked meat aroma, saturated and monounsaturated aldehydes have been shown to be higher in cooked ground beef muscle from cattle finished on concentrates (Larick and Turner, 1990 (649); Lorenz *et al.*, 2002; Raes, 2003). 12-methyltridecanal, a key aroma compound in cooked beef, was found to be slightly higher in the uncooked muscles of pasture-fed cattle than concentrate-fed cattle (Dannenberger, 2006). However, despite the differences that exist between the volatile compounds of beef from animals fed forage or cereal, Muir *et al.* (1998) concluded that sensory panellists cannot reliably detect flavour differences between the two.

Fisher *et al.* (2000) showed that British consumers preferred grilled chops from grass-fed Suffolk sheep to concentrates-fed; grass-fed chops were higher in lamb flavour and lower in abnormal flavour. Spanish consumers, however, preferred grilled lamb loins from concentrates-fed animals to those from forage-fed animals, even though they described both products in a similar way to the British consumers (Sañudo *et al.*, 1998). These results also suggest that consumer acceptability may depend to a large extent on what is considered the norm, as concentrates-fed lamb is usually consumed in Spain, whereas grass-fed lamb is eaten in the United Kingdom.

Koutsidis *et al.* (2008a) have studied the effects of diet and breed on the water-soluble compounds of beef. Free amino acids were higher in grass-fed animals than cereal-fed animals, possibly due to the higher rate of growth of the concentrates-fed animals, leading to a depletion of the free amino acid pool in the muscle, caused by the higher rate of protein synthesis relative to protein breakdown. Alternatively, differences in the rate of pH drop during the first hours of conditioning, or deficiencies in the silage diet of one or more limiting amino acids, may have affected *post-mortem* proteolysis, by regulating the enzymes involved. Changes in sugars, IMP and IMP breakdown products were relatively small but it was

observed that total reducing sugars were slightly higher in concentrates-fed animals than in grass-fed animals.

5.3.2 Diets high in polyunsaturated fatty acids

Pigs are often fed high energy diets containing elevated levels of linoleic acid, leading to rapid growth and no noticeable effects on flavour quality. Very little work has focused on the effect of such diets on aroma volatile composition, although Larick *et al.* (1992) showed that linoleic acid breakdown products, such as pentanal, hexanal, 1-octen-3-ol and 2-pentylfuran, increased as the linoleic acid composition of pork muscle increased. When diets high in C18:3 *n*-3, usually as linseed, were fed to pigs, levels of C18:3 *n*-3 and, through chain elongation, C20:5 *n*-3 and C22:5 *n*-3, increased significantly, while little effect on C22:6 *n*-3 was observed (Fontanillas, 1997). Linseed supplements may cause off-flavours in pork if fed at too high a level, although meat stability can be increased by the addition of vitamin E to the diet (Wood *et al.*, 2003). Fish oil is high in EPA and DHA, and levels of these PUFA will readily increase in the muscle of fish oil-fed pigs. Again, there is a risk of off-flavours if fish oil levels in the diet are too high (Øverland, 1996). Sárraga *et al.* (2007) fed pigs with an algal supplement rich in DHA, plus α -tocopherol as an antioxidant. Cooked hams prepared from these animals had acceptable sensory properties, but dry-cured hams tasted saltier and more fishy than control hams.

Rumen bacteria hydrogenate C₁₈ PUFA and also convert *cis* fatty acids to *trans*; neither phenomenon is nutritionally desirable to humans (Scott and Ashes, 1993). Scott *et al.* (1971) prepared formaldehyde-treated protein–lipid supplements that passed largely unchanged from the rumen into the abomasum, where the acidic conditions weakened the protein–aldehyde link, allowing the lipids in the supplements to be digested and absorbed. Later work by the same group (Ashes *et al.*, 1992) showed that C₂₀ and C₂₂ PUFA were hydrogenated to a much lesser extent in the rumen of both sheep and cattle, compared to C₁₈ fatty acids.

The effects of dietary lipid supplements, high in *n*-3 PUFA, on the composition of both beef and lamb muscle, have been studied. Four diets were compared: a control diet, the same diet supplemented with lightly bruised linseed, unprotected fish oil, and a linseed:fish oil diet (1:1). The seed case of the linseed provided some protection against rumen hydrogenation. Vitamin E was included in the diets, to increase the oxidative stability of the meat. In beef muscle, phospholipids linseed doubled the levels of C18:3 *n*-3 and also increased EPA, while fish oil doubled the levels of DHA and caused a 7-fold increase in both C20:4 *n*-3 and C22:4 *n*-3. The combination diet was particularly effective at increasing levels of DHA. All diets reduced *n*-6 levels in the phospholipids, and similar but smaller effects were observed in intramuscular triacylglycerols. However, these nutritionally beneficial effects were accompanied by an increase in *trans*-C18:1 (Vatansever *et al.*, 2000). Steaks from the fish oil-fed animals possessed an undesirable fishy taste, while the steaks from animals fed both linseed-containing diets were rated less tough; the linseed-only steaks were preferred to the control steaks. The shelf-life of the steaks

from fish oil-fed animals was significantly lower than the steaks from the other treatments, particularly when the steaks were made into burgers. The pressure-cooked steaks from the animals receiving the dietary supplements contained high levels of *n*-3 derived volatiles, such as 2-ethylfuran and 2-(2-pentenyl) furan (Elmore *et al.*, 1999), compared with the control. Compounds formed from the reaction of *n*-3 fatty acids with Maillard reaction intermediates, such as 2-ethyl-(2*H*)-thiapyran and 2-ethylthiophene (Elmore and Mottram, 2000) were also at higher levels in the supplemented steaks. Furthermore, some *n*-6 and *n*-9 derived compounds were also present in high amounts, suggesting that the fish oil *n*-3 acids had catalysed the breakdown of the more saturated fatty acids found in beef.

Pressure-cooked steaks from lambs fed the same diets exhibited similar effects to the beef steaks (Elmore *et al.*, 2000). Again fish oil raised levels of *n*-3 derived volatiles, in particular (*Z*)-4-heptenal, which has been associated with unpleasant pastoral aroma in cooked lamb (Young *et al.*, 1999). Levels of fat-derived volatiles were generally higher in lamb, possibly indicative of the higher concentration of PUFA in the total lipids of lamb compared to beef. In both experiments, Maillard-derived volatiles were unaffected by dietary treatment, although lipid-Maillard compounds were affected by diet more in lamb than in beef. Ponnampalam *et al.* (2002) showed that fish oil supplements fed to lambs on a mixed forage/concentrates diet, led to a doubling of *n*-3 fatty acids in muscle phospholipids but a decrease in overall palatability.

Another source of long-chain PUFA is marine algae. Suffolk-cross lambs were fed five diets high in PUFA, including two containing a high-DHA marine algae, one algae:fish oil (1:1) and one algae:protected lipid supplement (PLS; 1:1). PLS was high in C18:2 *n*-6 and C18:3 *n*-3, and, on its own, was another dietary treatment studied. The remaining supplements were fish oil alone (high in EPA and DHA) and linseed oil alone (high in C18:3 *n*-3) (Cooper *et al.*, 2004). Muscle phospholipids of lambs fed the algae diets contained at least twice as much DHA as the muscle phospholipids of lambs on the other diets, while the neutral lipids of the muscles of the lambs that were fed the PLS diet were five times higher than those from lambs fed diets without PLS. The PLS meat had the highest polyunsaturates:saturates ratio, while the fish oil:algae meat had the highest *n*-3:*n*-6 ratio, and the linseed diet provided the best balance of these nutritional attributes. Grilled lamb steaks from these animals were compared. Algae supplements led to massive increases in lipid-derived volatiles, particularly when combined with fish oil: levels of 1-penten-3-ol were 20 times higher in the fish/algae steaks, compared to the linseed. Other compounds over 10 times higher in the algae/fish steaks, compared with linseed, were (*E*)-2-pentenal, 2-penten-1-ol, (*E*)-2-hexenal, 3-hexenal, (*E,Z*)-2,4-heptadienal, (*E,E*)-2,4-heptadienal, 1,5-octadien-3-ol, (*E,E*)-2,4-decadienal, 2-ethylfuran, 2-(2-pentenyl)furan, 2-ethylbenzaldehyde, and numerous hydrocarbons. Many of these compounds have been reported as *n*-3 decomposition products. Not surprisingly, the meat from the algae diets was scored high for rancid flavour and abnormal flavour by a sensory panel, while meat from fish oil diets scored high for fishy flavours and meat from PLS-fed diets scored highest for grass flavour, due to its high hexanal content. Linseed-fed lamb

scored highest for lamb flavour and lowest for abnormal flavour (Elmore *et al.*, 2005).

5.4 Other pre-slaughter factors affecting meat flavour

5.4.1 Breed effect

When compared against dietary effects, breed effects on cooked meat flavour are relatively small. Raes *et al.* (2003) compared the sensory properties of four beef samples: two from intensively-grown Belgian breeds, an Irish beef sample and an Argentinian sample, the latter two assumed to be extensively reared. The Argentinian and Irish beef samples had a higher flavour intensity, which was probably due to their higher *n*-3 PUFA content, whereas the two Belgian breeds were not discriminated. Machiels *et al.* (2004) compared cooked steaks from double-musced Belgian Blue bulls with those from Limousin and Aberdeen Angus bulls, using GC-MS and GC-olfactometry. Twenty-one volatile compounds were affected by breed. 5-Methyl-2,3-diethylpyrazine was an important aroma compound present at higher levels in the Belgian Blue steaks, while the Strecker aldehyde 2-methylpropanal was at higher levels in the other two steaks (Fig. 5.8). Chambaz *et al.* (2003) compared four beef breeds for sensory quality. Flavour intensity was unaffected by breed, while juiciness and tenderness were. Campo *et al.* (1999) noted small effects on flavour and aroma when comparing four beef breeds aged over 21 days, but trends were difficult to ascertain.

It is often assumed in beef that the traditional breeds such as Aberdeen Angus have better flavour than dairy breeds such as Holstein–Friesian. Vatansever *et al.* (2000) compared meat from Welsh Black and Holstein–Friesian cross-breeds. The Welsh Black was significantly tougher than the Holstein–Friesian, but no differences in flavour were noted. Similar results were obtained by Monsón *et al.* (2005). They compared the sensory properties of four breeds at different conditioning times. This time the Holstein dairy breed was the least tender and the Limousin beef breed was the most tender, although differences were only significant at 7 days or earlier after slaughter. Nuernberg *et al.* (2005) compared German Holsteins with German Simmentals and found that steaks from Holsteins scored higher for bitter taste but overall liking was not significantly different.

Elmore *et al.* (2004b) compared the volatile aroma compounds of steaks from Aberdeen Angus and Holstein–Friesian steers. Only four compounds were affected by breed: 1-phytene, 2-phytene and S-methylthioacetate were higher in the Holstein–Friesian steaks, and acetone was higher in the Aberdeen Angus steaks (Fig. 5.8). Koutsidis *et al.* (2008a) showed that the effects of breed on the water-soluble compounds of these steaks were relatively small. Ribose, arginine and creatinine were slightly higher in Aberdeen Angus than Holstein–Friesian, while glycine and hypoxanthine were higher in the Holstein–Friesian.

The flavour and texture characteristics of Wagyu breed beef are highly regarded. Boylston *et al.* (1996) showed that lipid-derived volatiles in Wagyu

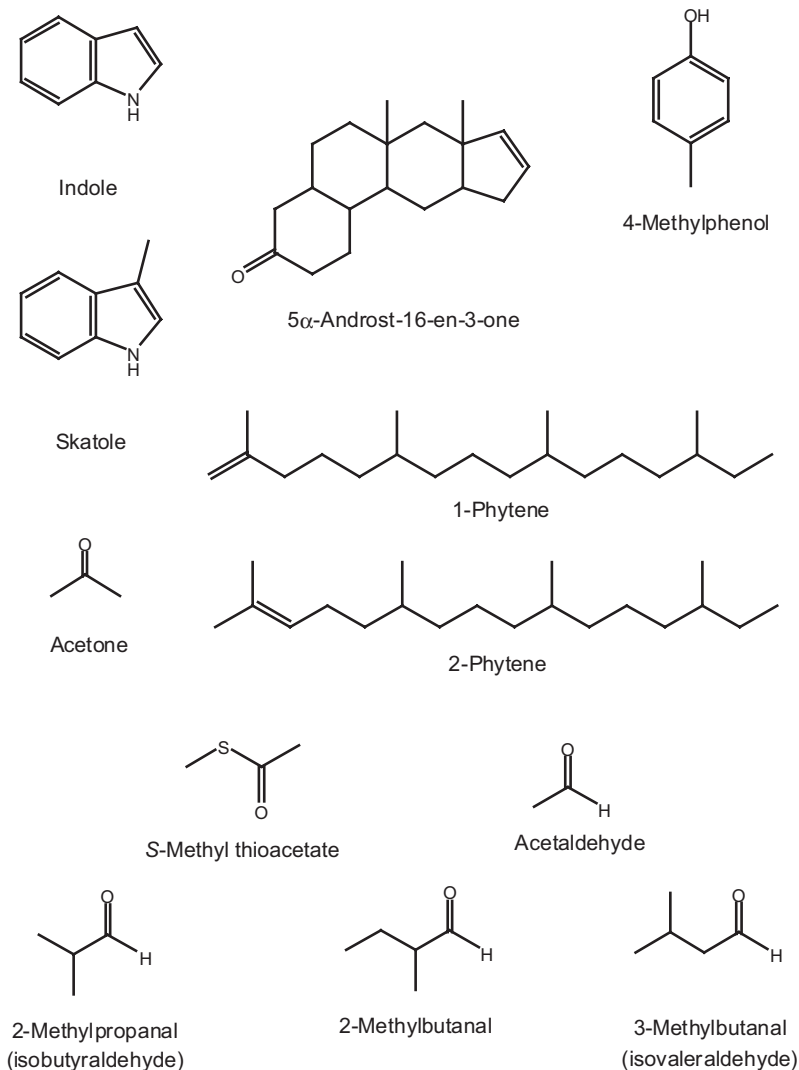


Fig. 5.8 Miscellaneous compounds found in meat, including those associated with boar taint.

steers were higher than in three common American breeds, but only after storing the cooked meat at 3 °C for 3 days. The higher neutral lipids content of the Wagyu meat may have been responsible for this effect. Larick *et al.* (1989) compared the flavour of bison steaks with those of Hereford and Brahman steers. Bison steaks scored higher for off-flavour and aftertaste; off-flavours included ammonia, bitter, gamey, liverish, metallic, old, rotten and sour. Off-flavours may have been due to the higher content, and more unsaturated composition, of bison phospholipids, compared to the two cattle breeds.

Wood *et al.* (2004) compared the sensory properties of cooked meat samples from two traditional (Berkshire, Tamworth) and two modern (Duroc, Large White) pig breeds. The modern breeds were heavier at slaughter with a lower intramuscular fat content, and were lower in pork flavour and desirable flavour than the traditional breeds.

Martinez-Cerezo *et al.* (2005) compared the flavour of cooked muscle from three lamb breeds, one of which was a dairy breed. Differences were small, with no effect on acceptability. Other workers have also shown little effect of breed on sheep meat flavour (Young *et al.*, 1993). However, when Elmore *et al.* (2000) compared the aroma volatiles of pressure-cooked steaks of Suffolk lambs with those of Soay, a rare Scottish semi-feral breed, over 50 compounds were present at higher levels in the cooked Soay steaks. Many of these compounds were derived from the Maillard reaction, including sensorially important alkylpyrazines, dimethyl disulphide and dimethyl trisulphide. When the sensory properties of grilled steaks from grass-fed Suffolk lambs were compared with those of grass-fed Soays, Soays scored lower for juiciness, sweetness, normal lamb flavour and overall liking, and higher for abnormal flavour, bitterness, toughness and rancidity (Fisher *et al.*, 2000).

5.4.2 Age/weight at slaughter

When the aroma volatiles of grilled beef steaks from cattle which had been fed either concentrates or grass-based diets were compared, higher levels of the important sulphur compounds dimethyl disulphide and dimethyl trisulphide were present in animals slaughtered at 24 months, compared with those slaughtered at 14 months (Elmore *et al.*, 2006). Differences in volatile composition due to the diets were smaller in the older animals, reflecting smaller differences in the fatty acid composition of the animals at 24 months. Guth and Grosch (1995) showed a linear increase with age of animal in the formation of the character impact compound 12-methyltridecanal from the plasmalogens of beef phospholipids.

Weller *et al.* (1962) examined lambs slaughtered at three different ages and three different slaughter weights. Roasts were compared and older lambs (over 6 months and 100 lb) were preferred, scoring higher for mildness and typical flavour. Sutherland and Ames (1996) reported higher levels of several fatty acids, including the odour-potent branched chain fatty acids, in the adipose tissue of lambs slaughtered at 30 weeks, compared to those slaughtered at 12 weeks. 4-methyloctanoic acid, in particular, was present at high enough levels to have an impact on lamb flavour. Ames and Sutherland (1999) found higher levels of alkylphenols in the adipose tissue of the same lambs slaughtered at 30 weeks. These compounds may contribute to pastoral flavour in ruminant meat (Young *et al.*, 1999).

Madrugá *et al.* (2000) studied the effect of slaughter age on goat meat flavour. Older animals were tougher with more goaty aroma and less flavour and juiciness, leading to a reduction in overall palatability.

5.4.3 Castration

The use of entire male animals in meat production has been limited, principally due to their aggressive nature, which may also result in glycogen depletion at slaughter, resulting in meat quality defects. In general, however, entire males have a greater lean-to-fat ratio and a better food conversion ratio (Moss, 1992). Boar taint is an off-flavour associated with entire male pigs and castration is seen as a means to minimise its formation.

Ames and Sutherland (1999) showed no effect of castration on the headspace aroma composition of cooked meat from lambs slaughtered at 12 weeks and 30 weeks of age. However, three thiophenols were identified in the adipose tissue of entire lambs, which were absent in castrates. In addition, the faecal-smelling semi-volatile compounds indole and skatole were found only in the muscle and adipose tissue of entire lambs, particularly those slaughtered at 30 weeks. Cooked meat with adipose tissue from entire animals was scored much higher for 'farmyard' aroma than meat with adipose tissue from castrates, and also scored higher for 'stale' and 'urine' aromas. Entire animals also scored higher for 'lamby', 'meaty' and 'roasty' notes, as did animals slaughtered at 30 weeks.

Madrugá *et al.* (2000) studied the effect of castration on goat meat flavour. Differences were small but entire animals scored higher for overall palatability. Lipid-derived aldehydes and hydrocarbons were present at higher levels in entire males, while lipid-derived ketones were at higher levels in castrates.

5.4.4 Stress and pH

Under ideal conditions, the pH of meat should be around 5.5 (Lawrie, 1991), and some problems with meat quality can be attributed to pH changes, often caused by animals becoming stressed immediately pre-slaughter. Elevated pH, i.e. ≥ 5.8 , due to insufficient lactic acid production in the muscle post-slaughter, leads to a reduction in shelf-life, and at its extreme, results in the phenomenon known as DFD, because the meat appears dark, firm and dry.

Braggins (1996) reported that high-pH (pH ~6.8) cooked lamb mince scored low for overall odour, overall flavour, sheepmeat flavour, and high for foreign odour, compared to ideal pH lamb mince. Nearly all aroma compounds were at lower levels in the high-pH meat, although due to relatively mild cooking conditions, few Maillard reaction-derived compounds were measured. Yancey (2005) found that steaks from high-pH cattle exhibiting DFD had less beef flavour, less brown-roasted flavour, and more rancid flavour than steaks from normal-pH muscle.

Dransfield *et al.* (1985) examined the relationship between pH post-slaughter and eating quality in pigs fed diets based on soya meal or rapeseed meal. Final pH values ranged from 5.3 to 7.1. Pork became darker as pH increased, with most of the samples being DFD above pH 6.0, while pork flavour was at a maximum and abnormal flavour at a minimum at pH 5.8.

5.5 Post-slaughter factors affecting meat flavour

5.5.1 *Post-mortem conditioning*

Wasserman (1972) emphasised the importance of conditioning, particularly of beef, in the formation of flavour precursors, and suggested that microbial and enzymatic changes in the muscle alter the flavour profile of the meat. Enzymatic changes in *post-mortem* muscle, which affect the water-soluble fraction, include the breakdown of ribonucleotides to yield free ribose, hypoxanthine and phosphate; the increase in free amino acids and peptides through proteolysis; and the depletion of glycogen to yield a pool of small molecular weight, sugar-related metabolites (Lawrie, 1991).

Daszkiewicz *et al.* (2003) concluded that the process of ageing had a positive effect on the organoleptic properties of beef *M. longissimus dorsi* (LD) conditioned at 0–2 °C. In particular, samples stored for 10 and 14 days were characterised by much better taste than those stored for 3 and 7 days. Miller *et al.* (1997) also concluded that ageing of beef for 14 days, compared to 7 days, increased flavour intensity, while initial differences, attributed to location of production and quality grade, disappeared. Campo *et al.* (1999) investigated the effects of breed and ageing time on the sensory characteristics of beef strip loin steaks and reported highly significant effects of ageing in all of the sensory properties studied, including overall odour/flavour intensity, liver odour/flavour intensity, acid flavour intensity, tenderness and juiciness. Jeremiah and Gibson (2003) concluded that ageing of beef ribs or short loins for up to four weeks appeared to increase tenderness, flavour intensity and desirability. Monsón *et al.* (2005) aged beef for up to five weeks and found that tenderness, beef odour intensity, bitter flavour intensity and overall acceptance were affected, with the highest overall quality observed between 7 and 14 days conditioning. Meinert *et al.* (2007) showed that pork tenderness increased with ageing but there was little effect on other sensory attributes. The same effect was also observed in lamb muscle (Martinez-Cerezo *et al.*, 2005).

When beef muscle was vacuum packed and conditioned at 4 °C for up to 21 days (Koutsidis *et al.*, 2008b), a 6-fold increase in free ribose occurred, accompanied by a large decrease in IMP. Some conversion of hexose phosphates to their hexoses occurred, while changes in pentose phosphates were not observed. Increases in most free amino acids occurred; in particular, levels of the sulphur-containing amino acid methionine increased 7 fold, and 3- to 4-fold increases were observed for several of those amino acids which have an important role in aroma formation during cooking, namely cysteine, isoleucine, leucine, phenylalanine, serine, threonine and valine.

5.5.2 *Preservation*

Curing involves the treatment of meat with sodium chloride and sodium nitrite, often in combination with ascorbic acid (Skibsted, 1992), as a brine or pickle. Nitrite is reduced to the free radical nitric oxide, which may contribute towards

typical cured flavour by stopping free-radical processes during lipid oxidation, forming stable nitriles and nitrates instead of aldehydes and alcohols. Nitric oxide may be stored in cured meat as nitrosylmyoglobin, preventing release of iron, a catalyst of oxidation. In southern Europe the curing ingredients are applied without added water and, in addition to the curing process, a long ageing period is involved, where lipolytic, proteolytic and chemical processes occur. This type of curing is usually known as dry curing. Flores (1997) discusses both types of curing, alongside additional preservation processes such as smoking and fermentation.

Timón *et al.* (2004) compared the aroma volatiles of fried pork and fried bacon. They found much lower levels of lipid-derived aldehydes in the bacon compared to the pork, alongside elevated levels of pyrazines and pyridines, as well as nitriles and nitrates, suggesting that the curing agent had reduced lipid oxidation, allowing more Maillard products to be formed. However, sulphur-containing compounds were at generally lower levels in bacon, suggesting that nitrate may be interacting with methanethiol and/or hydrogen sulphide from methionine and cysteine breakdown, respectively. Cross and Ziegler (1965) found that uncured beef, pork and chicken aroma extracts from which the aldehydes had been removed by derivatisation possessed an aroma similar to that of cured ham, and suggested that typical cured ham flavour is the aroma of cooked meat where no lipid oxidation has occurred.

Moulds have been used in the production of fermented meats for many centuries. Nowadays, specific mould strains are used which induce lipolysis, proteolysis and carbohydrate breakdown (Bruna *et al.*, 2001). Volatile compounds associated with fermented meat products include Strecker aldehydes and alcohols from amino acid breakdown, and straight-chain ketones, esters, and primary and secondary alcohols from lipid oxidation. Different microorganisms will confer particular aroma notes and the addition of herbs and spices will, of course, provide characteristic aroma properties. Formation of aroma compounds will also be affected by pH conditions and the water activity of the meat, while the antimicrobial effect of salt may harm some microorganisms more than others (Stahnke, 1995; Olesen *et al.*, 2004).

Ansorena and Astiasarán (2004) increased the *n*-3 content of dry-fermented chorizo sausage by including linseed oil in its formulation. Addition of antioxidants meant that the lipid profile of the product was improved, with no increase in volatile aldehydes formed as a result of lipid oxidation. Valencia *et al.* (2007) used algal oil instead of linseed. Again, the lipid profile was improved and the shelf-life of the product was acceptable when vacuum packed with added antioxidants.

Of course, there is a wide range of preserved meat products and this section can only provide a few examples of some of the work carried out on these products. Reviews of cured meat flavour exist (Gray and Pearson, 1984; Ramarathnam, 1998; Flores *et al.*, 1998), while Dainty and Blom (1995) have reviewed the flavour chemistry of fermented meats. Even so, there is a need for a comprehensive review of preserved meat flavour, to include cured, fermented and smoked meat.

5.6 Off-flavours in meat

As well as the off-flavours described in this section, the sensory quality of meat can also be impaired by microbial spoilage, irradiation and retorting. A comprehensive review of off-flavours in meat has been written by Bailey *et al.* (1992).

5.6.1 Lipid oxidation/warmed-over flavour

Lipid oxidation is a major cause of deterioration in meat quality, eventually resulting in rancidity. Hexanal formation is often monitored as a means of determining the onset of rancidity (Skibsted *et al.*, 1998). Meat high in unsaturated lipids should oxidise more rapidly than meat with low PUFA content, but the presence of natural antioxidants such as α -tocopherol will reduce oxidation rates. Campo *et al.* (2006) showed, in cooked steaks from animals fed diets varying substantially in PUFA composition, that beef flavour and overall liking scores decreased as oxidation progressed, while abnormal flavour and rancidity scores increased.

Warmed-over flavour (WOF) is a phenomenon caused by lipid oxidation observed in reheated meat, the aroma of which is characterised as 'rancid' and 'stale'. Konopka and Grosch (1991) reported that WOF in boiled beef, stored for 48 hours at 4 °C, was due mainly to increases in *trans*-4,5-epoxy-(*E*)-2-decenal and (*E,E*)-2,4-decadienal. Konopka *et al.* (1995) stated that hexanal and *trans*-4,5-epoxy-(*E*)-2-decenal were key contributors to WOF in cooked beef, pork and chicken, while 1-octen-3-one also contributed to WOF in stewed beef and cooked chicken. They also showed that WOF was not a problem in cured pork. Kerler and Grosch (1997) identified (*E,E*)-2,4-decadienal, butyric acid and furaneol as key compounds in fresh boiled chicken aroma, whereas hexanal, butyric acid and 1-octen-3-one were the key compounds in chicken reheated after 48 hours at 4 °C. However, Siegmund and Pfannhauser (1999) found that (*E,E*)-2,4-decadienal was a key compound of warmed-over pressure-cooked chicken, along with hexanal and several other lipid oxidation products.

O'Sullivan *et al.* (2003) showed that in pork, WOF was greater in *M. psoas major*, compared to *M. longissimus dorsi*, possibly due to the higher iron content of the former, and animals fed iron supplements produced meat with more WOF. Lipid-derived compounds correlated well with sensory attributes of WOF, such as 'rancid', 'fish' and 'vegetable oil'.

5.6.2 Boar taint

Three compounds that have been widely studied with regard to their contribution to the phenomenon known as boar taint, an unpleasant odour of boar found in pork from a proportion of uncastrated male pigs, are 5 α -androst-16-en-3-one, indole and skatole (3-methylindole). These compounds are mainly located in adipose tissue and have been associated with odours such as 'perspiration-like' or 'urine-like' in cooked pork. Their structures are shown in [Fig. 5.8](#).

Many workers have studied boar taint, although clear reasons for its presence in some animals are not always obvious, as it has been detected in castrated animals (Jeremiah *et al.*, 1999), and dietary effects have not been consistent. A contemporary approach to prevent boar taint is to inhibit sexual development in entire males by manipulating the activity of gonadotropin releasing hormone by immunisation, using a vaccine such as Improvac®. Dunshea *et al.* (2001) measured levels of androstenone and skatole in the fat of two sets of entire boars, one set fed with Improvac and one set with a placebo. No Improvac-immunised boars contained levels of either compound above its threshold, whereas 50% of the control boars had suprathreshold levels of androstenone and 11% had suprathreshold levels of skatole. Improvac-treated boars were leaner and grew more rapidly than the controls.

5.6.3 Pastoral flavour

A typical 'pastoral' or 'grassy' flavour has often been reported as a negative trait in pasture-fed ruminants, particularly in lamb meat. Although a number of compounds have been implicated in its formation, a definitive source of pastoral flavour has not yet been determined. Skatole and indole, compounds associated with boar taint, have been suggested, along with (Z)-4-heptenal and other breakdown products of C18:3 *n*-3, and 4-methylphenol (Fig. 5.8), which may be formed from rumen metabolism of tyrosine (Young *et al.*, 1999). Indole and skatole levels were highly correlated with 'barnyard' aroma in cooked lamb mince (Young *et al.*, 2003).

5.7 Laboratory analysis of meat aroma compounds

5.7.1 Aroma extraction techniques

The volatile components of meat, which are responsible for its aroma, are present in extremely small quantities compared with the major constituents, of which water is the most abundant. A number of isolation techniques exist, all based on utilising the physical properties of the aroma compounds, to separate them from the food matrix and from water. Four widely used extraction techniques that have been used to analyse meat aroma are simultaneous steam distillation/extraction (SDE), solvent-assisted flavour extraction (SAFE), headspace adsorption on Tenax, and solid-phase microextraction (SPME). By far the most widely used technique for the separation and identification of aroma compounds in extracts is gas chromatography–mass spectrometry (GC–MS).

Simultaneous distillation/extraction (SDE)

One of the most widely used techniques in the analysis of cooked meat aroma combines steam distillation with solvent extraction in a Likens–Nickerson (1964) apparatus. The extracting solvent is immiscible with and less dense than water. Upon heating, volatile compounds in the steam are transferred to the solvent and

both liquids condense. The glassware is constructed so that both solvent and water are returned to their starting vessels. After extraction, the extract is collected and dried before being concentrated to a volume of approximately 0.1 mL for analysis. A low-boiling extracting solvent that can be removed without substantial losses of compounds of interest is therefore desirable. In addition, the solvent should be of high purity so that impurities do not become major chromatographic peaks when the extract is concentrated. Appropriate solvents, which have been widely used, are pentane, diethyl ether, or a combination of the two.

Solvent-assisted flavour evaporation (SAFE)

Because SDE may lead to artefact formation and overcooking, high vacuum transfer was developed in the early 1990s as a way of removing the volatile aroma compounds from solvent extracts of food materials. However, there were numerous drawbacks with the technique and a robust alternative, known as solvent-assisted flavour evaporation (SAFE), was developed to supersede it (Engel *et al.*, 1999). Although high vacuum transfer and SAFE are similar techniques in principle, greater thermal control and a more compact arrangement of the glassware mean that SAFE is the more efficient technique, resulting in higher yields of high-boiling and polar compounds. It can also be used directly on the food, with no need of a solvent extraction step, producing an extract with typical aroma.

Since SAFE has only been recently developed, it has not been used regularly for the analysis of meat aroma (Rota and Schieberle, 2006). However, it is proving to be a popular technique for aroma analysis and may well be the most effective aroma isolation technique available.

Headspace adsorption

Headspace aroma volatiles can be collected on suitable adsorbent materials, the most widely-used of which is Tenax TA. These materials readily adsorb volatiles while having little affinity for water, making them particularly useful in the analysis of samples with high water content. In a typical collection, purified inert gas sweeps the volatiles from the sample flask into a 'trap', a small tube containing the adsorbent. Adsorbed volatiles can be heat desorbed directly onto a gas chromatographic column by placing the trap in a specially modified injection port. Cooling the front of the column with solid carbon dioxide or liquid nitrogen during this desorption will avoid any loss in chromatographic resolution (Elmore, 2008).

Solid phase microextraction (SPME)

A very popular and simple to use technique, solid phase microextraction (SPME), uses a small fused silica fibre coated in an adsorbent material, mounted inside a syringe-like device. The needle is pushed through a septum and the fibre is exposed to the headspace above the meat sample, which is sealed in a suitable container. Volatile compounds are adsorbed onto the fibre and at the end of the extraction the fibre can be removed from the sample vessel and directly desorbed into the split/splitless injection port of a gas chromatograph (Elmore, 2008).

5.7.2 Separation and identification of aroma components

To determine the important compounds in an aroma extract, the complex mixture needs to be separated into its components. The amount of isolated material is usually small, containing many compounds of diverse chemical structures varying greatly in concentration, and important components are often present in extremely low amounts. The success of any aroma analysis depends mainly upon the efficiency of separation and the sensitivity of detection. GC using fused silica capillary columns is universally used in aroma analysis to separate complex mixtures; the most commonly used stationary phases are Carbowax 20M, a polar phase, and the two non-polar phases, 100% poly(dimethylsiloxane) and poly(5% diphenylsiloxane/95% dimethylsiloxane). The retention times of an aroma compound on two columns with different stationary phases, relative to the retention times of a series of straight-chain alkanes, can be helpful in its identification; databases containing retention data for volatile compounds are available (Kondjoyan and Berdagué, 1996). GC is a widely used technique and will not be discussed here.

GC–MS allows direct spectral analysis of the separated components and provides the most efficient means of volatile identification. Compounds eluting from the GC column enter the ion source of the mass spectrometer, where they are bombarded with electrons. A compound will fragment and the fragments are separated by their mass-to-charge ratio, resulting in a characteristic spectrum that will provide structural information.

Quadrupole mass spectrometers obtaining at least one spectrum per second are ideal for low resolution GC-MS of aroma extracts and are the type most commonly used for aroma analysis. Recently, time-of-flight (TOF) machines have become increasingly popular as mass spectrometric detectors, with newer models offering rapid scan speeds (up to 500 spectra per second) and high resolution capabilities. High scan speeds are necessary when using fast GC techniques, such as two-dimensional GC (GC \times GC). A recent paper by Rochat *et al.* (2007) showed the potential of GC \times GC, hyphenated to a time-of-flight MS, as an unrivalled technique for the separation of meat aroma volatiles. The technique is extremely sensitive as a result of low background and exceptionally high peak resolution, allowing thousands of peaks to be separated in one GC–MS trace. At present, the cost of such equipment may place it beyond the reach of most analytical laboratories, but its potential is evident.

The characterisation of unknown compounds is greatly facilitated by comparing their mass spectra with those of known compounds in compiled libraries, which are supplied with the GC–MS data system. Confirmation of the identity of compounds should always be carried out, preferably by comparing their mass spectra and GC retention times with those of authentic samples.

5.7.3 Quantification of aroma components

Often, quantitative information on aroma compounds in meat is desired. The most effective means of quantification is isotope dilution assay using GC–MS. In order

to quantify its non-labelled equivalent, a known amount of a ^{13}C - or ^2H -labelled internal standard is added to a meat sample, which is then homogenised. As the labelled and unlabelled aroma compounds possess similar physical properties, the proportion of each extracted from the meat will be the same. The relationship between the labelled standard and the compound of interest can be used to calculate accurately the amount of the compound of interest in the food. If the labelled standard is homogeneously distributed in the meat, then quantitative extraction of the compound under study is not necessary (Milo and Blank, 1998).

Kerscher and Grosch (1998) quantified four sulphur compounds in various cooked meats. Of particular interest was 2-methyl-3-furanthiol because of its importance in meat aroma. When comparing meats boiled for 45 minutes, this compound was shown to be highest in beef, then at similar levels in lamb and pork, and lowest in chicken.

5.7.4 Detection of components of sensory significance

A widely used technique for determining components that contribute to aroma is GC-olfactometry (GCO). The column effluent is split between a conventional GC detector and a vent to the outside of the oven, where the odours emerging can be smelled and described. Aroma extract dilution analysis (AEDA) is a quantitative GCO technique, which has been used many times to estimate the relative contributions of volatile components towards the total aroma quality of cooked meat. A solvent extract of the volatile fraction under study is analysed by GCO, diluted two-fold and analysed by GCO again, and so on. After a certain number of dilutions of the extract, no aromas will be perceived. The flavour dilution factor for a particular compound is defined as the highest dilution at which that compound can be perceived by GCO. For example, if the seventh dilution was the last at which the compound could be detected, its flavour dilution factor would be 2^7 (128). Hence, if the aroma extract is representative of the meat sample from which it is derived, the most important contributors to the aroma of the meat are those with the highest flavour dilution factors (Gasser and Grosch, 1988).

5.7.5 The electronic nose

Often, a detailed analysis of the individual compounds in a meat sample may not be necessary. An analytical technique, which provides a 'fingerprint' of the sample under study may be all that is required. Such a tool is the electronic nose, which can be used, for example, to distinguish meat from different breeds or diets, or to determine if a piece of meat is of sufficient freshness (Santos *et al.*, 2004; Blixt and Borch, 1999).

Originally, the term 'electronic nose' was used to describe an array of chemical sensors connected to a pattern recognition system, which responded to odours passing over it. Different odours cause different responses in the sensors and these responses provide a signal pattern, characteristic of a particular aroma. The computer evaluates the signal pattern and can compare the aromas of different

samples, using pattern recognition. Sensors are usually made of metal oxides or organic polymers although lately, surface acoustic waves and piezoelectric crystals have been used.

More recently, electronic noses based on mass spectrometry have been developed, which are also known as mass sensors or MS-noses. Volatile compounds are introduced directly into the mass sensor without any pre-separation. With these instruments the mass sensor is amalgamating all of the spectral data that make up a GC–MS run, to provide a fingerprint of the food under study (Pavón *et al.*, 2006).

5.8 Future trends

DNA-based techniques have been developed for species and animal identification, and for genetic selection, while RNA- and protein-based techniques could some day be used to determine the health, physiological and nutritional status of meat animals (Hocquette *et al.*, 2007). These tools have the potential to change production methods in cattle breeding, husbandry and nutrition.

Genetic factors are known to affect the biological characteristics of muscles, with resulting effects on tenderness and flavour. Bernard *et al.* (2007) identified differentially expressed genes in the *Longissimus thoracis* muscles of 25 Charolais bulls, of which 18 were positively correlated with flavour and juiciness, and 1 was negatively correlated. These genes may provide indicators of beef quality.

Of interest to the meat industry are the differences in the meat quality of pigs that carry the *RN*-allele and those that do not. Animals that carry the *RN*-allele produce meat with higher drip and cooking losses and lower processing yield. They have higher levels of muscle glycogen than non-carriers, resulting in a reduction in muscle protein. Enfält and Hullberg (2005) also found that the meat of *RN*-carriers contained higher levels of combined glucose and glucose-6-phosphate than non-carriers.

Meinert *et al.* (2007) analysed glucose and glucose 6-phosphate separately and showed large increases of both in the meat of *RN*-carriers. They also measured ribose but found no effect of the *RN*-allele. When they analysed fried steaks of both genotypes, the steaks from the *RN*-carriers possessed higher fried meat odour and flavour, more burnt caramel, more umami and more sour flavour, but less cardboard flavour than the *RN*-non-carriers. The steaks from the *RN*-carriers were also more tender and juicier. Acetone, 2-methylbutanal and 3-methylbutanal were at higher levels in the fried steaks from the *RN*-carriers (Fig. 5.8).

A major challenge to meat flavour research is to forge an understanding of how changes in water-soluble flavour precursors, brought about by production and/or processing, affect the flavour of cooked meat. Although many papers were published in the 1960s and early 1970s on meat flavour precursors, interest in these compounds has only resurfaced in the past ten years. The evolution of analytical techniques such as capillary electrophoresis, ion chromatography and liquid chromatography-mass spectrometry, and the development of simple to use

derivatisation procedures for GC-MS, have provided meat researchers with the means to confidently identify and quantify trace levels of water-soluble compounds and perhaps measure how levels of these compounds change during cooking.

Scollan *et al.* (2006) have recently highlighted areas for research in meat quality, in particular, with regard to beef. Novel strategies to increase the PUFA content of meat are prescribed, along with the development of meat products with functional properties. Karamichou *et al.* (2006), for instance, have studied the genetic basis of intramuscular fatty acid composition in order to examine the possibility of breeding for altered fatty acid composition. Of course, novel meat products must have desirable flavour; otherwise the health benefits of such products will be overlooked.

5.9 Sources of further information and advice

Johnson D E, Knight M and Ledward D A (1992), *The Chemistry of Muscle-Based Foods*, Cambridge, Royal Society of Chemistry.

Maarse H (1991), *Volatile Compounds in Foods and Beverages*, New York, Marcel Dekker.

Nollet, L M L and Toldrá, F (2008), *Handbook of Muscle Foods Analysis*, Boca Raton, CRC Press.

Shahidi F (1998), *Flavor of Meat, Meat Products and Seafoods*, London, Blackie Academic and Professional.

Xiong Y L, Ho C-T and Shahidi F (1999), *Quality Attributes of Muscle Foods*, New York, Kluwer Academic.

5.10 References

- Aliani M and Farmer L J (2005), 'Precursors of chicken flavour. I. Determination of some flavor precursors in chicken muscle', *J. Agric. Food Chem.*, 53, 6067–6072.
- Aliani M, Farmer L J and Hagan T D J (2003), in Le Quéré J-L and Étiévant P X, *Flavour Research at the Dawn of the 21st Century*, Paris, Editions Tec & Doc, 220–223.
- Ames J M and Sutherland M M (1999), 'Effect of castration and slaughter age on the flavor of sheepmeat', in Xiong Y L, Ho C-T and Shahidi F, *Quality Attributes of Muscle Foods*, New York, Kluwer Academic, 147–156.
- Ansorena D and Astiasarán I (2004), 'The use of linseed oil improves nutritional quality of the lipid fraction of dry-fermented sausages' *Food Chem.*, 87 (1), 69–74.
- Ashes J R, Siebert B D, Gulati S K, Cuthbertson A Z and Scott T W (1992), 'Incorporation of *n*-3 fatty acids of fish oil into tissue and serum lipids of ruminants', *Lipids*, 27, 629–631.
- Bailey M E, Rourke T J, Gutheil R A and Wang C Y-J (1992), 'Undesirable flavors of meat', in Charalambous G, *Off-Flavors in foods and Beverages*, Amsterdam, Elsevier, 127–169.
- Bernard C, Cassar-Malek I, le Cunff M, Dubroeuq H, Renand G and Hocquette J F (2007), 'New indicators of beef sensory quality revealed by expression of specific genes', *J. Agric. Food Chem.*, 55 (13), 5229–5237.
- Blixt Y and Borch E (1999), 'Using an electronic nose for determining the spoilage of vacuum-packaged beef', *Intl. J. Food Microbiol.*, 46 (2), 123–134.
- Boylston T D, Morgan S A, Johnsen K A, Wright Jr. R W, Busboom J R and Reeves J J (1996), 'Volatile lipid oxidation products of Wagyu and domestic breeds of beef', *J. Agric. Food Chem.*, 44, 1091–1095.

- Braggins T J (1996), 'Effect of stress-related changes in sheepmeat ultimate pH on cooked odor and flavor', *J. Agric. Food Chem.*, 44 (8), 2352–2360.
- Bruna J M, Hierro E M, de la Hoz L, Mottram D S, Fernández M and Ordóñez J A (2001), 'The contribution of *Penicillium aurantiogriseum* to the volatile composition and sensory quality of dry fermented sausages', *Meat Sci.*, 59, 97–107.
- Buttery R G, Lin L C, Teranishi R and Mon T R (1977), 'Roast lamb fat: Basic volatile components', *J. Agric. Food Chem.*, 25, 1227–1229.
- Campo M M, Nute G R, Hughes S I, Enser M, Wood J D and Richardson R I (2006), 'Flavour perception of oxidation in beef', *Meat Sci.*, 72 (2), 303–311.
- Campo M M, Sañudo C, Panea B, Alberti P and Santolaria P (1999), 'Breed type and ageing time effects on sensory characteristics of beef strip loin steaks', *Meat Sci.*, 51 (4), 383–390.
- Cerny C and Grosch W (1992), 'Evaluation of potent odorants in roasted beef by aroma extract dilution analysis', *Z. Lebensm. -Untersuch. -Forsch.*, 194, 322–325.
- Chambaz A, Scheeder M R L, Kreuzer M and Dufey P A (2003), 'Meat quality of Angus, Simmental, Charolais and Limousin steers compared at the same intramuscular fat content', *Meat Sci.*, 63 (4), 491–500.
- Cooper S L, Sinclair L A, Wilkinson R G, Hallett K G, Enser M and Wood J D (2004), 'Manipulation of the *n*-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs', *J. Anim. Sci.*, 82 (5), 1461–1470.
- Cross, C K and Ziegler, P (1965), 'A comparison of the volatile fractions from cured and uncured meat', *J. Food Sci.*, 30, 610–614.
- Dainty RH and Blom J (1995), 'Flavour chemistry of fermented sausage' in Campbell-Platt G and Cook P E, *Fermented Meats*, Glasgow, Blackie Academic and Professional, 176–193.
- Dannenberger D, Lorenz S, Nuernberg G, Scollan N, Ender K and Nuernberg K (2006), 'Analysis of fatty aldehyde composition, including 12-methyltridecanal, in plasmalogens from longissimus muscle of concentrate- and pasture-fed bulls', *J. Agric. Food Chem.*, 54 (1), 182–188.
- Daszkiewicz T, Wajda S and Matusiewicz P (2003), 'Changing of beef quality in the process of storage', *Veterinarija ir Zootechnika. T.*, 21 (43), 62–65.
- Dransfield E, Nute G R, Mottram D S, Rowan T G and Lawrence T L J (1985), 'Pork quality from pigs fed on low glucosinolate rapeseed meal: Influence of level in the diet, sex and ultimate pH', *J. Sci. Food Agric.*, 36, 546–556.
- Dunshea F R, Colantoni C, Howard K, McCauley I, Jackson P, Long K A, Lopaticki S, Nugent E A, Simons J A, Walker J and Hennessy D P (2001), 'Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance', *J. Anim. Sci.*, 79, 2524–2535.
- Elmore JS (2008), 'Aroma', in Nollet L M L and Toldrá F, *Handbook of Muscle Foods Analysis*, Boca Raton, CRC Press, 242–262.
- Elmore J S and Mottram D S (1997), 'Investigation of the reaction between ammonium sulfide, aldehydes, and alpha-hydroxyketones or alpha-dicarbonyls to form some lipid–Maillard interaction products found in cooked beef', *J. Agric. Food Chem.*, 45 (9), 3595–3602.
- Elmore J S, Cooper S L, Enser M, Mottram D S, Sinclair L A, Wilkinson R G and Wood J D (2005), 'Dietary manipulation of fatty acid composition in lamb meat and its effect on the volatile aroma compounds of grilled lamb', *Meat Sci.*, 69, 233–242.
- Elmore J S and Mottram D S (2000), 'Formation of 2-alkyl-(2*H*)-thiapyrans and 2-alkylthiophenes in cooked beef and lamb', *J. Agric. Food Chem.*, 48, 2420–2424.
- Elmore J S, Mottram D S and Dodson A T (2004a), 'Meat aroma analysis: Problems and solutions', in Deibler K D and Delwiche J, *Handbook of Flavor Characterization: Sensory Analysis, Chemistry, and Physiology*, New York, Marcel Dekker, Inc., 295–310.
- Elmore J S, Mottram D S, Enser M and Wood J D (1997), 'Novel thiazoles and 3-thiazolines in cooked beef aroma', *J. Agric. Food Chem.*, 45, 3603–3607.

- Elmore J S, Mottram D S, Enser M and Wood J D (1999), 'Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles.', *J. Agric. Food Chem.*, 47, 1619–1625.
- Elmore J S, Mottram D S, Enser M and Wood J D (2000), 'The effects of diet and breed on the major volatiles present in lamb aroma', *Meat Sci.*, 55, 149–159.
- Elmore J S, Mottram D S, Enser M and Wood J D (2006), 'The effects of diet, breed and age of animal at slaughter on the volatile compounds of grilled beef', in Weenen H and Shahidi F, *Food Lipids: Chemistry, Flavor and Texture*, Washington, DC, American Chemical Society, 35–48.
- Elmore J S, Warren H E, Mottram D S, Scollan N D, Enser M, Richardson R I and Wood J D (2004b), 'A comparison of the aroma volatiles and fatty acid compositions of grilled beef muscle from Aberdeen Angus and Holstein–Friesian steers fed diets based on silage or concentrates', *Meat Sci.*, 68 (1), 27–33.
- Enfält A C and Hullberg A (2005), 'Glycogen, glucose and glucose-6-phosphate content in fresh and cooked meat and meat exudate from carriers and noncarriers of the RN-allele.', *J. Muscle Foods*, 16 (4), 330–341.
- Engel W, Bahr W, and Schieberle P (1999), Solvent assisted flavour evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices, *Eur. Food Res. Technol.*, 209, 237.
- Enser M, Hallett K G, Hewett B, Fursey G A J and Wood J D (1996), 'Fatty acid content and composition of English beef, lamb and pork at retail', *Meat Sci.*, 42, 443–456.
- Enser M, Hallett K G, Hewett B, Fursey G A J, Wood J D and Harrington G (1998), 'Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition', *Meat Sci.*, 49 (3), 329–341.
- Farmer L J (1992), 'Meat Flavour', in Ledward D A, Johnston D E and Knight M K, *The Chemistry of Muscle-based Foods*, Cambridge, Royal Society of Chemistry, 169–182.
- Farmer L J and Mottram D S (1994), 'Lipid–Maillard interactions in the formation of volatile aroma compounds', in Maarse H and van der Heij D G, *Trends in Flavour Research*, Amsterdam, Elsevier, 313–326.
- Farmer L J, Mottram D S and Whitfield F B (1989), 'Volatile compounds produced in Maillard reactions involving cysteine, ribose and phospholipid', *J. Sci. Food Agric.*, 49, 347–368.
- Fisher A V, Enser M, Richardson R I, Wood J D, Nute G R, Kurt E, Sinclair L A and Wilkinson R G (2000), 'Fatty acid composition and eating quality of lamb types derived from four diverse breed × production systems', *Meat Sci.*, 55, 141–147.
- Flores J (1997), 'Mediterranean vs northern European meat products. Processing technologies and main differences', *Food Chem.*, 59 (4), 505–510.
- Flores M, Spanier A M and Toldrá F (1998), 'Flavour analysis of dry-cured ham', in Shahidi F, *The Flavour of Meat, Meat Products and Seafood*, London, Blackie Academic and Professional, 320–341.
- Fogerty A C, Whitfield F B, Svoronos D and Ford G L (1990), 'Changes in the composition of the fatty acids and aldehydes of meat lipids after heating', *Intl. J. Food Sci. Technol.*, 25, 304–312.
- Fogerty A C, Whitfield F B, Svoronos D and Ford G L (1991), 'The composition of the fatty acids and aldehydes of the ethanolamine and choline phospholipids of various meats', *Intl. J. Food Sci. Technol.*, 26, 363–371.
- Fontanillas R, Barroeta A, Baucells M D and Codony R (1997), 'Effect of feeding high *cis*-monounsaturated, trans, or *n*–3 fats on lipid composition of muscle and adipose tissue of pigs', *J. Agric. Food Chem.*, 45, 3070–3075.
- Gasser U and Grosch W (1988), 'Identification of volatile flavour compounds with high aroma values from cooked beef', *Z. Lebensm. -Unters -Forsch.*, 186, 489–494.
- Gasser U and Grosch W (1990), 'Primary odorants of chicken broth – a comparative-study with meat broths from cow and ox', *Z. Lebensm. Unters Forsch.*, 190, 3–8.
- Gray J I and Pearson A M (1984), 'Cured meat flavour', in Chichester C O, Mrak E M and

- Schweigert B S, *Advances in Food Research*, Orlando, FL, Academic Press Inc., 2–86.
- Grosch W (1987), 'Reactions of hydroperoxides – products of low molecular weight', in Chan H W-S, *Autoxidation of Unsaturated Lipids*, London, Academic Press, 95–139.
- Guth H and Grosch W (1993), '12-Methyltridecanal, a species-specific odorant of stewed beef', *Lebensm. -Wiss. -Technol.*, 26, 171–177.
- Guth H and Grosch W (1994), 'Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies', *J. Agric. Food Chem.*, 42, 2862–2866.
- Guth H and Grosch W (1995), 'Dependence of the 12-methyltridecanal concentration in beef on the age of the animal', *Z. Lebensm. -Unters -Forsch.*, 201, 25–26.
- Ha J K and Lindsay R C (1990), 'Distribution of volatile branched-chain fatty acids in perinephric fats of various red meat species', *Lebensm. -Wiss. -Technol.*, 23, 433–440.
- Hocquette J F, Lehnert S A, Barendse W, Cassar-Malek I and Picard B (2007), 'Recent advances in cattle functional genomics and their application to beef quality', *Animal*, 1, 159–173.
- Jeremiah L E, Squires E J and Sather A P (1999), 'Gender and diet influences on pork palatability and consumer acceptance. II. Sex taint compounds and their relationship to sensory properties', *J. Muscle Foods*, 10 (4), 317–331.
- Jeremiah L E and Gibson L L (2003), 'The effects of *post-mortem* product handling and aging time on beef palatability', *Food Res. Intl.*, 36, 929–941.
- Karamichou E, Richardson R I, Nute G R, Gibson K P and Bishop S C (2006), 'Genetic analyses and quantitative trait loci detection, using a partial genome scan, for intramuscular fatty acid composition in Scottish Blackface sheep', *J. Anim. Sci.*, 84 (12), 3228–3238.
- Kerler J and Grosch W (1997), 'Character impact odorants of boiled chicken: Changes during refrigerated storage and heating', *Z. Lebensm. -Unters -Forsch.*, 205, 232–238.
- Kerscher R and Grosch W (1998), 'Quantification of 2-methyl-3-furanthiol, 2-furfurylthiol, 3-mercapto-2-pentanone, and 2-mercapto-3-pentanone in heated meat', *J. Agric. Food Chem.*, 46 (5), 1954–1958.
- Kerscher, R and Grosch, W (2000), 'Comparison of the aromas of cooked beef, pork and chicken', in Schieberle P and Engel K-H, *Frontiers of Flavour Science*, Garching, Deutsche Forschungsanstalt für Lebensmittelchemie, 17–21.
- Kondjoyan N and Berdagué J-L (1996), *A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds*, Saint Genes Champanelle, INRA de Theix.
- Konopka U C and Grosch W (1991), 'Potent odorants causing the warmed-over flavor in boiled beef', *Z. Lebensm. -Unters -Forsch.*, 193, 123–125.
- Konopka U C, Guth H and Grosch W (1995), 'Potent odorants formed by lipid peroxidation as indicators of warmed-over flavor (WOF) of cooked meat', *Z. Lebensm. -Unters -Forsch.*, 201, 339–343.
- Koutsidis G, Elmore J S, Oruna-Concha M J, Campo M M, Wood J D and Mottram D S (2008a), 'Water-soluble precursors of beef flavour: I. Effect of diet and breed', *Meat Sci.*, 79, 24–30.
- Koutsidis G, Elmore J S, Oruna-Concha M J, Campo M M, Wood J D and Mottram D S (2008b), 'Water-soluble precursors of beef flavour: II. Effect of conditioning', *Meat Sci.*, 79, 270–277.
- Larick D K and Turner B E (1990), 'Headspace volatiles and sensory characteristics of ground beef from forage-fed and grain-fed heifers', *J. Food Sci.*, 55 (3), 649–654.
- Larick D K, Turner B E, Koch R M and Crouse J D (1989), 'Influence of phospholipid content and fatty acid composition of individual phospholipids in muscle from bison, Hereford and Brahman steers on flavor', *J. Food Sci.*, 54 (3), 521–526.
- Larick D K, Turner B E, Schoenherr W D, Coffey M T and Pilkington D H (1992), 'Volatile compound content and fatty acid composition of pork as influenced by linoleic acid content of the diet', *J. Anim. Sci.*, 70, 1397–1403.
- Lawrie R A (1991) *Meat Science*, 5th edition, Pergamon, Oxford.

- Likens S T and Nickerson G B (1964), 'Detection of certain hop oil constituents in brewing products', *Proc. Am. Soc. Brew. Chem.*, 5.
- Lorenz S, Buettner A, Ender K, Nürnberg G, Papstein H-J, Schieberle P and Nürnberg K (2002), 'Influence of keeping system on the fatty acid composition in the *longissimus* muscle of bulls and odorants formed after cooking', *Eur. Food Res. Technol.*, 214, 112–118.
- Maarse H and Visscher C A (1996), *Volatile Compounds in Food – Qualitative and Quantitative Data*, Zeist, TNO-CIVO Food Analysis Institute.
- Machiels D, Istasse L and van Ruth S M (2004), 'Gas chromatography–olfactometry analysis of beef meat originating from differently fed Belgian Blue, Limousin and Aberdeen Angus bulls', *Food Chem.*, 86, 377–383.
- MacLeod G (1986), 'The scientific and technological basis of meat flavours', in Birch G C and Lindley M G, *Developments in Food Flavors*, London, Elsevier, 191–223.
- MacLeod G (1998), 'The flavour of beef', in Shahidi F, *The Flavour of Meat, Meat Products and Seafood*, London, Blackie Academic and Professional, 27–60.
- Macy Jr. R L, Naumann H D and Bailey M E (1964a), 'Water-soluble flavor and odor precursors of meat. I. Qualitative study of certain amino acids, carbohydrates, non-amino acid nitrogen compounds and phosphoric acid esters of beef, pork and lamb', *J. Food Sci.*, 29, 136–141.
- Macy Jr. R L, Naumann H D and Bailey M E (1964b), 'Water-soluble flavor and odor precursors of meat. II. Effects of heating on amino nitrogen constituents and carbohydrates in lyophilized diffusates from aqueous extracts of beef, pork and lamb', *J. Food Sci.*, 29, 142–148.
- Madrugá M S, Arruda S G B, Narain N and Souza J G (2000), 'Castration and slaughter age effects on panel assessment and aroma compounds of the 'Mestico' goat meat', *Meat Sci.*, 56, 117–125.
- Martinez-Cerezo S, Sanudo C, Medel I and Olleta J L (2005), 'Breed, slaughter weight and ageing time effects on sensory characteristics of lamb', *Meat Sci.*, 69 (3), 571–578.
- Meinert L, Andersen L T, Bredie W L P, Bjerregaard C and Aaslyng M D (2007), 'Chemical and sensory characterisation of pan-fried pork flavour: Interactions between raw meat quality, ageing and frying temperature', *Meat Sci.*, 75 (2), 229–242.
- Melton S L (1990), 'Effects of feeds on flavor of red meat: A review', *J. Anim. Sci.*, 68, 4421–4435.
- Miller, M F, Kerth, C R, Wise, J W, Lansdell, J L, Stowell, J E and Ramsey, C B (1997), 'Slaughter plant location, USDA quality grade, external fat thickness and aging time effects on sensory characteristics of beef loin strip steak', *J. Anim. Sci.*, 75, 662–667.
- Milo C and Blank I (1998), 'Quantification of impact of odorants in food by isotope dilution assay: Strengths and limitations'. In Mussinan C J and Morello M J, *Flavor Analysis: Developments in Isolation and Characterization*. Washington, DC, American Chemical Society, 69–77.
- Monsón F, Sanudo C and Sierra I (2005), 'Influence of breed and ageing time on the sensory meat quality and consumer acceptability in intensively reared beef', *Meat Sci.*, 71 (3), 471–479.
- Morton I D, Akroyd P and May C G (1960), 'Flavouring substances and their preparation', US Patent 2934437, April 26th.
- Moss (1992), 'Lean meat, animal welfare, and meat quality'. In Johnson D E, Knight M and Ledward D A, *The Chemistry of Muscle-based Foods*, Cambridge, Royal Society of Chemistry, 62–76.
- Mottram D S (1985), 'The effect of cooking conditions on the formation of volatile heterocyclic compounds in pork', *J. Sci. Food Agric.*, 36, 377–382.
- Mottram D S (1991), 'Meat', in Maarse H, *Volatile Compounds in Foods and Beverages*, New York, Marcel Dekker, 107–177.
- Mottram D S (1998), 'Flavour formation in meat and meat products: A review', *Food Chem.*, 62 (4), 415–424.

- Mottram D S and Edwards R A (1983), 'The role of triglycerides and phospholipids in the aroma of cooked beef', *J. Sci. Food Agric.*, 34, 517–522.
- Muir P D, Deaker J M and Bown M D (1998), 'Effects of forage- and grain-based feeding systems on beef quality: A review', *N. Z. J. Agric. Res.*, 41 (4), 623–635.
- Nuernberg K, Dannenberger D, Nuernberg G, Ender K, Voigt J, Scollan N D, Wood J D, Nute G R and Richardson R I (2005), 'Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of *longissimus* muscle in different cattle breeds', *Livestock Prod. Sci.*, 94 (1–2), 137–147.
- Olesen P T, Meyer A S and Stahnke L H (2004), 'Generation of flavour compounds in fermented sausages – the influence of curing ingredients, *Staphylococcus* starter culture and ripening time', *Meat Sci.*, 66 (3), 675–687.
- O'Sullivan M G, Byrne D V, Jensen M T, Andersen H J and Vestergaard J (2003), 'A comparison of warmed-over flavour in pork by sensory analysis, GC/MS and the electronic nose', *Meat Sci.*, 65 (3), 1125–1138.
- Øverland M, Taugbol O, Haug A and Sundstol E (1996), 'Effect of fish oil on growth performance, carcass characteristics, sensory parameters, and fatty acid composition in pigs', *Acta Agric. Scand. Sect. A – Anim. Sci.*, 46 (1), 11–17.
- Pavón, J L P. Sanchez M D, Pinto C G, Laespada M E F, Cordero B M and Pena A G (2006), 'Strategies for qualitative and quantitative analyses with mass-spectrometry-based electronic noses', *TRAC Trends Anal. Chem.*, 25, 257–266.
- Pearson A M, Wenham L M, Carse W A, McLeod K, Davey C L and Kirton A H (1973), 'Observations on the contribution of fat and lean to the aroma of cooked beef and lamb', *J. Anim. Sci.*, 36 (3), 511–515.
- Ponnampalam E N, Sinclair A J, Egan A R, Ferrier G R and Leury B J (2002), 'Dietary manipulation of muscle long-chain omega-3 and omega-6 fatty acids and sensory properties of lamb meat', *Meat Sci.*, 60, 125–132.
- Raes K, Balcaen A, Dirinck P, De Winne A, Claeys E, Demeyer D and De Smet S (2003), 'Meat quality, fatty acid composition and flavour analysis in Belgian retail beef', *Meat Sci.*, 65 (4), 1237–1246.
- Raes K, De Smet S and Demeyer D (2004), 'Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review', *Anim. Feed Sci. Technol.*, 113, 199–221.
- Ramarathnam, N (1998), 'The flavour of cured meat', in Shahidi F, *The Flavour of Meat, Meat Products and Seafood*, London, Blackie Academic and Professional, 290–319.
- Rochat S, de Saint Laumer J-Y and Chaintreau A (2007), 'Analysis of sulphur compounds from the in-oven roast beef aroma by comprehensive two-dimensional gas chromatography', *J. Chromatogr. A.*, 1147, 85–94.
- Rota V and Schieberle P (2006), 'Changes in key odorants of sheep meat induced by cooking', in Shahidi F and Weenen H, *Food Lipids: Chemistry, Flavor, and Texture (ACS Symposium Series 920)*, Washington, D.C., American Chemical Society, 73–83.
- Ruther J and Baltes W (1994), 'Sulphur-containing furans in commercial meat flavorings', *J. Agric. Food Chem.*, 42, 2254–2258.
- Santos, J.P. Garcia M, Aleixandre M, Horrillo M C, Gutierrez J, Sayago I, Fernandez M J and Ares L. (2004), 'Electronic nose for the identification of pig feeding and ripening time in Iberian hams', *Meat Sci.*, 66, 727–732.
- Sañudo C, Nute G R, Campo M M, María G, Baker A, Sierra I, Enser M E and Wood J D (1998), 'Assessment of commercial lamb meat quality by British and Spanish taste panels', *Meat Sci.*, 48 (1/2), 91–100.
- Sárraga C, Guardia M D, Díaz I, Guerrero L, Regueiro J A G and Arnau J (2007), 'Nutritional and sensory quality of porcine raw meat, cooked ham and dry-cured shoulder as affected by dietary enrichment with docosahexaenoic acid (DHA) and alpha-tocopheryl acetate', *Meat Sci.*, 76 (2), 377–384.
- Scollan N D, Hocquette J F, Nuernberg K, Dannenberger D, Richardson I and Moloney A (2006), 'Innovations in beef production systems that enhance the nutritional and health

- value of beef lipids and their relationship with meat quality', *Meat Sci.*, 74 (1), 17–33.
- Scollan N D, Choi N-J, Kurt E, Fisher A V, Enser M and Wood J D (2001), 'Manipulating the fatty acid composition of muscle and adipose tissue of beef cattle', *Brit. J. Nutr.*, 85, 115–124.
- Scott T W and Ashes J R (1993), 'Dietary lipids for ruminants: Protection, utilization and effects on remodelling of skeletal muscle phospholipids', *Austr. J. Agric. Res.*, 44, 495–508.
- Scott T W, Cook L J and Mills S C (1971), 'Protection of dietary polyunsaturated fatty acids against microbial hydrogenation in ruminants', *J. Amer. Oil Chem. Soc.*, 48, 358–364.
- Siegmund B and Pfannhauser W (1999), 'Changes of the volatile fraction of cooked chicken meat during chill storing: Results obtained by the electronic nose in comparison to GC-MS and GC olfactometry', *Z. Lebensm. -Unters. -Forsch.*, 208 (5–6), 336–341.
- Sitz B M, Calkins C R, Feuz D M, Umberger W J and Eskridge K M (2005), 'Consumer sensory acceptance and value of domestic, Canadian, and Australian grass-fed beef steaks', *J. Anim. Sci.*, 83 (12), 2863–2868.
- Skibsted L H (1992), 'Cured meat products and their oxidative stability'. In Johnson D E, Knight M and Ledward D A, *The Chemistry of Muscle-based Foods*, Cambridge, Royal Society of Chemistry, 266–286.
- Skibsted L H, Mikkelsen A and Bertelsen G (1998), 'Lipid-derived off-flavours in meat', in Shahidi F, *The Flavour of Meat, Meat Products and Seafood*, London, Blackie Academic and Professional, 217–256.
- Specht K and Baltes W (1994), 'Identification of volatile flavor compounds with high aroma values from shallow-fried beef', *J. Agric. Food Chem.*, 42, 2246–2253.
- Stahnke L H (1995), 'Dried sausages fermented with *Staphylococcus-xylosus* at different temperatures and with different ingredient levels. 2. Volatile components', *Meat Sci.*, 41 (2), 193–209.
- Sutherland M M and Ames J M (1996), 'Free fatty acid composition of the adipose tissue of intact and castrated lambs slaughtered at 12 and 30 weeks of age', *J. Agric. Food Chem.*, 44, 3113–3116.
- Tang J, Jin Q Z, Shen G H, Ho C-T and Chang S S (1983), 'Isolation and identification of volatile compounds from fried chicken', *J. Agric. Food Chem.*, 31, 1287–1292.
- Timón M L, Carrapiso A I, Jurado A and van de Lagemaat J (2004), 'A study of the aroma of fried bacon and fried pork loin', *J. Sci. Food Agric.*, 84 (8), 825–831.
- Valencia I, Ansorena D and Astiasaran I (2007), 'Development of dry fermented sausages rich in docosahexaenoic acid with oil from the microalgae *Schizochytrium* sp.: Influence on nutritional properties, sensorial quality and oxidation stability', *Food Chem.*, 104 (3), 1087–1096.
- Vasta V and Priolo A (2006), 'Ruminant fat volatiles as affected by diet. A review', *Meat Sci.*, 73 (2), 218–228.
- Vatansever L, Kurt E, Enser M, Nute G R, Scollan N D, Wood J D and Richardson R I (2000), 'Shelf life and eating quality of beef from cattle of different breeds given diets differing in n-3 polyunsaturated fatty acid composition', *Anim. Sci.*, 71, 471–482.
- Wasserman A E (1972), 'Thermally produced flavor components in the aroma of meat and poultry', *J. Agric. Food Chem.*, 20, 737–741.
- Wasserman A E and Talley F (1968), 'Organoleptic identification of roasted beef, veal, lamb and pork as affected by fat', *J. Food Sci.*, 33, 219–223.
- Weller M, Galgan M W and Jacobson M (1962), 'Flavor and tenderness of lamb as influenced by age', *J. Animal Sci.*, 21, 927–929.
- Werkhoff P, Brüning J, Emberger R, Güntert M, and Hopp R (1993), 'Flavor chemistry of meat volatiles: New results on flavor components from beef, pork and chicken', In Hopp R and Mori K, *Recent Developments in Flavor and Fragrance Chemistry*, Weinheim, VCH, 183–213.
- Whitfield F B (1992), 'Volatiles from interactions of Maillard reactions and lipids', *Crit. Rev. Food Sci. Nutr.*, 31 (1/2), 1–58.

- Wood J D, Nute G R, Richardson R I, Whittington F M, Southwood O, Plastow G, Mansbridge R, da Costa N and Chang K C (2004), 'Effects of breed, diet and muscle on fat deposition and eating quality in pigs', *Meat Sci.*, 67 (4), 651–667.
- Wood J D and Enser M (1997), 'Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality', *Brit. J. Nutr.*, 78, Suppl. 1, S49–S60.
- Wood J D, Richardson R I, Nute G R, Fisher A V, Campo M M, Kasapidou E, Sheard P R and Enser M (2003), 'Effect of fatty acids on meat quality: a review', *Meat Sci.*, 66, 21–32.
- Yancey C J, Dikeman M E, Hachmeister K A, Chambers E and Milliken G A (2005), 'Flavor characterization of top-blade, top sirloin, and tenderloin steaks as affected by pH, maturity, and marbling', *J. Anim. Sci.*, 83, 2618–2623.
- Young O A and Braggins T (1999), 'Sheepmeat odour and flavour', in Shahidi F, *The Flavour of Meat, Meat Products and Seafood*, London, Blackie Academic and Professional, 101–130.
- Young O A, Braggins T J, West J and Lane G A (1999), 'Animal production origins of some meat color and flavor attributes', in Xiong Y L, Ho C-T and Shahidi F, *Quality Attributes of Muscle Foods*, New York, Kluwer Academic, 11–28.
- Young O A, Lane G A, Priolo A and Fraser K (2003), 'Pastoral and species flavour in lambs raised on pasture, lucerne or maize', *J. Sci. Food Agric.*, 83 (2), 93–104.
- Young O A, Reid D H and Scales G H (1993), 'Effect of breed and ultimate pH on the odour and flavour of sheep meat', *N. Z. J. Agric. Res.*, 36, 363–370.

6

Fresh meat water-holding capacity

E. Huff-Lonergan, Iowa State University, USA

Abstract: Water-holding capacity and its corollary, drip-loss, are important to the meat industry as they affect both palatability traits and economic traits. Even though water-holding capacity/drip-loss of fresh meat (particularly pork) has been studied for years, there is still much that is not understood. Prevention of water loss and management of moisture retention are challenging subjects, both for processors and for researchers. The mechanism underlying the retention of water in fresh meat is rooted in the structure of the muscle cell and in the state of key proteins associated with the myofibril. This chapter explores some of the major causes of drip-loss in fresh meat and relates some of the current hypotheses regarding its development.

Key words: drip-loss, pork, beef, muscle structure, purge.

6.1 Introduction

One of the most important quality traits of pork is its ability to retain moisture. In fresh pork, the loss of moisture is commonly referred to as drip-loss and the fluid that is lost is often described as purge. Economically, drip-loss is important to the industry as it represents not only loss of product weight, but also loss of a significant amount of protein; more than one hundred milligrams of protein per milliliter of fluid can be lost (Savage *et al.*, 1990). Most of these proteins are water-soluble sarcoplasmic proteins and they include the pigment protein myoglobin, giving the purge a pinkish-red color that sometimes is misidentified by consumers as blood. The development of purge in a retail package produces an unattractive product and can lead to negative consumer bias and subsequent reduction in sales.

Even though water-holding capacity/drip-loss of fresh meat (particularly pork) has been studied for years (Offer and Knight, 1988a,b; Offer and Trinick, 1983),

there is still much that is not understood. Prevention and management of moisture retention is a challenging subject, both for processors and for researchers. This is evidenced by the fact that many products still have what processors consider greater than desired drip-loss (Stetzer and McKeith, 2003; Wright *et al.*, 2005; Person *et al.*, 2005). This chapter will explore some of the major causes of drip-loss in fresh meat and will detail some of the current hypotheses regarding its development.

6.2 Water-holding capacity defined

Water-holding capacity is broadly defined as the ability of meat to retain moisture. This includes the moisture inherent to the muscle tissue as well as any fluids that may be added to the meat during further processing. In fresh meat, a considerable amount of attention is given to what is commonly called drip-loss or purge. Drip and/or purge refers to the high protein fluid that is lost from fresh meat. This particular definition applies to that fluid that is lost from meat without any added mechanical force other than gravity.

Measurement of drip-loss or purge is typically reported as a percentage of the weight of the meat that is lost due to the fluid that is released from the tissue during storage. Thus, this measurement is time dependent. The amount of drip-loss will increase over time in a non-linear fashion. This fact underscores the need to standardize the time postmortem and the storage time at which the measurement is made. Other factors that must be considered when measuring drip-loss include:

- (i) *The temperature at which the product was stored.* Higher storage temperature will favor increased drip-loss.
- (ii) *The type of packaging.* Skin and/or vacuum packaging will impart some physical force on the sample and can cause greater drip-loss when compared to samples stored at atmospheric pressure or in modified atmosphere packaging systems.
- (iii) *The dimensions of the sample.* A thin slice, or a sample with multiple cut surfaces, will lose more fluid on a percentage basis than a thick slice or a sample with the fewest possible cut surfaces.

Therefore, in order to make the most accurate assessment, an evaluation of drip-loss must be made under very controlled conditions taking all of these factors into consideration (Honikel, 1998).

6.3 Inherent factors in postmortem muscle that influence water-holding capacity

A major cause of early postmortem changes in meat is the loss of a functioning circulatory system. When the muscle is converted to meat, lactic acid is produced

during anaerobic glycolysis. This would normally be removed via the blood-stream, but in postmortem muscle it builds up in the tissue, leading to a reduction in pH of the meat. Once the pH has reached the isoelectric point (pI) of the major proteins, especially myosin (pI = 5.4), the net charge of the protein is zero, meaning the numbers of positive and negative charges on the proteins are essentially equal. These positive and negative groups within the protein are attracted to each other and this results in a reduction in the amount of water that can be attracted and held by that protein. Additionally, since like charges repel, as the net charge of the proteins that make up the myofibril approaches zero (diminished net negative or positive charge), repulsion of structures within the myofibril is reduced allowing those structures to pack more closely together. The end result is a reduction of space within the myofibril. Partial denaturation of the myosin head at low pH (especially if the temperature is still high) is also believed to be responsible for a large part of the shrinkage in myofibrillar lattice spacing (Offer, 1991).

6.3.1 Influence of pH

The role of pH in water-holding capacity of pork has been well documented and it has a significant impact on the ultrastructure of muscle and on the state of denaturation of key muscle proteins (Scopes, 1964; Melody *et al.*, 2004; Offer, 1991; Offer and Trinick, 1983). The pH is an important indicator of the metabolic status of pre-rigor meat, and in some cases, can be an indicator of the metabolic 'history' of fresh, post-rigor pork (Offer, 1991). After the muscle shifts to using anaerobic glycolysis as a major energy-generating pathway, the pH reflects the accumulation of lactic acid within the muscle. In muscle, during periods of oxygen 'debt', anaerobic glycolysis becomes an important source of energy. The pH of muscle falls due to the formation of approximately 0.1 mol l⁻¹ of lactic acid per glycogen molecule that is used in this pathway (Brewer, 2004). In living muscle, lactic acid is primarily cleared from the muscle via the circulatory system and thus does not typically accumulate to the same extent that it does in postmortem muscle. Metabolism continues for some time in postmortem muscle. However, since the circulatory system is no longer functioning, lactic acid cannot be cleared from the muscle and thus builds up. The end result is a drop in pH from approximately 7.0–7.2 to 5.3–5.8 (ultimate pH). In pork with normal water-holding capacity, it typically takes about 6–8 hours to reach ultimate pH values (Gardner *et al.*, 2005, 2006; Melody *et al.*, 2004).

Normal ultimate pH of muscle is very close to the isoelectric point of many of the major proteins in muscle (5.3–5.5) (Huff-Lonergan and Lonergan, 2005). The electrostatic forces that help maintain myofilament spacing are reduced near the isoelectric point. When the normal repulsion forces are diminished, the space between the thick and thin filaments is reduced, thus limiting the interfilament spacing even beyond that caused by formation of rigor bonds. The result is a loss of space within the myofibril for water. Therefore, the water is expelled from the myofibril into the extramyofibrillar spaces, where it is eventually more easily lost from the muscle cell. This phenomenon is exaggerated in pork with abnormally

low ultimate pH values (Offer, 1991; Offer and Cousins, 1992; Offer and Knight, 1988a,b).

6.3.2 Muscle structure and water-holding capacity

Myofibrils make up a large proportion of the muscle cell. These organelles constitute over 80% of the volume of the muscle cell. Up to 85% of the water in a muscle cell is held in the myofibrils. Much of that water is held by capillary forces arising from the arrangement of the thick and thin filaments within the myofibril. In living muscle, it has been shown that sarcomeres remain isovolumetric during contraction and relaxation (Millman *et al.*, 1981, 1983). This would indicate that, under physiological conditions, the amount of water retained within the filamentous structure of the muscle cell would not necessarily change. However, the location of this water *can* be affected by changes in volume as muscle undergoes rigor. As muscle goes into rigor, cross-bridges form between the thick and thin filaments, thus reducing available space for water to reside (Offer and Trinick, 1983). It has been shown that as the pH of porcine muscle is reduced from physiological values to 5.2–5.5 (near the isoelectric point of myosin), the distance between the thick filaments declines an average of 2.5 nm (Diesbourg *et al.*, 1988). This may force sarcoplasmic fluid from between the myofilaments to the extramyofibrillar space. In fact, enough fluid may be lost from the intramyofibrillar space to increase the extramyofibrillar volume by as much as 1.6 times more than its pre-rigor volume (Bendall and Swatland, 1988).

During the development of rigor, the overall diameter of muscle cells decreases (Hegarty, 1970; Swatland and Belfry, 1985) and is likely the result of transmittal of the lateral shrinkage of the myofibrils to the entire cell (Diesbourg *et al.*, 1988). Additionally, during rigor development sarcomeres can shorten; this also further reduces the space available for water within the myofibril. In fact, it has been shown that drip-loss increases linearly with a decrease in the length of the sarcomeres in muscle cells (Honikel *et al.*, 1986). More recently, highly sensitive low field nuclear magnetic resonance (NMR) studies have been used to gain a more complete understanding of the relationship between muscle cell structure and water distribution (Straadt *et al.*, 2007; Bertram *et al.*, 2002; Bertram and Andersen, 2007). These studies suggest that, within the myofibril, proportionally less water is held in the protein dense A-band than in the less dense I-band. This observation may help explain why shorter sarcomeres (especially in cold-shortened muscle) are often associated with increased drip-losses. As the myofibril shortens and rigor sets in, the shortening of the sarcomere leads to shortening and subsequent lowering of the volume of the I-band region in the myofibril. Loss of volume in this myofibrillar region (where much water may reside), combined with the pH-induced lateral shrinkage of the myofibril, leads to expulsion of water from the myofibrillar structure into the extramyofibrillar spaces within the muscle cell (Bendall and Swatland, 1988). It is thus likely that the gradual mobilization of water from inside the myofibril to other spaces within the cell may be key in providing a significant source of drip.

It is important to note that shrinkage of the myofibrils alone could not be responsible for the movement of fluid to the extracellular space and ultimately out of the muscle. The myofibrils are linked to each other and to the cell membrane via proteinacious connections (Wang and Ramirez-Mitchell, 1983). These connections, if they are maintained intact in postmortem muscle, transfer the reduction in diameter of the myofibrils to the muscle cell (Kristensen and Purslow, 2001; Melody *et al.*, 2004; Morrison *et al.*, 1998; Diesbourg *et al.*, 1988). Myofibril shrinkage could thus lead to reduction in the overall diameter of the muscle cell, creating channels between cells and between bundles of cells that can funnel drip out of the product (Offer and Knight, 1988). Extracellular space around muscle fibers continually increases up to 24 hours postmortem, but gaps between muscle fiber bundles decrease slightly between nine and 24 hours postmortem, perhaps due to fluid outflow from these major channels (Schafer *et al.*, 2002). These linkages between adjacent myofibrils and between myofibrils and the cell membrane are made up of several proteins that are associated with intermediate filament structures and structures known as costameres. Costameres provide the structural framework responsible for attaching the myofibrils to the sarcolemma. Proteins that make up, or are associated with, the intermediate filaments and costameres include (among others) desmin, filamin, and synemin, dystrophin, talin and vinculin (Greaser, 1991). If costameres and costameric linkages remain intact during the conversion of muscle to meat, shrinkage of the myofibrils as the muscle goes into rigor would be transmitted to the entire cell via these proteinacious linkages and would ultimately reduce the volume of the muscle cell itself (Melody *et al.*, 2004; Offer and Knight, 1988b; Kristensen and Purslow, 2001). Thus, the rigor process could result in mobilization of water not only out of the myofibril, but also out of the extramyofibril spaces as the overall volume of the cell is constricted. In fact, reduction in the diameter of muscle cells has been observed in postmortem muscle (Offer and Cousins, 1992). This water that is expelled from the myofibril and ultimately the muscle cell eventually collects in the extracellular space. Several studies have shown that gaps develop between muscle cells and between muscle bundles during the post-rigor period (Offer and Cousins, 1992; Offer *et al.*, 1989). These gaps between muscle bundles are the primary channels by which purge is allowed to flow from the meat; some investigators have actually termed them 'drip channels'.

Recent research has suggested that *reduced* degradation of proteins that tie the myofibril to the sarcolemma (such as desmin) results in *increased* shrinking of the muscle cell, which is ultimately translated into drip-loss (Morrison *et al.*, 1998; Huff-Lonergan and Lonergan, 2005; Bee *et al.*, 2007, 2004). If those myofibrillar linkages are not degraded relatively early in the postmortem period, there is less space for water expelled from the myofibrils during the development of rigor to remain inside the cells. Degradation of the intermediate filament protein, desmin, can occur as early as 45 minutes post-exsanguination in some muscles with higher water-holding capacity (Melody *et al.*, 2004). This time frame is ideal for allowing the retention of fluid displaced from within the myofibril. Several studies have shown that there is a significant correlation between the degradation of desmin and

water-holding capacity of pork (Bee *et al.*, 2007, 2004; Gardner *et al.*, 2005, 2006; Melody *et al.*, 2004; Zhang *et al.*, 2006). The degradation of certain costameric proteins also may play a role in maintaining fluid within the cell (Kristensen and Purslow, 2001; Zhang *et al.*, 2006). Costameric proteins aid in tying the intermediate filament proteins to the sarcomere; thus, their degradation could also allow the removal of some structural constraints. The costameric proteins vinculin and talin have been shown to be more extensively degraded in pork that has higher water-holding capacity. Thus, it does seem that degradation of inter-myofibrillar and costameric connections relatively soon after exsanguination does have the potential to improve water-holding capacity (Bee *et al.*, 2007, 2004; Gardner *et al.*, 2005; Melody *et al.*, 2004; Huff-Lonergan and Lonergan, 2005). This could occur by limiting the extent to which lateral shrinkage of the myofibrils is transmitted to the overall muscle cell which could allow, at least to some extent, the intracellular space to be somewhat independent of myofibrillar volume or shrinkage (Huff-Lonergan and Lonergan, 2005).

It is important to note that generalized degradation of muscle proteins may not necessarily be beneficial to the water-holding capacity of fresh meat. For example, degradation of the protein integrin may not be beneficial to water-holding capacity (Lawson, 2004; Zhang *et al.*, 2006). Integrins are a family of adhesion receptors that mediate interactions between the extracellular matrix and the cell cytoskeleton. Degradation of integrin in pork has been shown to be associated with increased water loss in fresh pork (Zhang *et al.*, 2006). Degradation of integrin has also been shown to be associated with the formation of drip channels in pork (Lawson, 2004). Thus, drip formation is likely the result of specific structural changes in the muscle cell. Thus it is possible that degradation of intermediate filament proteins such as desmin, and membrane-associated proteins such as integrin may have very different roles in mediating the water-holding capacity of pork. For example, the degradation of desmin early postmortem may release constraints within the cell that limit the space available for water/fluid that has been forced from the myofibril during rigor. Conversely, degradation of a protein like integrin could actually contribute to the formation of drip channels and thus might actually improve the ability of moisture to 'escape' from the muscle cell (Lawson, 2004; Zhang *et al.*, 2006).

6.3.3 Role of the proteolytic enzymes – calpains

Increased postmortem degradation of specific muscle cell proteins is associated with an improvement in meat tenderness (Huff-Lonergan *et al.*, 1996). There is significant evidence to support the involvement of proteolysis in water-holding capacity (Huff-Lonergan and Lonergan, 2005; Melody *et al.*, 2004; Zhang *et al.*, 2006; Bee *et al.*, 2007). The endogenous sarcoplasmic calpain enzyme system (the enzymes μ -calpain, m-calpain and their inhibitor calpastatin) has been implicated as playing a major role in regulating postmortem protein degradation. The calpains require calcium for their activity *in vitro*. The two forms of the calpains (μ and m)

are differentiated by the amount of calcium they require for activation; μ -calpain requires micromolar concentrations for activity, while m-calpain requires near millimolar amounts (Goll *et al.*, 2003, 1992, 1998). μ -Calpain has been shown to degrade several proteins in skeletal muscle cells. Of particular interest is the fact that it degrades the proteins desmin (Huff-Lonergan *et al.*, 1996), vinculin and talin, which, as discussed previously, may be involved in drip-loss (Bee *et al.*, 2007; Kristensen and Purslow, 2001; Morrison *et al.*, 1998). Therefore, this system may play a significant role in the development of water-holding capacity. While the calpain enzymes and their inhibitor, calpastatin, are active under postmortem conditions, their activity is compromised by the low pH and high ionic strength conditions that develop within meat during storage (Maddock *et al.*, 2005; Huff-Lonergan *et al.*, 1996). However, the enzyme μ -calpain is likely active during the early postmortem period – the critical time for desmin to be degraded (Melody *et al.*, 2004). Also, during postmortem storage, the enzyme μ -calpain autolyzes and more μ -calpain ‘comes out of solution’ and becomes associated with the myofibril rather than remaining soluble in the sarcoplasm. This shift in location may have an effect on the activity of the enzyme. Additionally, the specific inhibitor calpastatin is progressively degraded and its activity is reduced as postmortem time increases (Boehm *et al.*, 1998).

The autolysis of μ -calpain in postmortem muscle has been used to indicate its prior activity (Melody *et al.*, 2004). μ -Calpain autolysis, as determined by a decrease in the percent 80 kDa (intact) large subunit and an increase in the percent 76 kDa autolysis product, appears to be hindered by low pH early postmortem, suggesting that low pH decreases the opportunity for activation of μ -calpain. A stepwise regression analysis demonstrated that pH at 6 hours postmortem explains approximately 34% of the variation in drip-loss. Adding pH and μ -calpain autolysis (percent of μ -calpain large subunit present as the 76 kDa autolysis product) to the model improves the model R^2 to 0.483. When less autolysis product is detected at 24 h postmortem, greater drip-loss is expected (Gardner *et al.*, 2005, 2006). These results suggest that μ -calpain autolysis is associated with variations in water holding capacity and that it may be partly independent of pH decline. These results support the proposed hypothesis, as greater activation of μ -calpain within the first 24 hours postmortem should correspond to greater proteolysis and less myofiber shrinkage.

6.4 Ante- and early postmortem factors that influence water-holding capacity

Many factors inherent to the live animal are responsible for the water-holding capacity of the product. The range of factors includes the genetics of the animal, the handling of the animal prior to slaughter and the handling of the carcass and meat after slaughter.

6.4.1 Pale, soft, exudative (PSE)

Both low ultimate pH and accelerated pH decline are related to the development of low water-holding capacity and unacceptably high purge loss. Very fast pH decline, resulting in ultimate or near ultimate pH while the muscle is still warm, causes the denaturation and the loss of functionality and water-binding ability of many proteins. The resulting product is pale in color, soft in texture and loses moisture easily (exudative). The common term for product exhibiting these characteristics is PSE (Pale, Soft, and Exudative). The major biochemical cause of this condition is rapid energy consumption by the muscle. In muscles that are prone to PSE, ATP breakdown and glycogen usage is accelerated, resulting in the rapid accumulation of lactic acid (byproduct of anaerobic glycolysis) (Bendall and Swatland, 1988).

The most severe purge or drip-loss is often found in product from pigs that have inherited a mutation in the ryanodine receptor/calcium release channel in the sarcoplasmic reticulum (Fujii *et al.*, 1991). The result of this mutation is an impairment of the ability of this channel to control calcium release from the sarcoplasmic reticulum into the sarcoplasm of the muscle cell. This deregulation is exaggerated under periods of physical stress. Accelerated release of calcium into the sarcoplasm causes an increase in muscle metabolism, rapid contraction, and an increased rate of pH decline (Lundstrom *et al.*, 1989; Bendall and Wismer-Pedersen, 1962). This particular mutation in the gene coding resulting in a defect in ryanodine receptor can be identified in breeding stock. Because a commercial test for this mutation exists, the United States industry has virtually eliminated this mutation in most commercial herds. Pigs with this specific mutation in the ryanodine receptor were once commonly identified by challenging them with the anesthesia, halothane. When pigs that carry this mutation are given halothane, they experience severe muscle contractions, muscle stiffening and blotching of the skin. For many years, this was used as a screening test and aided in the determination of the mode of inheritance of this condition. Therefore, it became common to refer to the defect as the halothane gene (Ewan and Topel, 1974; Topel *et al.*, 1974).

The halothane gene is but one example of a condition that can result in PSE. Other factors can cause PSE meat to occur. Before harvest, short-term stress in normal animals can accelerate their metabolism enough for the postmortem metabolism in the muscle to be accelerated, causing a more rapid accumulation of lactic acid and a corresponding acceleration in pH decline compared to non-stressed animals. While the condition may not be as severe as that caused by the halothane gene, protein denaturation does occur, and drip-losses can be greater than in muscles that have a normal, slower rate of pH decline. It should also be noted that while the pH of these muscles falls faster than normal, the ultimate pH may not be below normal ranges (Rosenvold and Andersen, 2003).

Another factor that can influence the development of PSE is the rate at which the carcass is chilled. Since the causative factor in the development of PSE is the combination of an acidic condition in the muscle *and* high muscle temperatures, rapid cooling techniques can aid in reducing the incidence of milder forms of PSE (Vada, 1977).

Other metabolic states and conditions of muscle often direct the extent of pH decline in postmortem muscle. Many studies have reported the effect of decreasing glycogen content in muscle to minimize lactate accumulation in postmortem muscle, reviewed by Rosenvold and Andersen (2003). Limiting the energy intake of the animal prior to slaughter can do this.

Classically, muscle pH early postmortem (45 min) has been used to monitor quality differences in fresh pork. Extremely low pH early postmortem is known to result in pale, soft and exudative pork (Briskey, 1964), likely due to denaturation of a number of proteins, including myosin (Penny, 1967). In pork that is not pale, soft and exudative (PSE), the correlation of pH to drip-loss is higher at 4 (0.499) and 6 (0.540) hours postmortem than pH at 45 minutes postmortem (0.398) (Gardner *et al.*, 2005). Therefore, these time points may be equally as good as, or even better than, the traditional 24 hour or ultimate pH values to predict water-holding capacity. This observation highlights the need to investigate factors that contribute to pH decline after the initial events within the first 60 minutes after exsanguination. For example, it has been demonstrated that glycogen debranching enzyme activity is influenced by temperature. It has been suggested that this may explain the variation in rate of glycogenolysis and, under some circumstances, the rate of pH decline (Kylä-Puhju *et al.*, 2005).

6.4.2 Rendement Napole (RN) gene

Genetic factors influencing basal metabolism also have the potential to similarly affect lactate accumulation and extent of pH decline. For many years, researchers noted that product from several lines of Hampshire pigs had low ultimate pH and poor water-holding capacity, but had near normal color and early postmortem pH decline, and so were clearly not PSE. This condition was referred to as Rendement Napole (RN), in reference to the method used to characterize the product (Monin and Sellier, 1985; Monin *et al.*, 1984). The discovery (Milan *et al.*, 2000) of a non-conserved substitution in protein kinase adenosine monophosphate-activated γ_3 -subunit gene (*PRKAG3*) has explained the dominant mutation (denoted *RN*⁻) that accounted for large differences in meat quality and processing yields in the Hampshire pig breed (Monin and Sellier, 1985). The substitution (*R200Q*) in the *PRKAG3* gene causes a 70% increase in muscle glycogen in *RN*⁻ homozygous and heterozygous pigs. This increase in glycogen *directly* results in greater production of lactate in postmortem muscle, a lower ultimate pH and poorer water-holding capacity in fresh pork.

The *PRKAG3* gene encodes one isoform of one of the regulatory subunits (γ) in mammalian adenosine monophosphate (AMP)-activated protein kinase (AMPK). When subjected to nutritional or environmental stress, the AMP/ATP ratio of eukaryotic cells will rise, triggering the 'AMPK cascade', stimulating the cells to conserve energy (Thornton *et al.*, 1998) and to begin ATP synthesis (Hardie *et al.*, 1998). While the dominant *RN*⁻ mutation that is found in the Hampshire breed of pigs, is a nonconservative substitution (*R200Q*) in the *PRKAG3* gene, other alleles within the same gene are associated with lower muscle glycogen content and

improved meat quality traits (Ciobanu *et al.*, 2001). From three missense mutations identified (*T30N*, *G52R*, *I199V*) in porcine *PRKAG3*, significant effects on pH and Minolta L values were noted between products from animals with the analyzed substitutions. (Ciobanu *et al.*, 2001). These observations establish a genetic basis for variation in ultimate pH across many breeds and commercial lines, not just Hampshire pigs.

6.4.3 Dark, firm and dry (DFD)

Another factor that can influence water-holding capacity is lack of glycogen in the muscle. This results in a very limited pH decline because the lack of glycogen does not allow significant production of lactic acid. Therefore, the ultimate pH of muscle with limited glycogen content at death is much higher than it is for pork within the normal pH range of 5.4–5.8. The product with higher than normal pH has very good water-holding capacity but is darker in color and has a firmer texture than does product with lower ultimate pH (Bendall and Swatland, 1988; Joo *et al.*, 1999; Karlsson *et al.*, 1994; Ryu and Kim, 2006; Shackelford *et al.*, 1994).

6.5 Future trends

Water-holding capacity is a very complex trait in meat. Many factors influence its development. Processors and researchers need to be aware of how not only the genetics and handling of the live animal impact the production traits but also how they may influence the early postmortem metabolism of the muscle and the subsequent quality of the final product. Future work in this area will likely focus on identifying more genetic markers for improving water-holding capacity. Additionally, live animal and carcass/meat handling procedures and processes need to be refined in order to provide a more consistent product. Further, defining the exact mechanisms in the muscle and early postmortem meat that are responsible for the development of water-holding capacity is needed in order to discover long-term solutions to water-holding capacity problems in meat.

6.6 Sources of further information and advice

The following books and reference articles are complementary to the material contained in this chapter and provide more information regarding the conversion of muscle to meat, the role of skeletal muscle proteins in meat quality and specifically, more background on earlier research on water-holding capacity.

Lawrie, R and Ledward, D (2006), *Lawrie's Meat Science*, New York, NY USA, CRC Press. This book provides a good, comprehensive background on muscle structure, antemortem and postmortem muscle metabolism.

Offer, G and Knight, P (1988), The structural basis of water-holding capacity in meat. Part 1: General principles and water uptake in meat processing. In Lawrie, R (Ed.) *Developments in Meat Science*. New York, Elsevier Applied Science.

Offer, G and Knight, P (1988) The structural basis of water-holding capacity in meat. Part 2: Drip-losses. In Lawrie, R (Ed.) *Developments in Meat Science*. London, UK, Elsevier Science Publications.

These two classic chapters by Offer and Knight provide an excellent, in-depth perspective on the biochemical and structural factors that influence not only the water-holding capacity of fresh meat but also a detailed discussion of other factors influencing water-holding capacity.

6.7 References

- Bee, G, Anderson, A L, Lonergan, S M and Huff-Lonergan, E (2007), Rate and extent of pH decline affect proteolysis of cytoskeletal proteins and water holding capacity in pork. *Meat Science*, 76, 359–365.
- Bee, G, Lonergan, S M and Huff-Lonergan, E (2004), Early postmortem pH influences proteolysis of cytoskeletal proteins during aging in porcine longissimus muscle. *Journal of Animal Science*, 82, 13.
- Bendall, J R and Swatland, H J (1988), A Review of the Relationships of pH with Physical Aspects of Pork Quality. *Meat Science*, 24, 85–126.
- Bendall, J R and Wismer-Pedersen, J (1962), Some properties of the fibrillar proteins of normal and watery pork muscle. *Journal of Food Science*, 27, 144–159.
- Bertram, H C and Andersen, H J (2007), NMR and the water-holding issue of pork. *Journal of Animal Breeding and Genetics*, 124, 35–42.
- Bertram, H C, Purslow, P P and Andersen, H J (2002), Relationship between meat structure, water mobility, and distribution: A low-field nuclear magnetic resonance study. *Journal of Agricultural and Food Chemistry*, 50, 824–829.
- Boehm, M L, Kendall, T L, Thompson, V F and Goll, D E (1998), Changes in the calpains and calpastatin during postmortem storage of bovine muscle. *Journal of Animal Science*, 76, 2415–2434.
- Brewer, M S (2004), Water-Holding Capacity. In Jensen, W K, Devine, C and Dikeman, M E (Eds.) *Encyclopedia of Meat Science*. 1st ed. New York, Elsevier.
- Briskey, E J (1964) Etiological status and associated studies of pale, soft, exudative porcine musculature. *Advances in Food Research*, 13, 89–178.
- Ciobanu, D C, Bastiaansen, J, Malek, M, Helm, J, Wollard, J, Plastow, G and Rothschild, M F (2001), Evidence for new alleles in the protein kinase adenosine monophosphate-activated g3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. *Genetics*, 159, 1151–1162.
- Diesbourg, L, Swatland, H J and Millman, B M (1988), X-Ray-Diffraction Measurements of Postmortem Changes in the Myofilament Lattice of Pork. *Journal of Animal Science*, 66, 1048–1054.
- Ewan, R C and Topel, D G (1974), Chemical Composition of Normal and PSE Pork Muscle. *Journal of Animal Science*, 39, 169–169.
- Fujii, J, Otsu, K, Zorzato, F, Deleon, S, Khanna, V K, Weiler, J E, O'Brien, P J and MacLennan, D H (1991), Identification of a Mutation in Porcine Ryanodine Receptor Associated with Malignant Hyperthermia. *Science*, 253, 448–451.
- Gardner, M A, Huff-Lonergan, E and Lonergan, S M (2005), Prediction of fresh pork quality using indicators of protein degradation and calpain activation. *51st International Congress of Meat Science and Technology*. Baltimore, MD, USA, American Meat Science Association.
- Gardner, M A, Huff-Lonergan, E, Rowe, L J, Schultz-Kaster, C M and Lonergan, S M (2006), Influence of harvest processes on pork loin and ham quality. *Journal of Animal Science*, 84, 178–184.
- Goll, D E, Thompson, V F, Li, H Q, Wei, W and Cong, J Y (2003), The calpain system. *Physiological Reviews*, 83, 731–801.

- Goll, D E, Thompson, V F, Taylor, R G and Christiansen, J A (1992), Role of the Calpain System in Muscle Growth. *Biochimie*, 74, 225–237.
- Goll, D E, Thompson, V F, Taylor, R G and Ouali, A (1998), The calpain system and skeletal muscle growth. *Canadian Journal of Animal Science*, 78, 503–512.
- Greaser, M L (1991), An overview of the muscle cell cytoskeleton. *Reciprocal Meats Conference Proceedings*. American Meat Science Association.
- Hardie, D G, Carling, D and Carlson, M (1998), The AMP-activated/SNF1 protein kinase subfamily: Metabolic sensors of the eukaryotic cell? *Annual Review of Biochemistry*, 67, 821–855.
- Hegarty, P V (1970), Differences in fibre size of histologically processed pre- and post-rigor mouse skeletal muscle. *Life Sciences*, 9, 443–449.
- Honikel, K O (1998), Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49, 447–457.
- Honikel, K O, Kim, C J, Hamm, R and Roncales, P (1986), Sarcomere Shortening of Prerigor Muscles and Its Influence on Drip-loss. *Meat Science*, 16, 267–282.
- Huff-Loneragan, E and Lonergan, S M (2005), Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, 71, 194–204.
- Huff-Loneragan, E, Mitsuhashi, T, Beekman, D D, Parrish, F C, Olson, D G and Robson, R M (1996), Proteolysis of specific muscle structural proteins by mu-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. *Journal of Animal Science*, 74, 993–1008.
- Joo, S T, Kauffman, R G, Van Laack, R L J M, Lee, S and Kim, B C (1999), Variations in rate of water loss as related to different types of post-rigor porcine musculature during storage. *Journal of Food Science*, 64, 865–868.
- Karlsson, A, Essengustavsson, B and Lundstrom, K (1994), Muscle Glycogen Depletion Pattern in Halothane-gene-free Pigs at Slaughter and its Relation to Meat Quality. *Meat Science*, 38, 91–101.
- Kristensen, L and Purslow, P P (2001), The effect of ageing on the water-holding capacity of pork: Role of cytoskeletal proteins. *Meat Science*, 58, 17–23.
- Kylä-Puhju, M, Ruusunen, M and Puolanne, E (2005), Activity of porcine muscle glycogen debranching enzyme in relation to pH and temperature. *Meat Science*, 69, 143–146.
- Lawson, M A (2004), The role of integrin degradation in post-mortem drip-loss in pork. *Meat Science*, 559–566.
- Lundstrom, K, Essen-Gustavsson, B, Rundgren, M, Edforslilja, I and Malmfors, G (1989), Effect of Halothane Genotype on Muscle Metabolism at Slaughter and its Relationship with Meat Quality – A Within-litter Comparison. *Meat Science*, 25, 251–263.
- Maddock, K R, Huff-Loneragan, E, Rowe, L J and Lonergan, S M (2005), Effect of pH and ionic strength on μ - and m-calpain inhibition by calpastatin. *Journal of Animal Science*, 83, 1370–1376.
- Melody, J L, Lonergan, S M, Rowe, L J, Huiatt, T W, Mayes, M S and Huff-Loneragan, E (2004), Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. *J. Anim. Sci.*, 82, 1195–1205.
- Milan, D, Jeon, J T, Looft, C, Amarger, V, Robic, A, Thelander, M, Rogel-Gaillard, C, Paul, S, Iannuccelli, N, Rask, L, Ronne, H, Lundstrom, K, Reinsch, N, Gellin, J, Kalm, E, Le Roy, P, Chardon, P and Andersson, L (2000), A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science*, 288, 1248–1251.
- Millman, B M, Racey, T J and Matsubara, I (1981), Effects of hyperosmotic solutions on the filament lattice of intact frog skeletal muscle. *Biophysical Journal*, 33, 189–202.
- Millman, B M, Wakabayashi, K and Racey, T J (1983), Lateral forces in the filament lattice of vertebrate striated muscle in the rigor state. *Biophysical Journal*, 41, 259–267.
- Monin, G, Gruaud, J, Laborde, D and Sellier, P (1984), The Hampshire Effect on the Technological Qualities of Pork. *Annales De Zootechnie*, 33, 394–394.

- Monin, G and Sellier, P (1985), Pork of Low Technological Quality with a Normal Rate of Muscle pH Fall in the Immediate Post-Mortem Period – The Case of the Hampshire Breed. *Meat Science*, 13, 49–63.
- Morrison, E H, Mielche, M M and Purslow, P P (1998), Immunolocalisation of intermediate filament proteins in porcine meat. Fibre type and muscle-specific variations during conditioning. *Meat Science*, 50, 91–104.
- Offer, G (1991), Modeling of the Formation of Pale, Soft and Exudative Meat – Effects of Chilling Regime and Rate and Extent of Glycolysis. *Meat Science*, 30, 157–184.
- Offer, G and Cousins, T (1992), The Mechanism of Drip Production – Formation of 2 Compartments of Extracellular-space in Muscle Postmortem. *Journal of the Science of Food and Agriculture*, 58, 107–116.
- Offer, G and Knight, P (1988a), The Structural Basis of Water-holding Capacity in Meat. Part 1: General Principles and Water Uptake in Meat Processing. In Lawrie, R (Ed.) *Developments in Meat Science*. New York, Elsevier Applied Science.
- Offer, G and Knight, P (1988b), The Structural Basis of Water-holding Capacity in Meat. Part 2: Drip-losses. In Lawrie, R (Ed.) *Developments in Meat Science*. London, UK, Elsevier Science Publications.
- Offer, G, Knight, P, Jeacocke, R, Almond, R, Cousins, T, Elsey, J, Parsons, N, Sharp, A, Starr, R and Purslow, P (1989), The Structural Basis of the Water-holding, Appearance and Toughness of Meat and Meat-products. *Food Microstructure*, 8, 151–170.
- Offer, G and Trinick, J (1983), On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. *Meat Science*, 8, 245–281.
- Penny, I F (1967), The influence of pH and temperature on the properties of myosin. *Biochemical Journal*, 104, 609–615.
- Person, R C, McKenna, D R, Ellebracht, J W, Griffin, D B, McKeith, F K, Scanga, J A, Belk, K E, Smith, G C and Savell, J W (2005), Benchmarking value in the pork supply chain: Processing and consumer characteristics of hams manufactured from different quality raw materials. *Meat Science*, 70, 91–97.
- Rosenvold, K and Andersen, H J (2003), Factors of significance for pork quality – A review. *Meat Science*, 64, 219–237.
- Ryu, Y C and Kim, B C (2006), Comparison of histochemical characteristics in various pork groups categorized by postmortem metabolic rate and pork quality. *Journal of Animal Science*, 84, 894–901.
- Savage, A W J, Warriss, P D and Jolley, P D (1990), The amount and composition of the proteins in drip from stored pig meat. *Meat Science*, 27, 289–303.
- Schafer, A, Rosenvold, K, Purslow, P P, Andersen, H J and Henckel, P (2002), Physiological and structural events postmortem of importance for drip-loss in pork. *Meat Science*, 61, 355–366.
- Scopes, R K (1964), The influence of post-mortem conditions on the solubilities of muscle proteins. *Biochemistry Journal*, 91, 201–204.
- Shackelford, S D, Koohmaraie, M, Wheeler, T L, Cundiff, L V and Dikeman, M E (1994), Effect of biological type of cattle on the incidence of the dark, firm, and dry condition in the longissimus muscle. *J. Anim. Sci.*, 72, 337–343.
- Stetzer, a J and McKeith, F K (2003), *Benchmarking value in the pork supply chain: Quantitative strategies and opportunities to improve quality: Phase I*. Savoy, IL, American Meat Science Association.
- Straadt, I K, Rasmussen, M, Andersen, H J and Bertram, H C (2007), Aging-induced changes in microstructure and water distribution in fresh and cooked pork in relation to water-holding capacity and cooking loss – A combined confocal laser scanning microscopy (CLSM) and low-field nuclear magnetic resonance relaxation study. *Meat Science*, 75, 687–695.
- Swatland, H J and Belfry, S (1985), Post-mortem Changes in the Shape and Size of Myofibrils from Skeletal-muscle of Pigs. *Mikroskopie*, 42, 26–34.
- Thornton, C, Snowden, M A and Carling, D (1998), Identification of a novel AMP-activated

- protein kinase beta subunit isoform that is highly expressed in skeletal muscle. *Journal of Biological Chemistry*, 273, 12443–12450.
- Topel, D G, Miller, J, Berger, P J, Rust, R E and Parrish, F C (1974), Palatability and Visual Acceptance of Dark, Normal and Pale Pork. *Journal of Animal Science*, 39, 176–176.
- Vada, M (1977), Effect of cooling rate upon processing characteristics of pork meat of different glycolysis type during postmortem aging. *Meat Science*, 1, 245–252.
- Wang, K and Ramirez-Mitchell, R (1983), A Network of Transverse and Longitudinal Intermediate Filaments Is Associated with Sarcomeres of Adult Vertebrate Skeletal-muscle. *Journal of Cell Biology*, 96, 562–570.
- Wright, L I, Scanga, J A, Belk, K E, Engle, T E, Tatum, J D, Person, R C, McKenna, D R, Griffin, D B, McKeith, F K, Savell, J W and Smith, G C (2005), Benchmarking value in the pork supply chain: Characterization of US pork in the retail marketplace. *Meat Science*, 71, 451–463.
- Zhang, W G, Lonergan, S M, Gardner, M A and Huff-Lonergan, E (2006), Contribution of postmortem changes of integrin, desmin and mu-calpain to variation in water holding capacity of pork. *Meat Science*, 74, 578–585.

The nutritional quality of meat

H. K. Biesalski and D. Nohr, University of Hohenheim, Germany

Abstract: Meat is often regarded as a high-fat, cancer-promoting part of our nutrition that also increases the risk of obesity and metabolic syndrome. Consequently, meat intake is more and more reduced, especially during weight-reducing diets. This chapter outlines the importance of meat as a part of a mixed and balanced diet to provide the organism with valuable macro- and micronutrients and their role in promoting or reducing the risk of cancer and other diseases.

Key words: macronutrients, micronutrients, vitamins, cancer, obesity, metabolic syndrome.

7.1 Introduction

At a time of health claims for everything we eat or drink, meat is suffering from a negative image due to its presumed high fat content and – especially in the case of red meat at a period of intensive barbecuing – from its image as a cancer-promoting food. In consequence, low meat intake is recommended to avoid the risks of cancer, obesity and metabolic syndrome. This rather narrow view of this nutrient overlooks the fact that some of the most important micronutrients are best available from meat, e.g. iron, selenium, and vitamins A, B12 and folic acid, either because they do not exist in plant-derived food or because they have poor bioavailability. Furthermore, meat is rich in proteins and poor in carbohydrates, and thus contributes to a low glycemic index, which is assumed to have positive effects with regard to obesity and the development of diabetes and cancer ('insulin resistance hypothesis').

In the following chapter and based on current knowledge, we will outline the importance of meat as part of a mixed and balanced diet, its major macro- and micronutrients and their role in developing or preventing cancer. Thus, for our purposes the term 'nutritional quality' of meat means its non-visible qualities, i.e. the more or less important (micro)nutrients it contains, rather than the characteristics seen by the consumer at the butcher's shop (e.g. colour or consistency).

7.2 Macronutrients in meat

7.2.1 Proteins and carbohydrates

Based on *per capita* protein intake and the risk of colon cancer, it has been assumed that total protein is related to this cancer risk (Youngman and Campbell, 1998). The majority of epidemiological cohort and case studies, however, do not confirm these assumptions, although there seems to be some evidence that red meat-derived proteins increase the risk if consumed twice or more a day (MacIntosh and Le Leu, 2001) or if they are processed (boiled or fried). It is still not understood whether the non-fat matrix of the meat (amino acid composition; amount of heme iron) plays a role in carcinogenesis. An increase – however non-significant – from 33% to 59% in the incidence of differential methylation hybridization (DHM)-induced intestinal tumors was observed in mature rats when barbecued beef was substituted for whey protein concentrate against a high fat (20%) diet background (MacIntosh *et al.*, 1998). However, the role of protein concentration in determining the risk of cancer remains controversial (MacIntosh and Le Leu, 2001), while there are also suggestions that the type of protein plays a role: a diet high in methionine (*type* of protein as well as ingested quantity) has been shown to increase circulating insulin, which has been assumed to promote colon carcinogenesis. The authors of that study hypothesized that insulin resistance leads to increased initiation and promotion of colon cancer by elevating insulin as a growth factor for raised glucose and triglycerides as fuels (McKeown-Eyssen, 1994). Additional reasons summarized by Bruce and his co-workers (2000) which strengthen this hypothesis were: colon cancer patients frequently show glucose intolerance and insulin resistance; cohorts of type 2 diabetes have been found to have a high mortality rate due to colon cancer; a clear association of early colon cancer, colon cancer and polyps with increased levels of fasting insulin, triglycerides, very low density lipoproteins (VLDL) and abdominal obesity was found in cohort and case studies; and the development of colonic polyps was more frequent in patients consuming more carbohydrates with a higher glycemic index than in controls. A further association with the risk of colon cancer concerns the plasma insulin-like growth factor I (IGF-I), which is often increased in insulin-resistant patients, and IGF binding protein.

High glycemic index diets are thought to favour insulin resistance (IR) (Frost *et al.*, 1998). However, red meat has a low glycemic index and should thus not contribute to metabolic syndrome as long as it does not contribute primarily to daily fat/energy intake. Interestingly, Slattery and colleagues (2005) could find only minor influences of polymorphisms in 4 insulin-related genes on the risk of colon cancer (IGF-I; IGFBP-III; IRS-I; IRS-II), while obesity, physical activity and energy intake seemed to be more important. This latter finding was also supported by Koohestani and co-workers (1997), who concluded from studies with a rat model for IR that a diet high in energy, saturated fat and glycemic carbohydrates, but low in n3-fatty acids, could negatively affect cell signalling in colonic cells by leading to IR and promoting aberrant crypt formation in IR rats, an important step in carcinogenesis. Thus, dietary intervention to reduce IR may also

reduce the risk of colon cancer and, in consequence, meat low in fat and carbohydrates does not seem to contribute to colon cancer. Hodgson and co-workers (2006) showed that replacement of carbohydrates by red-meat proteins could lead to a reduction in blood pressure in hypertensive patients, comparable to the effects of plant-derived proteins. Comparable results for pigs were described by Jönsson and colleagues (2006), who suggested that a paleolithic diet (vegetables, fruit, meat, tubers) confers higher insulin sensitivity, lower C-reactive protein and lower blood pressure when compared to a cereal-based diet, while another study showed a lower prevalence of hypertension and blood pressure in vegetarians compared to meat-eaters, although the differences were small and largely related to body mass index (BMI) (Appleby *et al.* 2002). With regard to proteins and carbohydrates, when meat is consumed in low amounts and as part of a mixed and balanced diet, it seems to be more beneficial than hazardous. It will be interesting to see the final health outcomes of the ongoing UK study of females, comparing vegetarians, fish-eaters and meat-eaters (Cade *et al.*, 2004) and the EPIC-Oxford study with comparable aims (Davey *et al.*, 2003).

Glucosamine is a derivative of glucose needed for the production of cartilage in the joints and is normally released by chondrocytes. Numerous studies have investigated the therapeutic effects on osteoarthritis of orally applied glucosamine preparations, mainly glucosamine-sulfate, often with chondroitin-sulfate (meta-analysis by Towheed *et al.*, 2005). However, the results are contradictory for the Lequesne-Index (dealing with pain, the ability to walk and perform all-day activities), the WOMAC scores (dealing with pain, stiffness, joint function) and pain relief. Nowadays, large amounts of glucosamine-containing supplements are ingested, that contain either glucosamine extracted from animal sources (cartilage) or synthetically prepared, mostly in the form of glucosamine-sulfate. Interestingly, it is possibly the sulfur component that is beneficial rather than the glucosamine (Parcell, 2002).

Meat contains glucosamine at concentrations of around 500 mg/100 g dry weight. Freudenreich (1984) used glucosamine concentration to try to distinguish qualitatively between various cuts (brisket, silverside, wing end) of heifer meat, very young cow meat and young bull meat. This method proved suitable as long as the same cuts of the different animals were compared. In conclusion, this type of meat seems to be a good 'natural' source of glucosamine as well as sulfur.

7.2.2 Fat and fatty acids

A number of epidemiological studies in the 1990s suggested a positive correlation between fat intake and the incidence of breast-, colon- and prostate cancer, while cohort, large case studies and the pooled analysis of case studies failed to detect a correlation between fat intake and colon cancer (Biesalski, 2005). For breast cancer, no overall correlation could be found for total fat intakes over the range of 15% to >45% of energy from fat. Furthermore, the risk of breast cancer was elevated twofold in the small group of women consuming less than 15% energy from fat (Hunter *et al.*, 1996). In a Spanish case-control study, the consumption of

meat seemed to reduce the relative risk (RR) (odds ratio (OR): 0.24) for lung cancer, whereas it was enhanced by the consumption of fish (OR: 1.67; Dosil-Diaz *et al.*, 2007). For prostate cancer, results are controversial and dependent on adjustment for energy intake. Meat intake, however, correlated with prostate cancer as consumption of 5 or more servings of red meat per week elevated the RR of prostate cancer to 1.47, while 2–4 servings reduced the RR to 0.96, indicating that a low to moderate consumption does not contribute to prostate cancer (Michaud *et al.*, 2001). Interestingly, a recent multiethnic study in Hawaii and Los Angeles (82 483 African Americans, Japanese Americans, Latinos and Whites) found no indication that intake of fat and meat substantially affects prostate cancer risk (Park *et al.*, 2007).

It is generally accepted that animal fat, rich in saturated fat, relates to cancerogenesis, while plant-derived mostly unsaturated fat (PUFA) is more canceroprotective. However, in animal models most PUFAS exhibited a tumor-promoting effect and other studies showed that *n*-6 fatty acids (linoleic acid) enhanced cancer development in rodents (Fay *et al.*, 1997). The prostaglandin E2 (PGE2), formed from *n*-6 acids (arachidonic acid), also seems to be involved in carcinogenesis. In mice deficient in EP1, the PGE2 receptor showed resistance to AOM induction of neoplastic colon lesions (Watanabe *et al.*, 1999). *n*-3-fatty acids (linolenic acid), however, suppressed colon carcinogenesis by inhibiting the arachidonic pathway (Takahashi *et al.*, 1997).

The rather simple ‘fat–colon–cancer hypothesis’, i.e. the view that the uptake of dietary fat through the consumption of red meat increases the risk of colon cancer, is based on the premise that dietary fat promotes excretion of bile acids, which are then converted to carcinogens (Reddy, 1981). However, there are different ways of interpreting or explaining the controversial results of meta-analyses since the fat content of red meat varies over a wide range and also shows different patterns (Biesalski, 2005):

- Palmitic, but not stearic, acid, present in different amounts in meat, has been shown to be a strong mitogen in cell culture.
- Red-meat-derived fat might be less absorbed due to its composition or to the matrix it is in (muscle).
- Polymorphisms of genes playing a role in fat metabolism might be involved in an individual’s susceptibility.
- Other components (heterocyclic amines (HCA); iron content) might be involved; e.g. dietary iron enhances lipid peroxidation in the mouse colon and increases the incidence of DMH-induced colorectal cancer in mice and rats.

Human studies also point to a relationship between body iron stores and the incidence of colon cancer. Finally, the processing of meat and the carcinogens formed (e.g. HCAs) may contribute to various types of cancer. HCAs are further converted by cytochrome P450s (CYP 1A2) into DNA adducts with guanines at the C8 position, leading to base substitution and a mutation. Oxidation of the DNA by reactive oxygen species (ROS) can lead to comparable effects.

The effects of processing meat are as complex as the effects described above.

Epidemiological studies revealed positive (Zheng *et al.*, 1998), as well as negative (Augustsson *et al.*, 1999), correlations between cancer risk and well-done meat or fish. The intensity of cooking led to conflicting results, e.g. a positive (Knekt *et al.*, 1994) or a negative (Ambrosone *et al.*, 1998) correlation with breast cancer.

Some genetic polymorphisms also have to be investigated. No correlation was found between N-acetyl-transferase (NAT2) (involved in HCA activation, its rate determined by a polymorphic gene) and breast cancer, nor any association between the intake of red meat and any degree of doneness and breast cancer (Ambrosone *et al.*, 1998). Genetic polymorphisms, including environmental aspects (gene-environment interactions), may also play a critical role in colorectal cancer with respect to red meat intake. Le Marchand (1999) investigated colorectal cancer rates in Japanese immigrants in Hawaii. The colorectal cancer incidence of these groups (214 000 immigrants between 1886 and 1924) was initially very low, but is now the highest in the world. The fast acetylator genotype (NAT2), without the polymorphism, is present in 90% of Japanese, compared with 45% of Caucasians; and the frequency of the CYP1A2 phenotype is similar in both groups. Consumption of well-done meat, together with a specific genotype of NAT2 and CYP1A2, may substantially increase colorectal cancer risk. Amongst the Japanese migrants who ate well-done red meat, those without the polymorphisms in both NAT2 and CYP1A2 had a 3.6-fold greater risk of developing colon cancer than those with the polymorphisms.

Another family of genes that might determine individual susceptibility are glutathione transferase M1 (GSTM1) and T1 (GSTT1). Both of these code for the cytosolic enzyme glutathione S-transferase, which is involved in phase 2 metabolism, especially in polycyclic aromatic hydrocarbon metabolism. The results of the few studies dealing with genetic polymorphisms of GST are inconsistent. Two suggest increased colon cancer risk in subjects with a high meat intake and GST non-null genotype, contrary to the underlying hypothesis. One study suggests a strong inverse relationship between colorectal adenomas and broccoli consumption, particularly in subjects who are GSTM1 null (for review, see Cotton *et al.*, 2000). Until genotypically defined cohorts are studied with respect to their susceptibility to meat intake (in different forms), the risk of red meat cannot be clearly identified. White meat and fish seem to offer no risk; red meat offers a risk only in a form where cytotoxic by-products such as HCA are produced.

Formation of HCAs can be significantly reduced by inexpensive and practical measures, such as avoiding the exposure of the meat surfaces to flames, use of aluminium foil to wrap the meat before oven roasting, and the use of microwave cooking. Another protective approach involves combination with protective bioactive constituents derived from plant food. For example, diallyl sulphide, an organosulfur compound in garlic, blocks HCA carcinogenesis (Hasegawa *et al.*, 1995; Morie *et al.*, 1999). Nevertheless, there may be some induction or promotion factors in meat that depend on the composition of the diet; for example, whether anti-carcinogenic factors from plants neutralize any harmful factors in meat. In addition, meat contains bioactive constituents known to be protective against cancer formation.

7.3 Meat micronutrients

Meat is a very good source of various micronutrients: low-fat pork contains 1.8 mg iron, 2.6 mg zinc; and pigs' liver contains 360 mg magnesium, 20 mg iron and 60 µg selenium per 100 g. A daily intake of 100 g of meat and liver can supply up to 50% of the RDA for iron, zinc, selenium, vitamins B1, B2, B6, B12 and 100% of vitamin A.

7.3.1 Meat as an important source for micronutrients

The importance of meat as an essential source of some micronutrients is due to the fact that it is either the only source of, or has a higher bioavailability of, some micronutrients. Vitamins A and B12 occur exclusively in meat and cannot be compensated for by plant-derived provitamins since there is no provitamin B12, and provitamin A, β -carotene, would have to be taken up in huge amounts due to its conversion rate (1:12). Iron has a higher bioavailability from meat than from plants (heme iron), as has folic acid which is nearly 10-fold more, especially from liver or eggs, compared to vegetables. In consequence, a low meat intake enhances the risk of deficiencies in some micronutrients, as shown below.

7.3.2 Vitamin A

Vitamin A is essential for the growth and development of various cells and tissues. Its active form, retinoic acid (RA) controls the regular differentiation as a ligand for retinoic acid receptors (RAR, RXR) and is involved in the integration of cell formation, i.e. the formation of gap junctions (Kurokawa *et al.*, 1994). It plays a very prominent role in lung development and function. The incidence of lung diseases is enhanced by moderate vitamin A deficiency and repeated respiratory infections can be treated therapeutically with moderate vitamin A supplementation (Biesalski, 2005). Vitamin A is also responsible for lung development and maturation and for the development of other tissues, and control of these processes seems to be dependent from the expressions of RA receptors.

Alveolar type II cells synthesize and secrete surfactant, a phospholipid needed for breathing as it reduces surface tension in the alveoles. RA is able, dose-dependently, to stop the expression of surfactant protein A (SP-A) in the human fetal lung, and the SP-A mRNA expression can be downregulated due to insulin, TGF- β and high concentrations of glucocorticoids (Odom *et al.*, 1988). On the other hand, SP-B mRNA expression is increased in human fetal lung explants (e.g. fetal lung tissue kept in cell culture) by dexamethasone and hyperoxia in rats (Metzler and Snyder, 1993). Thus, different surfactant proteins seem to be regulated differently and selectively by RA and glucocorticoids. RA can interfere in lung development by its modulating effect on the expression of epidermal growth factor, its increasing effect on the expression of the EGF receptor, and subsequent PGE2-induced surfactant formation (Sundell *et al.*, 1980; Haigh *et al.*, 1989). Continuous availability of RA, either from blood sources or local stores, is therefore pivotal, and it is not sufficient to determine blood levels because vitamin

A is regulated homeostatically as long as it is available from liver stores, independent of local sources which might be empty due to temporarily enhanced needs. For example, vitamin A stores in the respiratory epithelium of the fetus are nearly empty in the late prenatal phase of lung maturation, due to an enhanced demand for cellular differentiation and metabolic work (surfactant production). During this phase, the neonate is highly dependent on vitamin A from its mother to refill its own stores, i.e. the mother's stores also have to be sufficient for both their demands. A pilot study by Schulz and co-workers showed that, in mothers with twins or children born at short intervals, about 40% showed marginal serum vitamin A deficiencies, and had low β -carotene levels in their serum, cord blood and colostrum, although they took up high levels of β -carotene. The retinol and β -carotene levels in the cord blood and colostrum were even significantly lower than in the maternal serum. Interestingly, almost 50% of the women did not eat any liver for vitamin A supply (Schulz *et al.*, 2007). The situation becomes even more critical in premature babies, who have even lower retinol levels in the serum and liver than full-term babies (for an overview, see Biesalski, 2005). The disease bronchopulmonary dysplasia has to be seen in the context of vitamin A deficiency, although its pathology seems to be multifactorial. However, a comparable change in the respiratory epithelium can be observed in smokers with chronic obstructive pulmonary disease (COPD) (focal loss of ciliated cells with keratinizing metaplasia and an increase of mucous secreting cells). In a study by Kohlhäufel and co-workers, inhalation of retinyl-esters by smokers with COPD and dys- and metaplastic epithelia led to about 40% recovery to the normal situation (Kohlhäufl *et al.*, 2002). Interestingly, in contrast to the surrounding normal epithelial cells, the metaplastic cells contained no vitamin A.

Although liver is the best available source of vitamin A, it has a 'bad reputation' due to other potential constituents of this organ, such as heavy metals, hormones or xenobiotics. However, these contaminants have almost vanished during recent decades, whereas contamination of fruit and vegetables is rising. Additionally, in order to obtain the recommended 1 mg retinol per day from vegetables, 500 mg of mixed and β -carotene rich vegetables have to be eaten daily, while 100 g of liver twice a month is sufficient and is neither toxic nor teratogenic (see Biesalski, 2005; Nohr and Biesalski, 2007).

7.3.3 Iron

Iron supports oxidative metabolism; it is essential for gas exchange at the cellular and tissue level through oxygenation of hemoglobin in red blood cells and of myoglobin in skeletal muscle. In addition, enzymes containing iron are involved in energy metabolism and host defence responses (Beard *et al.*, 1996). These functions are due to the biological catalytic activity of iron. It possesses unfilled atomic orbitals that allow it to coordinate electron donors and participate in redox processes (Fraga and Oteiza, 2002).

Although iron is one of the most abundant elements in the Earth's crust, iron deficiency is the most common and widespread nutritional disorder. As a result of

biological losses, such as cyclical monthly bleeding of fertile women, blood-feeding parasites or poor bioavailability of iron from plant-based diets, it is estimated that about 4–5 billion people, 66–80% of the world's population, may be iron deficient (DeMaeyer and Adiels-Tegman, 1985; World Health Organization, 2004). At any given time, 2 billion people – over 30% of the world's population – are anemic, about 50% of them due to iron deficiency, and in developing countries this is frequently exacerbated by malaria and worm infections (World Health Organization, 2004). Iron deficiency is a particular risk for women and girls of child-bearing age because of menstrual losses. In a recent Irish food consumption survey, almost half of the women aged 18–50 had inadequate iron intakes when compared with national average requirements (Beard *et al.*, 1996). In the British National Diet and Nutrition Survey, iron intakes were found to be low in girls (aged 7–18), with intakes decreasing with age. Adolescent females (aged 15–18) were found to have extremely low intakes of iron when compared to UK dietary reference values. Depending on the composition of the individual diet, the bioavailability of iron can differ 5- to 10-fold. The bioavailability depends on the presence or absence of different ligands (phytates from cereal products, tannins from coffee and tea, and oxalates from vegetables) which form complexes with iron and zinc and block their absorption. A diet based mainly on vegetables, rice, beans and corn is associated with poor iron bioavailability, which explains the high incidence of anemia in developing countries. 100 g of pork added to the vegetarian diet described above increases iron absorption 3.6-fold. A recent study of ca. 50 000 Canadian women showed no association of intake of iron, heme iron or iron from meat with the risk of colorectal cancer (Kabat *et al.*, 2007)

7.3.4 Folic acid

The average folate intake in European adults is comparable in most countries, about 300 µg/day and 250 µg/day in men and women, respectively. This is below the recommended intake of 400 µg/day for both sexes, and far below the recommended intake for pregnant women (600 µg/day) and – even more important – for women planning pregnancy, in order to prevent neural tube defects, a disease that is correlated with folic acid deficiency. It is important for folate levels to be optimal about 4 weeks before conception. Currently, more than 80% of women of child-bearing age have dietary intakes below the recommended amounts. The bioavailability of folate differs widely between various nutrients, e.g. only 3–5% from vegetables, 55% from liver and almost 95–100% from supplements, the last two sources thus being highly recommended for women who are planning pregnancy.

7.3.5 Vitamin B12

Vitamin B12 (cobalamin) can be taken up only from animal products; it does not exist in plants. Thus, for example, in the UK about 25% of vegetarians and 50% of vegans had suboptimal intakes and, in consequence, low to very low serum levels

– about 25% below the threshold level (130 ng/L) for developing neurological signs (Lloyd-Wright *et al.*, 2000). In addition, elderly people are particularly at risk of vitamin B12 deficiency, mainly due to suboptimal intestinal absorption.

7.3.6 Selenium

Selenium is largely found in grains, fish and meats, and enters the food chain through plants at geographically variable rates, depending on the selenium concentration of the soil. Its best known biochemical role is as part of the active site of the enzyme glutathione peroxidase (GPx), which is involved in the metabolism and detoxification of oxygen. It is assumed that GPx can protect DNA from oxidative damage, and consequently from mutation and subsequent neoplastic transformation of cells (Combs and Clark, 1985). *In vitro* and *in vivo* studies have demonstrated that organic and inorganic selenium inhibit proliferation of normal and malignant cells and inhibit tumor growth (Griffin, 1982; Redman *et al.*, 1997). Apoptosis may result from the competition of selenium for s-adenosyl-methionine with ornithine decarboxylase (ODC), the activity of ODC indeed being critically involved in cancerogenesis. Geographical studies have shown that, in areas with a sufficient selenium concentration in the diet (depending on the selenium concentration of the soil), there is an inverse relationship between selenium status and cancer (Schrauzer *et al.*, 1977; Clark *et al.*, 1991). Epidemiological studies have shown inverse associations of selenium intake or plasma levels and cancers at different sites (prostate, colon, skin, etc.). In a double-blind, placebo-controlled cancer prevention trial, 200 µg selenium (approx. 3 times the RDA) was given daily to patients with histories of basal and squamous skin carcinoma (Clark *et al.*, 1996). Although selenium supplementation did not influence the primary endpoint prevention of recurrent skin cancers, it was surprisingly inversely associated with the incidence of, and mortality from, the overall incidence of prostate, lung and colorectal cancers. Yoshizawa and colleagues (1998) reported a strong inverse association of toenail concentration of selenium (reflecting long-term intake) and prostate cancer risk (65% reduced risk in the highest quintile). Recent surveys indicate that average intake of selenium may be as low as 30–40 µg/day (Rayman, 1997). Intake data, however, do not really reflect bioavailability. Consequently, diet has a strong influence on total selenium supply to tissues. Especially in areas with low soil selenium, dietary sources containing substantial amounts of selenium with good bioavailability should be recommended. In the US, selenium is mainly supplied by cereals, bread, meats and meat products. Two studies in humans showed that meat was as good a source of selenium as wheat (Van der Torre *et al.*, 1991) and that L-selenomethionine (SeMet) was absorbed more rapidly than selenite in selenium-deficient men (Xia *et al.*, 1992). In a recent study in rats, the bioavailability of selenium was estimated from various portions of fully cooked commercial cuts of beef, including liver, striploin, round, shoulder and brisket (Shi and Spallholz, 1994). The bioavailability from the beef diets was compared with that of selenium as selenite or SeMet. Liver GPx-recovery was highest with SeMet, lower with beef muscle and lowest with selenite, with the latter equaling beef liver.

Muscle deposition was highest from SeMet, lower from beef muscle, and even lower from comparable selenite and beef liver. From these results, the authors concluded that the bioavailability of selenium from beef is higher than – or at least equal to – that of selenite, and slightly lower than that of SeMet. In summary, meat as part of the diet is an important source of bioavailable selenium.

7.3.7 Zinc

Zinc-deficient individuals have slower wound-healing and are more sensitive to infections. However, studies of the effect of zinc supplementation aimed at the healing rate of venous leg ulcers have been inconclusive. A Cochrane review concluded that oral zinc did not appear to aid the healing of leg ulcers, as there was only weak evidence for a positive effect of zinc in patients with venous leg ulcers and low serum levels (Wilkinson and Hawke, 2002). An inhibitory effect on rhinovirus replication *in vitro* was described, and some studies described a possibility that zinc may beneficially affect cold symptoms. In contrast, a meta-analysis of randomized controlled trials found no evidence for such an effect (Jackson *et al.*, 2000). Brown and co-workers found that zinc supplementation may improve children's growth in situations with high rates of stunting and low plasma zinc concentrations (Brown *et al.*, 1998). A low intake of zinc is associated with a weakened immune system. Consequently, a recommended dietary intake of 10 mg zinc/day (125g liver; 320 g meat) increases the quantity of interferon-gamma-producing natural killer cells and strengthens the immune system against neoplasms and viral infections (Metz *et al.*, 2007). Especially in elderly people, a reduced zinc status is evident (Lukito *et al.* 2004). In a recent study, supplementation of elderly people with zinc (45 mg/day for 12 months) led to a significantly lower incidence of infections, significantly higher serum levels, and TNF- α (pro-inflammatory) and markers for oxidative stress were also significantly lower (Prasad *et al.*, 2007). During pregnancy and lactation, a higher need for zinc is documented, as also during chronic inflammatory diseases (Rink and Gabriel, 2000). With regard to cancer, the results are very controversial; some groups found lower zinc levels in cancer patients, others did not (Biesalski, 2005). Adequate biomarkers – not available at present – might help to estimate individual zinc status and individual risk, especially as food questionnaires about zinc are not very helpful because of the high variability in the zinc content of different sources, especially meat and sea food, and the much better bioavailability of zinc from meat than from vegetables (Groff and Gropper, 2000).

7.3.8 Potential risk groups for low intake of (meat-derived) micro-nutrients

There are several groups at risk of deficiencies of one or more micronutrients: elderly people for vitamins A, D, E, folate, iron and calcium, mostly because of diseases and an age-adapted lifestyle, less because of physiological problems (with the exception of iron and vitamin B12 uptake due to gastric mucosal atrophy).

In pregnant women, risk of deficiency of vitamin D, folic acid, zinc and iron is due to enhanced demands (Draper *et al.*, 1993; Fogelholm, 1999; Saletti *et al.*, 2000), especially when meat is avoided in the diet, as is often seen. Supplementation is recommended, especially for folic acid, in order to avoid serious birth defects. Vitamin A deficiency also seems to be a risk, as shown by Schulz *et al.* (2007) for women with twins or births at short intervals.

Vegans are at risk of deficiency of micronutrients which are found exclusively in animal-derived food, e.g. vitamin B12, riboflavin and selenium, and even supplementation with B12 and selenium is sometimes not sufficient (Andersson *et al.*, 1986; Boelsma *et al.*, 2001).

People dieting to lose weight could obviously be at risk of micronutrient deficiencies, but the results of a number of studies present only weak scientific evidence for this. However, the levels of iron, magnesium, zinc, fat soluble vitamins and essential fatty acids should be controlled during the diet. A recent meta-analysis showed that protein-rich diets that were low in carbohydrates but with a moderate-to-high fat content resulted in a better weight loss than diets low in protein and fat, but high in carbohydrates (Bravata *et al.*, 2003). Better satiety, higher energy expenditure and greater loss of fat cell mass were supposedly responsible for the weight loss.

Institutionalized and hospitalized elderly are another group at risk, the prevalence of malnutrition being associated with the severity of morbidity, functional impairments and mental state. This deficiency involves a number of micronutrients: vitamins B1, B6, folate, B12, vitamins C, D, E, essential fatty acids and selenium (Bates *et al.*, 1999; Brubacher *et al.*, 2000). Above all, thiamine and folate should be within a normal range, due to their role in depression, impaired cognition and dementia (Block *et al.*, 2001).

Chronic use of drugs may also induce micronutrient deficiency as a result of reduced appetite, reduced motility of the upper gastrointestinal tract, decreased bioavailability and interference with metabolism. However, this usually becomes relevant only in the case of prolonged use of specific drugs in high doses given to susceptible persons.

7.4 Laboratory analysis of the nutritional quality of meat

Today, analytical techniques allow the detection of smaller and smaller amounts of many substances, including nutrients and pollutants. For example, most of the harmful substances in liver (e.g. heavy metals, herbicides) have now reached concentrations that are more or less below the harmful threshold, while concentrations are rising in fruits and vegetables (DGE, 2004). All known micronutrients can be analyzed more or less easily in company and university laboratories (e.g. most vitamins by high performance liquid chromatography, zinc and selenium by atomic absorption spectroscopy). Future investigations will, therefore, be able to detect not only single substances, but also combinations of micronutrients in order to yield a better understanding of their concentrations in different circumstances

(healthy people, cancer patients, the elderly, etc.). However, it will become more and more complicated to discover the functional role of each micronutrient *per se*, or in any combinations of micronutrients that have developed over the tens of thousands of years of human evolution.

7.5 Future trends

It will be very important to direct future research concerning the nutritional quality of meat in a direction that will be helpful not only for basic sciences and scientists who want to understand the mechanisms that can be influenced by meat-typical combinations of macro- and micronutrients. However, it might be very helpful to be able to influence and enhance the quality of meat, beginning as early as during the periods of breeding (genetic influences) and feeding (supplements in the food of the parent's generation and in that of the offsprings) of the animals. Classical epidemiological studies on the retrospective effects of consuming meat will be less and less important and will be replaced by prospective and intervention studies, in order to control what really happens more effectively. The influence of epigenetic factors will also have to be investigated because of the growing number of people changing their habits and environment (e.g. increasing risk of colon cancer in Africans moving from rural regions to the bigger cities, and also intercontinental movements). It is erroneous to think that we understand what happens when we eat a food as complex as meat. It will take decades of research, prospective and interventional studies to obtain a better basis for understanding and, maybe more importantly, to influence our metabolic system not only preventively but also therapeutically.

In addition, it will be important to be able to provide reliable information ('health claims') about the quality, reliability and necessity of meat and other nutrients, and their optimization and uptake, to reduce modern diseases such as cancer and coronary heart diseases by preventive nutrition.

7.6 Conclusions

The overall impression and conclusion of nearly all (meta) analyses seems to be that a low to moderate meat intake seems to be more beneficial (due to its content of selected anti-oxidative and/or anti-carcinogenic micronutrients with a high bioavailability) than it is hazardous (due to its presumed pro-oxidative and/or pro-carcinogenic components (see Biesalski, 2005; Nohr and Biesalski, 2007; Ströhle *et al.*, 2007; Faramawi *et al.*, 2007; Tappel, 2007)). Thus, meat as part of a mixed and balanced diet, possibly adapted to local traditional nutrition, can be strongly recommended. Future research will hopefully help to give us a better understanding of the mechanisms and prerequisites for a healthy diet.

7.7 Sources of further information and advice

In an age of computers and internet communication, it is less and less necessary to have a good bookshop or library at hand, since almost any information can be found on the internet. In addition, numerous daily and weekly magazines propose optimal solutions to nutritional problems and anti-ageing strategies in this context. Although this sounds very easy, the huge amount of material of all kinds and qualities makes it very difficult for the layman (and even the experts) to filter the information, and so it is also not easy to give advice. However, the final outcome of the majority of the publications, and the most common opinion, is that the best diet is one that is mixed and balanced, and that it should be complemented, supplemented or adapted to any situation where people are at risk of nutritional deficiencies.

7.8 References

- Ambrosone C B, Freudenheim J L and Sinha R (1998), 'Breast cancer risk, meat consumption and N-acetyltransferase (NAT2) genetic polymorphisms.' *Journal of Cancer*, 75, 825–830.
- Andersson H, Bosaeus I, Brummer R J, Fasth S, Hultén L, Magnusson O and Strauss B (1986), 'Nutritional and metabolic consequences of extensive bowel resection'. *Digestive Diseases*, 4, 193–202.
- Appleby P N, Davey G K and Key T J (2002), 'Hypertension and blood pressure among meat eaters, fish eaters, vegetarians and vegans in EPIC-Oxford'. *Publ. Health Nutr.*, 5(5), 645–654.
- Augustsson K, Skog K and Jägestad M (1999), 'Dietary heterocyclic amines and cancer of the colon, rectum, bladder and kidney: A population based study.' *Lancet*, 353, 703–707.
- Bates C J, Prentice A, Cole T J, van der Pols J C, Doyle W, Finch S, Smithers G and Clarke P C (1999), 'Micronutrients: Highlights and Research Challenges from the 1994/5 National Diet and Nutrition Survey of People Aged 65 Years and Over'. *Brit. J. Nutr.*, 82, 7–15.
- Beard J L, Dawson H and Pinero D J (1996), 'Iron metabolism: A comprehensive review'. *Nutrition Reviews*, 54(10), 295–317.
- Biesalski H K (2005), 'Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet?' *Meat Sci.*, 70, 509–524.
- Block G, Norkus E, Hudes M, Mandel S and Helzlsouer K (2001), 'Which plasma antioxidants are most related to fruit and vegetable consumption?' *Amer. J. Epidemiol.*, 154, 1113–1118.
- Boelsma E, Hendriks H F and Roza L (2001), 'Nutritional skin care: Health effects of micronutrients and fatty acids'. *Am. J. Clin. Nutr.*, 73, 853–864.
- Bravata D M, Sanders L, Huang J, Krumholz H M, Olkin I, Gardner C D and Bravata D M (2003), 'Efficacy and safety of low-carbohydrate diets: A systematic review'. *J. Amer. Med. Assoc.*, 289(4), 1837–1850.
- Brown K H, Peerson J M and Allen L H (1998), 'Effect of zinc supplementation on children's growth: A meta-analysis of intervention trials'. *Bibliotheca Nutritio et Dieta*, 54, 76–83.
- Brubacher D, Moser U and Jordan P (2000), 'Vitamin C concentrations in plasma as a function of intake: A meta-analysis'. *Int. J. Vit. Nutr. Res.*, 70, 226–237.
- Bruce W R, Wolever T M S and Giacca A (2000), 'Mechanisms linking diet and colorectal cancer: The possible role of insulin resistance. *Nutrition and Cancer*, 37, 19–26.

- Cade J E, Burley V J and Greenwood D C, UK women's cohort study steering group (2004), 'The UK women's cohort study: Comparison of vegetarians, fish eaters and meat-eaters'. *Publ Health Nutr*, 7(7), 871–878.
- Clark L C, Cantor K P and Allaway W H (1991), 'Selenium in forage crops and cancer mortality in US countries'. *Arch Environmental Health*, 46, 37–42.
- Clark L C, Combs G F, Turnbull B W, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Leshner J L Jr, Park HK, Sanders BB Jr, Smith CL and Taylor JR. (1996), 'Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin'. *J. Amer. Med. Assoc.*, 276, 1957–1963.
- Combs G F and Clark L C (1985), 'Can dietary selenium modify cancer risk?' *Nutr. Reviews*, 43, 325–331.
- Cotton S C, Sharp L, Little J and Brockton N (2000), 'Glutathione S-transferase polymorphisms and colorectal cancer: A HuGE review'. *Am. J. Epidemiol.*, 151, 7–32.
- Davey G K, Spencer E A, Appleby P N, Allen N E, Knox K H and Key T J (2003), 'EPIC-Oxford: Lifestyle characteristics and nutrient intakes in a cohort of 33883 meat-eaters and 31546 non meat-eaters in the UK'. *Publ. Health Nutr.*, 6(3), 259–268.
- DeMaeyer E and Adiels-Tegman M (1985), The prevalence of anaemia in the world. *World Health Stat. Q.*, 38(3), 302–316.
- DGE (Deutsche Gesellschaft für Ernährung) (2004), *Ernährungsbericht 2004*. Bonn, Deutsche Gesellschaft für Ernährung.
- Dosil-Diaz O, Ruano-Ravina A, Gestal-Otero J J and Barros-Dios J M (2007), 'Meat and fish consumption and risk of lung cancer: A case-control study in Galicia, Spain.' *Cancer Lett.*, 252(1), 115–122.
- Draper A, Lewis J, Malhotra N and Wheeler E (1993), 'The energy and nutrient intakes of different types of vegetarian: A case for supplements?' *Brit. J. Nutr.*, 69, 3–19.
- Faramawi M F, Johnson E, Fry M W, Sall M and Yi Z (2007), 'Consumption of different types of meat and the risk of renal cancer: Meta-analysis of case-control studies.' *Cancer Causes Control*, 18(2), 125–133.
- Fay M P, Freedman L S, Clifford C K and Midthune D N (1997), 'Effect of different types and amounts of fat on the development of mammary tumors in rodents: A review.' *Cancer Research*, 57, 3979–3988.
- Fogelholm M (1999), 'Micronutrients: Interaction between physical activity, intakes and requirements'. *Public Health Nutrition*, 2, 349–356.
- Fraga G C and Oteiza P I (2002), 'Iron toxicity and antioxidant nutrients'. *Toxicology*, 180, 23–32.
- Freudenreich P (1984), Untersuchungen über die Beschaffenheit von Kalb- und Jungbullenfleisch. *Fleischwirtsch*, 64:609–612.
- Frost G, Leeds A, Trew, R, Margara R and Dornhorst A (1998), Insulin sensitivity in women at risk of coronary heart disease and the effect of a low glycemic diet. *Metabolism*, 47, 1245–51.
- Griffin A C (1982), 'The chemopreventive role of selenium in carcinogenesis'. In M S Arnott and J Van Eys, *Molecular interrelations of nutrition and cancer*. (pp. 401–408). New York, Raven Press.
- Groff J L and Gropper S S (2000), 'Zinc'. In J L Groff and S S Gropper, *Advanced nutrition and human metabolism*. (pp419–430). Belmont Wadsworth.
- Haigh R, D'Souza S W, Micklewright L, Gregory H, Butler S J, Hollingsworth M, Donnai P and Boyd R D (1989), 'Human amniotic fluid urogastrone (epidermal growth factor) and fetal lung phospholipids'. *British Journal of Obstetrics and Gynaecology*, 96, 171–178.
- Hasegawa R, Hirose M, Kato T, Hagiwara A, Boonyaphiphat P, Nagao M, Ito N and Shirai T. (1995), 'Inhibitory effect of chlorophyllin on PhIP induced mammary carcinogenesis in female F344 rats'. *Carcinogenesis*, 16, 2243–2246.
- Hodgson J M, Burke V, Beilin L J and Puddey I B (2006), 'Partial substitution of carbohydrate intake with protein intake from lean red meat lowers blood pressure in hypertensive persons'. *Am. J. Clin. Nutr.*, 83, 780–787.

- Hunter D J, Spiegelman D, Adami H O, Beeson L, van den Brandt PA, Folsom AR, Fraser GE, Goldbohm RA, Graham S and Howe GR *et al.* (1996), 'Cohort studies of fat intake and the risk of breast cancer: A pooled analysis'. *The New England Journal of Medicine*, 334, 356–361.
- Jackson J L, Lesho E and Peterson C (2000), 'Zinc and the common cold: A meta-analysis revisited'. *J. Nutr.*, 130, 1512S–1515S.
- Jönsson T, Åhrén B, Pacini G, Sundler F, Wierup N, Steen S, Sjöberg T, Ugander M, Frostegård J, Göransson L and Lindeberg S (2006), 'A paleolithic diet confers higher insulin sensitivity, lower C-reactive protein and lower blood pressure than a cereal-based diet in domestic pigs'. *Nutr. Metab.*, 3, 39–48.
- Kabat G C, Miller A B, Jain M and Rohan T E (2007), 'A cohort study of dietary iron and heme iron intake and risk for colorectal cancer in women'. *Brit. J. Cancer*, 97(1), 118–122, Epub 2007 June 5.
- Knekt P, Steineck G, Jarvinen R, Hakulinen T and Aromaa A. (1994), 'Intake of fried meat and risk of cancer: A follow up study in Finland.' *International Journal of Cancer*, 59, 756–760.
- Kohlhäufl M, Häussinger K, Stanzel F, Markus A, Tritschler J, Mühlhöfer A, Morresi-Hauf A, Golly I, Scheuch G, Jany B H and Biesalski H K (2002), 'Inhalation of aerosolized Vitamin A: Reversibility of metaplasia and dysplasia of human respiratory epithelia – A prospective pilot study'. *Eur. J. Med. Res.*, 7(2), 72–78.
- Koohestani N, Tran T T, Lee W, Wolever T M and Bruce W R (1997), 'Insulin resistance and promotion of aberrant crypt foci in the colons of rats on a high fat diet'. *Nutrition and Cancer*, 29, 69–76.
- Kurokawa R, DiRenzo J, Boehm M, Sugarman J, Gloss B, Rosenfeld MG, Heyman RA and Glass CK. (1994), 'Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding'. *Nature*, 371, 528–531.
- Le Marchand L (1999), 'Combined influence of genetic and dietary factors on colorectal cancer incidence in Japanese Americans'. *J. Natl. Cancer Inst. Monographs*, 26, 101–105.
- Lloyd-Wright Z, Allen N, Key T and Sanders T (2000), 'How prevalent is vitamin B12 deficiency among British vegetarians and vegans?' *Proc. Nutri. Society*, 60, 1–16.
- Lukito W, Wattanapenpaiboon N, Savage G S, Hutchinson P and Wahlqvist M L (2004), 'Nutritional indicators, peripheral blood lymphocyte subsets and survival in an institutionalised elderly population'. *Asia Pacific J. Clin. Nutr.*, 13(1), 107–112.
- MacIntosh G H and Le Leu R K (2001), 'The influence of dietary proteins on colon cancer risks'. *Nutrition Research*, 21, 1053–1066.
- MacIntosh G H, Royle P J, Le Leu R K, Regester G O, Johnson M A, Grinstead R L, Kenward R S and Smithers G W (1998), 'Whey protein as functional food ingredients?' *International Dairy Journal*, 8, 425–438.
- McKeown-Eyssen, G. (1994), Epidemiology of colorectal cancer revisited: Are serum triglycerides and/or plasma glucose associated with risk? *Cancer Epidemiology, Biomarkers & Prevention*, 3, 687–695.
- Metz C H, Schröder A K, Overbeck S, Kahmann L, Plümäkers B and Rink L (2007), 'T-helper type 1 cytokine release is enhanced by in vitro zinc supplementation due to increased natural killer cells'. *Nutrition*, 23(2), 157–163.
- Metzler M D and Snyder J M (1993), Retinoic acid differentially regulates expression of surfactant-associated proteins in human fetal lung. *Endocrinology*, 133(5), 1990–1998.
- Michaud D S, Augustsson K, Rimm E B, Stampfer M J, Willet W C and Giovannucci E. (2001), 'A prospective study on intake of animal products and risk of prostate cancer.' *Cancer Causes and Control*, 12, 557–567.
- Mori H, Sugie S, Rahman W and Suzui N (1999), Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary carcinogenesis in rats'. *Cancer Lett.*, 143, 195–198.
- Nohr D and Biesalski H K (2007), '“Mealthy” food: meat as a healthy and valuable source of micronutrients'. *Animal*, 1(2), 309–316.

- Odom M J, Snyder J M, Boggaram V and Mendelson C R (1988), 'Glucocorticoid regulation of the major surfactant associated protein (SP-A) and its messenger ribonucleic acid and of morphological development of human fetal lung in vitro'. *Endocrinology*, 123, 1712–1720.
- Parcell S (2002), Sulfur in human nutrition and applications in medicine. *Altern. Med. Rev.*, 7, 22–44.
- Park S Y, Murphy S P, Wilkens L R, Henderson B E and Kolonel L N (2007), 'Fat and meat intake and prostate cancer risk: The multiethnic cohort study'. *Int. J. Cancer*, 121(6), 1339–1345.
- Prasad A S, Beck F W, Bao B, Fitzgerald J T, Snell D C, Steinberg J D and Cardozo L J (2007), 'Zinc supplementation decreases incidence of infections in the elderly: Effect of zinc on generation of cytokines and oxidative stress'. *Am. J. Clin. Nutr.*, 85(3), 837–844.
- Rayman M P (1997), 'Dietary selenium: Time to act'. *Brit. Med. J.*, 314, 387–388.
- Reddy B S (1981), 'Diet and excretion of bile acids'. *Cancer Research*, 41, 3766–3768.
- Redman C, Xu M J, Peng Y M, Scott J A, Payne C, Clark L C and Nelson M A (1997), 'Involvement of polyamines in selenomethionine induced apoptosis and mitotic alterations in human tumor cells'. *Carcinogenesis*, 18, 1195–1202.
- Rink L and Gabriel P (2000), 'Zinc and the immune system'. *Proc. Nutr. Soc.*, 59(4), 541–52.
- Saletti A, Lindgren E Y, Johansson L and Cederholm T (2000), 'Nutritional status according to mini nutritional assessment in an institutionalized elderly population in Sweden'. *Gerontology*, 46, 139–145.
- Schrauzer G N, White D A and Schneider C J (1977), 'Cancer mortality correlation studies. III'. *Bioinorganic Chemistry*, 7, 23–34.
- Schulz C, Engel U, Kreienberg R and Biesalski H K (2007), Vitamin A and β -carotene supply of women with Gemini or short birth intervals: A pilot study', *Eur. J. Nutr.*, 46(1), 12–20.
- Shi B B and Spallholz J E (1994), 'Selenium from beef is highly bioavailable as assessed by liver glutathione peroxidase activity and tissue selenium'. *Brit. Med. J.*, 72, 873–881.
- Slattery M L, Murtaugh M, Caan B, Ni Ma K, Neuhausen S and Samowitz W (2005), 'Energy balance, insulin-related genes and risk of colon and rectal cancer', *Int. J. Cancer*, 115, 148–154.
- Ströhle A, Maike W and Hahn A (2007), 'Nutrition and colorectal cancer'. *Med. Monatsschr. Pharm.*, 30(1), 25–32.
- Sundell H W, Gray M E, Serenius F S, Escobedo M B and Stahlman M. T (1980), 'Effects of epidermal growth factor on lung maturation in fetal lambs'. *Am. J. Pathol.*, 100, 707–725.
- Takahashi M, Fukutake M, Isoi T, Fukuda K, Sato H, Yazawa K, Sugimura T and Wakabayashi K. (1997), 'Suppression of azoxymethane-induced rat colon carcinoma development by a fish oil component, docosahexaenoic acid (DHA)'. *Carcinogenesis*, 18, 1337–1342.
- Tappel A (2007), 'Heme of consumed red meat can act as a catalyst of oxidative damage and could initiate colon, breast and prostate cancers, heart disease and other diseases'. *Med. Hypotheses*, 68(3), 562–564.
- Towheed T E, Maxwell L, Anastassiades T P, Shea B, Houpt J, Robinson V, Hochberg M C and Wells G (2005), Glucosamine therapy for treating osteoarthritis. *Cochrane Database of Systematic reviews* 2005, Issue 2. Art. No. CD002946.
- van der Torre HW, Van Dokkum W, Schaafsma G, Wedel M and Ockhuizen T (1991), Effect of various levels of selenium in wheat and meat on blood Se status indices and on Se balance in Dutch men. *Br. J. Nutr.*, 65(1), 69–80.
- Watanabe K, Kawamori T, Natatsugi S, Ohta T, Ohuchida S, Yamamoto H, Maruyama T, Kondo K, Ushikubi F, Narumiya S, Sugimura T and Wakabayashi K. (1999), 'Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis'. *Cancer Res.*, 59, 5093–96.

- Wilkinson E A J and Hawke C I (2002), 'Oral zinc for arterial and venous leg ulcers' (Cochrane Review). In: *The Cochrane Library, Issue 2*, Oxford: Update Software.
- World Health Organization (2004), http://www.who.int/nutrition/publications/WHOandUNICEF_statement_anaemia.pdf (last checked October 2008).
- Xia Y, Zhao X, Zhu L and Whanger C D (1992), 'Metabolism of selenate and selenomethionine by a selenium-deficient population of men in China'. *J. Nutr. Biochem.*, 3, 202–210.
- Yoshizawa K, Willett W C, Morris S J, Stampfer M J, Spiegelman D, Rimm E B and Giovannucci E (1998), 'Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer'. *J. Natl. Cancer Inst.*, 90, 1219–2124.
- Youngman, L D and Campbell, T C (1992), The sustained development of preneoplastic lesions depends on high protein intake. *Nutr. Cancer*, 18: 131–142.
- Zheng W, Gustaffson D R, Sinha R, Cerhan J R, Moore D, Hong C P, Anderson K E, Kushi L H, Sellers T A and Folsom A R (1998), 'Well done meat intake and the risk of breast cancer'. *Journal of the National Cancer Institute*, 90, 1724–1729.

8

Sensory evaluation of fresh meat

M. G. O'Sullivan and J. P. Kerry, University College Cork, Ireland

Abstract: This chapter discusses the methods available for the sensory evaluation of fresh meat. It first reviews methods for the sensory evaluation of meat colour, then discusses the sensory evaluation of meat flavour, the sensory assessment of meat tenderness and concludes with future developments in sensory evaluation methods.

Key words: meat, sensory evaluation, colour, flavour, tenderness.

8.1 Introduction

Appearance determines how consumers perceive quality and it significantly influences purchasing decisions (Issanchou, 1996). The three sensory properties by which consumers most readily judge meat quality are appearance, texture and flavour (Liu *et al.*, 1995). Colour perception plays a major role in consumer evaluation of meat quality (Lanari *et al.*, 1995). Consumers need first to be entirely satisfied with the sensory properties of products, before other quality dimensions become relevant (Chambers and Bowers, 1993).

Following purchase of the meat product in question, then sensory evaluation with respect to flavour becomes a critical determinant of quality for the consumer. Carpenter and co-workers (2001) surveyed consumers' preferences for beef colour and found that the type of packaging used would likely sway their decision to purchase. However, the preferences for beef colour and packaging did not bias taste scores. They conclude that the initial perceptions of quality will likely not bias eating satisfaction once a decision to purchase is made and the meat is taken home, thereby hastening the acceptance of the newer packaging technologies. As the meat industry moves toward central processing that employs modified atmosphere packaging (MAP) and vacuum skin packaging (VSP), they may need to overcome consumer preference for fresh beef that is bright red in colour and packaged with the traditional PVC over-wrap (Carpenter *et al.*, 2001).

In addition to an acceptable flavour, the consumer desires meat to be palatable

and, consequently, meat tenderness is another critical determinant used by the consumer to determine meat quality. In fact, it has been demonstrated that the consumer would be willing to pay a higher price in the marketplace for beef as long as it is guaranteed tender (Miller *et al.*, 2001). Tenderness can be evaluated by objective methods (e.g. instrumental or sensorial with trained panels) or by subjective methods (e.g. using a consumer panel) (AMSA, 1995). Objective methods allow the comparison of different treatments as well as ascertaining their effect on a particular characteristic, but do not provide information concerning product acceptability or preference for one kind of meat over another (Wheeler *et al.*, 1997). Therefore, consumer opinion is a key factor to establish meat value and justify purchase decision.

In a later chapter, the sensory and quality properties of packaged meat will be described. However, this chapter presents specific methodologies, which can be employed to quantify the sensory properties of packaged meat with respect to colour, flavour and texture. Ultimately, meat products are consumed and it is important that they are assessed by human responses and that reproducible and reliable methods are available to accurately quantify them.

8.2 Sensory evaluation of meat colour

Colour stability of meats has been an important research area in meat quality assessment and improvement for many years. At the point of sale, colour and colour stability are the most important attributes of meat quality and various approaches have been used to meet consumer expectations that an attractive, bright-red colour indicates a long shelf-life and good eating quality (Hood and Mead, 1993). Carpenter *et al.* (2001) noted a strong association between colour preference and purchasing intent with consumers discriminating against beef that is not red (i.e. beef that is purple or brown). Therefore, visual determinations are the gold standard for assessing treatment effects and estimating consumer perception.

Several researchers have used direct colour sensory assessment of meat using sensory panellists. Chan *et al.* (1996) conducted a double-blind sensory evaluation experiment where test subjects were asked to evaluate steak for the extent of discolouration, appearance acceptability and intent to purchase. In addition, panellists assessed spoilage according to olfactory acceptability and indicated whether or not they would consume the steak. The steaks, *M. psoas major*, *M. gluteus medius*, and *M. longissimus lumborum* were from cattle supplemented with vitamin E levels at 57 and 1204 IU/head/day and were placed in a refrigerated display cabinet at 4 °C. Panellists evaluated the various steaks on days 0, 2, 4, and 8. Chan *et al.* (1996) concluded that panellists' responses for appearance acceptability and purchase-preference for steaks were similar, suggesting, that appearance was used as a major indicator for steak quality. Furthermore, the visual acceptance of steaks correlated well with estimated steak surface discolouration. Both subjective evaluation and objective measurement of beef in the sensory study

indicated that dietary vitamin E supplementation extended colour shelf-life and visual acceptance for the three muscles used. Zanardi *et al.* (1998) used a ten-member panel, trained to judge the appearance of the brown colour of metmyoglobin and carried out colour stability evaluations on pork chops from animals supplemented with either vitamin E, oleic acid (sunflower oil) or copper. Panellists evaluated the samples according to a 5-point scale of brown varying from 1 (very light) to 5 (very deep). These workers found that the sensory scores did not show significant differences in the rate of brown appearance in O₂ permeable packed chops. Increased colour stability was observed under protective atmosphere (80% O₂ and 20% CO₂) for groups supplemented with 100 and 200 ppm vitamin E. However, Zanardi *et al.* (1998) does not elaborate on the colour references, duration and the training methods employed to train sensory panellists. In another study, Brewer *et al.* (2001) evaluated the visual colour, marbling and overall acceptability of pork loins with varying degrees of marbling, using naïve sensory panellists. Panellists used a 5-point category scale where 1 = very light pink, very lean or very unacceptable and 5 = dark pink, highly marbled or very unacceptable, respectively. These authors found that, based on visual evaluation, chops with low and medium amounts of marbling had higher overall appearance and acceptability ratings. Furthermore, purchase intent paralleled lean appearance and acceptability. Wiklund *et al.* (2006) used a six-member trained panel to assess high O₂ MAP pork loin chops for visual raw lean colour, colour uniformity (striping) and sheen. Samples were evaluated through intact packages on the day immediately before the chops were placed in the retail case, and on each of the 2 days the chops were in display. Raw colour was evaluated using a 7-point scale (1 = dark, brownish pink; 7 = extremely bright pink) and colour uniformity was evaluated using a 5-point scale (1 = extreme two-toning; 5 = uniform colour). Surface sheen was evaluated on a 5-point scale (1 = extremely wet surface with excessively viscous exudates; 5 = dry to very slightly wet). Standards were provided for visual colour and colour uniformity (striping). They concluded that raw CO-MAP (carbon monoxide) chops were visually brighter pink than were high O₂ MAP chops, regardless of the inclusion of a phosphate treatment.

The use of structured line scaling, as presented, has been the convention in sensory evaluation of meat colour studies to date. However, unstructured line scaling may give a higher degree of discrimination between samples. O'Sullivan *et al.* (2002a) used unstructured line scaling to evaluate the colour stability of pork chops from four dietary treatments (control, vitamin E, iron/vitamin E and iron) under standard commercial retail display conditions by a trained sensory panel (Fig. 8.1). A higher degree of discrimination in visual assessment was determined relative to instrumental methods (Hunter L, a, b) as measured by a Minolta colorimeter. Colour references, which were the most red (day 0) and most brown (day 5) samples respectively, determined from a preliminary trial, were presented to test subjects during profiling. For both training and profiling, unstructured 15-cm line scales anchored on the left by the term 'none' and on the right by the term 'extreme' were used for the red and brown colour descriptors (Meilgaard *et al.*, 1999). Unstructured line scales may be said to allow for a greater degree of

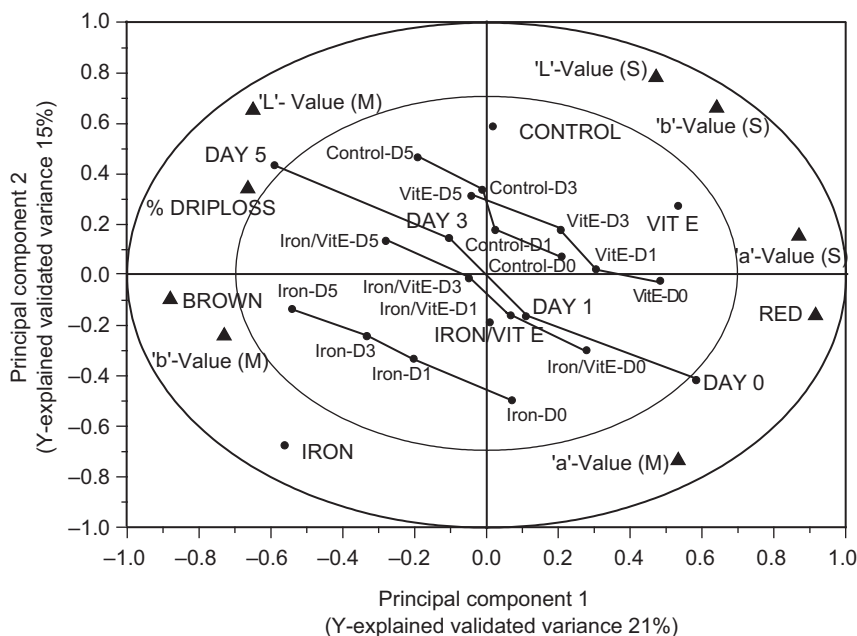


Fig. 8.1 An overview of the variation found in the mean data from the ANOVA-Partial Least Squares Regression (APLSR) correlation loadings plot for each of the four dietary treatment groups. Shown are the loadings of the X- and Y-variables for the first 2 PCs for level corrected data. ● = sample and ▲ = sensory and instrumental descriptor. S = sensory variable and M = Minolta colorimetric measurement. D = day in refrigerated display cabinet. The concentric circles represent 100% and 50% explained variance respectively. (Adapted from O'Sullivan *et al.*, 2002b.)

discrimination, compared to structured or category scales, by allowing assessors a greater degree of freedom with respect to their sensory assessment of a particular characteristic. However, sensory panellists will use the scale differently and it may be difficult to correlate their individual perception of a sensory stimulus. Using multivariate data analytical methodology, level correction may be employed to correct for assessor variation. Range correction may also be used to correct for individual assessor variation between different test days (O'Sullivan *et al.*, 2002b).

Package type can also influence red colour perception of meat. Meat packaged with film contact (PVC overwrap or vacuum) was perceived as more red than meat packaged with headspace (Carpenter *et al.*, 2001). These authors also noted that panellist descriptions of colour may depend on individual cognition when references are not used. This point re-emphasizes the need for visual panels to be trained, screened, and selected based on their abilities to consistently evaluate desired colour traits. Carpenter *et al.* (2001) concluded that consumer preference for bright-red coloured beef overwrapped in PVC might slow the industry's move toward central packaging (MAP and vacuum-skin packaging).

O'Sullivan *et al.* (2004) used a semi-trained panel of 15 people to examine the meat colour effect of conserved forage and concentrate feeding on the quality of beef held in overwrapped (aerobic) or MAP under simulated retail display for 17 days. Two displays of meat, Display X (aerobically packaged) and Display Y (MAP), were shown to panellists. Within each display there were five trays, with each tray containing meat cores from the nine steaks per dietary group. Panellists were asked to evaluate each display separately and score the colour of each tray of meat cores on a 10-point scale (1 = very poor colour to 10 = excellent colour). Panellists were also asked to indicate in terms of colour their most preferred dietary group. These authors conclude that the colour of fresh beef may be improved by feeding animals grass silage that has undergone extensive fermentation. Moreover, results indicated that packaging environment has a pronounced effect on the quality attributes of beef from differing feeding systems.

O'Sullivan *et al.* (2002a) showed that trained panellists were able to differentiate four experimental treatment groups (control, vitamin E, iron/vitamin E and iron) on each day of a retail display study of uncooked samples of whole cuts of pork *M. longissimus dorsi* muscle. These authors found that the trained panel was more effective in evaluating the colour quality of samples than instrumental methods as determined by a portable colorimeter (Minolta). In another study, O'Sullivan *et al.* (2003a) investigated whether training of sensory panellists for sensory visual assessment of meat gave a discriminative improvement in the subsequent evaluation compared to using an untrained sensory panel. In this study *M. longissimus dorsi* and *M. psoas major* minced pork patties from three dietary treatment groups (control, iron/vitamin E and iron) were packaged in polythene bags and placed in a retail refrigerated display cabinet at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$, under fluorescent light (1000 LUX) for up to 5 days. Samples were subjected to visual colour evaluation by a trained sensory panel ($n = 8$) and an untrained panel ($n = 8$) on days 0, 1, 3 and 5 of retail display. O'Sullivan *et al.* (2003a) found that the signal to noise (S/N) error for assessors and replicates for the trained assessor group were better than those of the untrained assessor group, indicating that their results were more reliable. The trained assessor group produced relatively normal percentile distributions for sensory terms in the assessment of both *M. longissimus dorsi* and *M. psoas major* muscles. The untrained assessor group displayed skewed or non-symmetric distributions for *M. longissimus dorsi*, but produced a normal distribution for *M. psoas major*. The qualitative and quantitative analysis of the sensory profiling by both the trained and untrained groups of test subjects showed that, in general, sensory training contributed to a more effective visual sensory evaluation of *M. longissimus dorsi*. However, this was not the case for *M. psoas major* where both groups of assessors produced relatively similar results (O'Sullivan *et al.*, 2003a). In order for a sensory term to be included in a sensory profile, it must be relevant to the product, discriminate between the products, be non-redundant, and have cognitive clarity (Byrne *et al.*, 1999b). The trained assessor group was provided with reference samples both during training and profiling. O'Sullivan *et al.* (2003a) further postulated that the trained panellists should have been cognitively clearer in their respective discrimination of the various products compared to the

untrained group of assessors. The trained group of assessors was better in the assessment of *M. longissimus dorsi*, a minced white porcine muscle. However, the untrained group discriminated between the various experimental treatments well for *M. psoas major*. *M. psoas major* (0.08% wet weight) has almost twice the concentration of myoglobin compared with *M. longissimus dorsi* (0.04% wet weight) (Lawrie, 1991). Thus, the colour changes in *M. longissimus dorsi* had lesser variation compared to *M. psoas major* and consequently, it may be said that greater sensitivity is required for a more discriminative evaluation. The trained group of assessors performed better than the untrained group in the sensory visual evaluation of this muscle because, with training and the provision of reference samples, their individual assessment of the colour changes in the various samples during the course of this study had a higher degree of sensitivity (O'Sullivan *et al.*, 2003a). Both groups of assessors during the course of this study expressed their unfamiliarity with the minced *M. longissimus dorsi* samples presented for evaluation. Although *M. psoas major* is a muscle that usually is not commercially minced and sold because of its prime nature as a meat cut, all assessors were familiar with comminuted red porcine muscles in the supermarket context. The untrained group could be postulated to have intuitively assessed the colour of both types of muscle samples and displayed better results for the red muscle *M. psoas major*, because they were more familiar with this type of product due to market-place exposure.

In conclusion, the sensory visual assessment of meat products can be done effectively without training when the product is familiar to the assessors. However, training of panellists and the provision of reference samples become relevant when an unfamiliar product is to be assessed. The trained group of assessors may be said to discriminate in a more cognitive manner, due to relevant sample exposure, compared to the untrained group in the objective sensory assessment of the white porcine muscle *M. longissimus dorsi* (O'Sullivan *et al.*, 2003a).

The lighting used for visual colour evaluation can dramatically influence visual colour perception. Lighting type and intensity must be standardized from sample to sample, panellist to panellist, and replication to replication. To maximize appearance yet minimize photo-oxidation, recommended lighting is 1614 lux (150 foot candles) of fluorescent lighting, which should have a colour temperature of 3000–3500 K (lamps such as Deluxe Warm White, Natural, Deluxe Cool White, SP 3000, SP 3500). Cool White or lamps giving unreal pink, blue, or green tints should be avoided (Mancini and Hunt, 2005). Barbut (2001) reported that incandescent light increased beef and pork colour desirability due to its spectrum consisting of more red wavelengths. Fluorescent lighting used in this study had virtually no luminance in the red region, and thus the beef was considered less desirable than beef displayed under incandescent lighting.

8.3 Sensory evaluation of meat flavour

Flavour research is concerned with developing improved methods of characterising and measuring overall flavour quality and individual attributes of food and

beverage products. This can be achieved by studying the influence on flavour of changes in food materials and procedures at all stages in the food chain, to protect established standards of flavour quality (Land, 1977). There are generally two types of sensory panels that can be used in the sensory evaluation of meat products, 'difference' testing panels and 'descriptive attribute testing' panels.

Difference testing can be categorised into overall difference tests and attribute-specific directional difference tests. Overall, difference tests provide a method to determine if a sensory difference is detectable between the samples, whereas attribute difference testing asks if a specified attribute is perceived as different between samples. Commonly used difference tests are the triangle test, duo-trio test, simple same-difference test and 'A'– 'not A' test (Lawless and Heymann, 1998; Piggott *et al.*, 1998). Attribute difference tests include the alternative forced choice (AFC) methods and simple ranking test. These latter tests are more sensitive to detect sensory differences. However, they are not practical in meat studies since they require that only one sensory attribute varies independently of the other meat sensory properties (Byrne and Bredie, 2002).

Descriptive analysis is a method where defined sensory terms are quantified by sensory panellists. A list of descriptive terms are determined initially and are referred to as a lexicon or descriptive vocabulary. They describe the specific sensory attributes in a meat sample and can be used to evaluate the changes in these attributes (Byrne and Bredie 2002). Two methods are generally used, the Quantitative Descriptive Analysis (QDA) and the Spectrum methods. In the QDA method, the vocabulary is based on the terms suggested by the panellists themselves, in discussions under supervision of a panel-leader. The Spectrum method prescribes the use of a strict technical sensory vocabulary using reference materials. These descriptive terms can be initially determined from lexicons of descriptive terms that have been developed and employed by a number of authors for the sensory evaluation of meat products: Johnson and Civille (1986) for beef, Lyon (1987); Byrne *et al.* (1999b) for chicken, and Byrne *et al.* (1999a,b) for pork. For the QDA method, along with such lexicons, experts with product knowledge can evaluate a sample set of the meat to be profiled in the laboratory and suggest descriptive terms that specifically describe the meat product to be tested and the sensory dimension to be examined, e.g. warmed-over flavour (WOF) (O'Sullivan *et al.* (2003b). Once an initial list of terms is decided upon, the next step is to reduce these terms through the training and term reduction process. In order to be included during subsequent profiling the sensory terms selected must be (i) relevant to the samples, (ii) discriminate between the samples, (iii) have cognitive clarity and (iv) be non-redundant (Byrne *et al.*, 1999a,b, 2001b; O'Sullivan *et al.*, 2002b, 2003b). Various means can be employed in this term reduction process and these have included principal component analysis (PCA) in conjunction with assessor suggestions (Byrne *et al.*, 1999a,b, 2001b; O'Sullivan *et al.*, 2003b). Free choice profiling (FCP) can also be used and this involves panellists developing their own descriptive terms (Delahunty *et al.*, 1997). The problem with this method is that the subjective correlation of terms derived by different assessors may not, in reality, be related. Generalised procrustes analysis (GPA) has also been used in term

Table 8.1 The final list of 21 descriptive terms developed for further sensory profiling (adapted from O'Sullivan *et al.*, 2002b)

Sensory term	Definition (with appropriate reference)
<i>Odour</i>	<i>Odour reference</i>
1. Cardboard-O	Wet cardboard
2. Linseed oil-O	Warmed linseed oil/linseed oil-based paint
3. Rubber/Sulphur-O	Warmed rubber/the white of a boiled egg
4. Nut-O	Crushed fresh hazel nuts
5. Green-O	Fresh green French beans
6. Fatty-O	Pig back fat (fresh, non-oxidised)
<i>Taste</i>	<i>Taste reference</i>
7. Sweet-T	Sucrose 1 g/l aqueous soln.
8. Salt-T	Sodium chloride 0.5 g/l aqueous soln.
9. Sour-T	Citric acid monohydrate 0.3 g/l aqueous soln.
10. Bitter-T	Quinine chloride 0.05 g/l aqueous soln.
11. MSG/Umami-T	Monosodium glutamate 0.5 g/l aqueous soln.
<i>Flavour</i>	<i>Flavour reference</i>
12. Metallic-F/Bloody-F	Ferrous sulphate 0.1 g/l aqueous soln.
13. Fresh cooked pork-F	Oven cooked pork without browning
14. Rancid-F	Oxidised vegetable oil
15. Lactic acid/fresh sour-F	Natural yoghurt
16. Vegetable oil-F	Fresh vegetable oil
17. Piggy/Animal-F	Skatole 0.06 µg/ml refined vegetable oil
18. Fish-F	Fish stock in boiling water
19. Tinny-F	Stainless steel strip
20. Livery-F	Cooked beef liver
<i>Aftertaste</i>	<i>Aftertaste reference</i>
21. Metallic/Bloody-AT	Ferrous sulphate 0.1 g/l aqueous soln.

Note: Suffix to sensory terms indicates method of assessment by panellists: -O = Odour, -F = Flavour, -T = Taste, -AT = Aftertaste.

reduction (Byrne *et al.*, 2001b) and a new method known as descriptor leverage (O'Sullivan *et al.*, 2002b). Both of these methods are similar in that they involve a level and range correction of assessors, but the descriptor leverage method does not include the rotational aspect of GPA. The descriptor leverage method is rapid and unambiguous and was investigated by O'Sullivan *et al.* (2002b) to determine whether it provided any discriminative improvement in the term reduction process. These authors concluded that the use of descriptor leverage provided a greater amount of information regarding the elimination of sensory terms as opposed to PCA and assessor suggestions alone. The final list of sensory terms selected in this study is seen in Table 8.1. Descriptor leverage displayed the uniqueness of sensory terms for all the relevant principal components in a model and provided a higher

Sensory terms	Session 1 36 terms	Session 2 36 terms	Session 3 29 terms	Session 4 27 terms	Session 5 22 terms	Final List 21 terms
Cardboard-F						
Cardboard-O						√
Linseed-F						
Linseed-O						√
Rubber-F						
Rubber-O						√
Green-O						√
Rancid-F						√
Vegetable Oil-F						√
Fish-F						√
Lactic-AT						
Lactic-O						
Lactic-F						√
Fatty-Mouthcoating-AT						
Fatty-O						√
Sweet-T						√
Sour-T						√
Salt-T						√
Bitter-T						√
MSG-T (Monosodium Glutamate)					<i>MSG-T/ Bouillon-O</i>	√
Bloody-F						
Metallic-F					<i>Metallic-F/ Bloody-F</i>	√
Metallic-AT						
Tinny-F						√
Livery-F						√
Nut-F						
Nut-O						√
Meat-F						
Fresh Cooked Pork-O						
Fresh Cooked Pork-F						√
Bouillon-AT						
Bouillon-O						
Roasted-F						
Piggy-O						
Piggy-F						√
Astringent-AT						√

Fig. 8.2 A schematic overview of the vocabulary development methodology over five training sessions. Blanks = removed sensory terms, italics = merged sensory terms, √ = sensory terms in final list, -O = Odour, -F = Flavour, -T = Taste, -AT = Aftertaste. (Adapted from O’Sullivan *et al.*, 2002b.)

degree of confidence with respect to sensory term reduction. O’Sullivan *et al.* (2002a) further suggested that a combination of descriptor leverage, graphical interpretation of bilinear models and assessor suggestions may be a useful strategy in future vocabulary development studies. A schematic overview of the vocabulary development methodology over five training sessions is presented in Fig. 8.2.

After training, sensory profiling is undertaken and a number of sensory profiles have been conducted to date on WOF in various meat products. Byrne *et al.* (2001a) investigated the effects of pre-slaughter stress on WOF development in porcine meat. They found that pre-slaughter stress appeared, in general, to manifest itself as a separate sensory dimension to WOF in meat samples, but there were indications that increasing pre-slaughter stress may have reduced perceived WOF development. Byrne *et al.* (2002a) evaluated the effects of cooking temperature on WOF development in chicken. Cooking at higher temperatures produces Maillard reaction products, which are known to have an antioxidant effect (Bailey, 1988). However, Byrne *et al.* (2002a) showed that temperature increased the formation of Maillard-derived compounds, but did not show strong effects on the prevention of WOF in the cooked chicken patties. Byrne *et al.* (2002b) investigated the effects of the RN-gene (Rendement Napole) on WOF development in pork. A higher glycogen content in muscle results in lower post-mortem ultimate pH in RN⁻ carriers, the dominant gene (Enfält *et al.*, 1997). Byrne *et al.* (2001a) concluded that WOF, cooking temperature and genotype in the meat samples profiled were independent phenomena and that lactic/fresh sour flavour was a significant descriptor for describing meat from RN⁻ carriers. O'Sullivan *et al.* (2003b) determined the sensory effects of iron supplementation on WOF development in pork meat patties made from *M. longissimus dorsi* and from *M. psoas major*. They concluded that *M. psoas major* was more susceptible to WOF development, as determined by sensory profiling, compared to *M. longissimus dorsi* for all treatments. *M. longissimus dorsi* development of WOF was highest in the iron supplemented groups, followed by the control and vitamin E supplemented groups. The development of WOF in *M. psoas major* was tentatively the highest in the iron supplemented groups but was less well defined between dietary treatments because the level of vitamin E uptake in *M. psoas major* was probably sufficient to protect the muscles from oxidative attack.

One phenomenon that is consistently described by the above researchers (Byrne *et al.*, 2001a,b, 2002b) with respect to WOF development is the perceived loss in the 'meatiness' of samples and the increase in the more oxidative profiling during days of WOF development. Authors such as Drumm and Spanier (1991) and St. Angelo *et al.* (1990) have suggested that reactions involving protein degradation and/or hetero-atomic compounds leading to a reduction in meatiness may, in addition to lipid oxidation, form an inherent part of WOF development. More specifically, the degradation of unstable sulphur-containing amino acids (in meat proteins) and sulphur-containing meaty aroma compounds may be important (Byrne *et al.*, 2002a).

Zakrys *et al.* (2008a) evaluated the sensory scores of MAP beef *M. longissimus dorsi* muscle stored under a range of O₂ atmospheres (0%, 10%, 20%, 50% and 80%). Each panellist was presented with five samples, randomly chosen from each of the treatments and asked to assess the attributes (colour, tenderness, juiciness, oxidised flavour, overall acceptability) presented according to a standard 9-point scale and found that the quality of steaks was best promoted by packaging under an atmosphere containing 50% oxygen. Furthermore, panellists ($n = 2 \times 8$) were

asked to choose their most preferred sample on each sensory evaluation day and chose steaks packed in 50% O₂ on days 3, 6, 9 and 12. Therefore, panellists regarded 50% O₂ packed beef steaks as the optimal sample of the five treatments presented. They proposed that periodic consumption and exposure to MAP meat products in the supermarket could result in consumers becoming more familiar with certain oxidized flavours, thus associating it with normality.

Detailed descriptions of sensory terminology and procedural guidelines for the identification and selection of descriptors for establishing a sensory profile by a multidimensional approach have been described in ISO (1992) and ISO (1994), respectively. As mentioned earlier, a number of defined methodologies have been proposed for sensory descriptive analysis of food products, e.g. the Spectrum method and Quantitative Descriptive Analysis (QDA) (Byrne and Bredie, 2002). The Spectrum method is pragmatic in that it provides the tools with which to design a descriptive procedure for a given product category. Its principal characteristic is that the panellist scores the perceived intensities with reference to pre-learned 'absolute' intensity scales. The purpose is to make the resulting profiles universally understandable. The method provides for this purpose an array of standard attribute names ('lexicons'), each with its set of standards that define a scale of intensity (Muñoz and Civille, 1992; Meilgaard *et al.*, 1999). The QDA method first proposed by Stone *et al.* (1974) relies heavily on statistical analysis to determine the appropriate terms, procedures and panellists to be used for the analysis of a specific product. The training and QDA panels require the use of product references to stimulate the generation of terminology. The panel leader acts as facilitator, but does not influence the group. Panellists do not discuss data, terminology or samples after each taste session, but must depend on the discretion of the panel leader for any information on their performance. Feedback is provided by the facilitator-based on the statistical analysis of the taste session data (Lawless and Heymann, 1998; Meilgaard *et al.*, 1999). Moreover, in relation to muscle food evaluation, QDA has been extensively discussed by Miller (1994).

8.4 Sensory assessment of meat tenderness

The US Beef Consumer Satisfaction Study (Lorenzen *et al.*, 1999; Neely *et al.*, 1998, 1999; Savell *et al.*, 1989) showed that tenderness is a major and contributing factor to consumers' perception of taste. The ability of consumers to discern varying tenderness levels is essential for establishing the value of beef tenderness. If consumers do not have the ability to select among differences in tenderness, then all efforts to improve the tenderness of beef are of little value. Assuming that consumers can detect variation in beef tenderness, then the need exists to measure and establish the value of tenderness to the marketplace (Boleman *et al.*, 1997). One of the earlier methods developed to assess meat texture included the chew count (Harrington and Pearson, 1962). This method requires panellists to count the number of chews to reduce the product to the necessary size needed for swallow-

ing. Panel members may differ widely in their chew count, but the method was an attempt to place texture on a quantitative basis. Several studies have been conducted in an attempt to establish threshold Warner–Bratzler (WB) values as indices of tenderness acceptability (Boleman *et al.*, 1997; Miller *et al.*, 1995, 2001; Shackelford *et al.*, 1991, 1997). Destefanis *et al.* (2007) used a sensory panel of 220 people to evaluate 60 samples of *M. longissimus thoracis* muscle, using a 5-point intensity scale (1: very tough; 5: very tender). Samples differed for commercial category, breed of animals and ageing length of meat. Shear force was measured by an Instron equipped with a WB device on 1.27 cm diameter cores. The principal aim of the study was to investigate the consumer's ability to discern different levels of tenderness indirectly established by WB shear force. They concluded that their results indicate that beef with WB values > 52.68 N and < 42.87 N is perceived by most consumers as 'tough' and 'tender', respectively. They further suggest that these values could represent reliable thresholds to classify beef for tenderness, with the great advantage of overcoming the practical problems of sensory evaluation. Several studies have shown that USDA quality grades were not very good at accurately classifying carcasses by texture (McDonald and Chen, 1991; Smith *et al.*, 1987). Certain researchers have used LD muscle colour measurements for this purpose (Canell *et al.*, 2002; Wulf *et al.*, 1997; Wulf and Page, 2000). However, no clear relationship has been found for beef tenderness. On the other hand, some studies have shown a relationship between ultimate muscle pH and (or) muscle colour and meat tenderness (Purchas, 1990; Jeremiah *et al.*, 1991; Watanabe *et al.*, 1996; Wulf *et al.*, 1997). Wulf and Page (2000) reported that measurements of muscle colour or pH could be used in a branded-beef program to increase the palatability consistency of its beef products. However, Lorenzen *et al.* (2003), using an eight-member trained descriptive attribute panel, evaluated each cooked steak for muscle fibre tenderness and found that there is an inherent difficulty in predicting consumer responses from objective laboratory procedures, such as trained sensory panels and WB shear force. These authors used the following descriptive terms to evaluate the products: Muscle fiber tenderness (MFT), connective tissue amount (CTA), overall tenderness (OTEND), juiciness (TJUIC), flavour intensity (FLAV), cooked beef flavour intensity (BEEFY), and cooked beef fat flavour intensity (FAT), using 8-point scales (8 = extremely tender, none, extremely tender, extremely juicy, extremely intense, extremely intense, and extremely intense; 1 = extremely tough, abundant, extremely tough, extremely dry, extremely bland, extremely bland, and extremely bland). Andrés *et al.* (2008), working with 120 muscles from a small number of animals ($n = 30$), suggest that visible and near infrared spectroscopy instruments (400–2500 nm) can accurately predict pH 24 and L^* parameters, and have a good potential to provide useful prediction of WBSF on intact beef muscle samples. Lorenzen *et al.* (2003) conclude that there are far too many factors that occur in households when preparing meat, such as cooking method, degree of doneness, seasonings added before, during and after cooking, and person-to-person differences in preferences and thresholds for tenderness, juiciness and flavour. This makes it difficult to predict from objective data how consumers will rate meat at home.

Consumer preference studies are important measures that assess product quality at the most important part of the supply chain, the end-user. Voges *et al.* (2006) recruited 713 panellists who were asked to complete a demographic questionnaire regarding a selection of over 10 beef cuts with these data being compared to Warner–Bratzler shear force (WBSF) measurements. Beef cuts from retail and foodservice establishments in 11 US cities were evaluated. Steaks were served randomly to individual panellists in sensory booths. Each consumer received two 1.27 cm cubes of each sample and evaluated eight random samples during the session. Samples were characterized using 10-point scales for overall liking (10 = like extremely; 1 = dislike extremely), flavour (10 = like extremely; 1 = dislike extremely), beef flavour (10 = an extreme amount; 1 = none at all), juiciness (10 = very juicy; 1 = not at all juicy), and tenderness (10 = very tender; 1 = not at all tender), and like tenderness (10 = like extremely; 1 = dislike extremely). These authors found that, for retail meat cuts, the three cuts from the round, top round, bottom round, and eye of round had the highest ($P < 0.05$) WBSF values compared to cuts from the chuck, rib, and loin. Top loin steaks had the lowest ($P < 0.05$) WBSF values compared with rib-eye and top sirloin foodservice steaks. Retail bone-in top loin, top loin, rib-eye, T-bone, and porterhouse received the highest ($P < 0.05$) ratings by consumers for overall like and tenderness like. Quality grade had little or no effect on foodservice sensory evaluations.

Rowe *et al.* (2004) reported that increased protein oxidation (PO) during the first 24 h post-mortem can substantially decrease beef tenderness, even in steaks aged 14 days. Zakrys *et al.* (2008a) evaluated the sensory scores of MAP beef *M. longissimus dorsi* muscle stored under a range of O₂ atmospheres (0%, 10%, 20%, 50% and 80% O₂). Each trained panellist was presented with five samples, randomly chosen from each of the treatments, and asked to assess the tenderness as well as other attributes (colour, tenderness, juiciness, oxidised flavour, overall acceptability) presented according to a standard 9-point scale. These workers found that the quality of steaks was best promoted by packaging using an atmosphere containing 50% O₂. Furthermore, panellists ($n = 16$) were asked to choose their most preferred sample on each sensory evaluation day and chose steaks packed in 50% O₂ on days 3, 6, 9 and 12. It appeared that samples packed with 50% and 80% O₂ were tougher than lower O₂ treated samples. Zakrys *et al.* (2008b) repeated the study using 134 consumers and 10 cm unstructured line scaling for the attributes liking of flavour, juiciness, toughness, oxidised flavour and overall acceptability. They also found that the consumer panel directionally found MAP beef steaks with the higher oxygen treatments tougher than lower oxygen treated samples.

Lund *et al.* (2007) investigated the effect of MAP (70% O₂/30% CO₂) and skin packaging (no O₂) on protein oxidation and texture of pork *M. longissimus dorsi* muscle during storage for 14 days at 4 °C and found that the high O₂ atmosphere resulted in reduced tenderness and juiciness of the experimental meat samples. The panel consisted of nine assessors, whom they describe as being part of a professional sensory panel at the Danish Meat Research Institute. All assessors had

undergone a basic training program in sensory assessment in accordance with ISO 4121, ASTM-MNL 13, DIN 10964 and were familiar with sensory assessments of meat. These researchers used a 15 cm unstructured line scale anchored with 'little' to 'much'. Descriptive terms of the texture of LD slices were: hardness (defined as hardness at first bite with molars), juiciness (amount of juice in the mouth after five chews), crumbliness (sense of grittiness and dryness during chewing), and tenderness (easiness whereby the meat is divided during chewing). Bertram and Aaslyng (2007) used two trained sensory panels, 9 and 8 people, respectively, to assess the effects on meat texture of pelvic suspension and pre-rigor excision on sarcomere length, water distribution, technological yield and sensory properties of pork. The assessors had undergone a basic training programme in sensory assessment in accordance with ISO 4121, ASTM-MNL 13, DIN 10964 and were all familiar with sensory assessment of pork. The samples were covered during serving and assessed at approximately 55–60 °C. All assessors tasted the loins in the same order. The attributes assessed in this study were: juiciness after 5 chews, juiciness after 15 chews, hardness at first bite with the molars, tenderness, crumbliness, fibrousness and chewing time. The scale was a 15 cm non-structured line scale anchored at the extremes (0 = slight and 15 = intense).

Aaslyng *et al.* (2007) used Danish consumers, from Roskilde ($n = 213$) and from Holstebro ($n = 162$), to assess nine different samples of pork on an unstructured hedonic scale from 'do not like at all' to 'like very much'. The samples represented variation in raw meat quality (pH, IMF and carcass weight), muscle, M. biceps femoris (LD and BF), origin (Danish/French Pay Basque), cooking method (pan/oven) and end point temperature (65 °C/75 °C). The meat was described by sensory profiling and chemical and physical analysis (pH, fat, water, colour, fatty acid composition). All the consumers preferred tender, juicy meat with a fried flavour and no off-flavours. However, within this description there were differences. The consumers from Holstebro put more emphasis on tenderness and the absence of off-flavours, while the consumers from Roskilde preferred the fried flavour. The young consumers put less emphasis on tenderness, compared with consumers aged over 30 years, but preferred instead some crumbliness in the meat. A segmentation of the consumers showed that about 6% of the consumers were influenced only by flavour attributes in their preference. In contrast, 12% of the consumers were influenced mainly by texture, irrespective of flavour attributes other than sour-like taste. However, most of the consumers were influenced by both flavour and texture, as well as appearance (Aaslyng *et al.* 2007).

Beilken *et al.* (1991) used two different methods for determining the sensory profiles for texture of meat patties compared with consumer ratings. The individual sensory profiles developed in free-choice profiling (FCP) were analysed using generalised Procrustes and principal components analysis techniques and converted by discussion to a single consensus profile. The free-choice profiling method allows panellists (consumers) to use any number of their own attributes to describe and quantify product attributes and is based on the assumption that panellists do not differ in their perceptions but merely in the way in which they describe them. The number of attributes generated is limited only by the perceptual

and descriptive skills of the panellist (Oreskovich *et al.*, 1991). The distinct advantage of FCP is the avoidance of panel training, participants need only to be able to use a scale and be consumers of the product under evaluation (Piggott *et al.*, 1989). However, sometimes the handling of individual ballots for each panellist can prove time-consuming and the interpretation of the resulting individual descriptors by the sensory analyst can also be challenging (Murray *et al.*, 2001).

In conclusion, chemical and instrumental measurements can offer important indices of meat quality, but the direct consumer response to meat quality, be that colour, flavour or texture, are the most important methods that we have in assessing meat quality. Each attribute has its particular role, colour for primary purchase followed by flavour and texture. However, once purchased, colour and packaging do not appear to bias taste scores. Also, it has been shown that, as beef steaks become tougher, flavour and juiciness have a greater effect on consumer satisfaction. It is clear that all three attributes (colour, flavour and texture) combine to give the overall consumer experience of a meat product and it is difficult or inappropriate to predict one from another.

8.5 Future trends

As described above, descriptive analysis is a method where defined sensory terms are quantified by sensory panellists. A list of descriptive terms is determined initially and is referred to as a lexicon or descriptive vocabulary. The terms describe the specific sensory attributes in a food sample and can be used to evaluate the changes in these attributes (Byrne and Bredie, 2002). A major strength of descriptive analysis is its ability to allow relationships between descriptive sensory and instrumental or consumer preference measurements to be determined. Knowledge of 'desired composition' allows for product optimisation and validated models between descriptive sensory and the relevant instrumental and/or preference measures are highly desirable and are increasingly being utilised within the food industry (Murray *et al.*, 2001). However, it is pre-supposed that a 'trained' set of assessors, become a reliable, and valid, measuring instrument. This supposition, of course, depends on the quality of the sensory training carried out prior to profiling. Moreover, generalisation of profiling results to a consumer population is very difficult because sensory profiling is limited in that it focuses on non-affective parts of food perception. Actual food choice behaviour is affective ('liking') based, and the ultimate challenge often is to predict food choice (Dijksterhuis and Byrne, 2005). Sensory profiling methods are constantly undergoing further development and optimisation. It is envisaged that, in the future, such developments will further increase the reliability of descriptive analysis as a tool in sensory profiling and also increase the capability of these methods to evaluate and predict consumer preferences.

8.6 References

- Aaslyng, M.D., Oksama, M., Olsen, E.V., Bejerholm, C., Baltzer, M., Andersen, G., Bredie, W.L.P., Byrne, D.V. and Gabrielsen, G. (2007), The impact of sensory quality of pork on consumer preference. *Meat Science*, 7, 661–73.
- AMSA (1995), *Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat*. Chicago, Illinois: American Meat Science Association in cooperation with National Live Stock and Meat Board.
- Andrés, S., Silva, A., Soares-Pereira, A.L., Martins, C., Bruno-Soares A.M. and Murray I. (2008), The use of visible and near infrared reflectance spectroscopy to predict beef *M. longissimus thoracis et lumborum* quality attributes. *Meat Science*, 78 (3), 217–224.
- Bailey, M.E. (1988), Inhibition of warmed-over flavour with emphasis on Maillard reaction products. *Food Technology*, 42, 123–126.
- Barbut, S. (2001), Effect of illumination source on the appearance of fresh meat. *Meat Science*, 59, 187–191.
- Beilken, S.L., Eadie, L.M., Griffiths, I., Jones, P.N. and Harris, P.V. (1991), Assessment of the sensory characteristics of meat patties. *Journal of Food Science*, 56 (6), 1470–1475.
- Bertram, H.C. and Aaslyng, M.D. (2007), Pelvic suspension and fast post-mortem chilling: Effects on technological and sensory quality of pork – A combined NMR and sensory study. *Meat Science*, 76, 524–535.
- Boleman, S. J., Boleman, S.L., Miller, R. K. Taylor, J. F., Cross, H. R., Wheeler, T. L., Koohmaraie, M., Shackelford, S. D., Miller, M. F. West, R. L., Johnson, D. D. and Savell, J.W. (1997), Consumer evaluation of beef of known categories of tenderness. *Journal of Animal Science*, 75, 1521–1524.
- Brewer, M.S., Zhu, L.G. and McKeith, F.K. (2001), Marbling effects on quality characteristics of pork loin chops: Consumer purchase intent, visual and sensory characteristics. *Meat Science*, 59, 153–163.
- Byrne, D.V., Bak, L.S., Bredie, W.L.P., Bertelsen, G. and Martens, M. (1999a), Development of a sensory vocabulary for warmed-over flavour I: In porcine meat. *Journal of Sensory Studies*, 14, 47–65.
- Byrne, D. V., Bredie, W. L. P. and Martens, M. (1999b), Development of a sensory vocabulary for warmed-over flavour: Part II. In chicken meat. *Journal of Sensory Studies*, 14, 67–78.
- Byrne, D.V., Bredie, W.L.P., Bak, L.S., Bertelsen, G., Martens, H. and Martens, M. (2001a), Sensory and chemical analysis of cooked porcine meat patties in relation to warmed-over flavour and pre-slaughter stress. *Meat Science*, 59, 229–249.
- Byrne, D.V., O'Sullivan, M.G., Dijksterhuis, G.B., Bredie and Martens, M. (2001b), Sensory panel consistency during development of a vocabulary for warmed-over flavour. *Food Quality and Preference*, 12, 171–187.
- Byrne, D.V. and Bredie, W.L.P. (2002), Sensory meat quality and warmed-over flavour: A Review. In F. Toldrá, *Research advances in the quality of meat and meat products*. Volume within Agriculture and Food Chemistry, Research Signpost, 95–212.
- Byrne, D. V., Bredie, W. L. P., Mottram, D. S. and Martens, M. (2002a), Sensory and chemical investigations on the effect of oven cooking on warmed-over flavour development in chicken meat. *Meat Science*, 61, 127–139.
- Byrne, D.V., O'Sullivan, M.G., Bredie, W.L.P. and Martens, M. (2002b), Descriptive sensory profiling and physical/chemical analyses of warmed-over flavour in meat patties from carriers and non-carriers of the RN- allele. *Meat Science*, 63, 211–224.
- Canell, R. C., Belk, K. E., Tatum, J. D., Wise, J. W., Chapman, P. L., Scanga, J. A. et al. (2002), Online evaluation of a commercial video image analysis system (Computer Vision System) to predict beef carcass red meat yield and for augmenting the assignment of USDA yield grades. *Journal of Animal Science*, 80, 1195–1201.
- Carpenter, C.E., Cornforth, D.P. and Whittier, D. (2001), Consumer preferences for beef

- colour and packaging did not affect eating satisfaction. *Meat Science*, 57 (2001) 359–363.
- Chambers IV, E. and Bowers, J. (1993), Consumer perception of sensory quality in muscle foods: Sensory characteristics of meat influence consumer decisions. *Food Technology*, 47, 116–120.
- Chan, W.K.M., Hakkarainen, K. Faustman, C., Schaefer, D.M., Scheller, K.K. and Liu, Q. (1996), Dietary vitamin E effect on colour stability and sensory assessment of spoilage in three beef muscles. *Meat Science*, 42, 387–399.
- Delahunty, C.M., McCord, A., O'Neill, E.E. and Morrissey, P.A. (1997), Sensory characterisation of cooked hams by untrained consumers using free-choice profiling. *Food Quality and Preference*, 8, 381–388.
- Destefanis, G., Brugiapaglia, A., Barge, M.T. and Dal Molin, E. (2008), Relationship between beef consumer tenderness perception and Warner–Bratzler shear force. *Meat Science*, 78, 153–156.
- Dijksterhuis, G.M. and Byrne, D.V. (2005), Does the Mind Reflect the Mouth? Sensory Profiling and the Future. *Critical Reviews in Food Science and Nutrition*, 45, 527–534.
- Drumm, T.D. and Spanier, A.M. (1991), Changes in the lipid content of autoxidation and sulphur-containing compounds in cooked beef during storage. *Journal of Agriculture and Food Chemistry*, 49, 336–343.
- Enfält, A.C., Lundström, K., Hansson, I., Johansen, S. and Nyström, P.E. (1997), Composition of non-carriers and heterozygous carriers of the RN[−] allele for carcass composition, muscle distribution and technological quality. *Meat Science*, 45, 1–45.
- Harrington, G. and Pearson, A.M. (1962), Chew count as a measure of tenderness of pork pork loins with various degrees of marbling. *Journal of Food Science*, 27, 106–110.
- Hood, D.E. and Mead, G.C. (1993), Modified atmosphere storage of fresh meat and poultry, In R.T. Parry, *Principles and applications of modified atmosphere packing of food* (pp 269–298). London: Blackie Academic and Professional.
- ISO (1992), International Standard. 5492. *Sensory analysis – Vocabulary*. Ref. No. ISO 5492:1992 (E/F). International Organization for Standardization, Genève.
- ISO (1994), International Standard. 11035. *Sensory analysis – Identification and selection of descriptors establishing a sensory profile by a multidimensional approach*. Ref. No. ISO 11035:1994 (E). International Organization for Standardization, Genève.
- Jeremiah, L.E., Tong, A.K.W. and Gibson, L.L. (1991), The usefulness of muscle color and pH for segregating beef carcasses into tenderness groups. *Meat Science*, 30, 97–114.
- Johnson, P.B. and Civille, G.V. (1986), A standardized lexicon of meat WOF descriptors. *Journal of Sensory Studies*, 1, 99–104.
- Lanari, M.C., Schaefer, D.M. and Scheller, K.K. (1995), Dietary vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. *Meat Science*, 41, 237–250.
- Land, D.G. (1977), Flavour research in the ARC. *ARC Research Review*, 3, 5–8.
- Lawless, H.T. and Heymann, H. (1998), Descriptive analysis. In H.T. Lawless and H. Heymann. *Sensory Evaluation of Food, Principles and Practices* (pp 117–138, pp 341–378). New York: Chapman and Hall.
- Lawrie R.A. (1991), Chemical and Biochemical Constitution of Muscle. In *Meat Science*, 5th edn., (pp 71–72). Oxford: Pergamon Press.
- Liu, Q., Lanari, M.C. and Schaefer, D.M. (1995), A review of dietary vitamin E supplementation for improvement of beef quality. *Journal of Animal Science*, 73, 3131–3140.
- Lorenzen, C. L., Neely, T. R., Miller, R. K., Tatum, J. D., Wise, J. W., Taylor, J. F. *et al.* (1999), Beef customer satisfaction: Cooking method and degree of doneness effects on the top loin steak. *Journal of Animal Science*, 77, 637–644.
- Lorenzen, C. L., R. K. Miller, J. F. Taylor, T. R. Neely, J. D. Tatum, J. W. Wise, M. J. Buyck, J. O. Reagan and J. W. Savell (2003), Beef customer satisfaction: Trained sensory panel ratings and WarnerBratzler shear force values. *Journal of Animal Science*, 81, 143–149.

- Lund, M.N., Hviid, M.S. and Skibsted, L.H. (2007), The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. *Meat Science*, 76, 226–233.
- Lyon, B.G. (1987), Development of chicken flavour descriptive attribute terms aided by multivariate statistical procedures. *Journal of Sensory Studies*, 2, 55–67.
- Mancini, R.A. and Hunt, M.C. (2005) Current research in meat colour. *Meat Science*, 71, 100–121.
- McDonald, T. P. and Chen, Y. R. (1991), Visual characterization of marbling in beef ribeyes and its relationship to taste parameters. *Transactions of ASAE*, 34(6), 2499–2504.
- Meilgaard, M.C., Civille, G.V. and Carr, B.T. (1999), In *Sensory evaluation techniques*, 3rd edition, Chapter 5 (pp 54–55). Florida: Academic Press.
- Miller, R. (1994), Sensory methods to evaluate muscle foods. In D.M. Klinsman, A.W. Kotula, B.C. Breidenstein, *Muscle Foods: Meat Poultry and Seafood Technology* (pp. 333–360). New York: Chapman and Hall.
- Miller, M. F., Hoover, L.C., Cook, A.L., Guerra, A.L., Huffman, K.L., Tinney, K.S. *et al.* (1995), Consumer acceptability of beef steak tenderness in home and restaurant. *Journal of Food Science*, 60, 963–965.
- Miller, M.F., Carr, M.A., Ramsey, C.B., Crockett, K.L. and Hoover, L.C. (2001), Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science*, 79, 3062–3068.
- Muñoz, A.M. and Civille, G.V. (1992), The spectrum descriptive analysis method. In R.C. Hootman, *ASTM Manual on Descriptive Analysis*. Pennsylvania: American Society for Testing and Materials.
- Murray, J.M., Delahunty, C.M. and Baxter, I.A. (2001), Descriptive sensory analysis: Past, present and future. *Food Research International*, 34 (6), 461–471.
- Neely, T.R., Lorenzen, C.L., Miller, R.K., Tatum, J.D., Wise, J.W., Taylor, J.F. *et al.* (1998), Beef customer satisfaction: Role of cut, USDA quality grade, and city on in-home consumer ratings. *Journal of Animal Science*, 76, 1027–1033.
- Neely, T.R., Lorenzen, C.L., Miller, R.K., Tatum, J.D., Wise, J.W., Taylor, J.F. *et al.* (1999), Beef customer satisfaction: Cooking method and degree of doneness effects on the top round steak. *Journal of Animal Science*, 77, 653–660.
- Oreskovich, D.C., Klein, B.P. and Sutherland, J.W. (1991), Procrustes analysis and its applications to free choice and other sensory profiling. In: Lawless, H.T. and Klein, B.P., Editors, 1991. *Sensory science theory and applications in foods*, Marcel Dekker, New York, pp. 353–394.
- O'Sullivan, A., O'Sullivan, K., Galvin K., Moloney A.P., Troy D.J. and Kerry J.P. (2004), Influence of concentrate composition and forage type on retail packaged beef quality. *Journal of Animal Science*, 82, 2384–2391.
- O'Sullivan, M.G., Byrne, D.V., Stagsted, J. Andersen, H.J. and Martens, M. (2002a), Sensory colour assessment of fresh meat from pigs supplemented with iron and vitamin E. *Meat Science*, 60, 253–265.
- O'Sullivan, M.G., Byrne D. V. and Martens, M. (2002b), Data analytical methodologies in the development of a vocabulary for evaluation of meat quality *Journal of Sensory Studies*, 17, 539–558.
- O'Sullivan, M.G., Byrne D. V. and Martens, M. (2003a), Evaluation of pork colour: Sensory colour assessment using trained and untrained sensory panellists. *Meat Science*, 63, 119–129.
- O'Sullivan, M.G., Byrne, D.V., Nielsen, J.H., Andersen, H.J. and Martens, M. (2003b), Sensory and chemical assessment of pork supplemented with iron and vitamin E. *Meat Science*, 64, 175–189.
- Piggott, J.R., Sheen, M.R. and Guy, C. (1989), Bridging the gap between laboratory flavour research and the consumer. In: Charalambous, G., Editor, 1989. *Flavours and off-flavours, Proceedings of the 6th International Flavour Conference*, Elsevier Science, Amsterdam, pp. 543–552.

- Piggott, J.R., Simpson, S.J. and Williams, S.A.R. (1998), Sensory analysis. *International Journal of Food Science & Technology*, 33 (1), 7–12.
- Purchas, R.W. (1990), An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. *Meat Science*, 27, 129–140.
- Rowe L.J., Maddock K.R., Lonergan S.M and Huff-Lonergan E. (2004), Influence of early post-mortem protein oxidation on beef quality. *American Society of Animal Science*, 82, 785–793.
- Savell, J.W., H.R. Cross, J.J. Francis, J.W. Wise, D.S. Hale, D.L. Wilkes and G.C. Smith (1989), National Consumer Retail Beef Study: Interaction of trim level, price and grade on consumer acceptance of beef steaks and roasts. *Food Quality*, 12, 251–274.
- Shackelford, S.D., Morgan, J.B., Cross, H.R. and Savell, J.W. (1991), Identification of threshold levels for Warner–Bratzler shear force in beef top loin steaks. *Journal of Muscle Foods*, 2, 289–296.
- Shackelford, S.D., Wheeler, T.L. and Koohmaraie, M. (1997), Tenderness classification of beef: 1 Evaluation of beef *longissimus* shear force at 1 or 2 days post mortem as a predictor of aged beef tenderness. *Journal of Animal Science*, 75, 2417–2422.
- Smith, G.C., Savell, J.W., Cross, H.R., Carpenter, Z.L., Murphey, C.E., Davis, G.W., Abraham, H.C., Parrish, F.C. and Berry, B.W. (1987), Relationship of USDA quality grades to palatability of cooked beef. *Journal of Food Quality*, 10, 269–287.
- St. Angelo, A.J., Crippen, K.L., Depuy H.P. and James, C., Jr. (1990), Chemical and sensory studies of antioxidant-treated beef. *Journal of Food Science*, 55, 1501–1539.
- Stone, H., Sidel, J., Oliver, S., Woolsey, A. and Singleton, R.C. (1974), Sensory evaluation by quantitative descriptive analysis. *Food Technology*, 28, 24–34.
- Voges, K.L., Mason, C.L., Brooks, J.C., Delmore, R.J., Griffin, D.B., Hale, D.S., Henning, W.R., Johnson, D.D., Lorenzen, C.L., Maddock, R.J., Miller, R.K., Morgan, J.B., Baird, B.E., Gwartney B.L. and Savell, J.W. (2006), National beef tenderness survey – 2006: Assessment of Warner–Bratzler shear and sensory panel ratings for beef from US retail and foodservice establishments. *Meat Science*, 77, (3), 357–36.
- Watanabe, A., Daly, C.C. and Devine, C.E. (1996), The effects of the ultimate pH of meat on tenderness changes during aging. *Meat Science*, 42, 67–78.
- Wheeler, T.L., Shackelford, S.D. and Koohmaraie, M. (1997), Standardizing collection and interpretation of Warner–Bratzler shear force and sensory tenderness data. In *Proceedings 50th Annual Reciprocal Meat Conference* (pp. 68–77), Ames, IA.
- Wicklund, R.A., Paulson, D.D., Tucker, E.M., Stetzer, A.J., DeSantos, F., Rojas, M., MacFarlane B.J. and Brewer M.S. (2006), Effect of carbon monoxide and high oxygen modified atmosphere packaging and phosphate enhanced, case-ready pork chops. *Meat Science*, 74, (4), 704–709.
- Wulf, D.M., O'Connor, S.F., Tatum, J.D. and Smith, G.C. (1997), Using objective measures of color to predict beef *longissimus* tenderness. *Journal of Animal Science*, 75, 685–692.
- Wulf, D.M. and Page, J.K. (2000), Using measurements of muscle color, pH, and electrical impedance to augment the current USDA beef quality grading standards and improve the accuracy and precision of sorting carcasses into palatability groups. *Journal of Animal Science*, 78, 2595–2607.
- Zakrys, P.I., Hogan, S.A., O'Sullivan, M.G., Allan, P. and Kerry, J.P. (2007), Effects of oxygen concentration on the sensory evaluation and quality indicators of beef muscle packed under modified atmosphere. *Meat Science*, 79, 648–655.
- Zakrys, P.I., O'Sullivan, M.G., Allan, P. and Kerry, J.P. (2008), Consumer acceptability and physiochemical characteristics of modified atmosphere packed beef steaks. *Meat Science* (submitted for publication).
- Zanardi, E., Novelli, E., Nanni, N., Ghiretti, G.P., Delbono, G., Campanini, G., Dazi, G., Madarana, G. and Chizzolini, R. (1998), Oxidative stability and dietary treatment with vitamin E, oleic acid and copper of fresh and cooked pork chops. *Meat Science*, 49, 309–320.

Part II

Improving the quality of fresh meat: genetic and genomic technologies

New insights into the biology of meat quality from genomic and proteomic perspectives, with particular emphasis on beef

A. M. Mullen, L. Pannier and R. Hamill, Ashtown Food Research Centre, Teagasc, Ireland

Abstract: Optimal management of meat systems requires an appreciation of the factors that impact on the quality of the final product, including animal feeding and management regimes, slaughtering, and both carcass handling and processing post-slaughter. Much research effort has focused on optimising these factors to reduce the variation in beef and pork tenderness, water-holding capacity, colour and flavour; however, much remains unexplained. Since 2002, whole genome sequences and associated genomic tools have entered the public domain for high priority domestic animal species. Emanating from this research, molecular biologists are working towards identifying and understanding the actual genes and proteins that co-ordinate and regulate meat quality with a view to improving prediction and control of meat for market specifications. In this paper we review approaches to, and provide a synopsis of, genomics, proteomics and other ‘omics’ research in the meat science arena.

Key words: single nucleotide polymorphism, candidate gene, microarray, 2-D electrophoresis, biomarker, meat management system, meat quality.

9.1 Introduction

Optimal management of meat systems along the whole meat chain requires an appreciation of the factors which impact on the quality of the final product. This becomes particularly critical with increasing consumer demand for meat which is healthier and which offers consistency and high standards in quality.

Meat quality has been defined in many ways and interpretations vary among countries and members of the production chain (Hocquette and Gigli, 2005). The consumer is concerned with safety issues, health-related aspects and the

characteristics of sensory enjoyment. From an industry perspective, features such as yield and colour are of particular relevance. Quality traits defined by sensory analysis include tenderness, flavour, juiciness, colour and overall palatability. The overall and relative importance of these traits varies, depending on both the end product and the consumer profile (Koochmaraie and Geesink, 2006). A UK based study has shown that the three most important determinants of sensory enjoyment are flavour, tenderness and juiciness (McIlveen and Buchanan, 2001). Of these attributes, among others, Ouali, 1990, Warkup *et al.*, 1995, and Szczesniak, 1998, all concluded that beef tenderness was the primary determinant of satisfaction among beef consumers. In Norway, a recent study found that beef consumers were willing to pay 50% more for very tender beef and 25% more for tender beef compared with less tender beef (Alfnes *et al.*, 2005). Therefore, providing consistently tender beef should be a key priority for the beef industry. While there have been many successful efforts at improving the tenderness of beef, research has shown that an unacceptable level of variability still remains in beef tenderness (Maher *et al.*, 2004). A large amount of variability is also evident in other quality traits, such as water holding capacity, colour and flavour.

Many factors affect the quality of meat, including the way animals are fed, managed and slaughtered, and the way carcasses are handled and processed post-slaughter. While there is often emphasis on the management systems that can be implemented to meet market specifications, there has, until recent years, been little emphasis on factoring in the molecular or biological components of meat quality. We are now in an exciting period where many new opportunities are presented to researchers through the application of genomics, proteomics and other 'omic' approaches. Extracting useful information from the large amounts of data stemming from this research is a major challenge being addressed by the field of bioinformatics. In this paper we review approaches and provide a synopsis of the current status of transcriptomic and proteomic research in the meat science arena. For the purposes of this paper, quality will, in general, refer to palatability, and technological and nutritive aspects of quality.

Since 2002, considerable progress has been achieved towards the goal of placing whole genome sequences and associated genomic tools into the public domain for high priority domestic animal species. As of today, with the bovine genome nearing completion, scientists can facilitate a better understanding of the bovine genome structure and rapidly identify genetic markers associated with economically important phenotypes. Today the 7.1 fold coverage (Build 3.1, based on Btau_3.1, released Jan. '07) sequence of the bovine genome is released and is free to public access on various databases such as GenBank (<http://www.ncbi.nih.gov/Genbank>) at NIH (National Institutes of Health) and at EMBL (the European Molecular Biology Laboratory) (<http://www.ebi.ac.uk/embl/index.html>). The Swine Genome Sequencing Consortium aims to achieve draft 4x sequence depth across the genome (SGSC, 2003). The Sino-Danish pig genome sequencing project has generated a resource of 3.8 million shotgun reads with 0.66x coverage of redundancy of the 3.15 billion base pair pig genome and approximately 1 million EST sequences (Wernersson *et al.*, 2005).

9.2 Genetic markers

Genetic markers are variations in the DNA sequence between individuals which are associated with a trait of interest. There are many types of markers, which range from a single base-pair change (single nucleotide polymorphisms) through to larger microsatellites (repeats of 1–4 base pairs). In recent years, researchers in the meat and animal arena have focused on identification of genetic markers for meat quality and production traits. The economic benefits of small improvements in production or meat quality traits are significant and may be achievable through unravelling the relationship between the genome and these traits.

9.2.1 Single nucleotide polymorphisms

A single nucleotide polymorphism (SNP) refers to a variation in the DNA sequence between individuals. For example, GGAATC and GGAACC differ by a single nucleotide. In this situation there are two alleles: T and C. SNP mutations can occur anywhere in the coding sequences of genes, non-coding regions of genes or in the intergenic region (between genes). SNPs found in protein coding regions (exons) of genes that alter the coded protein sequence are termed non-synonymous and are of particular interest as they may alter protein function. Similarly, SNP in promoter regions may impact on gene expression levels and are also of particular interest. However, in some cases apparently synonymous SNPs may also influence the phenotype due to alternative splicing, and SNPs in the non-coding sequence may also be influential, or alternatively SNP may be linked to a causative SNP; thus, without having direct effects, such SNP may be used as markers for a trait in a particular population. There are several approaches to identifying novel marker SNP, including sequencing of candidate genes and the use of gene mapping techniques. In practice, the candidate gene approach is often combined with the mapping technique (Williams, 2005), for example, the localisation of a quantitative trait locus for subcutaneous fat in a region of chromosome 19, containing a candidate gene, the growth hormone locus (Taylor *et al.*, 1998).

9.2.2 SNP and meat quality

Many SNP markers have been identified (for examples, see [Table 9.1](#)) and some have been patented (Hocquette, 2005; Hocquette *et al.*, 2007). Several markers for tenderness in beef have been developed from the calpain I gene, a proteolytic enzyme (Page *et al.*, 2002 and 2004; White *et al.*, 2005; Casas *et al.*, 2006; Costello *et al.*, 2007), and its inhibitor, calpastatin (Barendse, 2002; Schenkel *et al.*, 2006). Candidate SNP for beef intramuscular fat level or marbling are found in the leptin gene (Buchanan *et al.*, 2007; Kononoff *et al.*, 2005; Lagonigro *et al.*, 2003; Schenkel *et al.*, 2005), the thyroglobulin gene (Barendse, 1999), the DGAT1 gene, which is also involved in the regulation of milk fat level (Grisart *et al.*, 2001; Thaller *et al.*, 2003), and the FABP4 gene (Michal *et al.*, 2006). SNP in the growth hormone gene are also associated with these traits (Barendse *et al.*, 2006; Di Stasio

et al., 2003). The stearoyl-coA desaturase gene (SCD), the fatty acid synthase gene (FASN) and the retinoic acid receptor-related orphan receptor C gene (RORC) are involved in fat metabolism in cattle. SCD is an enzyme catalysing the dehydrogenation of saturated fatty acids in mammalian adipocytes. The FASN gene plays a role in biosynthesis of long-chain fatty acids in mammals and SNPs in this gene are associated with variation in the fatty acid composition of adipose fat and milk fat (Morris *et al.*, 2007). RORC is a steroid and thyroid hormone receptor and binds retinoic acid as well as thyroid hormone. Polymorphisms in these genes have been identified and associated with marbling scores, MUFA content and milk fat content (Barendse *et al.*, 2007). Recently, genome-wide scans have led to the identification of SNP in novel candidate genes with fat metabolism traits, e.g. the novel nuclear-encoded mitochondrial poly (A) polymerase or PAPD1 gene, which contributes to extreme fat deposition in cattle (Michal *et al.*, 2008; Xiao *et al.*, 2006).

With pork, SNP markers have been successfully applied to reducing the frequency of undesirable traits in the population, ensuring more consistent meat quality (van der Steen *et al.*, 2005). The occurrence of a recessive mutation at the CRC1 (or halothane gene), which governs Ca^{2+} transport across muscle cell membranes, results in greater lean meat yield but reduced meat quality and increased susceptibility to stress-induced death in pigs, or porcine stress syndrome (Fujii *et al.*, 1991). A dominant mutation in the PRKAG3 gene results in meat that has a low pH and is associated with poor appearance and taste, and variation in cook loss (Milan *et al.*, 2000), and more recently a number of novel SNP mutations have been identified in this gene, some of which confer improved quality characteristics (Ciobanu *et al.*, 2001). Markers for this mutation are being used to select for more consistent quality meat (Milan *et al.*, 2000; van der Steen *et al.*, 2005). A particular haplotype in the calpastatin (CAST) gene is associated with quantitative variation in pork eating quality, including reduced shear force, lower cook loss and greater juiciness (Ciobanu, 2004). Traditionally in Europe, there has been strong selection for lean growth, whereas in China, pork meat with a high fat content has been selected. Mapping inter-breed crosses of Chinese and European pigs resulted in the identification of IGF-2 as a likely candidate present in the QTL region (Van Laere *et al.*, 2003). A single SNP was identified (G to A transition in intron 3 of the gene) that appears to be either a causative mutation or a quantitative trait nucleotide (QTN) (Van Laere *et al.*, 2003). Genes in the leptin pathway are proving profitable in association studies with growth and backfat in pork, e.g. leptin, the leptin receptor gene and the MC4R gene (Kim *et al.*, 2004; Ovilo *et al.*, 2006; Rothschild *et al.*, 2004). An important candidate gene family for muscle growth is the myogenic regulatory factor (MRF) gene family. The Myo-D and myf-5 genes regulate proliferation of myoblasts and satellite cells (te Pas, 2003) and are associated with growth traits in pigs (te Pas *et al.*, 1999).

Markers for lamb quality are fewer to date. The callipyge phenotype is related to a SNP on ovine chromosome 18 with a complex mode of inheritance (Freking *et al.*, 2002), and causes hypertrophy in sheep buttocks, which are, however, less tender and palatable as a consequence. A marker for the Texel breed-related

hypertrophy has also been described (Marcq *et al.*, 2002). Recently, an SNP in the ovine myostatin (GDF8) gene has been found to influence intramuscular fat and eating quality (Kijas *et al.*, 2007).

9.2.3 Applications of SNP markers

Current commercial applications

At present, the increased profit due to the incorporation of molecular markers in selection programmes is derived mainly from bulls with favourable allelic combinations achieving increased market share of breeding stock (Dekkers, 2004). There are a number of companies currently marketing commercial tests for polymorphisms in genes that are related to particular meat quality phenotypes in beef and sheep production. Companies include Igenity® Merial, Catapult Genetics and MMI genomics. For tenderness tests such as GeneSTAR® Tenderness and Igenity TenderGENE®, the genes typed include calpain, an enzyme that breaks down the fibres of meat, increasing tenderness, and calpastatin, its inhibitor (Barendse, 2002; Page *et al.*, 2002, 2004; Schenkel *et al.*, 2006; White *et al.*, 2005). Tests for intramuscular fat, or marbling, are based on genes linked to pathways involved in fat metabolism, e.g. thyroglobulin and leptin. Genotyping companies recommend breeders select sires with high average scores across markers (i.e. high proportion of desirable variants) or subgroup according to genotype and thus manage animals for different goals, e.g. Igenity® Carcass composition. Although many markers have been identified, and some independently validated, the proportion of phenotypic variance explained has tended to be small (Van Eenennaam *et al.*, 2007). More recently, MMI genomics in collaboration with Cargill, have developed large-scale SNP tests (MMI Genomics TruMarbling™ – 128 markers for Marbling, TruTenderness™ – 11 for tenderness) that purport to explain a larger proportion of the total variance in these traits (up to 25% of total phenotypic variation in quality grade, or 70% of heritable variation, in their own studies of Angus herds). Implementation of MAS on a commercial basis requires careful consideration of issues, ranging from sample collection and storage to genotyping and data analysis (Dekkers, 2004).

Potential applications

One of the main potential applications of genomic discoveries is in the area of marker-assisted selection. Alleles that have significant physiological associations with meat quality may be combined with estimated breeding values (EBVs) in a process known as marker-assisted selection (MAS; Kuhn *et al.*, 2005). MAS has particular advantages when the trait under consideration is difficult or impossible to measure in the live animal; for example, those that are assessed after the end of an individual's reproductive period and those that are measured post-mortem (e.g. many meat quality traits) (Dekkers, 2004). Important meat quality traits that may benefit from MAS could include meat pH, marbling, and tenderness (Kuhn *et al.*, 2005). Meuwissen and Goddard (1996) considered the impact of MAS for

different types of traits. For meat quality, an additional response of up to 64% could be achieved in selection programmes with the incorporation of marker information. This contrasts with 8% additional response for growth traits, which are measurable in life. Additionally, precision management systems could be greatly enhanced using the tools of genomics. Meat management systems have, to date, focused largely on optimising the processing and production environments (Thompson, 2002; Thompson *et al.*, 2006; Polkinghorne, 2006) but the incorporation of genetic markers into such systems could increase the accuracy of models and is currently being explored. The ability to precisely control the timing and extent of gene expression for growth-related genes such as myostatin or growth hormone also has great potential.

SNP chips and whole genome selection

The Affymetrix® targeted genotyping bovine 25K SNP Panel provides genotype analysis of approximately 25 000 SNPs identified through the bovine genome project and validated over 15 *Bos taurus* and *Bos indicus* breeds. In January 2008, a 12-sample genotyping platform (Infinium® BovineSNP50 BeadChip), developed in collaboration with the USDA-ARS, universities and other industry partners, was released by Illumina®, Inc. This chip features more than 54 000 SNPs, evenly spaced across the entire bovine genome (Sellner *et al.*, 2007). These platforms will accelerate both genetic mapping of meat quality traits and the implementation of genomic or 'whole genome' selection (Dekkers, 2007; Meuwissen *et al.*, 2001; Muir, 2007; Windig and Meuwissen, 2004). Currently, one of the most exciting prospects for cattle genomics, 'genomic selection' could revolutionise quantitative genetic improvement in dairy and beef cattle herds (Sellner *et al.*, 2007). The ability to predict the total genetic merit of livestock using molecular or biochemical markers would allow the opportunity to completely redesign animal breeding and management programmes. The selection process aims to capitalise on the availability of dense SNP maps, coupled with associated haplotype information, and select animals based on their genetic profile at thousands of SNP loci (Meuwissen *et al.*, 2001). It is a tool that could be used to enable rapid genetic progress to be made in economically important traits such as meat quality traits, which have always proved difficult to improve by conventional means, while simultaneously reducing costs of progeny testing for industry (USDA Animal Genomics Strategic Planning Task Force Report, 2007).

With minor exceptions, the cattle genome sequence will serve as a platform for the genome of goats, sheep, buffalo and other artiodactyls (Gallagher *et al.*, 1994) and the porcine genome project is currently under way with 0.66× coverage already published. It is likely that progress in transcriptomics will further stimulate the path to candidate gene and SNP discovery. The importance of genetic markers in selection is certain to grow in the coming years and the new focus is on a balanced approach to ensure improvements across multiple economically important meat quality, production, health and welfare and reproductive traits (Williams, 2005).

9.3 Functional genomics

Functional genomics is the study of the collective activities and dynamic interaction of the set of genes and proteins in the cell. Transcriptomics refers to the study of the set of all messenger RNA (mRNA) molecules, or 'transcripts', produced in one or a population of cells. Proteomics refers to the large-scale study of proteins (the proteome is the full complement of proteins in a cell, tissue or organism under particular conditions). Bioinformatics is an essential aspect of functional genomics due to the challenges presented in extracting biological meaning from the large quantity of data generated.

9.3.1 Transcriptomics

Techniques for evaluating gene expression have progressed over the past 2–3 decades, from methods developed for the analysis of single specific genes (northern, slot and dot blotting, semi-quantitative Polymerase Chain Reaction, PCR) to those focused on identifying a range of genes that differ in expression between experimental samples. Broad-spectrum approaches to identify differences in gene expression include suppressive subtractive hybridisation (SSH; Wan *et al.*, 2002; Mohan *et al.*, 2004), differential display (Liang and Pardee, 1992; Davis *et al.*, 1996), serial analysis of gene expression (SAGE; Velculescu *et al.*, 1995; Graff *et al.*, 2006) and microarray hybridisation (Schena *et al.*, 1995; Moody, 2001; Rinaudo and Schultz, 2004).

Microarray-based technologies have become the most widely used analytical techniques for the study of gene expression patterns on a genome-wide scale (Leung and Cavalieri, 2003; Schena *et al.*, 1995). Microarray analysis provides the potential for linking specific changes in gene expression events to a phenotype of interest. Microarrays consist of sequences of thousands of genes attached to small solid supports, e.g. glass microscope slides. The sequences, or spots, can be DNA, RNA-complementary cDNA or oligonucleotides and are attached at fixed locations. Array dimensions range from hundreds of probes, to tens of thousands. This contrasts with 'single gene' studies where throughput is very limited. Over the past decade, DNA microarray technology has become more affordable. Progress in ongoing genome projects for cow (Womack, 2006), pig (Wernersson *et al.*, 2005), chicken (Antin and Konieczka, 2005) and sheep (Cockett *et al.*, 2001) projects, which are at various stages of advancement, has resulted in the development of species-specific microarrays which have become an invaluable exploratory tool for food scientists. For example, the GeneChip®Bovine Genome Array contains 24 027 probe sets which can be used to study gene expression of over 23 000 bovine transcripts. In recent years, the focus of much research has turned to the functional aspects of genes and how expression controls protein production and ultimately the phenotypic characteristics of a trait.

While microarrays provide information on differentially expressed genes and enhance our understanding of the biological pathways which underlie the delivery of consistent quality meat, they are not without their limitations or technical difficulties. The number of sequences or genes represented on the array

immediately determines or limits the number of genes that can be assessed, and reproducibility is an issue which has been raised in relation to microarrays (Hocquette, 2005). Data normalisation is a major component of any microarray experiment, with the goal of removing non-biological influences on biological data, thus permitting comparison between arrays. Many inconsistencies within microarray experiments can be overcome through good initial experimental design, standardisation of laboratory practice and assessment of the quality of the samples at critical points throughout the process. The Minimum Information About a Microarray Experiment (MIAME) is a protocol that attempts to address the issue of reproducibility in order to enable the unambiguous interpretation of the results of individual microarray experiments and to permit reproduction of the experiment (Brazma *et al.*, 2001). For reviews on features of microarray experiments, see, for example, Allison *et al.* (2006); Breitling (2006); Pan *et al.* (2003); Wilson *et al.* (2003).

9.3.2 Transcriptomics and meat research

Functional genomic studies allow the detection of genes which are actively transcribed at any given time, depending on environmental factors. Several research efforts to construct cDNA libraries from different tissues, including liver and intestine (Dorroch *et al.*, 2001), bovine embryos (Potts *et al.*, 2003), uterus and ovaries and bovine muscles (Sudre *et al.*, 2005) or bovine muscle and adipose tissue (Lehnert *et al.*, 2004) have been completed. From these bovine cDNA libraries, arrays have been constructed (Cho *et al.*, 2002; Lehnert *et al.*, 2004). For example, Cooperative Research Centre (CRC) for Cattle and Beef Quality in Australia developed a bovine cDNA microarray of 9600 elements, printed in duplicate, comprising 1915 bovine expressed sequence tags selected from various library sources and 7291 anonymous cDNAs clones from bovine skeletal muscle and fat cDNA libraries (Reverter *et al.*, 2003; Lehnert *et al.*, 2004). A bovine muscle-specific microarray was developed in Ireland as part of collaborative research between the Ashtown Food Research Centre – Teagasc, University College Dublin and the National Diagnostics Centre. Using this cDNA array, 61 differentially expressed genes that may be associated with beef tenderness and intramuscular fat were identified (Pannier, 2008).

Microarrays have been used to examine gene expression profiles of numerous biological systems in livestock. Campbell *et al.* (2001) demonstrated that microarrays can provide insights into muscle biology. They compared white and mixed red fibre types and developed a global gene expression profile which identified 49 genes as being differentially expressed between the muscle types. Carson *et al.* (2002) used rat muscles to investigate the global changes in gene expression induced by work overload. Using their bovine cDNA microarray, Reverter *et al.* (2003) compared gene expression profiles of muscle in steers fed varying quality diets. Secondly, the expression profiles between two breeds of cattle were compared at three time points in development: 11, 15 and 20 months of age. Thirdly, mechanisms underlying *in vitro* adipogenesis were studied in fibroblast

Table 9.2 Examples of bovine muscle studies carried out with microarray technology

Array	Identification of differentially expressed genes	Reference
Bovine 0.5K cDNA array (NDC)	Between tough and tender <i>longissimus</i> from cross-bred cattle	Pannier, 2008
Bovine 0.5K cDNA array (NDC)	Between high IMF and low IMF <i>longissimus</i>	Pannier, 2008
Human skeletal muscle cDNA	During muscle ontogenesis	Sudre <i>et al.</i> , 2003
Bovine skeletal muscle and fat cDNA array	Between different feeding regimes	Reverter <i>et al.</i> , 2003
Bovine skeletal muscle and fat cDNA array	Between Brahman steers fed with different diets	Byrne <i>et al.</i> , 2005
Bovine skeletal muscle and fat cDNA array	Between Japanese Black and Holstein cattle	Wang <i>et al.</i> , 2005
Bovine skeletal muscle and fat cDNA array	Between foetuses from Wagyu × Hereford and Piedmontese × Hereford crosses	Lehnert <i>et al.</i> , 2007
Human and murine oligonucleotide array	Between normal and double-muscled cattle	Cassar-Malek <i>et al.</i> , 2007
Human and murine oligonucleotide array	Between beef meat cuts differing in tenderness, juiciness and flavour	Bernard <i>et al.</i> , 2007

cell cultures during muscle ontogenesis (Sudre *et al.*, 2003) and between different feeding regimes (Reverter *et al.*, 2003; reviewed by Hocquette *et al.*, 2005).

Several bovine studies carried out with microarray technology are listed in Table 9.2 adapted from (Pannier, 2008). Some studies have investigated the genomic basis of meat quality traits, such as intramuscular fat and tenderness. Some of these have shown the genetic potential of fat in the early stages of life (Wang *et al.*, 2005; Lehnert *et al.*, 2007) and confirm that the cellular development of adipocytes is fixed relatively early in life. Wang *et al.* (2005) investigated differential gene expression in the *longissimus* muscle of Japanese Black and Holstein cattle at 11.5 months of age. The results indicated that, at 11 months, the genes associated with adipogenesis, mono-unsaturated fatty acid synthesis and fatty acid accumulation were highly expressed in Japanese Black cattle. Differential expression has been monitored in the *longissimus* muscle from foetuses from both Wagyu × Hereford and Piedmontese × Hereford crosses, sampled at 4 different time points (60, 135, 195 days and at birth) during foetal development (Lehnert *et al.*, 2007). The majority of changes in gene expression in foetal *longissimus* muscle comprised structural and metabolic components of extracellular matrix and muscle fibres. Genes associated with adipogenesis/lipogenesis (e.g. FABP4 and FABP5) were differentially expressed between Wagyu and Piedmontese sires at both foetal and new-born stages of development. A new potential candidate gene for sensory tenderness has been identified: the DNAJA1 gene showed a strong negative correlation with meat tenderness in Charolais cattle (Bernard *et al.*, 2006, 2007).

Examples of gene expression discovery studies in porcine research include the

construction of a porcine *biceps femoris* muscle cDNA library, which identified 72 unique clones (Davoli *et al.*, 1999). Two porcine cDNA libraries (MARC 1PIG and MARC 2PIG), derived from embryonic and reproductive tissues, respectively, were also constructed, sequenced and analysed (Fahrenkrug *et al.*, 2002). A *Sus scrofa* Gene Index (SsGI), including all sequences in public repositories, was developed to facilitate further characterisation of porcine genes (Fahrenkrug *et al.*, 2002). From the *longissimus dorsi* of a 50-day porcine foetus and the gastrocnemius of a three-day old pig, 5500 clones were created to form the basis of a skeletal muscle cDNA microarray (Bai *et al.*, 2003). Also, a porcine brain cDNA library was generated and a cDNA microarray produced using 877 unique porcine brain EST amplicons spotted in triplicate on glass slides (Nobis *et al.*, 2003). Prior to this, only two publicly available porcine cDNA microarrays existed, both constructed from skeletal muscle cDNA libraries (Yao *et al.*, 2002, Bai *et al.*, 2003). cDNA, which was created based on porcine ovary and follicular RNA from an index line selected for higher litter size or a control line, was co-hybridized with 4600 follicle-derived probes to study gene expression patterns related to reproductive efficiency (Pomp *et al.*, 2001; Caetano *et al.*, 2004). Recently, prenatal muscle tissue expression profiles of two pig breeds (Duroc and Pietrain) differing in muscle characteristics were compared (Cagnazzo *et al.*, 2006). Samples from each breed were hybridised onto arrays containing more than 500 genes affecting myogenesis, energy metabolism, muscle structural genes and other genes from a porcine muscle cDNA library (Davoli *et al.*, 1999, 2002). Currently, an Affymetrix array is available covering 20 201 genes in the porcine genome (www.affymetrix.com) and expression patterns of adult Pietrain and Duroc breeds have been profiled on these chips (Hughes *et al.*, 2007).

Once a microarray experiment is completed, it is necessary to biologically validate the results. Real-time PCR is often referred to as the 'gold standard' for gene expression measurements due to its advantages in sequence specificity, detection sensitivity and large dynamic range, as well as its high precision and reproducible quantitation compared to other techniques (Wang *et al.*, 2006). For microarray validation, a subset of genes may be chosen to represent the variation in differential gene expression in the experiment, and transcript abundance may be estimated using either relative or absolute quantification (Wong and Medrano, 2005). Absolute quantification relies on a standard curve of known cDNA concentration to estimate the precise number of mRNA copies present in a given sample (Bustin, 2000). Relative quantification determines the relative expression ratio from the real-time PCR efficiencies and the crossing point deviation of an unknown sample versus a control (Pfaffl, 2001). Control samples are included in the model to standardise each reaction run with respect to RNA integrity, sample loading and inter-PCR variations. The selection of suitable endogenous controls is challenging for non-model species, and currently, the use of a single gene to normalise real-time PCR is no longer considered sufficient. Software has been developed to identify the most stable endogenous controls in a panel, e.g. geNorm, BestKeeper and Norm Finder (Vandesompele *et al.*, 2002; Pfaffl *et al.*, 2004; Robinson *et al.*, 2007).

Quantitative Real-time PCR can also be used for exploratory analysis of candidate genes, and several transcriptomic studies have permitted insight to be gained into the response of individual candidate genes and biochemical pathways underlying variation in animals and tissues differing in growth, muscling and meat quality traits, including intramuscular fat level (Gerbens *et al.*, 2001; Listrat *et al.*, 2005; te Pas *et al.*, 2003; Thue *et al.*, 2001; Yang *et al.*, 2003).

9.4 Proteomics

Proteomics is the study of the whole cell protein content or proteome. Since proteins are frequently the functional molecules, they are most likely to reflect differences in gene expression. Proteomics can be defined as the systematic determination of protein sequence, quantity, modification state, interaction partners, activity, subcellular localisation, and structure in a given cell type at a particular time (Anon, 2003). Proteome analysis is a direct measurement of proteins in terms of their presence and relative abundance (Wilkins *et al.*, 1996). Neither genomic DNA code nor the amount of mRNA that is expressed for each protein yields an accurate picture of the state of a cell. This is because genes may be present but not transcribed and the number of mRNA copies does not always reflect the number of functional proteins present (Celis *et al.*, 2000). The aim of proteomics is to obtain information about cellular protein expression and hence to reveal the function of genes, with the ultimate goal of explaining how heredity and environment interact to control cellular functions (Bendixen, 2005). However, global proteome analysis is a difficult task, as described by Ghaemmaghami *et al.* (2003), who successfully reported a complete protein census for yeast. Equally, this approach is beneficial when focusing on a selected set of proteins. For example, with regard to proteins relevant to meat quality, working with myofibrillar, exudate or sarcoplasmic extracts may be more manageable than attempting to examine the whole protein complement and can facilitate detection of some of the lower abundance proteins (Sierra *et al.*, 2005; Sayd *et al.*, 2006). Proteomics can address problems that cannot be approached using DNA analysis. As well as functional aspects, these problems include estimation of the relative abundance of the protein product, its post-translational modification, subcellular localization, turnover and interaction with other proteins (Celis *et al.*, 2000; Stagsted *et al.*, 2004).

There are two approaches to proteome characterisation, namely comparative proteomics and mapping proteomics. Mapping proteomics is similar to genome sequencing projects and aims to characterise and make comprehensive databases of 'cellular proteomes' (Bendixen *et al.*, 2005). However, this is a huge task, partly due to the complex variety of modification forms most proteins possess (Mann and Jensen, 2003), and also because the proteome constantly changes with time and physiological state. In every sense, every single cell and organism has an infinite number of proteomes. Comparative proteomics aims to characterise the biological mechanisms which form the link between observable phenotypes and genotypes,

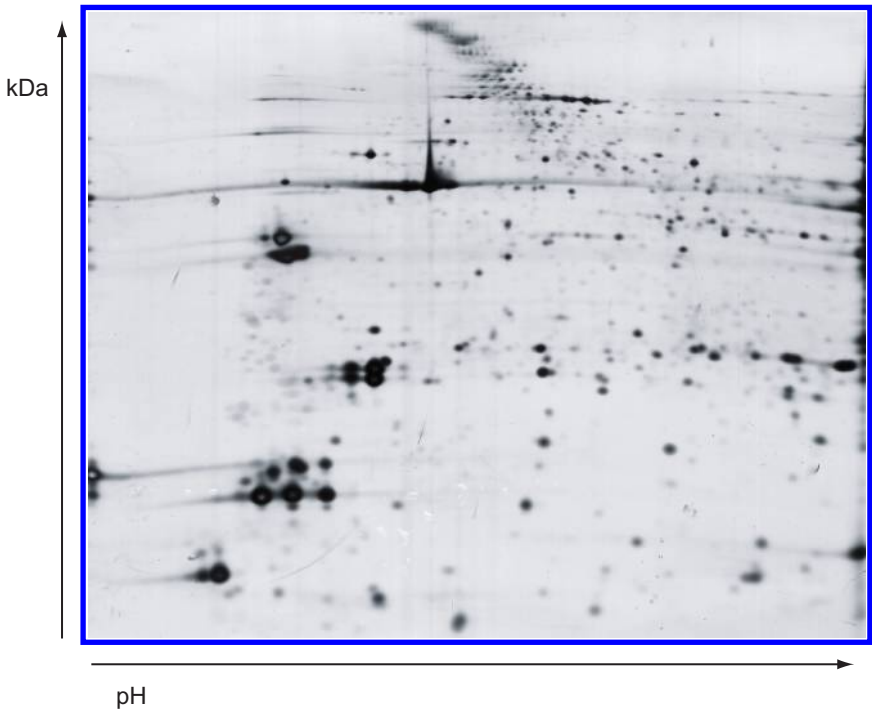


Fig. 9.1 2-D electrophoretic separation of bovine myofibrillar proteins from *M. longissimus thoracis et lumborum* at day 14 post-mortem over a pH range of 4–7 (Downey *et al.*, 2008).

thereby taking moment-by-moment snapshots of cellular responses at the protein level (Hunter *et al.*, 2002).

9.4.1 Protein technologies

Due to the abundance and diversity of proteins and the vast amount of data that can be generated, the production, processing and interpretation of proteomic data are complex. Mammalian tissue samples typically contain between 10 000 and 30 000 different protein species, hence a wide range of technologies must be used to prepare, separate and quantify the relative expression levels of thousands of proteins in parallel (Bendixen, 2005).

Two-dimensional electrophoresis (2DE) is commonly used in studying the proteome and involves two separation parameters, isoelectric point and molecular weight, which can improve resolution in the fractionation of complex mixtures of proteins, allowing multiple proteins to be separated for parallel analysis. Difference Gel Electrophoresis (DiGE) is a modification of 2-D PAGE (Unlu *et al.*, 1997). Two or three separate protein samples are labelled with different fluorescent dyes prior to separation, enabling a more accurate analysis of differences in protein abundance between samples. 2DE has some limitations which the

researcher needs to be aware of, e.g. co-migration of proteins and discrimination against basic and hydrophobic proteins. Some of these limitations may, however, be advantageous, for example, biomarker discovery discriminating against some proteins limits the amount of data to be processed at the initial stages while still producing a range of protein spots to be analysed. A further shortcoming of 2DE analysis is that, when it is applied to complex unfractionated samples, the limited dynamic range of 2DE only allows for the most abundant proteins to be analysed (Pedersen *et al.*, 2003). However, pre-fractionation of complex samples may be used to by-pass this problem (Gorg *et al.*, 2002; Spandidos and Rabbitts, 2002).

Two main applications of mass spectrometry (MS) have emerged in proteomic studies. The first is the identification of protein spots from 2DE analysis or cruder extracts, the second is comparative proteomics. Other techniques, such as electrospray ionisation (ESI) (Fenn *et al.*, 1989) and matrix-assisted laser desorption ionisation (MALDI) (Karas, 1996), are used for protein identification. MALDI can then be combined with a time-of-flight (ToF) mass analyser. This technology is ideally suited to protein mass fingerprinting (PMF) which analyses proteolytically digested proteins, e.g. trypsin digest. MS of peptides is not quantitative, but qualitative, as the ionisation capabilities of peptides are unpredictable (Lim *et al.*, 2003). Quantitative MS-based comparative proteomics methods, for example, Isotope Coded Affinity Tag labelling (ICAT), can overcome the shortcomings of 2DE analysis. ICAT allows for chemical tagging of proteins from different samples. New technologies with increased speed and sensitivity allow MS-based proteomics to become a more powerful tool. Examples of these include hybrid linear ion trap and fourier transform ion resonance cyclotron (FTICR; Le Blanc *et al.*, 2003; Belov *et al.*, 2004).

Protein microarrays are highly sensitive methods of proteome analysis that are miniaturised solid phase ligand binding assay systems using immobilised proteins. The technique involves using a probe that is specific for a particular analyte, which is placed at a defined position on a surface. The basic principles have been discussed by Elkins *et al.* (1989). However, the application of protein microarrays to proteomics is not very advanced when compared to that of DNA, 2DE and MS. While highly effective, it has limitations, including the fact that proteins expressed at low abundance may be missed. It is possible that many of the changes affecting protein levels may involve low abundance peptides (Celis *et al.*, 2000). Microarray technology may be advantageous in this respect.

9.4.2 Data analysis

Developments in the area of proteomics have increased the amount of data produced for analysis. An efficient use of the large amount of data generated is vital to achieve the most from proteomic research. Patterson (2003) stated that our ability to generate data now outstrips our ability to analyse it. For this reason, image and data analysis is of major importance to proteome research. The task of image analysis by comparing the relative volumes of individual spots on different gels in order to identify differentially expressed proteins is time consuming;

therefore, methods for automated image and data analysis have been suggested. Meunier *et al.* (2005) reported that statistical methods used for microarray analysis which identify a small number of differentially expressed genes may provide a useful method for quantitative determination of differentially expressed proteins. Recent developments in mathematical approaches to data analysis are helping to decode complex 2D-PAGE maps (Jessen *et al.*, 2002). Examples include the statistical model of peak overlapping (SMO), which is used for the statistical quantification of the degree of spot overlapping present in a map, and the 2-D auto covariance function (2D-ACVF) method which enables simple display of a comprehensive description of the whole map and offers simplified qualitative and quantitative information on the composition of the complex mixture (Pietrogrande *et al.*, 2006). Computational analysis for proteomics has also been developed, e.g. automated trend analysis (Malone *et al.*, 2006). As recently described by Hollung *et al.* (2007), several statistical approaches have been used to analyse proteomics data. Multivariate analyses, such as principal component analysis (PCA) (Martens and Martens, 2000; Næs *et al.*, 2002), are now included in several software packages for analysis of 2-DE experiments. Multivariate approaches have also been used (Jessen *et al.*, 2002; Jia *et al.*, 2006; K *et al.*, 2006), as have assessment of hierarchical clustering methodologies (Meunier *et al.*, 2007). The divergent statistical methods will shed light on divergent aspects of the proteomics data, as has been discussed in several papers (Jacobsen *et al.*, 2007; Maurer *et al.*, 2005).

Data handling from non-gel based systems must also be considered. Difficulties in data handling include the fact that many MS/MS spectra are not of peptides, but of instrument noise or minor contaminants, and their analysis consumes considerable computing time (Patterson, 2003). Thousands of MS spectra are generated during a comparative proteome study and extracting information from the data involves a series of analytical steps. However, improved algorithms and software are continuously being created (Bendixen, 2005; Chamrad *et al.*, 2003). For ease of visual inspection of large data sets and immediate identification of relevant differences, it may be a useful technique to represent MS-based data in a similar way to a 2DE gel. For these reasons 2D-MS mapping may be used, which is the visualisation of MS data in a pseudo-two-dimensional map (Roesli *et al.*, 2006).

9.4.3 Proteomics and meat research

Applications of proteomics in meat research have recently been reviewed (Hollung *et al.*, 2007). In a study focusing on changes in the muscle proteome in relation to Warner–Bratzler shear force, Lametsch and colleagues (2003) identified six proteins of interest. In particular, a quantitative increase in fragments of actin was observed, over the post-mortem ageing period (Lametsch *et al.*, 2002). Correlations with shear force were observed between protein fragments (actin, myosin heavy chain), myosin light chain II and the glycolytic enzyme triose phosphate isomerase. Some of these fragments were also associated with tenderness in a study by Hwang *et al.* (2005). In another study, proteome analysis of SM muscle from normal hams and from PSE-zones of defective hams demonstrated a

reduced proteolysis of troponin T, MLC 1, and alpha-crystallin in the defect muscles (Laville *et al.*, 2005).

Earlier studies using 1-D electrophoresis have presented researchers with insight into the proteolytic degradation that occurs in muscle during the post-mortem period. This knowledge has been greatly expanded through the advent of more sensitive tools of proteomic technologies. For example, while it was generally considered that actin was not degraded during the post-mortem period (Bandman and Zdanis, 1988; Huff-Lonergan *et al.*, 1995; Koohmaraie, 1994), recent 2-D based proteomic studies have highlighted the post-mortem appearance of actin fragments. Proteomics has been used to study changes occurring in muscle during post-mortem storage. Total protein extracts from porcine *longissimus* samples collected from slaughter through to 2 days post-mortem have revealed that 15 proteins were altered during this period (Lametsch and Bendixen, 2001; Lametsch *et al.*, 2002). Several of these proteins were identified as fragments of structural proteins such as actin, myosin heavy chain and troponin T.

During post-mortem storage, the calpain system is believed to be important for degradation of myofibrillar proteins and development of tenderness (Koohmaraie, 1994; Koohmaraie and Geesink, 2006). In a proteome study in pork LD muscle, several of the myofibrillar substrates for μ -calpain were identified and include desmin, actin, myosin heavy chain, myosin light chain I, troponin T, tropomyosin a1, tropomyosin a4, thioredoxin and CapZ (Lametsch *et al.*, 2004). Changes in the muscle proteome were observed between slaughter and 24 h storage in bovine *longissimus* and semitendinosus muscles (Jia *et al.*, 2006). In this study, five proteins were changed in both muscles, namely coWlin, lactoylglutathione lyase, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP27KDa and HSP20KDa. However, 15 proteins were changed in either *longissimus* or *semitendinosus* muscles. These divergences reflect distinct metabolic and physiological functions of the different muscles. Proteomic studies have also been targeted at understanding how environmental (transport, lairage, stunning, etc.) factors can influence the proteome and ultimately meat quality. Jia *et al.* (2006) identified 24 protein spots and demonstrated that metabolic enzymes and stress-related proteins were increased after slaughter.

As this research expands, we can anticipate a greater elucidation of the molecular processes underlying meat quality traits. In addition, we can gain valuable information regarding the mechanisms through which environmental factors (pre- and post-slaughter) influence quality through their impact on the proteome. Knowledge gained through these approaches will contribute to the optimisation of whole chain management systems for consistent quality meat.

9.5 Summary

Many exciting discoveries have been made through investigation of the genome and proteome in relation to meat quality, which are relevant to the meat industry. Potential applications of this research encompass improvements to traditional

breeding programmes, diagnostic tests for quality and management systems for quality. While there is often emphasis on the management systems that can be implemented to meet market specifications, there has, until recent years, been little emphasis on factoring in the molecular or biological components of meat quality. Through our ongoing appreciation of muscle molecular signatures and how they interact with environmental stimuli, management systems can be optimised on the basis of genotype, to deliver consistent quality meat.

The expanding development and rapid advances in molecular and quantitative genetics, reproduction technologies, animal nutrition and muscle science, carry with them a huge potential. Genes and proteins do not function independently; they participate in complex networks that ultimately give rise to cellular functions, tissues, organs and organisms. We have gained great insights through investigating single proteins or single pathways within the muscle cell. However, we now need to adopt a more holistic approach to understand how cellular processes interact within an animal, in response to environmental factors and in the delivery of consistent quality meat. Knowledge gained will benefit scientists and industry alike. Incorporation of this data into a beef management system such as Meat Standards Australia (MSA) will assist in defining management systems which are designed for genotype.

9.6 Acknowledgements

We wish to acknowledge the Irish National Development Plan under the Food Institutional Research Measure (FIRM), the Teagasc Biotechnology Initiative and the Sixth Framework Programme of the European Union. Thanks to E. Downey for providing images for [Fig. 9.1](#).

9.7 References

- Alfnes, F., Rickerten, K. and Ueland, O. (2005), Experimental evidence of risk aversion in consumer markets: The case of beef tenderness. In *Proceedings of the 11th International Congress of European Association of Agricultural Economists*. 1–12.
- Allison, D.B., Page, G.P., Beasley, T.M. and Edwards, J.W. (Eds.) (2006), *DNA Microarrays and Related Genomics Techniques: Design, Analysis, and Interpretation of Experiments*, Boca Raton: Chapman and Hall/CRC, 2006, ISBN 0-8247-5461-1, 371 pp.
- Anon. (2003), Biotechnology editorial, *Nature*, 21 (3), 213.
- Antin, P.B. and J.H. Konieczka (2005), Genomic resources for chicken. *Developmental Dynamics*, 232, 877–882.
- Bai, Q., McGillivray, C., Da Costa, N., Dornan, S., Evans, G., Stear, M.J. and Chang, K.C. (2003), Development of a porcine skeletal muscle cDNA microarray: Analysis of differential transcript expression in phenotypically distinct muscles. *BMC Genomics*, 4(8), E1–E13.
- Bandman, E. and Zdanis, D. (1988), An immunological method to assess protein-degradation in post-mortem muscle. *Meat Science*, 22, 1–19.
- Barendse, W. (1999), Assessing lipid metabolism. Int. Pat. Appl. PCT/AU98/00882, Int. Pat. Publ. WO99/23248.

- Barendse, W., Bunch, R., Harrison, B. and Thomas, M. (2006), The growth hormone GH1:c.457C>G mutation is associated with intramuscular and rump fat distribution in a large sample of Australian feedlot cattle. *Animal Genetics*, 37, 211–214.
- Barendse, W. (2002), DNA markers for meat tenderness. Pat. Appl. WO 02064820.
- Barendse, W., Bunch, R.J., Kijas, J.W. and Thomas, M.B. (2007), The effect of genetic variation of the retinoic acid receptor-related orphan receptor C gene on fatness in cattle. *Genetics*, 175, 843–853.
- Belov, M.E., Anderson, G.A., Wingerd, M.A., Udseth, H.R., Tang, K., Prior, D.C., Swanson, K.R., Buschbach, M.A., Strittmatter, E.F., Moore, R.J. and Smith, R.D. (2004), An automated high performance capillary liquid chromatography-Fourier transform ion cyclotron resonance mass spectrometer for high-throughput proteomics. *Journal of the American Society for Mass Spectrometry*, 15 (2), 212–232.
- Bendixen, E. (2005), The use of proteomics in meat science. *Meat Science*, 71, 138–149.
- Bendixen, E., Taylor, R., Hollung, K., Hildrum, K.I., Picard, B. and Bouley, J. (2005), Proteomics, an approach towards understanding the biology of meat quality. In *Indicators of milk and beef quality*. Eds. J.F. Hocquette and S. Gigli. EAAP Publication No. 112, 2005.
- Bernard C., Cassar-Malek I. and Hocquette J.F. (2006), *Genomic marker for meat tenderness*. Patent 06 300943.5. 12 September 2006.
- Bernard C., Cassar-Malek I., Le Cunff M., Dubroeuq H., Renand G. and Hocquette J.F. (2007), New indicators of beef sensory quality revealed by expression of specific genes. *Journal of Agricultural and Food Chemistry*, 55, 5229–5237.
- Brazma, A., Hingamp, P., Quackenbush, J., Sherlock, G., Spellman, P., Stoeckert, C., Aach, J., Ansorge, W., Ball, C., Causton, H., Gaasterland, T., Glenisson, P., Holstege, F., Kim, I., Markowitz, V., Matese, J., Parkinson, H., Robinson, A., Sarkans, U., Schulze-Kremer, S., Stewart, J., Taylor, R., Vilo, J. and Vingron, M. (2001), Minimum information about a microarray experiment (MIAME) – toward standards for microarray data. *Nature Genetics*, 29, 365–371.
- Breitling, R. (2006), Biological microarray interpretation: The rules of engagement. *Biochimica et Biophysica Acta*, 1759, 319–327.
- Buchanan, F.C., Kessel, A.G., Boisclair, Y.R., Block, H.C. and McKinnon, J.J. (2007), The leptin arg25cys affects performance, carcass traits and serum leptin concentrations in beef cattle. *Canadian Journal of Animal Science*, 87, 153–156.
- Bustin, S.A. (2000), Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *Journal of Molecular Endocrinology*, 25, 169–193.
- Caetano, A.R., Johnson, R.K., Ford, J.J. and Pomp, D. (2004), Microarray profiling for differential gene expression in ovaries and ovarian follicles of pigs selected for increased ovulation rate. *Genetics*, 168(3), 1529–37.
- Cagnazzo, M., te Pas, M.F., Priem, J., de Wit, A.A.C., Pool, M.H., Davoli, R. and Russo, V. (2006), Comparison of prenatal muscle tissue expression profiles of two pig breeds differing in muscle characteristics. *Journal of Animal Science*, 84, 1–10.
- Campbell, W.G., Gordon, S.E., Carlson, C.J., Pattison, J.S., Hamilton, M.T. and Booth, F.W. (2001), Differential global gene expression in red and white skeletal muscle. *American Journal of Physiology – Cell Physiology*, 280, C763–C768.
- Carson, J.A., Nettleton, D. and Reecy, J.M. (2002), Differential gene expression in the rat soleus muscle during early work overload-induced hypertrophy. *The Federation of American Societies for Experimental Biology Journal*, 16(2), 207–9.
- Casas, E., White, S.N., Wheeler, T.L., Shackelford, S.D., Koohmaraie, M., Riley, D.G., Chase Jr., C.C., Johnson D.D. and Smith, T.P.L. (2006), Effects of calpastatin and mu-calpain markers in beef cattle on tenderness traits. *Journal of Animal Science*, 84, 520–525.
- Celis, J.E., Kruhoffer, M., Gromova, I., Frederiksen, C., Ostergaard, M., Thykjaer, T., Gromov, P., Yu, J., Palsdottir, H., Magnusson, N. and Orntoft, T.F. (2000), Gene

- expression profiling: Monitoring transcription and translation products using DNA microarrays and proteomics. *FEBS Letters*, 480, 2–16.
- Chamrad, D.C., Koerting, G., Gobom, J., Thiele, H., Klose, J., Meyer, H.E. and Blueggel, M. (2003), Interpretation of mass spectrometry data for high-throughput proteomics. *Analytical and Bioanalytical Chemistry*, 376, 1014–1022.
- Cho, K.K., Han, K.H., Kang, S.K., Lee, S.H. and Choi, Y.J. (2002), Applications of cDNA microarray in ruminants. In *Proceedings of the 4th Korea–Japan Joint Symposium on Rumen Metabolism and Physiology*, Jeju, Korea.
- Ciobanu, D.C., Bastiaansen, J.W.M., Lonergan, S.M., Thomsen, H., Dekkers, J.C.M., Plastow, G.S. and Rothschild, M.F. (2004), New alleles in calpastatin gene are associated with meat quality traits in pigs. *Journal of Animal Science*, 82, 2829–2839.
- Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G. and Rothschild, M. (2001), Evidence for new alleles in the protein kinase monophosphate-activated γ 3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. *Genetics*, Vol. 159, pp. 1151–1162.
- Cockett, N.E., Shay, T.L. and Smit M. (2001), Analysis of the sheep genome. *Physiological Genomics*, 7, 69–78.
- Costello, S., O'Doherty, E., Troy, D.J., Ernst C.W., Kim, K.S., Stapleton, P., Sweeney T and Mullen A.M. (2007), Association of polymorphisms in the calpain I, calpain II and growth hormone genes with tenderness in bovine *M-longissimus dorsi*. *Meat Science*, 75, 551–557.
- Davis Jr., W., De Sousa, P.A. and Schultz, R.M. (1996), Transient expression of translation initiation factor eIF-4C during the 2-cell stage of the preimplantation mouse embryo: Identification by mRNA differential display and the role of DNA replication in zygotic gene activation. *Developmental Biology*, 174, 190–201.
- Davoli, R., Zambonelli, P., Bigi, D., Fontanesi, L. and Russo, V. (1999), Analysis of expressed sequence tags of porcine skeletal muscle. *Gene*, 233, 181–188.
- Davoli, R., Fontanesi, L., Zambonelli, P., Bigi, D., Gellin, J., Yerle, M., Milc, J., Braglia, S., Cenci, V., Cagnazzo, M. and Russo, V. (2002), Isolation of porcine expressed sequence tags for the construction of a first genomic transcript map of the skeletal muscle in pig. *Animal Genetics*, 33(1), 3–18.
- Dekkers, J. (2004), Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. *Journal of Animal Science*, 82(E. suppl.), E313–E328.
- Dekkers, J.C.M. (2007), Marker-assisted selection for commercial crossbred performance. *Journal of Animal Science*, 85, 2104–2114.
- Di Stasio, L., Brugiapaglia, A., Destefanis, G., Albera, A. and Sartore, S. (2003), GH1 as candidate gene for variability of meat production traits in Piemontese cattle, *Journal of Animal Breeding and Genetics*, 120, 358–361.
- Dorroch, U., Goldammer, T., Brunner, R.M., Kata, S.R., Kuhn, C., Womack, J.E. and Schwerin, M. (2001), Isolation and characterization of hepatic and intestinal expressed sequence tags potentially involved in trait differentiation between cows of different metabolic type. *Mammalian Genome*, 12, 528–537.
- Downey, E., Mullen, A.M., Brandon, K., White, A. and Pennington, S.R. (2008), Molecular analysis of Tenderstretch on post-mortem bovine skeletal muscle protein composition using 2-dimensional electrophoresis based proteomics. In book of abstracts of BSPR-EBI Meeting 2008 'Proteomics, From Technology to New Biology', Cambridge, UK, 98.
- Elkins, R., Chu, F. and Biggart, E. (1989), Development of microspot multi-analyte radiometric immunoassay using dual fluorescent-labelled antibodies. *Analytical Chemistry Acta*, 227, 73–96.
- Fahrenkrug, S.C., Smith, T.P., Freking, B.A., Cho, J., White, J., Vallet, J., Wise, T., Rohrer, G., Pertea, G., Sultana, R., Quackenbush, J. and Keele, J.W. (2002), Porcine gene discovery by normalized cDNA-library sequencing and EST cluster assembly. *Mammalian Genome*, 13(8), 475–8.
- Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F. and Whitehouse, C.M. (1989), Electrospray

- ionisation for mass spectrometry of large biomolecules. *Science*, 246, 64–71.
- Freking, B.A., Murphy, S.K., Wylie, A.A., Rhodes, S.J., Keele, J.W., Leymaster, K.A., Jirtle, R.L. and Smith, T.P.L. (2002), Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals. *Genome Research*, 12, 1496–1506.
- Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V., Weiler, J., O'Brien, P. and MacLennan, D. (1991), Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science*, 253, 448–451.
- Gallagher, D.S., Derr, J.N. and Womack, J.E. (1994), Chromosome conservation among the advanced pecorans and determination of the primitive bovid karyotype. *Journal of Heredity*, 85, 204–210.
- Gerbens, F., Verburg, F.J., van Moerkerk, H.T.B., Engel, B., Buist, W., Veerkamp, J.H. and te Pas, M.F.W. (2001), Associations of heart and adipocyte fatty acid-binding protein gene expression with intramuscular fat in pigs. *Journal of Animal Science*, 79, 347–354.
- Ghaemmaghami, S., Huh, W.K., Bower, K., Howson, R.W., Belle, A., Dephoure, N., O'Shea, E.K. and Weissman, J.S. (2003), Global analysis of protein expression in yeast. *Nature*, 425, 737–741.
- Gorg, A., Boguth, G., Kopf, A., Reil, G., Parlar, H. and Weiss, W. (2002), Sample prefractionation with Sephadex isoelectric focusing prior to narrow pH range two-dimensional gels. *Proteomics*, 2(12), 1652–1657.
- Graff, J.C., Behnke, M., Radke, J., White, M. and Jutila, M.A. (2006), A comprehensive SAGE database for the analysis of $\{\gamma\}\{\delta\}$ T cells. *International Immunology*, 18, 613–626.
- Grisart, B., Coppieeters, W., Farnir, F., Karim, L., Ford, C., Berzi, P., Cambisano, N., Mni, M., Reid, S., Simon, P., Spelman, R., Georges, M. and Snell, R. (2001), Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition, *Genome Research*, 12, 222–231.
- Hocquette, J.F., Lehnert, S., Barendse, W., Cassar-Malek, I. and Picard, B. (2007), Recent advances in cattle functional genomics and their application to beef quality. *Animal*, 1, 159–173.
- Hocquette, J.F. (2005), Where are we in genomics? *Journal of Physiology and Pharmacology*, 56(Suppl 3), 37–70.
- Hocquette, J.F., Cassas-Malek, I., Listrat, A. and Picard, B. (2005), Current genomics in cattle and application to beef quality. In *Indicators of Milk and Beef Quality*. Eds. J.F. Hocquette and S. Gigli. EAAP Publication No. 112, Wageningen Academic Publishers, Wageningen, The Netherlands.
- Hocquette, J.F. and Gigli, S. (2005), The challenge of quality. In *Indicators of Milk and Beef Quality*, J.F. Hocquette, S. Gigli (editors), EAAP Publication 112, Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 13–22. <http://www.cattlenetwork.net/docs/forum/eaap112.pdf>
- Hollung, K., Veiseth, E., Jia, X., Fargstad, E.M. and Hildrum, K.I. (2007), Application of proteomics to understand the molecular mechanisms behind meat quality. *Meat Science*, 77, 97–104.
- Huff-Lonergan, E., Parrish, F.C. and Robson, R.M. (1995), Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine *longissimus* muscle. *Journal of Animal Science*, 73, 1064–1073.
- Hughes L., Corcoran D., Nugent S., Hamill R.M. and Mullen A.M. (2007), Examining gene expression in two pig breeds divergent for meat quality traits using the Affymetrix GeneChip® Porcine Genome Array. In *Proceedings, 37th Annual Research Conference, Food, Nutrition and Consumer Science 2007*, Cork, p 70–71.
- Hunter, T.C., Andon, N.L., Koller, A., Yates III, J.R. and Haynes, P.A. (2002), The functional proteomics toolbox: Methods and applications. *Journal of Chromatography B*, 782, 165–181.

- Hwang, I.H., Park, B.Y., Kim, J.H., Cho, S.H. and Lee, J.M. (2005), Assessment of postmortem proteolysis by gel-based proteome analysis and its relationship to meat quality traits in pig *longissimus*. *Meat Science*, 69, 79–91.
- Jacobsen, S., Grove, H., Jensen, K.N., Sørensen, H.A., Jessen, F. and Hollung, K. (2007), Multivariate analysis of two-dimensional gel electrophoresis protein patterns – practical approaches. *Electrophoresis*, 28, 1289–1299.
- Jessen, F., Lametsch, R., Bendixen, E., Kjaersgard, I.V. and Jorgensen, B.M. (2002), Extracting information from two-dimensional electrophoresis gels by partial least squares regression. *Proteomics*, 2, 32–35.
- Jia, X., Hildrum, K.I., Westad, F., Kummen, E., Aass, L. and Hollung, K. (2006), Changes in enzymes associated with energy metabolism during the early post-mortem period in *longissimus thoracis* bovine muscle analyzed by proteomics. *Journal of Proteome Research*, 5, 1763–1769.
- Karas, M. (1996), Matrix-assisted laser desorption MS: A progress report. *Biomedical Society Transactions*, 24(3), 897–900.
- Kijas, J.W., McCulloch, R., Edwards, J.E.H., Oddy, V.H., Lee, S.H. and van der Werf, J. (2007), Evidence for multiple alleles affecting muscling and fatness at the ovine GDF8 locus. *BMC Genetics*, 8, 80.
- Kim, K., Kim, J., Dekkers, J.C.M. and Rothschild, M. (2004), Polar overdominant inheritance of a DLK1 polymorphism is associated with growth and fatness in pigs. *Mammalian Genome*, 15, 552–559.
- Kjaersgard, I.V., Norrelykke, M.R. and Jessen, F. (2006), Changes in cod muscle proteins during frozen storage revealed by proteome analysis and multivariate data analysis. *Proteomics*, 6, 1606–1618.
- Kononoff, P.J., Deobald, H.M., Stewart, E.L., Laycock, A.D. and Marquess, F.L.S. (2005), The effect of a leptin single nucleotide polymorphism on quality grade, yield grade, and carcass weight of beef cattle. *Journal of Animal Science*, 83, 927–932.
- Koohmaraie, M. (1994), Muscle proteinases and meat ageing. *Meat Science*, 36, 93–104.
- Koohmaraie, M. and G.H. Geesink. (2006), Contribution of post mortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science*, 74, 34–43.
- Kuhn, C., Leveziel, H., Renand, G., Goldammer, T., Schwerin, M. and Williams, J.L. (2005), Genetic markers for beef quality, In *Indicators of Milk and Beef Quality*. Eds. J.F. Hocquette and S. Gigli. EAAP Publication No. 112, 2005.
- Lagonigro, R., Wiener, P., Pilla, F., Woolliams, J.A. and Williams, J.L. (2003), A new mutation in the coding region of the bovine leptin gene associated with feed intake. *Animal Genetics*, 34, 371–374.
- Lametsch, R., Karlsson, A., Rosenvold, K., Anderson, H.J., Roepstorff, P. and Bendixen, E. (2003), Postmortem proteome changes of porcine muscle related to tenderness. *Journal of Agricultural and Food Chemistry*, 51, 6992–6997.
- Lametsch, R., Roepstorff, P., Moller, H.S. and Bendixen, E. (2004), Identification of myofibrillar substrates for μ -calpain. *Meat Science*, 68, 515–521.
- Lametsch, R. and Bendixen, E. (2001), Proteome analysis applied to meat science: Characterizing postmortem changes in porcine muscle. *Journal of Agricultural and Food Chemistry*, 49, 4531–4537.
- Lametsch, R., Roepstorff, P. and Bendixen, E. (2002), Identification of protein degradation during post-mortem storage of pig meat. *Journal of Agricultural and Food Chemistry*, 50, 5508–5512.
- Laville, E., Sayd, T., Sante-Lhoutellier, V., Morzel, M., Labas, R. and Franck, M. *et al.* (2005), Characterisation of PSE zones in semimembranosus pig muscle. *Meat Science*, 70, 167–172.
- Le Blanc, J.C., Hager, J.W., Ilisiu, A.M., Hunter, C., Zhong, F. and Chu, I. (2003), Unique scanning capabilities of a new hybrid linear ion trap mass spectrometer (Q TRAP) used for high sensitivity proteomics applications. *Proteomics*, 3, 859–896.

- Lehnert, S.A., Y.H. Wang, Y.H. and Byrne, K.A. (2004), Development and application of a bovine cDNA microarray for expression profiling of muscle and adipose tissue. *Australian Journal of Experimental Agriculture*, 44, 1127–1133.
- Lehnert, S., Reverter, A., Byrne, K.A., Wang Y-H., Natrass, G.S., Hudson, N.J. and Greenwood, P.L. (2007), Gene expression studies of developing bovine *longissimus* muscle from two different beef cattle breeds. *BMC Developmental Biology*, 2007, 7, 95.
- Leung, Y.F. and Cavalieri, D. (2003), Fundamentals of cDNA microarray data analysis. *Trends in Genetics*, 19(11): 649–659
- Liang, P. and Pardee, A.B. (1992), Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science*, 257, 967–971.
- Lim, H., Eng, J., Yates, J.R., Tollaksen, S.L., Giometti, C.S., Holden, J.F., Adams M.W.W., Reich C.I., Olsen G.L. and Hays, L.G. (2003), Identification of 2D-gel proteins: A comparison of MALDI ToF peptide mass mapping to μ LC-ESI tandem mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 14(9), 957–970.
- Listrat, A., Hocquette, J.F., Picard, B., Menissier, F.O., Djiane, J. and Jammes H. (2005), Growth hormone receptor gene expression in the skeletal muscle of normal and double-muscled bovines during foetal development. *Reproduction Nutrition Development*, 45, 393–403.
- Maher, S.C., Mullen, A.M., Moloney, A.P., Buckley, D.J. and Kerry J.P. (2004), Quantifying the extent of variation in the eating quality traits of the *M. longissimus dorsi* and *M. semimembranosus* of conventionally processed Irish beef. *Meat Science*, 66, 351–360.
- Malone, J., McMarry, K. and Bowerman, C. (2006), Automated trend analysis of proteomics data using an intelligent data mining architecture. *Expert Systems with Applications*, 30, 24–33.
- Mann, M. and Jensen, O.N. (2003), Proteomic analysis of post-translational modifications. *Nature Biotechnology*, 21, 255 – 261.
- Marcq, F., Larzul, C., Marot, V., Bouix, J., Eychenne, F., Laville, E., Bibé, B., Leroy, P., Georges, M. and Elsen, J.M. (2002), Preliminary results of a whole-genome scan targeting QTL for carcass traits in Texel \times intercross. In *Proceedings. 7th World Congress on Genetic Applied to Livestock Production*. (pp323–326), Montpellier, France.
- Martens, H., and Martens, M. (2000), ModiWed Jack.knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). *Food Quality and Preference*, 11, 5–16.
- Maurer, M.H., Feldmann, R.E., Jr., Bromme, J.O. and Kalenka, A. (2005), Comparison of statistical approaches for the analysis of proteome expression data of differentiating neural stem cells. *Journal of Proteome Research*, 4, 96–100.
- McIlveen and Buchanan (2001), The impact of sensory factors on beef purchase and consumption. *Nutrition and Food Science*, 31(6), 286–292.
- Meunier, B., Dumas, E., Piec, I., Bechet, D., Hebraud, M. and Hocquette, J. F. (2007). Assessment of hierarchical clustering methodologies for proteomic data mining. *Journal of Proteome Research*, 6, 358–366.
- Meunier, B., Bouley, J., Piec, I., Bernard, C., Picard, B. and Hocquette, J.F. (2005), Data analysis methods for detection of differential protein expression in two-dimensional gel electrophoresis. *Analytical Biochemistry*, 340, 226–230.
- Meuwissen, T.H.E. and Goddard, M.E. (1996), The use of marker haplotypes in animal breeding schemes. *Genetics Selection Evolution*, 28, 161–176.
- Meuwissen, T.H.E., Hayes, B.J. and Goddard, M.E. (2001), Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, 157, 1819–1829.
- Michal, J.J., Jiang, Z. and Xiao, Q. (2008), *Identifying animals having desirable intramuscular fat deposition by determining the presence of single nucleotide polymorphisms (SNPs) in a PAPD1 gene of the animal, which indicates intramuscular fat deposition*. Patent WO2007134162-A2 2008.
- Michal, J.J., Zhang, Z.W., Gaskins, C.T. and Jiang, Z. (2006), The bovine fatty acid binding

- protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu \times Limousin F-2 crosses. *Animal Genetics*, 37, 400–402.
- Milan, D., J.T. Jeon, J.T. Looft, C., Amarger, V., Robic, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L., Ronne, H., Lundstrom, K., Reinsch, N., Gellin, J., Kalm, E., Roy, P., Chardon, P. and Andersson, L. (2000), A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science*, 288, 1248–1251.
- Mohan, M., Hurst, A.G. and Malayer, J.R. (2004), Global gene expression analysis comparing bovine blastocysts flushed on day 7 or produced *in vitro*. *Molecular Reproduction and Development*, 68, 288–298.
- Moody, D.E. (2001), Genomics techniques: An overview of methods for the study of gene expression. *Journal of Animal Science*, 79(E. Suppl.), E128–E135.
- Morris, C.A., Cullen, N.G., Glass B.C., Hyndman, D.L., Manley, T.R., Hickey, S.M., McEwan, J.C., Pitchford, W.S., Bottema, C.D.K. and Lee, M.A.H. (2007), Fatty acid synthase effects on bovine adipose fat and milk fat. *Mammalian Genome*, 18, 64–74.
- Muir, W.M. (2007), Comparison of genomic and traditional BLUP-estimated breeding value accuracy and selection response under alternative trait and genomic parameters. *Journal of Animal Breeding and Genetics*, 124, 342–355.
- Næs, T., Isaksson, T., Fearn, T. and Davies, T. (2002), *A user-friendly guide to multivariate calibration and classification*. Chichester: NIR Publications.
- Nobis, W., Ren, X., Suchyta, S.P., Suchyta, T.R., Zanella, A.J. and Coussens, P.M. (2003), Development of a porcine brain cDNA library, EST database, and microarray resource. *Physiological Genomics*, 16, 153–159.
- Ouali, A. (1990), Meat tenderisation: Possible causes and mechanisms. A review. *Journal of Muscle Foods*, 50, 129–165.
- Ovilo, C., Fernandez, A., Rodriguez, M.C., Nieto, M. and Silio, L. (2006), Association of MC4R gene variants with growth, fatness, carcass composition and meat and fat quality traits in heavy pigs. *Meat Science*, 73, 42–47.
- Page, B.T., Casas, E., Heaton, M.P., Cullen, N.G., Hyndman, D.L., Morris, C.A., Crawford, A.M., Wheeler, T.L., Koohmaraie, M., Keele, J.W. and Smith, T.P.L. (2002), Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *Journal of Animal Science*, 80, 3077–3085.
- Page, B.T., Casas, E.R., Quaas, L., Thallman, R.M., Wheeler, T.L., Shackelford, S.D. *et al.* (2004), Association of markers in the bovine CAPN1 gene with meat tenderness in large crossbred populations that sample influential industry sires. *Journal of Animal Science*, 82, 3474–3481.
- Pan, W., Lin, J. and Le, C.T. (2003), A mixture model approach to detecting differentially expressed genes with microarray data. *Functional and Integrative Genomics*, 3, 117–124.
- Pannier, L. (2008), *A search for DNA polymorphisms and signature gene expression profiles associated with meat quality*. Thesis submitted in fulfillment of requirements for the Degree of Doctor of Philosophy of University College Dublin, May 2008.
- Patterson, S.D. (2003), Data analysis – the Achilles heel of proteomics. *Nature Biotechnology*, 21, 221–222.
- Pedersen, S.K., Harry, J.L., Sebastian, L., Baker, J., Traini, M.D., McCarthy, J.T., Manoharan, A., Wilkins, M.R., Gooley, A.A., Righetti, G.R., Packer, N.H., Williams, K.L. and Herbert, B.R. (2003), Unseen proteome: Mining below the tip of the iceberg to find low abundance and membrane proteins. *Journal of Protein Research*, 2, 300–311.
- Pfaffl, M.W. (2001), A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, 29(9), e45.
- Pfaffl, M.W., Tichopad, A., Prgomet, C. and Neuvians, T.P. (2004), Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper–Excel-based tool using pair-wise correlations. *Biotechnology Letters*, 26(6), 509–515.
- Pietrogrande, M.C., Marchetti, N., Dondi, F. and Righetti, P.G. (2006), Decoding 2D-PAGE complex maps: Relevance to proteomics. *Journal of Chromatography B*, 833 (1), 51–62.

- Polkinghorne, R.J. (2006), Implementing a palatability assured critical control points approach to satisfy consumer demands. *Meat Science*, 74 (1), 180–187.
- Pomp, D., Caetano, A.R., Bertani, G.R., Gladney, C.D. and Johnson, R.K. (2001), Applying functional genomics research to the study of pig reproduction. *Reproduction Supplement*, 58, 277–92.
- Potts, J.K., Echternkamp, S.E., Smith, T.P.L. and Reecy, J.M. (2003), Characterization of gene expression in double-muscled and normal-muscled bovine embryos. *Animal Genetics*, 34, 438–444.
- Reverter, A., Byrne, K.A., Bruce, H.L., Wang, Y.H., Dalrymple, B.P. and Lehnert, S.A. (2003), A mixture model-based cluster analysis of cDNA microarray gene expression data on Brahman and Brahman composite steers fed high, medium and low quality diets. *Journal of Animal Science*, 81, 1900–1910.
- Rinaudo, P. and Schultz, R.M. (2004), Effects of embryo culture on global pattern of gene expression in preimplantation mouse embryos. *Reproduction*, 128, 301–311.
- Robinson, T.L., Sutherland, I.A. and Sutherland, J. (2007), Validation of candidate bovine reference genes for use with real-time PCR. *Veterinary Immunology and Immunopathology*, 115, 160–165.
- Roesli, C., Elia G. and Neri, D. (2006), Two-dimensional mass spectrometric mapping. *Current Opinion in Chemical Biology*, 10, 1–7.
- Rothschild, M.F., Kim, K.S. and Emnett, R.S. (2004), *Genetic markers for improved meat characteristics in animals (MC4R)*. US Patent Application 20040261138A1 2004.
- Sayd, T., Morzel, M., Chambon, C., Franck, M., Figwer, P., Larzul, C., Le Roy, P., Monin, G., Chérel, P. and Laville, E. (2006), Proteome analysis of the sarcoplasmic fraction of pig *semimembranosus* muscle: Implications on meat color development. *Journal of Agriculture and Food Chemistry*, 54(7), 2732 – 2737.
- Schena, M., Shalon, D., Davis, R. W. and Brown, P.O. (1995), Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*, 270, 467–470.
- Schenkel, F.S., Miller, S.P., Jiang, Z., Mandell, I.B., Ye, X., Li, H. and Wilton, J.W. (2006), Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science*, 84, 291–299.
- Schenkel, F.S., Miller, S.P., Ye, X., Moore, S.S., Nkrumah, J.D., Li, C., Yu J., Mandell I.B., Wilton, J.W. and Williams, J.L. (2005), Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science*, 83, 2009–2020.
- Sellner, E.M., Kim, J.W. McClure, M.C., Taylor, K.H., Schnabel, R.D. and Taylor, J.F. (2007), Applications of genomic information in livestock. *Journal of Animal Science*, 85, 3148–3158 SGSC (2003) Swine Genome Sequencing Consortium <http://www.piggenome.org/index.php>
- Sierra, V., O'Reilly, K., White, A., Mullen, A.M. and Troy, D.J. (2005), Proteolytic fragments in bovine exudate as potential tenderisation markers. In *Proceedings 52nd International Congress of Meat Science and Technology*. Baltimore, Maryland USA. 7–12 August.
- Spandidos, A. and Rabbitts, T.H. (2002), Sub-proteome differential display: Single gel comparison by 2D electrophoresis and mass spectrometry. *Journal of Molecular Biology*, 318, 21–31.
- Stagsted, J., Bendixen, E. and Andersen, H.J. (2004), Identification of specific oxidatively modified proteins in chicken muscles using a combined immunologic and proteomic approach. *Journal of Agriculture and Food Chemistry*, 52, 3967–3974.
- Sudre, K., Cassar-Malek, I., Leroux, C., Listrat, A., Ueda, Y., Jurie, C., Renand, G., Martin, P. and Hocquette, J.F. (2003), Transcriptome analysis of muscle in order to identify genes which determine muscle characteristics and sensory quality traits of beef. *Sciences des Aliments*, 23, 65–69.
- Sudre, K., Leroux, C., Cassar-Malek, I., Hocquette, J.F. and Martin, P. (2005), A collection

- of bovine cDNA probes for gene expression profiling in muscle. *Molecular and Cellular Probes*, 19, 61–70.
- Szczesniak, A.S. (1998), Sensory texture profiling – historical and scientific perspectives. *Food Technology*, 52 (8), 54–57.
- Taylor, J., Eggen, A., Aleyasin, A., Mrmitage, S., Barendse, W., Beever, J., Bishop, M.D., Breneman, R., Burns, B., Davis, S., Elo, K., Harlizius, B., Kappes, S.M., Keele, J.W., Kemp, S., Kirkpatrick, B., Lewin, H.A., Ma, R., McGraw, R., Pomp, D., Stone, R.T., Sugimoto, Y., Teale, A., Vaiman, D., Vilkkilä, J., Williams, J.L., Yeh, C. and Zanotti, M. (1998), Report of the first workshop on the genetic map of bovine chromosome 1. *Animal Genetics*, 29, 228–235.
- te Pas, M.F., Soumilion, A., Harders, F.L., Verburg, F.J., van den Bosch, T.J., Galesloot, P. and Meuwissen, T.H. (1999), Influences of myogenin genotypes on birth weight, growth rate, carcass weight, backfat thickness, and lean weight of pigs. *Journal of Animal Science*, 77, 2352–2356.
- te Pas, M.F.W. (2003), Candidate genes for meat production and meat quality – the MRF genes. *Animal Science Papers and Reports*, 22, 115–118.
- te Pas, M.F.W., C.L.M. Gerritsen, A.H. Visscher, and K.H. De Greef (2003), Relationships between performance traits and the expression of growth hormone, insulin-like growth factor-I, and insulin in pigs selected for growth or leanness. *Journal of Animal Breeding and Genetics*, 120, 346–357.
- Thaller, G., Kuhn, C., Winter, A., Ewald, G., Bellmann, O., Wegner, J., Zuhlke, H. and Fries, R. (2003), DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. *Animal Genetics*, 34, 354–357.
- Thompson, J. (2002), Managing meat tenderness. *Meat Science*, 62, 295–308.
- Thompson, J.M., Perry, D., Daly, B., Gardner, G.E., Johnston, D.J., and Pethick, D.W. (2006), Genetic and environmental effects on the muscle structure response post-mortem. *Meat Science*, 74, 59–65.
- Thue, T.D., Goldale, B.G., Van Kessel, A., Schmutz, S.M., Laarveld, B. and Buchanan, F.F. (2001), Quantification of mRNA from adipose in beef cattle selected on genotype at the obese gene. *Plant and Animal Genome IX Conference*, San Diego, California.
- Unlu, M., Morgan, M.E. and Minden, J.S. (1997), Difference gel electrophoresis: A single gel method for detecting changes in protein extracts. *Electrophoresis*, 18, 2071–2077.
- USDA Animal Genomics Strategic Planning Task Force. (2007). *Blueprint for USDA Efforts in Agricultural Animal Genomics 2008–2017*. Report: U.S. Department of Agriculture, Agricultural Research Service and Cooperative State Research, Education, and Extension Service, Washington, DC.
- Van der Steen, H., Prall, G. and Plastow, G. (2005), Application of genomics to the pork industry. *Journal of Animal Science*, 83 (e-supplement), E1–E8.
- Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A. and Speleman, F. (2000), Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, 3, Research 0034.
- Van Eenennaam, A.L., Li, J., Thallman, R.M., Quaas, R.L., Dikeman, M.E., Gill, C.A., Franke, D.E. and Thomas, M.G. (2007), Validation of commercial DNA tests for quantitative beef quality traits. *Journal of Animal Science*, 85(4), 891–900.
- Van Laere, A., Nguyen, M., Braunschweig, M., Nezer, C., Collette, C., Moreau, L., Archibald, A.L., Haley, C.S., Buys, N., Tally, M., Andersson, G., Georges, M. and Andersson, L. (2003), A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. *Nature*, 425, 832–835.
- Velculescu, V.E., Zhang, L., Vogelstein, B. and Kinzler, K.W. (1995), Serial analysis of gene expression. *Science*, 270, 484–487.
- Wan, J., Wright, M.B., Cai, L., Flament, A. and Lindpaintner, K. (2002), Efficacy of SSH PCR in isolating differentially expressed genes. *BMC Genomics*, 3, p. 12.

- Wang, Y-H, Byrne, K., Reverter, A., Harper, G., Taniguchi, M., McWilliam, S., Mannen, H., Oyama, K. and Lehnert, S. (2005), Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. *Mammalian Genome*, 15, 201–210.
- Wang, Y., Barbacioru, C., Hyland, F., Xiao, W.M., Hunkapiller, K.L., Blake, J., Chan, F., Gonzalez, C., Zhang, L. and Samaha, R.R. (2006), Large scale real-time PCR validation on gene expression measurements from two commercial long-oligonucleotide microarrays. *BMC Genomics* 2006, 7:59, doi:10.1186/1471-2164-7-59
- Warkup, C., Marie, S. and Harrington, G. (1995), Consumer perceptions of texture; the most important quality attribute of meat? In *Expression of tissue proteinases and regulation of protein degradation as related to meat quality*. Ouali, A., Demeyer, D.I. and Smulders, F.J.M., eds., ECCEAMST, Utrecht, The Netherlands, 225.
- Wernersson, R., Schierup, R.H., Jørgensen, F.G., Gorodkin, J., Panitz, F., Staerfeldt, H.H., Christensen, O.F., Mailund, T., Hornshøj, H., Klein, A., Wang, J., Liu, B., Hu, S., Dong, W., Li, W., Wong, G.K., Yu, J., Wang, J., Bendixen, C., Fredholm, M., Brunak, S., Yang, H. and Bolund, L. (2005), Pigs in sequence space: a 0.66X pig genome survey based on shotgun sequencing. *BMC Genomics*, 6, 70.
- White, S. N., Casas, E., Wheeler, T.L., Shackelford, S.D., Koohmaraie, M., Riley, D.G., Chase, Jr., C.C., Johnson, D.D., Keele, J.W. and Smith, T.P.L. (2005), A new SNP in *CAPN1* is associated with tenderness in cattle of *Bos indicus*, *Bos taurus*, and crossbred descent. *Journal of Animal Science*, 83, 2001–2008.
- Wilkins, M.R., Pasquali, C., Appel, R.D., Ou K., Golaz, O., Sanchez, J.C., Yan, J.X., Gooley, A.A., Hughes, G., Humphrey-Smith, I., Williams, K.L. and Hochstrasser, D.F. (1996), From proteins to proteomes: Large scale protein identification by two-dimensional electrophoresis and amino acid analysis. *Biotechnology* (N.Y.), 14, 61–66.
- Williams, J.L. (2005), *The use of marker-assisted selection in animal breeding and biotechnology*. Scientific and Technical Review, Office International Des Epizooties 24, 379–391.
- Wilson, D.L., Buckley, M.J., Helliwell, C.A. and Wilson, I.W. (2003), New normalization methods for cDNA microarray data. *Bioinformatics*, 19(11): 1325–1332.
- Windig, J.J. and Meuwissen, T.H.E. (2004), Rapid haplotype reconstruction in pedigrees with dense marker maps. *Journal of Animal Breeding and Genetics*, 121, 26–39.
- Womack, J.E. (2006), The impact of sequencing the bovine genome. *Australian Journal of Experimental Agriculture*, 46, 151–153.
- Wong, M.L. and Medrano, J.F. (2005), Real-time PCR for mRNA quantitation. *Biotechniques*, 39(1), 75–85.
- Xiao, Q., Wu, X., Michal, J., Reeves, J., Busboom, J., Thorgaard, G. and Jiang, Z. (2006), A novel nuclear-encoded mitochondrial poly(A) polymerase PAPD1 is a potential candidate gene for the extreme obesity related phenotypes in mammals. *International Journal of Biological Sciences*, 2, 171–178.
- Yang, S.H., Matsui, T., Kawachi, H., Yamada, T., Nakanishi, N. and Yano, H. (2003), Fat depot-specific differences in leptin mRNA expression and its relation to adipocyte size in steers. *Animal Science Journal*, 74, 17–21.
- Yao, J., Coussens, P.M., Saama, P., Suchyta, S. and Ernst, C.W. (2002), Generation of expressed sequence tags from a normalized porcine skeletal muscle cDNA library. *Animal Biotechnology*, 13(2), 211–222.

Genetic and genomic approaches to improving pork quality

M. T. Cairns, NUI Galway, Ireland

Abstract: Pork quality is predicted upon gene expression at the time of pig slaughter and is considered the complex cumulative effect of a large number of genes, each playing a small part. Although pork has generally been stigmatised by the poor consistency of the product, large-scale studies at the mRNA expression, proteome and genome levels are providing new insights into the relationships between meat quality, individual genes and functional processes within the muscle tissue. The expected release of the complete pig genome sequence at 3× coverage in 2008 should consolidate these studies. Several DNA markers are already in use in the pig industry to select for improved meat quality.

Key words: gene expression, genotype, candidate gene, marker-assisted selection, muscle.

10.1 The importance of genetic and genomic approaches in improving pork quality

The pig is believed to be one of the first animals to be domesticated. Archaeological evidence suggests this was approximately 9000 years ago, in eastern Turkey and around the same time in China. In the last 40 years the pig industry has made substantial progress in improving efficiency in pork production: each sow produces 50% more offspring, which eat 33% less feed to produce 33% more lean meat (Plastow, 2003). Despite this improvement, surveys suggest that meat quality has actually fallen (Peterson *et al.*, 1997; Barton-Gade, 1990). Animal husbandry, slaughterhouse practice, diet and carcass processing have obviously an enormous amount to do with the quality of meat (Rosenvold and Andersen, 2003) that arrives on our supermarket shelves, but since the start of the genomic age it has become clear that meat quality is often associated with the genetic make-up of the pig and it is these issues that we will be dealing with in this review.

Meat quality is a difficult concept to define precisely. It will differ, for example, depending on the purpose for which the meat is to be used – whether it is to be eaten as fresh meat or whether it is to be processed (cured, smoked, etc.). It will differ between countries and continents: the hams favoured by Spain, Italy and France are not as popular in the UK, the high intramuscular fat content (IMF) desired by the Chinese for its tastiness is off-putting to the Westerner. Meat quality can be considered a combination of technological measurement and sensory perception. The major technological measurements are pH, drip loss/water holding capacity, colour, cooking losses and IMF/marbling, but these are complemented by a variety of sensorial factors such as smell, taste and tenderness, which require trained taste panels to subjectively score. Other traits such as carcass composition, growth rate, litter size and disease resistance are often more important to the meat production chain than meat quality.

Pale, soft, exudative (PSE) meat has been linked to the halothane (Hal) gene for several decades and genetic tests are available to test for the undesirable allele. However, PSE meat is still common and it is clear that other factors (some genetic) play a role. High cooking losses have been associated with the Rendement Napole gene in the Hampshire pig breed. Selective breeding for a favourable allele of the PRKAG3 gene can reduce quality control losses by 50 per cent (from 26% to 13%) (van der Steen *et al.*, 2005). However, although both the Hal and RN genes could be bred out of populations, heterozygote sires are kept for breeding stock as there are some (not insignificant) beneficial characteristics which must be weighed against the adverse effects of the gene.

The human genome sequence was published in 2004 (Human Genome Sequencing, 2004) and, to date, 27 eukaryotic genomes have been completely sequenced and a further 139 are at the assembly stage (<http://www.ncbi.nlm.nih.gov/genomes/leuks.cgi>). Genome sequences usually aim for at least 6× coverage and currently the pig genome as published by the ‘Sino-Danish Pig Genome Project’ was at approximately 0.66× coverage in 2005 (Wernersson *et al.*, 2005). It is expected that a published version of the pig genome at 3× coverage will be available some time in 2008. The speed of sequencing, alignment and annotation has been greatly increased by the evolutionary relationship of pigs and humans, allowing pig sequences to be mapped to the detailed human and mouse genome maps (Wernersson *et al.*, 2005). The pig genome sequencing project is based on pooled DNA from five different breeds of domestic pig (Wernersson *et al.*, 2005), which allows identification of potential polymorphisms that can be further collaborated by comparative whole genome analysis of other Mammalia. With this information we can examine the source of variability within species, the variability across breeds of pig and the variability within breeds. Beneficial phenotypes such as pork tenderness will be associated with specific genotypes, and marker-assisted breeding programmes will accurately be able to predict the meat qualities of the offspring. In a limited way this is already happening (e.g. with the Hal and RN genes), but only a few markers have such high heritability that they can be predictive. For meat quality traits it is usually assumed that a large number of alleles each play a small part.

In this review we will discuss (Section 10.2) some of the genes that are known (or at least suspected) to play a part in meat quality. These candidate genes have become candidates for a number of reasons, from QTL studies, from functional studies, from links with other species (especially human) or from functional genomics studies. Sections 10.3 to 10.5 will cover some of these discovery approaches. In Section 10.6 the future prospects for meat quality improvement will be discussed in relation to molecular and functional genetics, and, to conclude, in Section 10.7 some detail on useful resources (databases, additional reviews, websites, etc.) is provided.

10.2 Progress with identifying genes responsible for the meat quality traits in pigs

As stated previously, meat quality is considered a complex trait that is the result of the cumulative effect of a large number of alleles each playing a small part. A gene can be defined as a major gene when the difference between the mean value of the individuals homozygous for that gene and non-carrier individuals is equal to, or higher than, one phenotype standard deviation of that trait. There are several major genes that play a part in meat quality traits. The symptoms of Porcine Stress Syndrome (PSS) are malignant hyperthermia (MH), hypermetabolism and muscle rigidity (O'Brien, 1987). It can be induced by anaesthetic gases such as halothane and by stress. The disease may not be manifest before slaughter, however, susceptible pigs will yield an inferior meat product referred to as pale, soft, exudative (PSE) meat. As early as 1983 it was clear that the ryanodine receptor was involved (Ohnishi *et al.*, 1983) but it was only in 1993 that the causative mutation on *Sus scrofa* chromosome (SSC) 6 (6q1.1–q2.1) was identified (Fujii *et al.*, 1991). This receptor is a Ca^{2+} transporter of the sarcoplasmic reticulum and the mutation identified is a C to T transition coding for the amino acid substitution arginine to cysteine (R615C). Today the Hal mutation, the first marker developed for pigs, is screened for using the Hal1843 DNA Test, licensed by the University of Toronto Innovations Foundation.

Although the majority of pigs with PSS are homozygous positives (n, n), not all homozygous positives can be induced to develop PSS. Furthermore, not all PSE meat is the result of being homozygous positive (n, n); heterozygotic carriers (N, n) show a higher incidence of PSE meat than homozygous negatives (N, N) (Leach *et al.*, 1996). Although the n allele could be totally removed from pig lines, the n allele has some positive effects on carcass composition and meat quality. (N, n) and (N, N) animals grow at the same rates, yet the (N, n) carrier has a higher weight gain:feed ratio, a higher shoulder and ham yield and a higher lean cut yield (Leach *et al.*, 1996). Generally though, (N, n) heterozygotic carrier meat gives lower meat quality scores: higher drip loss, higher cooking loss, higher shear force and overall lower eating quality. Increased selection for feed efficiency and lean growth is probably the reason why the n allele became established in some breeds in the first place. Selection for economically important traits, even in (N, N) animals, can

compromise meat quality and lead to an increased incidence of PSE meat (Lonergan *et al.*, 2001). In pure breeds the Hal mutation is most prevalent in the Pietrain breed (<http://extension.tennessee.edu/publications/pbfiles/PB1606.pdf>, Agricultural Extension Service of the University of Tennessee) which has an n allele frequency of approximately 50%, whereas in Duroc, Landrace, Yorkshire and Hampshire it is between 5 and 8%. Calsequestrin (CSQ), ATP2A1 and ATP2A2 (also known as fast-twitch and slow-twitch SR calcium ATPases, SERCA1 and SERCA2) which are known to be part of the ryanodine signalling pathway are other candidate genes for pork meat quality. It has been shown at the protein level that levels of RyR, CSQ and ATP2A2 decrease between Hal⁻ (N, N) and Hal⁺ (n, n) pigs (Schulz *et al.*, 2006). Environmental factors (most probably with a genetic component) also have an effect on meat quality. High pre-slaughter stress clearly leads to impaired pork quality, even in an (N, N) background, with high muscle energy levels aggravating the negative effects of pre-slaughter stress. Monitoring stress level by blood lactate measurement in combination with strategies to control muscle energy present at slaughter may help to improve meat quality (Hambrecht *et al.*, 2004).

The Rendement Napole (RN⁻) phenotype is almost exclusive to Hampshire pigs and is characterised by a 70% increase in glycogen content in skeletal muscle and large effects on meat characteristics (pH, water content, technological yield and lean meat content). The phenotype is controlled by an autosomal dominant allele designated RN⁻. The protein kinase AMP-activated gamma 3 subunit gene, PRKAG3 (SSC15 15q2.1–q2.3), which encodes the gamma 3 isoform of AMP-activated protein kinase (AMPK), was identified as the causative gene for this phenotype by a positional cloning approach (Milan *et al.*, 2000). A missense mutation (R200Q) was determined as the causative mutation. But again the RN⁻ allele has advantages in some carcass and meat quality traits. Although samples of *longissimus* muscle from carrier pigs (RN⁻, rn+) have greater glycolytic potential values, drip loss percentages, and a* values, and lower pH values at fabrication than normal pigs (Carr *et al.*, 2006), carcasses of carrier pigs have less fat depth and a greater percentage carcass lean than carcasses of normal pigs (rn+, rn+).

PRKAG3 is not just implicated in the Rendement Napole condition: several quantitative trait loci (QTL) affecting muscle glycogen content and related traits were mapped to pig chromosome 15 using a three-generation intercross where all animals were normal for the RN locus (rn+, rn+). Three missense mutations (T30N, G52S and I199V) and a polymorphic short interspersed element (SINE) were identified (Ciobanu *et al.*, 2001). Therefore, there are other economically important alleles of the PRKAG3 gene affecting the glycogen content in the muscle and the resulting meat quality, which suggests that additional alleles of genes involved in major mutations may play a significant role in quantitative trait variation. Haplotypes I/I–Q/Q and I/V–Q/Q have not been observed in the Hampshire population, supporting the hypothesis that allele 200Q is tightly linked with allele 199V.

Boar taint is a condition that results in an unpleasant smell during cooking. It only affects meat from entire male pigs and has generally been avoided by slaughtering pigs at a younger age, by using females only, by using males before

they reach sexual maturity or by castrating the male pigs. However, male pigs have considerable economic value and, furthermore, castration is becoming a much more unacceptable practice for animal welfare reasons and is banned within the EU. Since pigs with high skatole levels were associated with a few sires, Lundstrom *et al.* (1994) first suggested a genetic link and a recessive allele for high skatole. The two agents responsible for boar taint are androstenone and skatole. Skatole is metabolised in the liver and high skatole levels correlate with low expression of the cytochrome p450 CYP2E1. Skatole also induces the expression of CYP2E1 in pig hepatocytes (Doran *et al.*, 2002) and androstenone can inhibit this induction. QTLs linked to these effects have been suggested on SSC3, SSC4, SSC6, SSC7, SSC9, SSC13 and SSC14 (Quintanilla *et al.*, 2003; Lee *et al.*, 2005). As CYP2E1 is mapped to SSC14, CYP2E1 was investigated as a candidate gene (Skinner *et al.*, 2005). Two coding region SNPs and four promoter SNPs were identified but none of these were associated with skatole levels (Skinner *et al.*, 2005). Furthermore, the mapped position of the gene appeared to be outside the confidence interval for the QTL. CYP2C18 was shown to map within the QTL but again no association with skatole levels was noted (Skinner *et al.*, 2006). Other candidate genes such as CYP2A6, SULT1A1 and SRD5A2 (all SSC3) and CYP11a and CYP21 (both SSC7) have been suggested but appear to be unlikely, at least in the breeds investigated (Skinner *et al.*, 2006; Quintanilla *et al.*, 2003): accurate mapping puts them outside the QTL region, the known SNPs are fixed in the investigated breeds, or no coding region SNPs were identified. As yet no gene mutation has been definitively proven causative for boar taint.

Tenderness (see Chapter 3) is measured both subjectively by a panel of tasters and instrumentally by, for example, the Instron 1222 Universal Testing Machine (shear force). A major determinant of tenderness is believed to be the rate of post-mortem proteolysis of the larger structural proteins of the muscle fibre (Taylor *et al.*, 1995). Calpains (both m and μ) are believed to be major proteases involved, together with calpastatin (CAST) the major inhibitor of these calpains. QTL analysis had indicated a region of SSC2 that was associated with meat tenderness and CAST and had been suggested as a possible candidate gene (Lonergan *et al.*, 1995; Malek *et al.*, 2001a,b). Additional markers and a known CAST polymorphism (*Msp*I) refined this area of the map (Ernst *et al.*, 1998), though no missense mutations were initially identified (Kocwin-Podsiadla *et al.*, 2003). Sequencing of the full CAST cDNA for a large number of F2 animals from a Berkshire \times Yorkshire cross, together with commercial animals, identified three missense mutations and several silent mutations (Ciobanu *et al.*, 2004). Two of the three missense mutations (at amino acid positions 66 and 249) were in linkage disequilibrium, which allowed association of tenderness data with three major haplotypes (Haplotype 4 was present only in Meishan pigs). It was clear that Haplotype 1 (N66/K249, R638) had much improved meat quality features over Haplotypes 2 (S66/R249, R638) and 3 (S66/R249, S638) (Ciobanu *et al.*, 2004). The restriction enzymes *Hpy*188I and *Pvu*II allowed simple identification of the three haplotypes (Ciobanu *et al.*, 2002a). Since serine residues are affected at two of these positions, it has been suggested that phosphorylation by protein kinase A

has an effect on the calpastatin activity in the different haplotypes (Ciobanu *et al.*, 2004). Although this marker is used commercially in marker-assisted selection strategies, it has yet to be determined which mutation is causative or whether the actual causative mutation is in linkage disequilibrium with the above mutations.

As mentioned, tenderness has been associated with enzyme activities of the calpains and their inhibitors (including calpastatin), especially in bovine and ovine studies. It has also been associated with other proteases (cathepsins, caspases, matrix metalloproteinases and the 20S proteasome) and inhibitors (cystatins and TIMPs) (Sentandreu *et al.*, 2002; Toldra and Flores, 2000). It has been suggested that calpains are responsible for up to 95% of all post-mortem tenderisation in sheep (Delgado *et al.*, 2001a,b), but that the ratio of calpain to inhibitor correlates better with meat tenderness than the absolute levels of calpain (Ouali and Talmant, 1990; Shackelford *et al.*, 1991). A study by Russo *et al.* (2002) identified a number of SNPs within the pork cathepsin B and cystatin B genes, with one in cystatin B producing a missense mutation coding for an aspartic to asparagine conversion. They found association with average daily gain (cystatin B) and backfat thickness (cathepsin B), but no association with meat eating qualities; neither did they find any association of the polymorphisms with cathepsin B activities.

Other regions strongly associated with tenderness are SSC4 (Harmegnies *et al.*, 2006; Rohrer *et al.*, 2006) and SSC 14 (Harmegnies *et al.*, 2006; Malek *et al.*, 2001b), while regions with suggestive association for tenderness are SSC3 (Harmegnies *et al.*, 2006), SSC7 (Harmegnies *et al.*, 2006), SSC10 (Malek *et al.*, 2001b), SSC12 (Malek *et al.*, 2001b), SSC15 (Harmegnies *et al.*, 2006; Rohrer *et al.*, 2006) and SSC17 (Malek *et al.*, 2001b), but no clear suggestions for candidate genes have been made.

Intramuscular fat (often termed marbling) is generally considered a positive attribute to flavour though, if too high, it has a negative effect on appearance. A value of 2% is considered a lower limit and 3% an upper limit, though for some forms of processing this upper limit is extended. Intramuscular fat is a highly heritable trait with values ranging from 0.26 to 0.86 (reviewed by Sellier, 1998) and recently recorded in *longissimus* at 0.69 (Newcom *et al.*, 2005a). A problem is that loin IMF is associated with subcutaneous backfat depth and decreasing the latter is a definite production goal. Newcom *et al.* investigated individual backfat depths (outer, middle and inner) with IMF and suggest that there is potential to increase IMF independently of inner backfat depth (Newcom *et al.*, 2005b).

The main genes that have been associated with IMF are RYR1 (SSC6), ATP2A1 (SSC3) and H-FABP (SSC6), but there is still much debate about this latter association of H-FABP with a QTL on SSC6 (see below). A major recessive gene (as yet unidentified) in the Meishan pig, if present as one copy of the allele, yields an IMF of 0.18%, whereas, if two copies of the allele are present, IMF reaches 0.36% (Janss *et al.*, 1994, 1997). RYR1 is also implicated because (N, n) animals have 0.07% higher IMF than homozygous normal (N, N) animals. ATP2A1 was implicated by Ciobanu *et al.* (2002b) and corroborated by Otto *et al.* (2007): the polymorphism recognised by *DpnII* is a C to T change, where T/T is favoured with 1.28% IMF compared to 1.18% for C/C. Other loci have been

implicated on SSC1, SSC8 and SSC10 (Malek *et al.*, 2001b), on SSC2, SSC4, SSC6 and SSC7 (de Koning *et al.*, 1999, 2000), on SSC13 (Yu *et al.*, 1999), on SSC15 (Nii *et al.*, 2005), on SSC12 (Paszek *et al.*, 2001), on SSC14 (van Wijk *et al.*, 2006a), on SSCX (Perez-Enciso *et al.*, 2002) and on SSC1, SSC8 and SSC17 (Rohrer *et al.*, 2006). The two QTLs on SSC6 identified by de Koning *et al.* (2000) are imprinted genes – one maternally expressed and one paternally expressed (de Koning *et al.*, 2000). These authors suggested MC5R, FABP3 and UOX as potential candidate genes but pointed out that there was no evidence of imprinting for any of these genes in any species. Near this region, two additional genes, p73 and PEG3, were known to be imprinted, at least in mice. On SSC12, a SNP in ESTL147 is associated with a lower fat/higher lean content, with the T allele favoured (Paszek *et al.*, 2001). On SSCX, the acyl-CoA synthetase 4 (ACSL4) gene has been investigated as a potential candidate (Mercade *et al.*, 2006; Perez-Enciso *et al.*, 2002, 2005) and a new marker associated with IMF on SSCX has been developed (Gaboreanu *et al.*, 2004).

The link between IMF and H-FABP (FABP3) was initially suggested by Gerbens *et al.* (2000) and Grindflek *et al.* (2001), based on the position of a QTL and the role of H-FABP in fatty acid metabolism. However, it appears that the polymorphisms in H-FABP may not be the causal mutations but are instead closely linked. Ovilo *et al.* (2000, 2002) have mapped H-FABP to a position 11 cM outside the confidence interval of the QTL for IMF. Another candidate on SSC6 is a small heterodimer partner (SHP) (Arnyasi *et al.*, 2006), an orphan nuclear receptor which represses and inhibits liver X receptor and retinoid X receptor, both of which play a role in lipid homeostasis (Brendel *et al.*, 2002; Chawla *et al.*, 2001).

Drip loss represents a potential high loss to the producer because of reduced weight. It is related to water holding capacity, cooking loss and thaw loss. High drip loss is also associated with less pork flavour, more off-flavours and less tenderness. Again, both the RYR1 and PRKAG3 genes are associated with this trait: (N, n) heterozygotes for the Hal gene have a higher drip loss than (N, N) homozygotes. There is also an interaction of the Agouti-related protein (AGRP) with RYR1: the T/T and C/C genotypes of AGRP differ in their effects on drip loss, depending on whether the animal is (N, n) or (N, N) (Otto *et al.*, 2007). In PRKAG3, the II homozygote at amino acid 199 is associated with lower drip loss than the IV or VV forms.

Melanocortin type 4 receptor (MC4R), a G protein coupled receptor that controls appetite in mice, is associated with growth and performance, carcass composition and drip loss traits in pigs (Kim *et al.*, 2000; Ovilo *et al.*, 2006; Otto *et al.*, 2007). The polymorphism G1426A that codes for the D298N missense mutation results in increased backfat, increased feed intake and increased drip loss. The favourable allele (G), if homozygous, has a reduced drip loss of 0.43% compared to the A/A unfavourable homozygote (Otto *et al.*, 2007). Rather surprisingly, Iberian pigs, which are used for the production of dry-cured hams, are not A/A homozygotes but instead are G/G homozygotes (Burgos *et al.*, 2006). This was taken as evidence that this was the ancestral gene and that the G allele came about as a result of selection for leanness. It also suggests that selection of the A

allele in Iberian pigs could improve the quality of cured hams by increasing the fat levels (Burgos *et al.*, 2006).

Many meat quality traits are related to each other with the result that QTLs for different traits often associate with the same chromosomal region and are ultimately shown to be caused by mutations in the same gene. Ultimate pH (24 h or 48 h) is the most commonly used trait to assess pork quality, and although it is not a direct measure of meat quality, it correlates with traits that are (Malek *et al.*, 2001a,b). Colour and pH are linked to tenderness and drip loss, and pH and tenderness are related to sensory measurements such as firmness and juiciness. Both pH₂₄ (24 h post-mortem) and colour are again associated with RYR1, PRKAG3 and MC4R. The pH₂₄ of ham is higher for the II genotype at PRKAG3 I199V than for the IV genotype, and both are higher than the VV genotype (Otto *et al.*, 2007). The MC4R A/A genotype is significantly darker than the G/G genotype and this again is related to the lesser drip loss of the A/A genotype. QTL studies have suggested strong associations of Minolta L* with SSC1 (Rohrer *et al.*, 2006) and with SSC4 (van Wijk *et al.*, 2006a) – the latter supporting three earlier studies (Ovilo *et al.*, 2002; de Koning *et al.*, 2000; Malek *et al.*, 2001b). One candidate gene is protoporphyrinogen oxidase, which is involved in the haem biosynthesis pathway (Ovilo *et al.*, 2002), and a second is glutamate–cysteine ligase modifier subunit, which may regulate the oxidation state of myoglobin through the glutathione synthesis pathway (van Wijk *et al.*, 2006b). Other suggestive QTL regions are on SSC2 (van Wijk *et al.*, 2006b; Malek *et al.*, 2001b; Rohrer *et al.*, 2006), SSC3 (Geldermann *et al.*, 2003; de Koning *et al.*, 2001), SSC4 (Ovilo *et al.*, 2002; Wang *et al.*, 1998) SSC5 (Harmegnies *et al.*, 2006; Malek *et al.*, 2001b; Rohrer *et al.*, 2006), SSC6 (Geldermann *et al.*, 2003; Nii *et al.*, 2005), SSC7 (Wang *et al.*, 1998; Ovilo *et al.*, 2002) SSC8 (van Wijk *et al.*, 2006b; Rohrer *et al.*, 2006; Geldermann *et al.*, 2003), SSC14 (Rohrer *et al.*, 2006), SSC17 (Malek *et al.*, 2001b; Rohrer *et al.*, 2007) and SSCX (Harmegnies *et al.*, 2006).

The Rendement Napole gene exemplifies a major approach that has been taken to identify candidate genes, that of QTL analysis (see [Section 10.5](#)) followed by positional cloning. Other candidate genes that follow this example would be ASIP and CEBPB (Rohrer *et al.*, 2006), SHP and FABP3 (Arnyasi *et al.*, 2006), CAST (Ciobanu *et al.*, 2004) and IGFII (Jungerius *et al.*, 2004). A second approach has been to look at key genes that are known to regulate major pathways (without necessarily having been suggested by QTL analysis), such as that of fatty acid metabolism, e.g. acyl CoA synthetase (Mercade *et al.*, 2006) or metabolic potential, e.g. succinate dehydrogenase complex subunit D (Zhu *et al.*, 2005). Similarly, several genes (PRKAA2, PRKAB1, PRKAB2, PRKAG3, GAA, GYS1, PYGM, ALDOA, GPI, LDHA, PGAM2 and PKM2), chosen according to their role in the regulation of energy balance and of glycogen metabolism and glycolysis of the skeletal muscle, were investigated for polymorphic sites (Fontanesi *et al.*, 2003). After being genotyped at the RN locus, significant associations were found for the PRKAG3, T30N and G52S polymorphic sites with meat colour and PGAM2 and PKM2 with drip loss percentage and glycogen content at one hour

post-mortem, respectively (Fontanesi *et al.*, 2003). Significant differences in allele frequencies of the three substitutions (T30N, G52S and V199I) between Chinese indigenous pigs and Western commercial pigs have been observed. Obvious high frequencies of the favourable alleles 30T and 52G in terms of meat quality were detected in Chinese indigenous pigs; however, the frequency of the favourable allele 199I, which was reported to have a greater effect on meat quality in comparison with 30T and 52G, was very low in all of the Chinese indigenous pigs except for the Min pig (Huang *et al.*, 2004).

A third approach, as exemplified by PRKAG3, is that, once a mutation in a gene has been linked to a meat quality trait, it is possible that other mutations in the same gene will affect meat quality. The Rendement Napole mutation R200Q implicated PRKAG3 in meat quality and subsequently the novel I199V mutation was identified. Taking this further, Demeure *et al.* investigated the three isoforms of AMP-activated protein kinase gamma chain (PRKAG1, PRKAG2 and PRKAG3) in order initially to identify polymorphisms (Demeure *et al.*, 2004). The *ryr-1* locus and other genes associated with the functioning of the ryanodine receptor would be obvious places to expect to find other mutations associated with meat quality, and specifically PSE meat. Considering the prevalence of mutations in the human ryanodine receptor (see review by Zalk *et al.*, 2007), it is perhaps surprising that very few other mutations have been recorded in pigs (Beja-Pereira *et al.*, 2001).

A good review of the current state of candidate genes associated with meat quality traits (especially drip loss) is that of Otto *et al.* (2007). The genes listed by Otto *et al.* (2007) include MC4R, LDHA, CAST, ATP2A1, AGRP, RYR1, CKM, HMGA1, GLUT4, Agouti/ASIP and PRKAG3.

10.3 Functional genomics and improving pork quality

Meat quality is predicted upon gene expression at the time of slaughter, where each meat quality trait is determined by the cumulative effect of all genes expressed in the tissue. Functional genomics allows the ability to look at the full complement of genes expressed in a tissue or organism at a particular time and under a particular set of circumstances. It is estimated that approximately 15 000 genes (mRNAs) are expressed in any one tissue at any time: therefore, with a carefully constructed microarray, it should be possible to get a snapshot of gene expression within muscle tissue (near slaughter) and predict the resulting meat quality.

A microarray starts from a collection of pig clones that should represent all relevant genes expressed in the pig. Although this would be all-encompassing, clones have instead often been derived from muscle-specific EST libraries, where the major issue is the confidence that all genes, including weakly expressed genes, are represented. Normalised libraries improve upon EST libraries by using the kinetics of self-hybridisation to allow multiple copies of the same gene to self-hybridise while leaving single copies non-hybridised and available for library construction: in theory, all genes should be represented at the same level in these libraries. Another alternative is the Suppression Subtractive Hybridisation (SSH)

library where only genes differentially regulated between two conditions (e.g. high drip loss animals vs. low drip loss animals) are enriched after subtraction of unchanging genes (Plastow *et al.*, 2005; Diatchenko *et al.*, 1996). All approaches have been used to generate pig microarrays and each approach has its supporters and detractors.

Clones can be spotted onto the microarray (cDNA array) or oligos representing these clones can be spotted (or synthesised directly) onto the array (Lockhart *et al.*, 1996; Schena *et al.*, 1995, 1996). The array (basically a glass slide) is hybridised with a labelled cDNA or cRNA isolated from a tissue of interest, and can be compared to other similar samples. The manipulation and statistical analysis of the digitised scanned images are complicated and require careful selection of background subtraction and normalisation methods (for reviews see Quackenbush, 2001; Butte, 2002). Selection of differentially expressed genes is carried out by various statistical tests about which there is still much debate. One complication is a necessary correction for false discovery rate because the number of tests is very high; a 10 000 spot array will produce 500 false positives if the *P* value is set at 0.05. A variety of corrections is available but their stringency varies. Because of the variety of analysis approaches, and demonstrations that quite different lists of differentially expressed genes can be generated from the same samples in different laboratories, most microarray analysis requires validation by a more robust technique such as qPCR, though this generally can be carried out only on individual genes.

Individual variation in animals is such a confounding factor in expression analysis that experimental design and animal numbers are a crucial and expensive part of it. To date, not many microarray analyses of pig muscle have been published, though this probably reflects the complexity of these approaches and the relatively recent availability of comprehensive clone collections.

The pig and human genomes are quite similar, which allows the assignment of gene ontology (GO) terms to most genes on a pig array. These describe the processes the genes are involved in, their molecular function and their cellular compartment. Statistical analysis can then compare a list of differentially expressed genes to the 'whole genome' of genes and determine statistically significant processes (e.g. 'ion balance' or 'response to stimulus') that are taking place in the cell as a result of the different compared conditions.

Initially, the use of human whole genome arrays was necessitated by a lack of porcine EST collections. Moody *et al.* (2002) showed that cross-species hybridisation of porcine targets hybridised to human probes was a possible approach if a slight decrease in washing stringency was adopted. This study used human nylon arrays (GF 211 Human GeneFilters from ResGen with 4324 human genes of known function) and the target cDNA was radioactively labelled with ³³P (Moody *et al.*, 2002). More recent studies have developed this approach using Cy5/Cy3-labelled cDNAs and higher density arrays such as the Incyte human uniGEM V2 array with 9182 genes (Lin and Hsu, 2005). An extreme version of this is the use of a human titin oligo array to examine the differentially splicing of a single gene (titin) in porcine skeletal muscle samples (Lahmers *et al.*, 2004).

The development of porcine resources continued alongside the use of human

arrays. As porcine skeletal muscle cDNA libraries became available (Davoli *et al.*, 2002; Bai *et al.*, 2003), so too did new porcine arrays. A macroarray representing 260 genes isolated from whole embryo, adult skeletal muscle and differential display products from a foetal–post-natal muscle comparison was used to compare foetal and post-natal muscle cDNAs labelled with ^{32}P on nylon membranes (Zhao *et al.*, 2003). Higher resolution microarrays have been developed to use the Cy5/Cy3 dual labelling approach to look specifically at expression in the muscle (Bai *et al.*, 2003; Te Pas *et al.*, 2005), but others such as the GeneChip Porcine Genome Array (Affymetrix) and the Qiagen-NRSP8 Array (Zhao *et al.*, 2005) are whole genome arrays that incorporate several thousands of probes. The Affymetrix array presents 23 937 probe sets for 20 201 *S. scrofa* genes (www.affymetrix.com), whereas the Qiagen array (discontinued) presents 13 297 cDNAs and ESTs. These two latter arrays are based on oligo sets compiled by analysis of existing database information (NCBI or TIGR) rather than being a development from in-house cDNA library construction and analysis. This emphasises the current strength of the information now available in the EST databases and, with the near completion of the pig genome sequencing project, indicates that library construction is no longer a prerequisite for studying porcine expression patterns.

Few reports that relate directly to the use of microarrays in the study of meat quality have been published to date, though the paper by Plastow *et al.* (2005) presents a resource that is being developed for that specific purpose. Arrays that compare red muscle to white muscle (da Costa *et al.*, 2004; Bai *et al.*, 2003), and those that investigate muscle development from the foetus to the post-natal, are meat quality related but do not identify differentially expressed genes that can be associated with meat quality.

10.4 Proteomics and improving pork quality

While RNA-based microarray approaches have proven easier to develop than proteomic approaches, protein is the normal functional molecule that effects change in the system. For interpretative simplicity it is assumed that an increase in RNA expression leads to an increase in protein expression, but this cannot be taken for granted nor can fold change in RNA expression be directly related to fold change in protein levels since a single RNA molecule can be translated multiple times to yield many protein molecules. Microarray expression profiles should therefore be validated first by qPCR and then by protein analysis if possible. This requires either clear separation of the protein from other proteins followed by definitive identification by sequencing, mass spectroscopy, etc., or identification by using specific antibodies (which may not be available).

The proteomic approach is more often used in isolation from transcriptomic approaches with the expectation that the results will be complementary. Loin muscle from five different breeds of pig were subjected to two-dimensional gel electrophoresis (2DE) over the *pI* range 3–10 and over the molecular weight range 10–200 kDa (Plastow *et al.*, 2005). These breeds were compared for various meat

quality traits and were shown to have clear differences, e.g. Duroc pigs showed significantly lower drip loss than Landrace pigs, and Duroc and Pietrain pigs had higher ultimate pH (pHu) than Landrace pigs. Approximately 750 spots were separated on this system and 21 were shown to be significantly differentially expressed between breeds (Plastow *et al.*, 2005). No report yet identifies these proteins. In another proteome approach, pigs fed *ad libitum* were compared to pigs fed on a restricted diet followed by compensatory growth on return to *ad libitum* feeding (Lametsch *et al.*, 2006). Compensatory growth had been previously shown to increase the tenderness of meat (Kristensen *et al.*, 2004). Muscle samples were fractionated and only sarcoplasmic reticulum proteins were loaded on the gel: this has the advantage of removing the highly expressed proteins (myosin heavy chain, titin, actin and nebulin) and thereby allowing better and more reproducible separation of the proteins. Comparing *ad libitum* and compensatory growth animals at slaughter identified a number of proteins that were all down-regulated in conditions of compensatory growth; these included the heat shock proteins HSC70 and HSP27 (A and B), enolase 3, glycerol-3-phosphate dehydrogenase, aldehyde dehydrogenases E2 and E3 and 2, 3 biphosphoglycerate mutase (Lametsch *et al.*, 2006). Comparing the two groups of animals 48 h post-mortem showed up-regulation of myosin light chains II and III, sulphite oxidase, chloride intracellular channel 1, 14-3-3 protein γ , elongin B and phosphohistidine phosphatase 14 in compensatory growth animals and only down-regulation of glycerol-3-phosphate dehydrogenase (Lametsch *et al.*, 2006).

A different approach to proteome analysis is that of reverse phase microarray analysis (RPMA). In this procedure (Espina *et al.*, 2003; Chan *et al.*, 2004), complex protein samples are immobilised on slides in a format similar to cDNA arrays and the slide is probed with fluorescently labelled antibodies to the protein of interest. Seven target proteins of the sarcoplasmic reticulum calcium regulation system in 24 halothane genotyped porcine muscle samples were investigated (Schulz *et al.*, 2006) by this method. It was shown that Hal⁺ animals (n, n) had significantly lower levels of ryanodine receptor than Hal⁻ animals (N, N) and that levels of calsequestrin, triadin, dihydropyridine receptor and slow-twitch calcium ATPase were also significantly lower in the Hal⁺ animals (Schulz *et al.*, 2006).

10.5 Quantitative trait loci analysis and improving pork quality

A recent review (Rothschild *et al.*, 2007) on QTL mapping in pigs highlights the information held in the database PigQTLdb (<http://www.animalgenome.org/QTLdb/pig.html>). This database holds information on all general traits (production, reproduction, health, exterior), not just meat quality traits (Hu *et al.*, 2005, 2007). Searching in this database with the term 'meat quality traits' points the user to all the QTLs that have been associated with any meat quality trait (e.g. pH, drip loss, colour and IMF), to which chromosomes they are mapped, which markers delineate their position and which publications report the findings. It also

allows a view of pig and human chromosome synteny. Searches with other terms such as chromosome number, marker or gene are also allowed. Readers are referred to this database as a more comprehensive repository of all QTL information, map and marker position than is detailed in this section. Some information discussed below is suggestive and has not been corroborated sufficiently to be included in the database, especially where candidate genes for QTL are proposed.

Meat quality traits hold a special place in the development of breeding programmes: meat quality, by its very nature, can be phenotyped only after the slaughter of the animal. Breeding is therefore not of the individuals tested but of their sibs, and marker assisted selection has a more crucial role to play. Ten years ago, QTL analysis generally followed the approach of crossing divergent breeds to produce large F2 families and searching for traits that segregated with DNA markers (RFLP, AFLP, microsatellites, SNPs). Exotic breeds, such as the European or Japanese wild boar, the Chinese Meishan, the Berlin Miniature, the Iberian boar, for example, would be crossed with more commercial breeds such as the Large White, or Duroc to produce 300–700 offspring with diverse phenotypes (Andersson *et al.*, 1994; Wimmers *et al.*, 2006; Nii *et al.*, 2005). A major limitation in early studies was the relatively low density of informative markers and hence any of hundreds of genes could carry the causative mutation. An obvious solution was to develop new markers for any area of interest. Ramos *et al.* (2006) developed an additional 21 PCR-RFLP markers for a QTL on SSC17, close to the known gene positions for melanocortin 3 receptor (MC3R) and agouti signalling protein (ASIP), both of which are associated with meat quality. The causative mutation for the Rendement Napole gene (see earlier) was similarly identified through increasing the density of markers between *SLC11A1* and SNP *S1010* on SSC15 (Milan *et al.*, 2000). More recently, studies have concentrated on using large commercial breed collections and association analysis with SNPs, and also using the candidate gene approach where the candidate gene may come from QTL analysis or some other relevant characteristic of the gene.

Carcass composition, growth, disease resistance, longevity and litter size were used in many of the early QTL studies but it was 1998 before the first study was carried out that included meat quality traits (Andersson-Eklund, 1998). Commercial breeds had been selected for many generations on production traits such as lean growth and muscle size; indeed, this selection, as mentioned previously, was the likely reason why the Hal and RN alleles were established in the pig population. In 2001 the first whole genome scan to detect QTL for meat quality traits in pigs was published (de Koning *et al.*, 2001). This examined 785 F2 animals of a Meishan boar and Dutch sow cross for eight meat quality traits. The genome was broadly covered by 132 microsatellite markers, ranging from 15 markers on SSC2 to two on SSC18. Three significant QTLs for colour were identified on SSC3, SSC4 and SSC13, together with 26 suggestive QTLs for all the meat quality traits. No candidate genes were suggested in the paper though, since a paternally expressed QTL on SSC4 was identified for both drip loss and pH, and since these two traits are related (oppositely correlated), it was suggested that the

same gene might be involved. This was also the case for a maternally expressed QTL for shearing force and pH on SSC9.

There followed a number of whole genome scan QTL studies that increasingly included meat quality traits (van Wijk *et al.*, 2006a; Ovilo *et al.*, 2002; Malek *et al.*, 2001a; Nii *et al.*, 2005; Harmegnies *et al.*, 2006; Rohrer *et al.*, 2006; Geldermann *et al.*, 2003). Most of the QTLs identified in these various studies showed a considerable degree of overlap, adding strength to the conclusions. For example, tenderness was linked strongly to SSC4 and SSC14, and suggestively to SSC3, SSC7 and SSC15 (Harmegnies *et al.*, 2006). This agreed closely with other reports on SSC4 and SSC15 (Rohrer *et al.*, 2006) and SSC14 (Malek *et al.*, 2001b). Breed-specific effects might be responsible for situations where a QTL identified in one study was not confirmed in a second study. There was some movement away from the exotic breeds to the commercial breeds in order to ensure that findings were relevant to the industry. Outbred populations ensured a high level of heterozygosity but this level of heterozygosity could not be assumed in commercial inbred lines. However, two papers that addressed this question (Evans *et al.*, 2003; Nagamine *et al.*, 2003) clearly showed that data from divergent crosses was equally relevant to commercial animals. The identification of significant and suggestive QTLs can be dependent on the various genetic and statistical models employed to analyse the results. The reader is directed to papers elsewhere where these different models are discussed (de Koning *et al.*, 2001; Haley *et al.*, 1994; Knott *et al.*, 1998).

Most of the above meat quality traits relate to measurements on muscle itself and expression of genes within the muscle; however, meat quality is not only governed by expression in the muscle and associated fat tissue, but also by expression in other tissues of the body. The liver, for example, plays a central role in metabolic balance in the body and is involved in carbohydrate, amino acid and lipid metabolism, as well as vitamin, mineral and electrolyte homeostasis. A candidate gene approach to liver investigation was taken by first identifying genes expressed preferentially in the liver (Ponsuksili *et al.*, 2001a,b) and then mapping these against a somatic cell hybrid panel (Wimmers *et al.*, 2002). SNPs were identified in these genes and segregation in the DUMI resource animals was determined (Ponsuksili *et al.*, 2005). An SNP in an EST (ESTL147) on SSC12 was associated with fat/lean meat content and another in the homogenisate 1, 2-dioxygenase (HGD) gene on SSC13 was associated with meat colour. The serious effects of stress on pig meat quality implicate hypothalamic–pituitary–adrenal axis (HPA) function; therefore a similar approach would be expected to suggest novel genes that may map to QTL loci.

Sequencing of the porcine genome has been published at a coverage of 0.66× (Wernersson *et al.*, 2005). This has opened up new opportunities using the power of comparative genomics. When Ramos *et al.* (2006) increased the density of markers on SSC17 around the ASIP and MC3R markers, they identified 21 new microsatellite markers by analysing the synteny between SSC17 and human chromosome (HSC) 20. This has been taken further by Karlskov-Mortensen *et al.* (2007), who have used the pig–human comparative map in combination with

shotgun sequences from the Sino-Danish Pig Genome Sequencing Consortium (<http://piggenome.dk>) to identify 10 882 new pig microsatellite markers and position 4528 of them on the pig map. This should be an invaluable source of markers to be used in linkage studies, in fine mapping and positional cloning of quantitative trait loci.

It has been suggested that too blinkered an approach is possibly being taken to QTL analysis (Rothschild *et al.*, 2007) and that biochemical and environmental factors, for example, should be considered as part of the whole picture. Furthermore, there are many overlapping QTL datasets available and much could be gained by pooling information; however, there is no standard design approach that facilitates pooling datasets. One of the difficulties with QTLs is that there are relatively few measurable phenotypic traits. A new approach that promises much is that of combining microarray expression data with QTL information. In genomic genotyping (see Section 10.6), as it has been referred to, expression data for each gene on an array is considered a different phenotypic trait thereby enormously increasing the number of traits that can be analysed.

10.6 Future trends

Some time during 2008, the porcine genome is expected to be published at 3× coverage. This is going to be a significant landmark in the development of pig and pork meat quality traits. One development already from the Sino-Danish Pig Genome Sequencing Consortium is the identification of 10 882 new porcine microsatellite sequences which can be used for marker development, linkage studies and positional cloning of QTL (Karlskov-Mortensen *et al.*, 2007). Comparative genomics approaches with pig, human and mouse will facilitate the identification of novel SNPs and precisely locate these SNPs (and existing SNPs) in relation to QTL markers. This will clarify the role of some candidate genes.

The comparative maps will also allow access to promoter and intronic sequences which have, until now, been more difficult to access than EST sequences. Functional genomics, through expression microarrays, currently highlights genes that are differentially expressed between pork samples of differing quality, but the underlying cause of the differential regulation is more complex. The promoter of the gene governs the rate at which new transcripts are produced and promoter SNPs may cause differential regulation by affecting the binding of transcription factors or accessory proteins. This view, however, is probably too simplistic. First of all, the steady-state level of transcripts may be determined by transcript stability as well as *de novo* transcript synthesis; therefore, other DNA binding proteins and nucleases may be involved that are not linked physically to the differentially expressed gene. Secondly, effects on transcription may result from cumulative effects on the complex catalogue of transcription factors and accessory proteins that regulate expression from the promoter. Again, the cause will be a *trans* effect, not a *cis* effect in the promoter.

Most of the candidate genes mentioned in Section 10.2 were identified with

coding region SNPs that produced a missense mutation in the gene. These genes would not necessarily be identified in a microarray analysis because there is no *a priori* reason why transcription of the gene should be affected. However, if the gene plays an essential role, one might imagine that the system will be perturbed as a result of the decreased functionality. If this occurs at the transcription level, then the gene (or other genes interacting with it) may still be identified on an expression microarray.

Microarray analysis will also lead to the identification of clusters of genes that are co-regulated. Co-regulation implies similarity in promoter regulator sites from which common transcription factor binding site modules or frameworks can be deduced (Cohen *et al.*, 2006). As well as allowing an improved understanding of the regulation of these genes, these frameworks can then be used to search the genome for novel genes that may also be part of this co-regulated network (Cohen *et al.*, 2006). In summary, many of the bioinformatics approaches that have been developed for the human genome will now be available to interrogate the porcine genome.

Another development will be the integration of expression and QTL data. QTL analysis depends on the number and variety of phenotypic traits for its efficacy, yet this number is quite limiting. Expression data yields information on thousands of genes and, by treating the expression of each gene as a phenotype, the power of QTL analysis can be greatly enhanced. In practice, both transcriptional profiling and QTL mapping are carried out on the same individuals in a F2 segregating population. This eQTL ('e' for expression) approach, variously called genetical genomics and transcriptome mapping, is now being applied to both human and livestock studies and has the ability to identify both *cis*- and *trans*- effects (see for references Kadarmideen *et al.*, 2006). For *cis*-effects, transcription and QTL peaks coincide, suggesting that expression of the gene is controlled by a polymorphism within the gene (e.g. a promoter SNP). For *trans*-effects, the QTL and transcription peaks do not coincide, so the suggestion is that the QTL represents a *trans*-acting factor (e.g. a transcription factor). Since the current belief is that *trans*-eQTLs outnumber *cis*-eQTLs, less time will be wasted in the search for causative mutations when eQTL suggests the cause is an unlinked gene.

Porcine whole genome arrays and SNP arrays are available currently and, in the case of the genome arrays, are probably quite comprehensive, but the completion of the porcine sequence will doubtless allow additions to both these arrays. As mentioned, most candidate genes identified to date have been linked to missense mutations and, for some, the functional consequences of the mutation can at least be guessed at. In the future, as gene regulation is understood better, more and more candidate genes will be identified where the causative mutation will be the result of synonymous mutation in the coding region, or mutation in the 3'UTR or mutation in intronic regions. Recently an SNP in the 3'UTR of the myostatin gene in Texel sheep was identified (Clop *et al.*, 2006). One form of the SNP created a site for microRNAs (miRNAs) that caused significant translational inhibition of the protein. A recent article identified 58 new miRNAs in the porcine genome on top of the existing 54 miRNAs (Kim *et al.*, 2006); since many are expected to be

expressed in skeletal muscle, a new level of gene regulation is becoming apparent that will no doubt have an impact on meat quality.

10.7 Sources of further information and advice

Several molecular aspects of meat quality are omitted in this review and some are only lightly touched upon. For example, no mention is made of the role muscle fibres play in meat quality. For further detail, the reader is referred to other chapters in this volume. An excellent series of six recent reviews are available in *Int. J. Biol. Sci.* (2007) 3 (3) pp. 129–197 and should be consulted for further references. So too should the pig QTL database (PigQTLdb), which is one of the three genomes available at www.animalgenome.org/QTLdb/ (Hu *et al.*, 2005, 2007).

There are many genomic and proteomic resources available on the internet. A major interdisciplinary collaboration in Denmark is available at <http://genome.dk/> which includes the Sino-Danish pig genome project. In the US, the NCBI holds pig genome resources at <http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig/>, and in Japan resources are held at <http://animal.dna.affrc.go.jp>. For more comprehensive lists of genomic and proteomic resources (though these sites generally have links to other relevant sites) see Chen *et al.* (2007) and Fadiel *et al.* (2005).

Commercially available porcine arrays are available at <http://www.pigoligoarray.org/> and at <http://www.affymetrix.com/>. The first array is a 70-mer oligonucleotide protein-annotated microarray that has been developed as an open source collaboration and can be purchased at a reasonable price.

Other useful sites are <http://www.pic.com/>, the commercial site for PIC which is now part of Genus plc; <http://www.nppc.org/about/index.html>, the site for the National Pork Producers Council (USA); <http://www.complextait.org/>, for more information on complex trait studies; <http://www.thepigsite.com/>, for general information of many aspects of pig science and welfare; and <http://isu.porkgateway.com/>, a site at Iowa State University. Many of the universities in the US have 'Extensions', and these sites are full of useful information.

10.8 References

- Andersson, L., Haley, C. S., Ellegren, H., Knott, S. A., Johansson, M., Andersson, K., Andersson-Eklund, L., Edfors-Lilja, I., Fredholm, M., Hansson, I. *et al.* (1994), Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science*, 263, 1771–1774.
- Andersson-Eklund, L., Marklund, L., Lundström, K., Haley, C. S., Andersson, K., Hansson, I., Moller, M. and Andersson, L. (1998), Mapping quantitative trait loci for carcass and meat quality traits in a wild boar × Large White intercross. *J. Anim. Sci.*, 76, 694–700.
- Arnyasi, M., Grindflek, E., Javor, A. and Lien, S. (2006), Investigation of two candidate genes for meat quality traits in a quantitative trait locus region on SSC6: The porcine short heterodimer partner and heart fatty acid binding protein genes. *J. Anim. Breed. Genet.*, 123, 198–203.
- Bai, Q., McGillivray, C., Da Costa, N., Dornan, S., Evans, G., Stear, M. and Chang, K.-C. (2003), Development of a porcine skeletal muscle cDNA microarray: Analysis of

- differential transcript expression in phenotypically distinct muscles. *BMC Genomics*, 4, 8.
- Barton-Gade, P. (1990), Danish experience in meat quality improvement. *Proceedings of the 4th Congress of Genetics Applied to Livestock Production*. Edinburgh, UK.
- Beja-Pereira, A., Bento, P., Ferrand, N. and Brenig, B. (2001), Genetic polymorphism of the 17th exon at porcine RYR1 locus: A new variant in a local Portuguese pig breed demonstrated by SSCP analysis. *J. Anim. Breed Genet.*, 118, 271–274.
- Brendel, C., Schoonjans, K., Botrugno, O. A., Treuter, E. and Auwerx, J. (2002), The Small Heterodimer Partner Interacts with the Liver X Receptor alpha and Represses its Transcriptional Activity. *Molecular Endocrinology*, 16, 2065–2076.
- Burgos, C., Carrodeguas, J. A., Moreno, C., Altarriba, J., Tarrafeta, L., Barcelona, J. A. and Lopez-Buesa, P. (2006), Allelic incidence in several pig breeds of a missense variant of pig melanocortin-4 receptor (MC4R) gene associated with carcass and productive traits; Its relation to IGF2 genotype. *Meat Science*, 73, 144–150.
- Butte, A. (2002), The use and analysis of microarray data. *Nature Reviews Drug Discovery*, 1, 951–960.
- Carr, C. C., Morgan, J. B., Berg, E. P., Carter, S. D. and Ray, F. K. (2006), Growth performance, carcass composition, quality, and enhancement treatment of fresh pork identified through deoxyribonucleic acid marker-assisted selection for the Rendement Napole gene. *J. Anim. Sci.*, 84, 910–917.
- Chan, S. M., Ermann, J., Su, L., Fathman, C. G. and Uuz, P. J. (2004), Protein microarrays for multiplex analysis of signal transduction pathways. *Nature Medicine*, 10, 1390–1396.
- Chawla, A., Repa, J. J., Evams, R. M. and Mandelsdorf, D. J. (2001), Nuclear Receptors and Lipid Physiology: Opening the X-Files. *Science*, 294, 1866–1870.
- Chen, K., Baxter, T., Muir, W. M., Grpemem, M. A. and Schook, L. B. (2007), Genetic resources, genome mapping and evolutionary genomics of the pig (*Sus scrofa*). *Int. J. Biol. Sci.*, 3, 153–165.
- Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G. and Rothschild, M. (2001), Evidence for new alleles in the protein kinase adenosine monophosphate-activated gamma(3)-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. *Genetics*, 159, 1151–1162.
- Ciobanu, D. C., Bastiaansen, J. W., Lonergan, S. M., Thomsen, H., Dekkers, J. C., Plastow, G. S. and Rothschild, M. F. (2004), New alleles in calpastatin gene are associated with meat quality traits in pigs. *J. Anim. Sci.*, 82, 2829–2839.
- Ciobanu, D. C., Lonergan, S. M., Bastiaansen, J. W. M., Woollard, J. R., Malek, M. and Huff-Lonergan, E. J. E. A. (2002a), Evidence for new alleles in calpastatin gene associated with meat quality traits in pigs. *Proceedings of the 7th World Congress on Genetics Applied to Livestock Production*. Montpellier, France.
- Ciobanu, D. C., Zhang, Y. and Rothschild, M. F. (2002b), Rapid communication: Mapping of the Ca2+ ATPase of fast twitch 1 skeletal muscle sarcoplasmic reticulum (ATP2A1) gene to porcine chromosome 3. *J. Anim. Sci.*, 80, 1386–1387.
- Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibe, B., Bouix, J., Caiment, F., Elsen, J.-M., Eychenne, F., Larzul, C., Laville, E., Meish, F., Milenkovic, D., Tobin, J., Charlier, C. and Georges, M. (2006), A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nat. Genet.*, 38, 813–818.
- Cohen, C. D., Klingenhoff, A., Boucherot, A., Nitsche, A., Henger, A., Brunner, B., Schmid, H., Merkle, M., Saleem, M. A., Koller, K.-P., Werner, T., Grone, H.-J., Nelson, P. J. and Kretzler, M. (2006), Comparative promoter analysis allows de novo identification of specialized cell junction-associated proteins. *Proc. Natl. Acad. Sci. USA*, 103, 5682–5687.
- Da Costa, N., McGillivray, C., Bai, Q., Wood, J. D., Evans, G. and Chang, K. C. (2004), Restriction of dietary energy and protein induces molecular changes in young porcine skeletal muscles. *J. Nutr.*, 134, 2191–2199.

- Davoli, R., Fontanesi, L., Zambonelli, P., Bigi, D., Gellin, J., Yerle, M., Milc, J., Braglia, S., Cenci, V., Cagnazzo, M. and Russo, V. (2002), Isolation of porcine expressed sequence tags for the construction of a first genomic transcript map of the skeletal muscle in pig. *Anim. Genet.*, 33, 3–18.
- De Konning, D. J., Rattink, A. P., Harlizius, B., Van Arendonk, J. A. M., Brascamp, E. W. and Groenen, M. A. M. (2000), Genome-wide scan for body composition in pigs reveals important role of imprinting. *Proc. Natl. Acad. Sci. USA*, 97, 7947–7950.
- De Konning, D. J., Harlizius, B., Rattink, K. A. P., Groenen, M. A., Brascamp, E. W. and Van Arendonk, J. A. (2001a), Detection and characterization of quantitative trait loci for meat quality traits in pigs. *J. Anim. Sci.*, 79, 2812–2819.
- De Konning, D. J., Janss, L. L. G., Rattink, A. P., Van Oers, P. A. M., De Vries, B. J., Groenen, M. A. M., Van Der Poel, J. J., De Groot, P. N., Brascamp, E. W. and Van Arendonk, J. A. M. (1999), Detection of Quantitative Trait Loci for Backfat Thickness and Intramuscular Fat Content in Pigs (*Sus scrofa*). *Genetics*, 152, 1679–1690.
- Delgado, E. F., Geesink, G. H., Marchello, J. A., Goll, D. E. and Koohmaraie, M. (2001a), The calpain system in three muscles of normal and callipyge sheep. *J. Anim. Sci.*, 79, 398–412.
- Delgado, E. F., Geesink, G. H., Marchello, J. A., Goll, D. E. and Koohmaraie, M. (2001b), Properties of myofibril-bound calpain activity in longissimus muscle of callipyge and normal sheep. *J. Anim. Sci.*, 79, 2097–2107.
- Demeure, O., Liaubet, L., Riquet, J. and Milan, D. (2004), Determination of PRKAG1 coding sequence and mapping of PRKAG1 and PRKAG2 relatively to porcine back fat thickness QTL. *Anim. Genet.*, 35, 123–125.
- Diatchenko, L., Lau, Y.-F. C., Campbell, A. P., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S., Lukyanov, K., Gurskaya, N., Sverdlov, E. D. and Siebert, P. D. (1996), Suppression subtractive hybridization: A method for generating differentially regulated or tissue-specific cDNA probes and libraries. *Proc. Natl. Acad. Sci. USA*, 93, 6025–6030.
- Doran, E., Whittington, F. W., Wood, J. D. and McGivan, J. D. (2002), Cytochrome P450III_{E1} (CYP2E1) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes. *Chemico-Biological Interactions*, 140, 81–92.
- Ernst, T. C. W., Robic, A., Yerle, M., Wang, L. and Rothschild, M. F. (1998), Mapping of calpastatin and three microsatellites to porcine chromosome 2q2.1–q2.4. *Anim. Genet.*, 29, 212–215.
- Espina, V., Mehta, A. I., Winters, M. E., Calvert, V., Wulfkühle, J., Petricoin, E. F. and Liotta, L. A. (2003), Protein microarrays: Molecular profiling technologies for clinical specimens. *Proteomics*, 3, 2091–2100.
- Evans, G. J., Giuffra, E., Sanchez, A., Kerje, S., Davalos, G., Vidal, O., Illan, S., Noguera, J. L., Varona, L., Velander, I., Southwood, O. I., De Koning, D. J., Haley, C. S., Plastow, G. S. and Andersson, L. (2003), Identification of Quantitative Trait Loci for Production Traits in Commercial Pig Populations. *Genetics*, 164, 621–627.
- Fadiel, A., Anidi, I. and Eichenbaum, K. D. (2005), Farm animal genomics and informatics: An update. *Nucleic Acids Research*, 33, 6308–6318.
- Fontanesi, L., Davoli, R., Nanni Costa, L., Scotti, E. and Russo, V. (2003), Study of candidate genes for glycolytic potential of porcine skeletal muscle: Identification and analysis of mutations, linkage and physical mapping and association with meat quality traits in pigs. *Cytogenet. Genome. Res.*, 102, 145–51.
- Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J. and MacLennan, D. H. (1991), Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science*, 253, 448–451.
- Gaboreanu, A. M., Grapes, L., Ramos, A. M., Kim, J. J. and Rothschild, M. F. (2004), Characterization of an X-chromosome PCR-RFLP marker associated with fat deposition and growth in the pig. *Anim. Genet.*, 35, 401–403.
- Geldermann, H., Muller, E., Moser, G., Reiner, G., Bartschlag, H., Cepica, S., Sratil, A., Kuryl, J., Moran, C., Davoli, R. and Brunsch, C. (2003), Genome-wide linkage and QTL

- mapping in porcine F2 families generated from Pietrain, Meishan and Wild Boar crosses. *J. Anim. Breed. Genet.*, 120, 363–393.
- Gerbens, F., De Koning, D. J., Harders, F. L., Meuwissen, T. H., Janss, L. L., Groenen, M. A., Veerkamp, J. H., Van Arendonk, J. A. and te Pas, M. F. (2000), The effect of adipocyte and heart fatty acid-binding protein genes on intramuscular fat and backfat content in Meishan crossbred pigs. *J. Anim. Sci.*, 78, 552–559.
- Grindflek, E., Szyda, J., Liu, Z. and Lien, S. (2001), Detection of quantitative trait loci for meat quality in a commercial slaughter pig cross. *Mamm. Genome.*, 12, 299–304.
- Haley, C. S., Knott, S. A. and Elsen, J. M. (1994), Mapping Quantitative Trait Loci in Crosses between Outbred Lines Using Least-Squares. *Genetics*, 136, 1195–1207.
- Hambrech, E., Eissen, J. J., Nooijent, R. I., Ducro, B. J., Smits, C. H., Den Hartog, L. A. and Verstegen, M. W. (2004), Preslaughter stress and muscle energy largely determine pork quality at two commercial processing plants. *J. Anim. Sci.*, 82, 1401–1409.
- Harmegnies, N., Davin, F., De Smet, S., Buys, N., Georges, M. and Coppeters, W. (2006), Results of a whole-genome quantitative trait locus scan for growth, carcass composition and meat quality in a porcine four-way cross. *Anim. Genet.*, 37, 543–553.
- Hu, Z.-L., Dracheva, S., Jang, W., Maglott, D., Bastiaansen, J., Rothschild, M. and Reecy, J. (2005), A QTL resource and comparison tool for pigs: PigQTLDB. *Mammalian Genome*, 16, 792–800.
- Hu, Z.-L., Fritz, E. R. and Reecy, J. M. (2007), AnimalQTLdb: A livestock QTL database tool set for positional QTL information mining and beyond. *Nucleic Acids Res.*, 35, (Database issue) D604–D609.
- Huang, L. S., Ma, J. W., Ren, J., Ding, N. S., Guo, Y. M., Ai, H. S., Li, L., Zhou, L. H. and Chen, C. Y. (2004), Genetic variations of the porcine PRKAG3 gene in Chinese indigenous pig breeds. *Genet. Sel. Evol.*, 36, 481–486.
- Human Genome Sequencing Consortium (2004), Finishing the euchromatic sequence of the human genome. *Nature*, 431, 931–945.
- Janss, L. L., Van Arendonk, J. A. and Brascamp, E. W. (1997), Bayesian statistical analyses for presence of single genes affecting meat quality traits in a crossed pig population. *Genetics*, 145, 395–408.
- Janss, L. L. G., Van Arendonk, J. A. M. and Brascamp, E. W. (1994), Identification of a single gene affecting intramuscular fat in crossbreds using Gibbs sampling. *Proceedings 5th World Congress Genetics Applied to Livestock Production*, Guelph, Ontario, Canada.
- Jungerius, B. J., Van Laere, A. S., te Pas, M. F., Van Oost, B. A., Anderssen, L. and Groenen, M. A. (2004), The IGF2-intron3-G3072A substitution explains a major imprinted QTL effect on backfat thickness in a Meishan × European white pig intercross. *Genet. Res.*, 84, 95–101.
- Kadarmideen, H. N., Von Rohr, P. and Janss, L. L. (2006), From genetical genomics to systems genetics: Potential applications in quantitative genomics and animal breeding. *Mamm. Genome.*, 17, 548–564.
- Karlskov-Mortensen, P., Hu, Z. L., Gorodkin, J., Reecy, J. M. and Fredholm, M. (2007), Identification of 10 882 porcine microsatellite sequences and virtual mapping of 4528 of these sequences. *Anim. Genet.*, 38, 401–405.
- Kim, H. J., Cui, X. S., Kim, E. J., Kim, W. J. and Kim, N. H. (2006), New porcine microRNA genes found by homology search. *Genome*, 49, 1283–1286.
- Kim, K. S., Larsen, N., Short, T., Plastow, G. and Rothschild, M. F. (2000), A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. *Mamm. Genome*, 11, 131–135.
- Knott, S. A., Marklund, L., Haley, C. S., Andersson, K., Davies, W., Ellegren, H., Fredholm, M., Hansson, I., Hoyheim, B., Lundstrom, K., Moller, M. and Andersson, L. (1998), Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and large white pigs. *Genetics*, 149, 1069–1080.
- Kocwin-Podsiadla, M., Kuryl, J., Krzeczio, E., Zybert, A. and Przybylski, W. (2003), The

- interaction between calpastatin and RYR1 genes for some pork quality traits. *Meat Science*, 65, 731–735.
- Kristensen, L., Therkildsen, M., Aaslyng, M. D., Oksbjerg, N. and Ertbjerg, P. (2004), Compensatory growth improves meat tenderness in gilts but not in barrows. *J. Anim. Sci.*, 82, 3617–3624.
- Lahmers, S., Wu, Y., Call, D. R., Labeit, S. and Granzier, H. (2004), Developmental control of titin isoform expression and passive stiffness in fetal and neonatal myocardium. *Circ. Res.*, 94, 505–513.
- Lametsch, R., Kristensen, L., Larsen, M. R., Therkildsen, M., Oksbjerg, N. and Ertbjerg, P. (2006), Changes in the muscle proteome after compensatory growth in pigs. *J. Anim. Sci.*, 84, 918–924.
- Leach, L. M., Ellis, M., Sutton, D. S., McKeith, F. K. and Wilson, E. R. (1996), The growth performance, carcass characteristics, and meat quality of halothane carrier and negative pigs. *J. Anim. Sci.*, 74, 934–943.
- Lee, G. J., Archibald, A. L., Law, A. S., Lloyd, S., Wood, J. and Haley, C. S. (2005), Detection of quantitative trait loci for androstenone, skatole and boar taint in a cross between Large White and Meishan pigs. *Anim. Genet.*, 36, 14–22.
- Lin, C. S. and Hsu, C. W. (2005), Differentially transcribed genes in skeletal muscle of Duroc and Taoyuan pigs. *J. Anim. Sci.*, 83, 2075–2086.
- Lockhart, D. J., Dong, H. L., Byrne, M. C., Follettie, M. T., Gallo, M. V., Chee, M. S., Mittmann, M., Wang, C. W., Kobayashi, M., Horton, H. and Brown, E. L. (1996), Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nature Biotechnology*, 14, 1675–1680.
- Loneragan, S. M., Ernst, C. W., Bishop, M. D., Calkins, C. R. and Koohmaraie, M. (1995), Relationship of restriction fragment length polymorphisms (RFLP) at the bovine calpastatin locus to calpastatin activity and meat tenderness. *J. Anim. Sci.*, 73, 3608–3612.
- Longergan, S. M., Huff-Loneragan, E., Rowe, L. J., Kuhlers, D. L. and Jungst, S. B. (2001), Selection for lean growth efficiency in Duroc pigs influences pork quality. *J. Anim. Sci.*, 79, 2075–2085.
- Lundstrom, K., Malmfors, B., Stern, S., Rydhmer, L., Eliassonselling, L., Mortensen, A. B. and Mortensen, H. P. (1994), Skatole Levels in Pigs Selected for High Lean Tissue-growth Rate on Different Dietary-protein Levels. *Livestock Production Science*, 38, 125–132.
- Malek, M., Dekkers, J. C., Lee, H. K., Baas, T. J., Prusa, K., Huff-Loneragan, E. and Rothschild, M. F. (2001a), A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition. *Mamm. Genome*, 12, 637–645.
- Malek, M., Dekkers, J. C., Lee, H. K., Baas, T. J., Prusa, K., Huff-Loneragan, E. and Rothschild, M. F. (2001b), A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. I. Growth and body composition. *Mammalian Genome*, 12, 637–645.
- Mercade, A., Estelle, J., Perez-Enciso, M., Varona, L., Silio, L., Noguera, J. L., Sanchez, A. and Folch, J. M. (2006), Characterization of the porcine acyl-CoA synthetase long-chain 4 gene and its association with growth and meat quality traits. *Anim. Genet.*, 37, 219–224.
- Milan, D., Jeon, J. T., Looft, C., Amarger, V., Robic, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L., Ronne, H., Lundstrom, K., Reinsch, N., Gellin, J., Kalm, E., Roy, P. L., Chardon, P. and Andersson, L. (2000), A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science*, 288, 1248–1251.
- Moody, D. E., Zou, Z. and McIntyre, L. (2002), Cross-species hybridisation of pig RNA to human nylon microarrays. *BMC Genomics*, 3, 27.
- Nagamine, Y., Haley, C. S., Sewalem, A. and Visscher, P. M. (2003), Quantitative trait loci variation for growth and obesity between and within lines of pigs (*Sus scrofa*). *Genetics*, 164, 629–635.
- Newcom, D. W., Baas, T. J., Schwab, C. R. and Stalder, K. J. (2005a), Genetic and phenotypic relationships between individual subcutaneous backfat layers and

- percentage of longissimus intramuscular fat in Duroc swine. *Animal Genetics*, 83, 316–323.
- Newcom, D. W., Baas, T. J., Stalder, K. J. and Schwab, C. R. (2005b), Comparison of three models to estimate breeding values for percentage of loin intramuscular fat in Duroc swine. *J. Anim. Sci.*, 83, 750–756.
- Nii, M., Hayashi, T., Mikawa, S., Tani, F., Niki, A., Mori, N., Uchida, Y., Fujishima-Kanaya, N., Komatsu, M. and Awata, T. (2005), Quantitative trait loci mapping for meat quality and muscle fiber traits in a Japanese wild boar \times Large White intercross. *J. Anim. Sci.*, 83, 308–315.
- O'Brien, P. J. (1987), Etiopathogenetic defect of malignant hyperthermia: Hypersensitive calcium-release channel of skeletal muscle sarcoplasmic reticulum. *Vet. Res. Commun.*, 11, 527–559.
- Ohnishi, S. T., Taylor, S. and Gronert, G. A. (1983), Calcium-induced Ca^{2+} release from sarcoplasmic reticulum of pigs susceptible to malignant hyperthermia. The effects of halothane and dantrolene. *FEBS Lett.*, 161, 103–107.
- Otto, G., Roehe, R., Looft, H., Thoelking, L., Knap, P. W., Rothschild, M. F., Plastow, G. S. and Kalm, E. (2007), Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. *Meat Science*, 75, 185–195.
- Ouali, A. and Talmant, A. (1990), Calpains and calpastatin distribution in bovine, porcine and ovine skeletal muscles. *Meat Science*, 28, 331–348.
- Ovilo, C., Clop, A., Noguera, J. L., Oliver, M. A., Barragan, C., Rodriguez, C., Silio, L., Toro, M. A., Coll, A., Folch, J. M., Sanchez, A., Babot, D., Varona, L. and Perez-Enciso, M. (2002), Quantitative trait locus mapping for meat quality traits in an Iberian \times Landrace F2 pig population. *J. Anim. Sci.*, 80, 2801–2808.
- Ovilo, C., Fernandez, A., Rodriguez, M. C., Nieto, M. and Silio, L. (2006), Association of MC4R gene variants with growth, fatness, carcass composition and meat and fat quality traits in heavy pigs. *Meat Science*, 73, 42–47.
- Ovilo, C., Oliver, J. L., Noguera, J. L., Clop, A., Barragan, C., Varona, L., Rodriguez, C., Toro, M. A., Sanchez, A., Perez-Enciso, M. and Silio, L. (2000), H-FABP gene association study for body composition in pigs. *Proceedings 27th International Conference on Animal Genetics*. Minneapolis, MN, July 22–26.
- Paszek, A. A., Wilkie, P. J., Flickinger, G. H., Miller, L. M., Louis, C. F., Rohrer, G. A., Alexander, L. J., Beattie, C. W. and Schook, L. B. (2001), Interval mapping of carcass and meat quality traits in a divergent swine cross. *Anim. Biotechnol.*, 12, 155–165.
- Perez-Enciso, M., Clop, A., Folch, J. M., Sanchez, A., Oliver, M. A., Ovilo, C., Barragan, C., Varona, L. and Noguera, J. L. (2002), Exploring Alternative Models for Sex-linked Quantitative Trait Loci in Outbred Populations: Application to an Iberian \times Landrace Pig Intercross. *Genetics*, 161, 1625–1632.
- Perez-Enciso, M., Mercade, A., Bidanel, J. P., Geldermann, H., Cepica, S., Bartenschlager, H., Varona, L., Milan, D. and Folch, J. M. (2005), Large-scale, multibreed, multitrait analyses of quantitative trait loci experiments: The case of porcine \times chromosome. *J. Animal Science*, 83, 2289–2296.
- Peterson, J. S., Henckel, P. and Stoier, S. (1997), Muscle physiology traits and meat quality in Danish Landrace pigs anno 1976 and 1995. *Book of Abstracts 48th Annual Meeting EAAP*. Vienna, Austria.
- Plastow, G. S. (2003), The changing world of genomics and its impact on the pork chain. *Advances in Pork Production*, 14, 67–71.
- Plastow, G. S., Carrion, D., Gil, M., Garcia-Regueiro, J. A., Furnols, M. F. I., Gispert, M., Oliver, M. A., Velarde, A., Guardia, M. D., Hortos, M., Rius, M. A., Sarraga, C., Diaz, I., Valero, A., Sosnicki, A., Klont, R., Dornan, S., Wilkinson, J. M., Evans, G., Sargent, C., Davey, G., Connolly, D., Houeix, B., Maltin, C. M., Hayes, H. E., Anandavijayan, V., Foury, A., Gevrink, N., Cairns, M., Tilley, R. E., Mormede, P. and Blott, S. C. (2005), Quality pork genes and meat production. *Meat Science*, 70, 409–421.
- Ponsuksili, S., Chomdej, S., Murani, E., Blaser, U., Schreinemachers, H. J., Schellander, K.

- and Wimmers, K. (2005), SNP detection and genetic mapping of porcine genes encoding enzymes in hepatic metabolic pathways and evaluation of linkage with carcass traits. *Anim. Genet.*, 36, 477–483.
- Ponsuksili, S., Wimmers, K. and Schellander, K. (2001a), Application of differential display RT-PCR to identify porcine liver ESTs. *Gene*, 280, 75–85.
- Ponsuksili, S., Wimmers, K., Yerle, M. and Schellander, K. (2001b), Mapping of 93 porcine ESTs preferentially expressed in liver. *Mammalian Genome*, 12, 869–872.
- Quackenbush, J. (2001), Computational analysis of microarray data. *Nature Reviews Genetics*, 2, 418–427.
- Quintanilla, R., Demeure, O., Bidanel, J. P., Milan, D., Iannuccelli, N., Amigues, Y., Gruand, J., Renard, C., Chevalet, C. and Bonneau, M. (2003), Detection of quantitative trait loci for fat androstenone levels in pigs. *J. Anim. Sci.*, 81, 385–394.
- Ramos, A. M., Helm, J., Sherwood, J., Rocha, D. and Rothschild, M. F. (2006), Mapping of 21 genetic markers to a QTL region for meat quality on pig chromosome 17. *Anim. Genet.*, 37, 296–297.
- Rohrer, G. A., Freking, B. A. and Nonneman, D. (2007), Single nucleotide polymorphisms for pig identification and parentage exclusion. *Anim. Genet.*, 38, 253–258.
- Rohrer, G. A., Thallman, R. M., Shackelford, S., Wheeler, T. and Koohmaraie, M. (2006), A genome scan for loci affecting pork quality in a Duroc-Landrace F2 population. *Anim. Genet.*, 37, 17–27.
- Rosenvold, K. and Andersen, H. J. (2003), Factors of significance for pork quality – A review. *Meat Science*, 64, 219–237.
- Rothschild, M. F., Hu, Z. L. and Jiang, Z. (2007), Advances in QTL mapping in pigs. *Int. J. Biol. Sci.*, 3, 192–197.
- Russo, V., Fontanesi, L., Davoli, R., Nanni Costa, L., Cagnazzo, M., Buttazzoni, L., Virgili, R. and Yerle, M. (2002), Investigation of candidate genes for meat quality in dry-cured ham production: The porcine cathepsin B (CTSB) and cystatin B (CSTB) genes. *Anim. Genet.*, 33, 123–131.
- Schena, M., Shalon, D., Davis, R. W. and Brown, P. O. (1995), Quantitative Monitoring of Gene-expression Patterns with a Complementary-DNA Microarray. *Science*, 270, 467–470.
- Schena, M., Shalon, D., Heller, R., Chai, A., Brown, P. O. and Davis, R. W. (1996), Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 10614–10619.
- Schulz, J. S., Palmer, N., Steckelberg, J., Jones, S. J. and Zeece, M. G. (2006), Microarray profiling of skeletal muscle sarcoplasmic reticulum proteins. *Biochimica et Biophysica Acta (BBA) – Proteins & Proteomics*, 1764, 1429–1435.
- Sellier, P. (1998), Genetics of meat and carcass traits. In Rothschild, M. F. and Ruvinsky, A. (Eds.) *The genetics of the pig*. Wallingford, UK, CABI.
- Sentandreu, M. A., Coulis, G. and Ouali, A. (2002), Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science and Technology*, 13, 400–421.
- Shackelford, S. D., Koohmaraie, M., Whipple, G., Wheeler, T. L., Miller, M. F., Crouse, J. D. and Reagan, J. O. (1991), Predictors of Beef Tenderness – Development and Verification. *Journal of Food Science*, 56, 1130–1135.
- Skinner, T. M., Anderson, J. A., Haley, C. S. and Archibald, A. L. (2006), Assessment of *SULT1A1*, *CYP2A6* and *CYP2C18* as candidate genes for elevated backfat skatole levels in commercial and experimental pig populations. *Anim. Genet.*, 37, 521–522.
- Skinner, T. M., Doran, E., McGivan, J. D., Haley, C. S. and Archibald, A. L. (2005), Cloning and mapping of the porcine cytochrome-p450 2E1 gene and its association with skatole levels in the domestic pig. *Anim. Genet.*, 36, 417–422.
- Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M. and Goll, D. E. (1995), Is Z-disk degradation responsible for postmortem tenderization? *J. Anim. Sci.*, 73, 1351–1367.
- te Pas, M. F., De Wit, A. A., Priem, J., Cagnazzo, M., Davoli, R., Russo, V. and Pool, M.

- H. (2005), Transcriptome expression profiles in prenatal pigs in relation to myogenesis. *J. Muscle Res. Cell. Motil.*, 26, 157–165.
- Toldra, F. and Flores, M. (2000), The use of muscle enzymes as predictors of pork meat quality. *Food Chemistry*, 69, 387–395.
- Van Der Steen, H. A. M., Prall, G. F. W. and Plastow, G. S. (2005), Application of genomics to the pork industry. *J. Anim. Sci.*, 83, E1–E8.
- Van Wijk, H. J., Dibbitts, B., Baron, E. E., Brings, A. D., Harlizius, B., Groenen, M. A., Knol, E. F. and Bovenhuis, H. (2006a), Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross. *J. Anim. Sci.*, 84, 789–799.
- Van Wijk, H. J., Dibbitts, B., Baron, E. E., Brings, A. D., Harlizius, B., Groenen, M. A. M., Knol, E. F. and Bovenhuis, H. (2006b), Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross. *J. Anim. Sci.*, 84, 789–799.
- Wang, L., Yu, T. P., Tuggle, C. K., Liu, H. C. and Rothschild, M. F. (1998), A directed search for quantitative trait loci on chromosomes 4 and 7 in pigs. *J. Anim. Sci.*, 76, 2560–2567.
- Wernersson, R., Schierup, M. H., Jorgensen, F. G., Gorodkin, J., Panitz, F., Staerfeldt, H. H., Christensen, O. F., Mailund, T., Hornshøj, H., Klein, A., Wang, J., Liu, B., Hu, S., Dong, W., Li, W., Wong, G. K., Yu, J., Wang, J., Bendixen, C., Fredholm, M., Brunak, S., Yang, H. and Bolund, L. (2005), Pigs in sequence space: a 0.66x coverage pig genome survey based on shotgun sequencing. *BMC Genomics*, 6, 70.
- Wimmers, K., Fiedler, I., Hardge, T., Murani, E., Schellander, K. and Ponsuksili, S. (2006), QTL for microstructural and biophysical muscle properties and body composition in pigs. *BMC Genet.*, 7, 15.
- Wimmers, K., Ponsuksili, S., Blaser, U., Gellin, J. and Schellander, K. (2002), Chromosomal assignments for porcine genes encoding enzymes in hepatic metabolic pathways. *Animal Genetics*, 33, 255–263.
- Yu, T. P., Wand, L., Tuggle, C. K. and Rothschild, M. F. (1999), Mapping genes for fatness and growth on pig chromosome 13: A search in the region close to the pig PIT1 gene. *Journal of Animal Breeding and Genetics*, 116, 269–280.
- Zalk, R., Lehnart, S. E. and Marks, A. R. (2007), Modulation of the Ryanodine Receptor and Intracellular Calcium. *Annu. Rev. Biochem.*, 76, 367–385.
- Zhao, S. H., Nettleton, D., Liu, W., Fitzsimmons, C., Ernst, C. W., Raney, N. E. and Tuggle, C. K. (2003), Complementary DNA macroarray analyses of differential gene expression in porcine fetal and postnatal muscle. *J. Anim. Sci.*, 81, 2179–2188.
- Zhao, S. H., Recknor, J., Lunney, J. K., Nettleton, D., Kuhar, D., Orley, S. and Tuggle, C. K. (2005), Validation of a first-generation long-oligonucleotide microarray for transcriptional profiling in the pig. *Genomics*, 86, 618–625.
- Zhu, Z. M., Zhang, J. B., Li, K. and Zhao, S. H. (2005), Cloning, mapping and association study with carcass traits of the porcine SDHD gene. *Anim. Genet.*, 36, 191–195.

11

Genetic and genomic approaches to improving sheep meat quality

S. C. Bishop and E. Karamichou, The Roslin Institute and R(D)SVS, University of Edinburgh, UK

Abstract: This chapter addresses how genetic improvement in sheep meat quality can be achieved in practice, selection goals that should be considered, and mechanisms underlying genetic differences in sheep meat quality. It begins by reviewing, both within and between breed, genetic variation in sheep meat quality. It then surveys known genes and quantitative trait loci influencing meat quality, and considers how functional genomics can assist in elucidating these effects. Finally, it discusses future genetic improvement options for sheep meat quality.

Key words: quantitative trait locus, selection, fatty acids, gene, functional genomics.

11.1 Introduction

This chapter concentrates on genetic aspects of sheep meat quality, and opportunities to genetically improve the quality of sheep meat. It covers both conventional genetic approaches, and genomic approaches aimed at uncovering the genes that underlie genetic variation in meat quality. Definitions of meat quality and aspects of particular importance are well covered elsewhere in this book, so it is genetic opportunities that are considered, rather than the biological properties of the meat quality traits.

There is now ample evidence of genetic variation in many traits describing meat quality in sheep, at both the between- and within-breed levels as described below, implying that genetic improvement or alteration of a variety of meat quality traits is possible, in principle. Important questions to be addressed are therefore:

- How can genetic improvement be achieved in practice?
- What are reasonable selection goals (i.e. in which direction should we seek to take meat quality traits)?
- What are the mechanisms underlying genetic differences in sheep meat quality?

In principle, genetic improvement can be achieved by selection based on phenotypic measurements or on genetic markers. Difficulties and challenges associated with utilising phenotypic measurements for meat quality traits are covered elsewhere in this volume (Chapter 12); however, the general principles are important for understanding the utility of genomic (genetic marker) approaches to improving meat quality. In most cases, phenotypic assessment of meat quality requires the slaughter of the animal; therefore, data can be collected only on relatives of the candidates for selection. This is clearly time-consuming, expensive and somewhat inefficient for breeding purposes. However, sheep have one specific advantage insofar as *in vivo* prediction of some aspects of meat quality is now possible. Specifically, muscle density, as assessed by computerised tomography (CT), is strongly genetically correlated with intramuscular fat content and with taste panel-assessed 'juiciness', 'flavour' and 'overall liking' (Karamichou *et al.*, 2006a), making *in vivo* prediction of these traits feasible, albeit still expensive.

Genetic markers supply an obvious means of circumventing the phenotype measurement issue: if markers can be defined that are consistently associated with desirable meat quality attributes, then these markers can be directly used in a breeding programme. Exploring this concept of marker-assisted selection, Meuwissen and Goddard (1996) found that meat and carcass traits are the trait category for which marker-assisted selection offers the greatest potential for improvement over conventional selection based on phenotypic measurements, with rates of genetic gains increased by up to 60%. Although this theoretical advantage of marker-assisted selection may be somewhat less if CT muscle-density measurements are available, potential benefits from genetic markers are nevertheless substantial. Moreover, as has been seen in beef cattle, genetic markers can be implemented at the breeder level more easily than selection schemes requiring expensive phenotypic measurement and complex logistics, as would be the case for selection based on CT characteristics.

This chapter has several aims. It reviews evidence for genetic variation in meat quality traits in sheep, concentrating mainly on within-breed variability; it considers the utility of CT measurements as proxies for meat quality assessments; it considers evidence for genes underlying genetic variation in sheep meat quality and the process of developing genetic markers for meat quality; and it briefly considers approaches that may be taken to understand the underlying genetic control. Lastly, it briefly considers future options for improving sheep meat quality.

11.2 Genetic variation in sheep meat quality

11.2.1 Between-breed comparisons

Most published information on genetic variation in sheep meat quality comes from between-breed studies. Although these studies are useful for establishing the

principles of genetic variation, they do not help so much with the definition of breeding goals, or the development of genetic markers for meat quality. In general, whilst a growing number of studies have investigated between-breed differences in meat quality, the resulting breed differences are not always consistent or convincing. This is partly a function of the empirical and often arbitrary nature of breed comparisons: what is the hypothesis being tested; is there any *a priori* evidence that the breeds being compared should differ in meat characteristics; can specific results be extrapolated to other circumstances?

Published results for the main meat quality trait categories are now presented. Some evidence exists of between-breed differences in aspects of meat colour (e.g. Carson *et al.*, 1999, 2001; Dawson *et al.*, 2002; Martinez-Cerezo *et al.*, 2005) and fat colour. For example, Legrand *et al.* (1995) suggested that breed differences may occur with regard to fat quality, especially fat colour. They showed that ram lambs sired by Texels had a more acceptable colour score for the subcutaneous fat when compared with lambs sired by Charollais. Crouse *et al.* (1981) also reported breed effects on fat colour, with Suffolk sired lambs having yellower fat than lambs sired by Rambouillets.

For tenderness, Young *et al.* (1993) reported consumer-assessed tenderness of the loins of terminal progeny from Merino dams mated to six sire breeds. They showed that the pure Merinos had a higher overall tenderness score than Oxford Down, Suffolk, Poll Dorset and Texel cross lambs. Sobrinho *et al.* (2005) found Romney lambs to have more tender meat than lambs from crosses between East Friesian, Finn and Texel sheep. Sañudo *et al.* (1998) reported that the meat of Spanish lambs was significantly ($P < 0.05$) less tender than meat of British lambs. Sañudo *et al.* (1998) also presented some evidence that juiciness was higher for Spanish lamb than for British lamb. These differences were large and statistically significant ($P < 0.01$) in the Spanish panel (17.8%) but less so in the British panel ($P < 0.05$), which judged the Spanish meat to be only 3% more juicy than the British meat.

Lamb flavour has been investigated in many studies, but again the breed difference results are variable. Jacobson and Koehler (1963) examined volatile compounds from roasting lamb from three breeds (South-down, Hampshire, and Columbia). They found carbonyl compounds contributed to aroma, but no differences were detected between breeds for these compounds or for other volatiles. Cramer *et al.* (1970a) compared three breeds (Rambouillet, Targhee and Columbia) for mutton flavour intensity. Mutton flavour intensified as the fineness of the wool increased with breeds. In a second study (Cramer *et al.*, 1970b), five breeds (Romney, Hampshire, Columbia, Rambouillet and Merino) were compared for intensity of mutton flavour. Mutton flavour intensity was similar between the breeds, but unsaturated fatty acid content was higher in the finer-wool breeds. Several other studies comparing breeds or sires (Fox *et al.* 1962, 1964; Dransfield *et al.*, 1979; Mendenhall and Ercanbrack, 1979; Crouse *et al.*, 1981) have been conducted, but differences in lamb flavour due to breed or sire were not observed. In a comparison of sire breeds (Dorper vs. Suffolk), Duckett *et al.* (1999) reported fatty acid compositional differences between sire breeds and a greater flavour

preference for Dorper-sired lamb. Elmore *et al.* (2000) reported higher levels of Maillard-derived volatiles and certain PUFAs in intramuscular fat from Soays compared to Suffolks, when fed various oils. Sañudo *et al.* (2000) reported that finishing system was more important than breed in determining fatty acid composition and flavour.

Cameron *et al.* (1994) examined the changes that occurred in the lipid content of the adipose tissue and fatty acid profiles of subcutaneous fat from lines of Texel–Oxford and Scottish Blackface rams that had been divergently selected for carcass lean content. They showed that, although back-fat depth responded to selection, there were no significant changes due to selection in the individual fatty acid concentrations of subcutaneous fat or in the proportion of unsaturated fatty acids. They did, however, show that there were breed differences for the concentration of myristic acid (C14:0), with Scottish Blackface rams having higher concentrations than Texel–Oxford rams. Further, Cameron *et al.* (1994) showed that across breeds and lines, the concentration of unsaturated fat in the subcutaneous depot was positively correlated with subcutaneous fat depth. Webb and Casey (1995) showed that there were genetic differences in the fatty acid composition of subcutaneous adipose tissue. After correction for differences in maturity, the concentrations of palmitic, palmitoleic and stearic acids differed between South Australia Mutton Merinos and Dorpers. Breed influenced the proportions of myristic, heptadecenoic and oleic acids; however, when compared at equivalent levels of fatness, the breed differences in the proportion of C17:1 and C18:1 in the subcutaneous tissue were negligible.

11.2.2 Within-breed studies

Within-breed studies, such as those that quantify heritabilities for traits of interest, give information that is often more easily interpreted or extrapolated to other circumstances than between-breed studies. However, because of the requirement for measurements on relatively large numbers of animals, i.e. hundreds rather than tens of animals, they are undertaken less often. In fact, the comprehensive review by Safari *et al.* (2005) found only two published sets of results for sheep meat quality traits, these being for pH and colour attributes. For these traits, mean heritabilities were generally low, averaging 0.18 for pH and 0.09 for various colour attributes.

More comprehensive results have been recently published by Karamichou *et al.* (2006a and c, 2007), using data collected on Scottish Blackface lambs. Heritability values are shown in Table 11.1 for general meat quality attributes and in Table 11.2 for fatty acids extracted from intramuscular fat (Karamichou *et al.*, 2006a). Results presented in these tables are promising as they indicate that substantial genetic variation occurs within a mainstream breed, e.g. the Scottish Blackface, for many attributes of meat quality. This is indicated by the moderate to high heritabilities, particularly for combinations of fatty acids, as well as substantial phenotypic variances and hence coefficients of variation for many of the traits. The results are backed by heritabilities for taste panel evaluations of meat quality, presented by

Table 11.1 Means, heritabilities (h^2), the standard error (s.e.) of the estimated heritabilities and phenotypic variances (σ_p^2) for meat quality traits in Scottish Blackface lambs (adapted from Karamichou *et al.*, 2006a)

Trait	Mean	h^2	s.e. (h^2)	σ_p^2
Shear force (kg)	5.25	0.39	0.16	3.84
Redness (a*) (+ve→red)	17.2	0.45	0.19	2.17
Yellowness (b*) (+ve→yellow)	7.89	0.33	0.17	1.02
Lightness (L*) (0 = black, 100 = white)	40.9	0.15	0.12	3.43
Hue (0° = red, 90° = yellow)	24.7	0.30	0.15	3.96
Saturation	19.0	0.45	0.18	2.78
pH ₄₅	6.70	0.54	0.18	0.03
Ultimate pH	5.72	0.21	0.14	0.01
Juiciness (units)	42.1	0.21	0.12	35.1
Toughness (units)	36.4	0.15	0.13	99.7
Overall liking (units)	22.0	0.22	0.13	51.7
Flavour (units)	26.1	0.11	0.11	23.8
Dry matter proportion	0.251	0.51	0.16	0.98
Intramuscular fat (mg/100 g muscle)	2467	0.32	0.09	664000

Table 11.2 Heritabilities (h^2) for intramuscular fatty acid contents in Scottish Blackface lambs (adapted from Karamichou *et al.*, 2006c)

	Trait	h^2	s.e.(h^2)
<i>Saturated</i>	Myristic acid – 14:0	0.19	0.14
	Palmitic acid – 16:0	0.29	0.17
	Stearic acid – 18:0	0.24	0.15
<i>Monounsaturated</i>	Palmitoleic acid – <i>cis</i> 16:1 (<i>n</i> -7, <i>n</i> -9)	0.31	0.18
	Oleic acid – <i>cis</i> 18:1 <i>n</i> -9	0.27	0.17
	<i>cis</i> -Vaccenic acid – <i>cis</i> 18:1 <i>n</i> -7	0.67	0.16
	Vaccenic acid – <i>trans</i> 18:1 <i>n</i> -7	0.49	0.17
	Gadoleic acid – 20:1	0.30	0.17
<i>Polyunsaturated</i>	Linoleic acid – <i>cis</i> 18:2 <i>n</i> -6	0.10	0.09
	Linolenic acid – <i>cis</i> 18:3 <i>n</i> -3	0.30	0.02
	Dihomo- γ -linolenic acid – 20:3 <i>n</i> -6	0.12	0.10
	Arachidonic acid – 20:4 <i>n</i> -6	0.60	0.17
	EPA (Eicosapentanoic acid) – 20:5 <i>n</i> -3	0.21	0.13
	Adrenic acid – 22:4 <i>n</i> -6	0.22	0.13
	DPA (Docosapentaenoic acid) – 22:5 <i>n</i> -3	0.13	0.12
	DHA (Docosahexaenoic acid) – 22:6 <i>n</i> -3	0.16	0.10
<i>Conjugated fatty acid</i>	Conjugated linoleic acid (CLA) – 9- <i>cis</i> , 11- <i>trans</i> 18:2	0.48	0.16
<i>Totals</i>	Total fatty acids (mg/100 g)	0.32	0.09
	SFA (saturated)	0.90	0.16
	MUFA (monounsaturated)	0.73	0.18
	PUFA (polyunsaturated)	0.40	0.16

Karamichou *et al.* (2007). Although these heritability values were variable, they were generally moderate (i.e. greater than 0.2) to high, and usually low only when then the trait was seldom given a high score – e.g. for fishy, metallic or soapy flavours.

In principle, the existence of substantial heritable between-animal variation indicates that a trait can be genetically improved. However, genetic improvement using phenotypes requires either measurements on the candidate for selection or substantial information captured from relatives of the selection candidate. The latter is likely to be expensive and logistically difficult for meat quality traits. However, an opportunity does exist to develop *in vivo* predictors of various meat quality attributes, using muscle density, as assessed by computerised tomography (CT). Karamichou *et al.* (2006a) reported that this trait was strongly genetically correlated with intramuscular fat content (genetic correlation of -0.67), and, as a consequence, it was also genetically correlated with juiciness, flavour and overall linking (genetic correlations of -0.71 , -0.73 and -0.80 , respectively). Upon further investigation, muscle density was genetically negatively correlated with total SFA, MUFA and PUFA contents. However, the correlations with the proportions of total fatty acids that were SFA, MUFA and PUFA were -0.23 , -0.54 and 0.39 , respectively (Karamichou *et al.*, 2006c), indicating that selection on muscle density could alter both the perceived desirability of the meat and the fatty acid profile, i.e. the ‘healthiness’ of the meat.

11.3 Genes impacting on meat quality

Problems of assessing meat quality in live animals may be partially circumvented if polymorphisms in genes underlying genetic differences in meat quality are known. Selection on such polymorphisms, i.e. using gene tests, will enable animals to be selected for improved meat quality attributes without the need for extensive phenotype information. However, the success of such gene tests depends on several factors, e.g. the genetic test having a sufficiently large effect on the trait of interest to be worthwhile, and the polymorphism not having negative attributes on other traits of economic or welfare importance.

It is a difficult task to demonstrate that an apparent gene effect on a trait of interest is due to the gene in question, and not due to other linked genes. Typically, evidence is developed either through the testing of polymorphisms in candidate genes that have been demonstrated as having an effect in other species, or by the process of quantitative trait (QTL) mapping, and subsequent fine mapping of regions that have evidence for containing genes with large effects on the trait of interest. QTL mapping for meat quality traits in sheep is described below.

A growing number of gene tests are now commercially available in beef cattle for various aspects of meat quality, and considerable effort has gone into both the development and verification of these tests, as described in Chapter 13 of this book by Barendse. Currently, genetic tests in sheep for meat quality traits, as opposed to carcass composition, lag considerably behind those in beef cattle. Cases where genes are known to have a major impact on meat quality have arisen through

testing the pleiotropic effects of genes affecting carcass composition. For example, leptin is a gene known to affect feed intake, growth and lipid metabolism, and in Suffolk sheep a polymorphism associated with reduced muscle thickness was also associated with significantly increased shear force (Boucher *et al.*, 2006).

The most remarkable single gene effect on meat quality known in sheep, perhaps in any mammalian species, is that associated with the Callipyge gene. This gene was detected due to the highly significant effect it has on muscular hypertrophy, with some individual muscles being more than 40% heavier in lambs expressing the effects of this gene compared to controls (Duckett *et al.*, 2000). The mode of inheritance of this gene is unique; only animals that inherit the callipyge allele from their sire and the wildtype allele from their dam display the phenotype (i.e. any animal inheriting the Callipyge allele from its dam does not display the phenotype) (Cockett *et al.*, 1996). The effect on meat quality is large; animals expressing the Callipyge phenotype have remarkably tough meat as assessed through shear-force measures or sensory panels, and the meat is associated with high calpastatin levels (Freking *et al.*, 1999; Duckett *et al.*, 2000). Fine mapping of the Callipyge locus, and its impact on associated imprinted genes was described by Charlier *et al.* (2001), leading to the identification of the causative mutation (Freking *et al.*, 2002). Subsequently, and with opportunities arising from developments in function genomics, this has led to possibilities of describing networks of genes affected by the Callipyge mutation (Vuocolo *et al.*, 2007). The effect of the Callipyge locus on tenderness is so large that it effectively precludes the sale of fresh meat from these animals (unless substantial ameliorative treatment can be logistically included in the meat production process).

The Callipyge effect was not discovered from meat quality genomics studies, but as an outcome of investigations into pleiotropic effects of genes influencing carcass quality. This has led to a recognition of the need to assess the impact on meat quality of other genes, or QTL impacting on musculature. Examples of these include muscling QTL found on chromosome 18 in Poll Dorset sheep in New Zealand, the so-called Carwell gene (McLaren *et al.*, 2001), and in Texel sheep in the UK (Walling *et al.*, 2004). Both QTL are located in the same region of chromosome 18 as Callipyge, although in both cases the effect is not due to the Callipyge mutation. However, it has been demonstrated that the Carwell gene is not associated with decreased tenderness, provided that the meat is aged appropriately (Jopson *et al.*, 2001). Currently, extensive investigations are being undertaken in the UK to quantify any possible impacts of the Texel chromosome 18 muscling QTL on meat quality.

A second known major gene affecting muscling in sheep is myostatin, and a mutation associated with this gene is thought to be the causative effect of the double muscling phenotype seen in Texel sheep. In essence, a mutation has been found in the 3'-UTR of *GDF8* (myostatin) (Clop *et al.*, 2006), and it is believed that this mutation leads to miRNA-mediated translational inhibition of *GDF8*, hence the double muscling phenotype in animals homozygous for the mutation. Recently, this mutation has also been demonstrated to segregate at intermediate frequencies in UK Charollais sheep, with a pronounced effect on muscle depth

(Hadjipavlou *et al.*, 2007). Again, it is clearly necessary to assess any impacts of this mutation on meat quality traits.

11.4 Quantitative trait loci approaches to improving meat quality

Usually, the first step towards detecting genes affecting traits of interest is through QTL mapping. QTL mapping comprises genome scan studies in which regions of the genome containing polymorphic genes affecting the target trait are inferred. Such studies tend to be costly and demanding of resources, both in terms of animals and genotyping resources; hence, applications to sheep are fewer than for economically more important species. Also, current genotyping technologies based on microsatellite markers generally require demonstration of within-family linkage; hence, large family sizes are a necessary requirement. This is generally at odds with quantitative genetic studies designed to estimate heritabilities, where the requirement is for a large number of smaller-sized families. A dense ‘Single nucleotide polymorphism (SNP) chip’ is due to be available in autumn 2008. Using this SNP chip, it will be possible to simultaneously genotype animals for tens of thousands of SNP markers. This will remove the restriction on population structure, as linkage studies will be replaced by genome-wide association studies in which it is assumed that the genotyped polymorphisms are in population-wide linkage disequilibrium with the causative mutation. This will make it possible to map QTL with greater precision than was possible with linkage studies. Furthermore, it will mean that studies can be designed that are efficient both for heritability estimation and for QTL detection.

The only sheep meat quality QTL results readily available in the public domain at the time of writing are those of Karamichou *et al.* (2006b and c). These papers present results for a wide variety of meat and carcass quality traits, including taste panel assessment, fatty acid composition and CT-assessed carcass composition, arising from a partial genome scan covering chromosomes 1, 2, 3, 5, 14, 18, 20 and 21. An inference that may reasonably be made is that, with a full genome scan, more QTL would have been found. The population studied comprised Scottish Blackface lambs which were *ca.* 5 months of age at the time of CT assessment and 8 months when the meat quality traits were measured. CT measurements were made on 600 lambs, males and females, from 9 half-sib families, and the meat quality traits were assessed on the 300 male lambs from these families. Significant QTLs for general meat quality traits are shown in Table 11.3, and those for fatty acids are shown in Table 11.4.

The most significant result presented in these tables is for lamb flavour on chromosome 1. It may be hypothesised that this is a function of intramuscular fat content, as a QTL for meat yellowness is also reported in the same region in Table 11.3, and several fatty acid QTL were also seen in this region (Table 11.4). However, muscle density, which is strongly genetically correlated with flavour, did not have a significant QTL in this region. Interestingly, the region on

Table 11.3 Quantitative trait loci for meat quality traits in Scottish Blackface lambs (adapted from Karamichou *et al.*, 2006b)

Trait	Chromosome	Position cM	F-ratio	5% Chromosome wide threshold
Lamb flavour	1	119	4.80	3.15
CT-assessed muscle density	2	28	3.45	3.45
CT-assessed muscle density	3	172	3.16	2.60
Colour b* (yellowness)	1	165	2.55	2.55
Colour a* (redness)	3	113	3.31	2.68
Colour L* (lightness)	18	80	2.74	2.24
Colour L* (lightness)	20	42	2.94	2.43

Table 11.4 Quantitative trait loci for intramuscular fatty acid contents in Scottish Blackface lambs (adapted from Karamichou *et al.*, 2006c)

	Trait	Chromo- some	Position cM	F-ratio	5% chromo- some wide threshold
<i>Saturated</i>	Myristic acid – 14:0	21	57	3.14	3.02
	Palmitic acid – 16:0	21	57	3.17	2.94
	Stearic acid – 18:0	21	58	3.25	3.13
<i>Monounsaturated</i>	Palmitoleic acid – <i>cis</i> 16:1 (<i>n</i> -7, <i>n</i> -9)	5	12	2.77	2.63
	Oleic acid – <i>cis</i> 18:1 <i>n</i> -9	21	58	3.23	2.98
	<i>cis</i> -Vaccenic acid – <i>cis</i> 18:1 <i>n</i> -7	21	58	3.26	2.99
	Gadoleic acid – 20:1	21	21	3.49	3.03
		18	97	2.38	2.01
<i>Polyunsaturated</i>	Linoleic acid – <i>cis</i> 18:2 <i>n</i> -6	21	58	3.22	3.14
	Linolenic acid – <i>cis</i> 18:3 <i>n</i> -3	2	269	3.97	2.89
		21	57	3.17	3.08
	Arachidonic acid – 20:4 <i>n</i> -6	21	58	3.84	2.41
		2	21	2.75	2.73
	EPA (Eicosapentaenoic acid) – 20:5 <i>n</i> -3	2	229	3.05	2.70
		1	79	3.02	3.00
	DPA (Docosapentaenoic acid) – 22:5 <i>n</i> -3	1	168	3.52	3.19
		2	87	3.49	3.32
		21	0	3.07	2.66
	DHA (Docosahexaenoic acid) – 22:6 <i>n</i> -3	18	105	2.32	2.31
<i>Conjugated fatty acid</i>	Conjugated linoleic acid	3	159	3.22	2.51
<i>Totals</i>	PUFA	1	85	2.84	2.59

chromosome 1 containing the flavour QTL shares conservation of synteny with a region on pig chromosome 13 containing a QTL for pork flavour (Lee *et al.*, 2004).

The most striking results shown in Tables 11.3 and 11.4 are the QTL for fatty acid contents of the meat, including a cluster of QTL on chromosome 21. These QTL tended to locate to the same position and segregate within the same families (Karamichou *et al.*, 2006c), strongly suggesting that it is the same QTL simultaneously affecting all of these fatty acids. These fatty acid QTL on chromosome 21 warrant further study, both in terms of finer mapping and investigation of gene functions that could lead to simultaneous effects on so many fatty acids. Elucidation of such effects using microsatellite markers is likely to be difficult, and dense SNP markers would be required to perform the fine mapping with sufficient precision. Additionally, gene expression microarray studies would almost certainly be required to reveal the cascade of pathways underlying the observed QTL effects.

QTL mapping is generally seen as an intermediate step in a larger goal, either gene discovery or marker-assisted selection. Marker-assisted selection aims to utilise molecular genetic information to increase the precision of selection, or to enable selection in the absence of phenotypic information. For sheep, the discovery that CT muscle density can predict aspects of meat quality does provide an alternative to the use of genetic markers in some circumstances but, in principle, marker-assisted selection still has an important role to play in genetically improving meat quality. The state-of-the-art is considerably more advanced in beef cattle, where specific gene tests are available for genetic improvement; hence, gene-assisted selection (see Barendse, Chapter 13), although the *ad hoc* use of such tests means that they are seldom used optimally within breeding programmes.

The QTL results shown in Tables 11.3 and 11.4 are not results that can be immediately applied in practical situations, as they describe only within-family linkage between genetic markers and traits of interest. To be generally applicable, markers must be found that are in population-wide linkage disequilibrium with causative mutations, or substantial quantities of phenotypic information has to be available to establish within-family linkage phases. These are challenging problems in the sheep industry for two reasons. Firstly, research investment in sheep is considerably less than that in other major livestock species; hence, progress in genomic tools and dissection of quantitative traits inevitably lags behind that seen in cattle, chickens and pigs. As a result, many of the advances in sheep are likely to come as a by-product of developments in cattle. Secondly, the general lack of vertical integration in sheep industries means that it is often difficult for the benefits of improved meat quality to be fed back to the breeder, i.e. to the person who incurs the cost of the genetic improvement. Restructuring within the sheep industry would be required to take full advantage of advances in genomics.

11.5 The contribution of functional genomics

From the perspective of genetically improving meat quality, the ultimate aim is to

identify and utilise specific polymorphisms which have beneficial effects on aspects of meat quality and which do not impinge on other economic or welfare traits. However, as described, fine mapping alone is seldom sufficient to achieve this, and other technologies must be used to gain the necessary insight. Within the broad categorisation of functional genomics, two options currently being used in sheep meat quality studies are gene expression studies using microarrays and proteomics.

Microarray-based transcriptional profiling can be used to study gene expression changes in a large proportion of the genes in the genome, and sheep studies often use microarray resources borrowed from cattle studies. Gene expression profiling may be used to compare changes in gene expression, e.g. across time; between tissues; in responses to specific experimental treatments; or between animals of different defined genotypes. An approach that may be used to aid the detection of genes underlying genetic variation in meat quality is to compare animals of known but different QTL genotypes for some meat or muscling attribute. RNA may then be collected from muscle tissues from animals at an appropriate age, e.g. the commencement of observable muscular hypertrophy, and used to quantify gene expression differences on a microarray. It is likely that many genes will be differentially expressed between the two genotypes – because they are directly associated with the polymorphism; because they occur downstream in a metabolic pathway that is influenced by the polymorphism; because they are linked with the causative polymorphism; or through chance. Genes that are differentially expressed and which map to the chromosomal region containing the QTL then become good candidates for further study, i.e. one of these genes may be the one within which the causative mutation resides. Further fine mapping and sequencing will be required, but the microarray results potentially assist in refining the shortlist of candidates.

Similar experimental designs can be used simply to gain greater insight into the biological processes underlying phenotypic differences, and hence predict the impact of other meat quality genes or identify new approaches for improving meat quality. An excellent example is given by studies into the gene networks contributing to hypertrophy in lambs expressing the callipyge phenotype (Vuocolo *et al.*, 2007). In this case, genes were identified that were differentially expressed as a function of genotype, and not simply as a consequence of changes in muscle fibre types. By comparing results from different ages, e.g. birth and 12 weeks of age, gene expression changes that led to the altered phenotype were identified as occurring before expression of the phenotype became apparent. These results were then used to describe a network of genes likely to underlie callipyge-induced muscular hypertrophy. In principle, an identical approach could be used to identify genetic changes underlying the large-scale alterations in fatty acid profiles described above and outlined by Karamichou *et al.* (2006c).

It must be appreciated that gene expression studies alone will not identify genes leading to genetic variation in phenotypic characteristics, as gene expression studies are not dependent upon genetic polymorphisms. Thus, it is a combination of gene expression and mapping studies that is required. Experience from

comparative functional genomic studies suggests that, whilst metabolic pathways are generally conserved between species, mutations are not. Therefore, similar phenotypic effects in different populations may be due to mutations in different genes in the same pathway, or even to different mutations in the same gene.

An alternative approach to gene expression studies is proteomics, where actual quantities and types of protein are quantified, rather than RNA levels. Applications of proteomic techniques are still in their infancy in sheep meat quality studies; however, interesting results are starting to emerge from large coordinated programmes. For example, in an Australian study comparing lambs of high muscling, high growth and control genotypes, observable phenotypic differences were reflected in levels of proteins involved in cellular mechanisms of protein and energy metabolism (McDonagh *et al.*, 2006).

11.6 Future trends

In many respects the sheep industry is in a fortunate and promising situation regarding the application of genetic and genomic techniques to the improvement of meat quality. It is clear that substantial genetic variation exists for a wide variety of meat quality attributes, the tools are becoming available that will enable much greater progress to be achieved in the mapping of genes underlying genetic differences in these traits and the way in which they influence the phenotype, and the genetic similarity of sheep to cattle ensures that many of the advances made in cattle can be translated relatively easily to sheep. Further, the use of CT measurements for *in vivo* prediction of meat quality is an exciting advance, and sheep are a convenient experimental animal with the advantage of being considerably less costly than cattle for large-scale genetic studies.

However, exploitation of these advantages will require considerable investment from funders, both in terms of the large-scale use of the genomic tools and the requirement for extensive phenotyping. This will require large-scale experimental programmes that cannot be expected to result in short-term paybacks. Additionally, as described above, capturing the benefits of the genetic improvement may require some restructuring within sheep industries towards more vertically integrated systems, as the means of rewarding breeders for improved meat quality attributes do not currently exist.

A big issue still to be resolved is the precise trait goals for improving sheep meat quality. There are many possible directions that the genetic improvement could take and there is no consensus yet on the optimal direction, which may well depend on the production system and the target market. Genetic improvement of meat quality in beef cattle has tended to focus on toughness or tenderness, as this is an identifiable issue with beef. Toughness is not yet considered to be a major problem with sheep meat, hence this may not be an appropriate direction. The results summarised above suggest that it is components of intramuscular fat that are the most amenable to genetic change, and which may offer the greatest advantages. Not only is the intramuscular fat profile strongly correlated with taste panel

perception of the meat quality (Karamichou *et al.*, 2007), but it is also associated with the perceived health properties of the meat. Consumer trends are away from saturated fatty acids (SFA) towards mono- and polyunsaturated fatty acids (MUFA and PUFA). Therefore, attempts to increase the meat MUFA content would improve both the perception of the meat (Karamichou *et al.*, 2007) as well as the 'healthiness' of the meat. In principle, this can be achieved by making joint use of CT muscle density and genetic markers. Phenotypic verification can be obtained using samples taken from carcasses and, perhaps, from biopsies on a small number of live animals.

In summary, future genomic research in sheep meat quality is potentially exciting. If funding allows, the genomic tools are becoming available to gain a much greater understanding of the genetic factors underlying meat quality, and also to develop various genetic tests to improve desirable meat quality attributes. It will also be necessary to test the effects on meat quality of genetic tests designed to improve other traits, particularly those directed towards carcass traits. From the available published evidence, it is suggested that manipulation of fatty acid profiles may be a rewarding and tractable means of improving the overall appeal of sheep meat.

11.7 Acknowledgements

We wish to thank the BBSRC, Defra, MLC, EBLEX and HCC for funding various aspects of the research described in this article. This work would not have been possible without collaboration from colleagues at Bristol University and SAC, and without inputs from David Wallace and Dougie McGavin at Roslin Institute's Blythbank Farm.

11.8 References

- Boucher D, Palin M F, Castonguay F, Garipey C and Pothier F (2006), 'Detection of polymorphisms in the ovine leptin (LEP) gene: Association of a single nucleotide polymorphism with muscle growth and meat quality traits', *Can. J. Anim. Sci.*, 86, 31–35.
- Cameron ND, Bishop S C, Speake B K, Bracken J and Noble R C (1994), 'Lipid composition and metabolism of subcutaneous fat in sheep divergently selected for carcass lean content', *Anim. Prod.*, 58, 237–242.
- Carson A F, Moss B W, Dawson L E R and Kilpatrick D J (2001), 'Effects of genotype and dietary forage to concentrate ratio during the finishing period on carcass characteristics and meat quality of lambs from hill sheep systems', *J. Agric. Sci.*, 137, 205–220.
- Carson A F, Moss B W, Steen R W J and Kilpatrick D J (1999), 'Effects of the percentage of Texel or Rouge de l'Ouest genes in lambs on carcass characteristics and meat quality', *Anim. Sci.*, 69, 81–92.
- Charlier C, Segers K, Karim L, Shay T, Gyapay G, Cockett N and Georges M (2001), 'The callipyge mutation enhances the expression of coregulated imprinted genes in *cis* without affecting their imprinting status', *Nature Gen.*, 27, 367–369.
- Clop A, Marcq F, Takeda H, Pirottin D, Tordoir X, Bibe B, Bouix J, Caiment F, Elsen J M, Eychenne F, Larzul C, Laville E, Meish F, Milenkovic D, Tobin J, Charlier C and

- Georges M (2006), 'A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep', *Nature Genet.*, 38, 813–818.
- Cockett N E, Jackson S, Shay T L, Farnir F, Berghmans S, Snowden G, Nielsen D and Georges M (1996), 'Polar overdominance at the ovine callipyge locus', *Science*, 273, 236–238.
- Cramer D A, Pruett J B, Kattnig R M and Schwartz W C (1970a), 'Comparing breeds of sheep. I. Flavor differences', *Proc. West. Sect. Am. Soc. Anim. Sci.*, 21, 267–269.
- Cramer, D A, Pruett J B, Swanson V B, Schwartz W C, Kattnig R M, Phillips B L and Wookey L E (1970b), 'Comparing breeds of sheep. II. Carcass Characteristics', *Proc. West. Sect. Am. Soc. Anim. Sci.*, 21, 270–272.
- Crouse J D, Busboom J R, Field R A and Ferrell C L (1981), 'The effect of breed, diet, sex, location and slaughter weight on lamb growth, carcass composition and meat flavour' *J. Anim. Sci.*, 53, 376–387.
- Dawson L E R, Carson A F and Moss B W (2002), 'Effects of crossbred ewe genotype and ram genotype on lamb meat quality from the lowland sheep flock', *J. Agric. Sci.*, 139, 195–204.
- Dransfield E, Nute G R, MacDougall D B and Rhodes D N (1979), 'Effect of sire breed on eating quality of crossbred lambs', *J. Sci. Food Agric.*, 3, 805–808.
- Duckett S K, Cuvala S L and Snowden G D (1999), 'Effects of Dorper genetics on tenderness, fatty acid and cholesterol content of lamb', *J. Anim. Sci.*, 77, (S1) 168.
- Duckett S K, Snowden G D and Cockett N E (2000), 'Effect of the callipyge gene on muscle growth, calpastatin activity, and tenderness of three muscles across the growth curve', *J. Anim. Sci.*, 78, 2836–2841.
- Elmore J S, Mottram D S, Enser M and Wood J D (2000), 'The effects of diet and breed on the volatile compounds of cooked lamb', *Meat Sci.*, 55, 149–159.
- Fox C W, McArthur J A B and Sather L (1962), 'Effect of sire and breed on flavor scores from weanling lamb', *J. Anim. Sci.*, 21, 665.
- Fox C W, Eller R, Sather L and McArthur J A B (1964), 'Effects of sire and breed on eating qualities from weanling lambs', *J. Anim. Sci.*, 23, 596.
- Freking B A, Keele J W, Shackelford S D, Wheeler T L, Koohmaraie M, Nielsen M K and Leymaster K A (1999), 'Evaluation of the ovine callipyge locus: III. Genotypic effects on meat quality traits', *J. Anim. Sci.*, 77, 2336–2344.
- Freking B A, Murphy S K, Wylie A A, Rhodes S J, Keele J W, Leymaster K A, Jirtle R L and Smith T P L (2002), 'Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals', *Genome Res.*, 12, 1496–1506.
- Hadjipavlou G, Matika O, Clap A and Bishop S C (2007), 'Two myostatin single nucleotide polymorphisms have significant effects on carcass traits of UK commercial Charollais sheep populations', *Proc. 3rd Int. Conf. Quantitative Genetics*, Zhejiang University, Hangzhou, 2007.
- Jacobson M and Koehler H H (1963), 'Components of the flavor of lamb', *Ag. Food Chem.*, 11, 336–339.
- Jopson N B, Nicoll G B, Stevenson-Barry J M, Duncan S, Greer G J, Bain W E, Gerard E M, Glass B C, Broad T E and McEwan J C (2001), 'Mode of inheritance and effects on meat quality of the rib-eye muscling (REM) QTL in sheep', *Proc. Assoc. Adv. Anim. Breed. Genet.*, 14, 111–114.
- Karamichou E, Nute G R, Richardson R I, McLean K and Bishop S C (2006a), 'Genetic Analyses of Carcass Composition, as Assessed by X-ray Computer Tomography, and Meat Quality Traits in Scottish Blackface Sheep', *Anim. Sci.*, 82, 151–162.
- Karamichou E, Nute G R, Richardson R I, McLean K and Bishop S C (2006b), 'A Partial Genome Scan to Map Quantitative Trait Loci for Carcass Composition, as Assessed by X-ray Computer Tomography, and Meat Quality Traits in Scottish Blackface Sheep', *Anim. Sci.*, 82, 301–309.
- Karamichou E, Richardson R I, Nute G R, Gibson K P and Bishop S C (2006c), 'Genetic

- analyses and QTL detection for fatty acid composition in Scottish Blackface sheep', *J. Anim. Sci.*, 84, 3228–3238.
- Karamichou E, Richardson R I, Nute G R, Wood J D and Bishop S C (2007), 'Genetic analyses of sensory characteristics and relationship with fatty acid composition in the meat from Scottish Blackface lambs' *Animal*, 1, 1524–1531.
- Lee G J, Archibald A L, Law A S, Lloyd S, Wood J D and Haley C S (2004), 'Detection of quantitative trait loci for androstenedione, skatole and boar taint in a cross between Large White and Meishan pigs', *Anim. Genet.*, 36, 14–22.
- Legrand I, Denoyelle C, Quilichini Y (1995), 'Effects of breed and rationing concentrates on lambs subcutaneous adipose tissue quality', *Proc 41st Ann. Int. Congr. Meat Sci. Tech.*, 11, 116–117.
- Martinez-Cerezo S, Sanudo C, Panea B, Medel I, Delfa R, Sierra I, Beltran J A, Cepero R, Olleta J L (2005), 'Breed, slaughter weight and ageing time effects on physico-chemical characteristics of lamb meat', *Meat Sci.*, 69, 325–333.
- McDonagh M B, Ferguson K L, Bacic A, Gardner G E and Hegarty R S (2006), 'Variation in protein abundance profiles in the M-semitendinosus of lambs bred from sires selected on the basis of growth and muscling potential', *Austr. J. Agric. Res.*, 57, 671–682.
- McLaren R J, Broad T E, McEwan J C, Jopson N B, Robertson T R, Glass, B C, Gerard E M, Greer G J, Bain W E and Nicoll G B (2001), 'Identification of positional candidates for the Carwell locus for rib-eye muscling in sheep', *Proc. Pl. Anim. Genome IX*, W46. San Diego, CA, USA (www.intl-pag.org/9/abstracts/W17_03.html).
- Mendenhall V T and Ercanbrack S K (1979), 'Effect of carcass weight, sex, and breed on consumer acceptance of lamb', *J. Food Sci.*, 44, 1063–1066.
- Meuwissen T H E and Goddard M E (1996), 'The use of marker haplotypes in animal breeding schemes', *Genet. Sel. Evol.*, 28, 161–176.
- Safari E, Fogarty N M and Gilmour A R (2005), 'A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep', *Livest. Prod. Sci.*, 92, 271–289.
- Sañudo C, Nute G R, Campo M M, Maria G, Baker A, Sierra I, Enser M E and Wood J D (1998), 'Assessment of commercial lamb meat quality by British and Spanish taste panels', *Meat Sci.*, 48, 91–100.
- Sañudo C, Enser M E, Campo M M, Nute G R, Maria G, Sierra I and Wood J D (2000), 'Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain', *Meat Sci.*, 54, 339–346.
- Sobrinho A G D, Purchas R W, Kadim I T and Yamamoto S M (2005), 'Meat quality in lambs of different genotypes and ages at slaughter', *Braz. J. Anim. Sci.*, 34, 1070–1078.
- Vuocolo T, Byrne K, White J, McWilliam S, Reverter A, Cockett N E and Tellam R L (2007), 'Identification of a gene network contributing to hypertrophy in callipyge skeletal muscle', *Phys. Genomics*, 28, 253–272.
- Walling G A, Visscher P M, Wilson A D, McTeir B L, Simm G and Bishop S C (2004), 'Mapping of quantitative trait loci for growth and carcass traits in commercial sheep populations', *J. Anim. Sci.*, 82, 2234–2245.
- Webb E C and Casey N H (1995), 'Genetic differences in fatty acid composition of subcutaneous adipose tissue in Dorper and SA Mutton Merino wethers at different live weights', *Small Rum. Res.*, 18, 81–88.
- Young O A, Reid D H, Scales G H (1993), 'Effects of breed and ultimate pH on the odour and flavour of sheep meat', *N. Z. J. Agric. Res.*, 36, 363–370.

Use of meat quality information in breeding programmes

G. Simm, N. Lambe, L. Bünger, E. Navajas and R. Roehe, Scottish Agricultural College (SAC), UK

Abstract: This chapter discusses the inclusion of meat quality information in livestock breeding programmes which could provide permanent and cumulative genetic improvements in these traits with continued selection. Reasons why meat quality information is not more widely used in current breeding programmes are considered. Measurement techniques, genetic parameters and breeding programme designs that could be exploited to successfully incorporate meat quality traits are discussed, as well as suggestions of possible future directions for the inclusion of meat quality traits in livestock breeding programmes.

Key words: breeding programme, genetics, breeding values, meat quality.

12.1 Introduction

Breeding programmes for livestock have resulted in substantial genetic improvements in production efficiency, quantity and quality of food products. These are permanent, and with continuous selection, cumulative (Dekkers and Hospital, 2002; Simm *et al.*, 2006). In the past few decades, considerable selection responses have been achieved in production traits such as growth and carcass composition using modern breeding methods. However, livestock breeding schemes designed to improve meat quality (MQ – chemical composition, mechanical properties, etc.) or meat eating quality (MEQ – sensory characteristics) are limited in number, despite the increasing importance of these traits to consumers.

In this chapter we discuss some of the reasons why MQ information is not more widely used in current breeding programmes, including technical, financial and biological limitations. The design of breeding programmes to successfully incorporate these traits is considered. Evidence for genetic variation in MQ traits is provided and relationships between MQ and other important economic or animal

welfare traits summarised. Knowledge of these associations is vital before incorporation of MQ traits into breeding programmes, to identify possible deleterious effects on other important traits. Possible methods to access MQ information for successful inclusion into breeding programmes are discussed, in terms of current and potential techniques for measuring MQ traits (*in vivo* or *post-mortem*). Finally, possible future directions for the inclusion of MQ traits in livestock breeding programmes are suggested, including tackling the increasing demand for uniformity in MQ and the use of progressively more sophisticated techniques for including genomic information in selection programmes to improve MQ.

12.2 Issues affecting the inclusion of meat quality information in breeding programmes

Genetic change in a population is currently brought about in three ways: by selection between breeds, by cross-breeding or by selection within a breed (e.g. Cartwright, 1970; Simm, 1998). (In future, direct genetic modification may provide an additional means to create genetic change, but this is not considered in this chapter.) Breeds of the main livestock species are known to differ in their MQ characteristics, often as a result of different selection histories. This has been exploited in some cases by crossing breeds or lines with superior MQ with lines that are specialised for other important traits, such as lean growth or maternal ability, to produce the slaughter generation. However, once a decision has been made on the breed or cross-breed to use, further genetic improvement can be achieved within-breed using selective breeding. Hence, breeding programmes designed to improve MQ or MEQ within-breed will be the focus of this chapter.

Before MQ traits can be incorporated into livestock breeding programmes, a clear definition of MQ and an understanding of the traits to use for characterising this composite and complex trait, are required. A summary of the main traits affecting MQ or MEQ is given in Fig. 12.1. In addition, many interactions also occur between these traits to affect MQ. MQ has been defined in various ways, since the traits conferring a good product differ between producers, processors, retailers and consumers, resulting in unclear signals within the industry (Bray, 1966; Sellier, 1998). Traits associated with increased quality differ at each level of the meat-marketing chain, and between countries and cultures, and associations between these traits are often poor. For example, low correlations have been found between aspects of visual appearance of meat and MEQ (Russell *et al.*, 2005). It is critical to define clear breeding goals that are relevant for the species and genotypes involved, as well as for the markets they intend to supply, in the design of breeding schemes that incorporate MQ.

The most relevant assessment of MEQ is through sensory analysis by taste panels, because these traits need to be quantified based on people's perceptions. Despite this, it is also the most difficult method to standardise, due to the problem of calibrating human judgements (Honikel, 1991), and incurs a high cost.

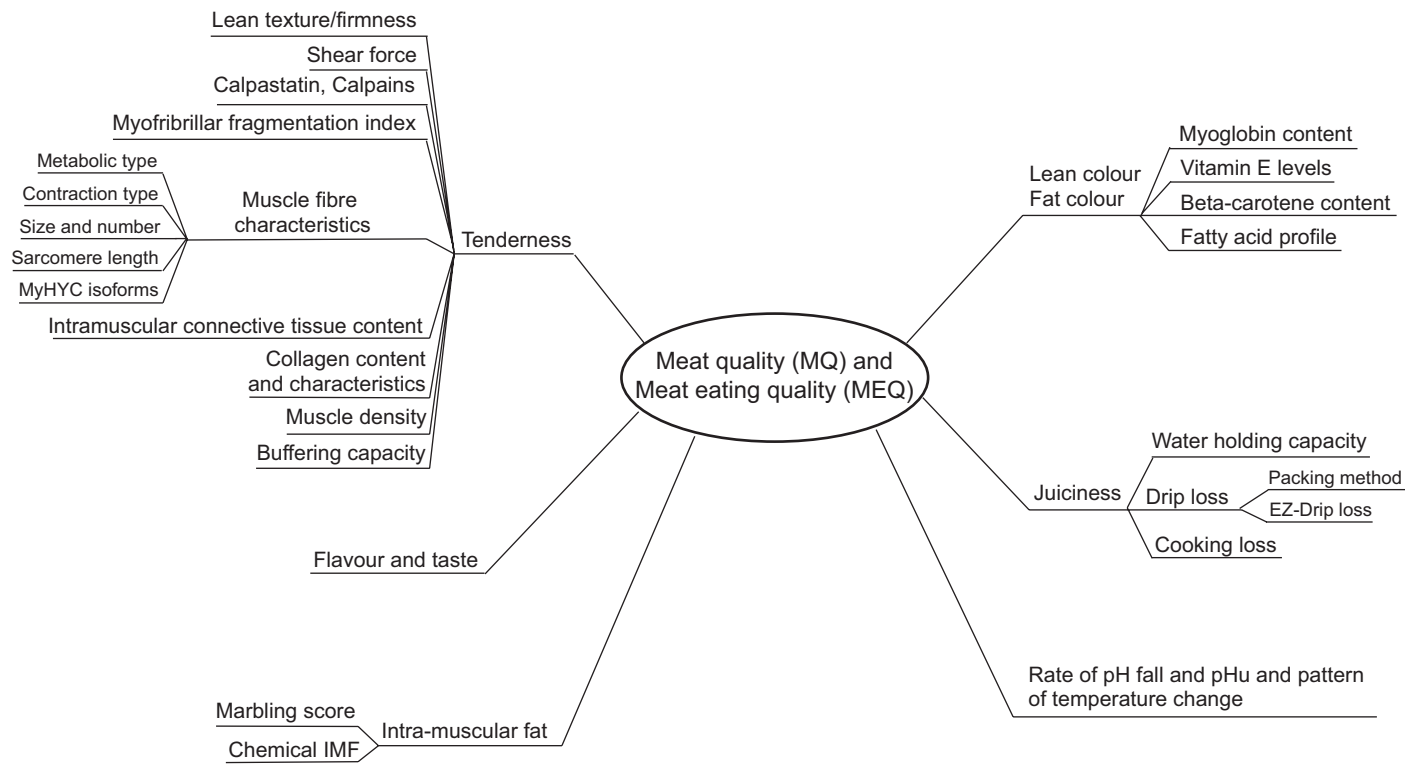


Fig. 12.1 Traits associated with MQ and MEQ.

However, because of their importance, mechanical or chemical measurements are usually compared to taste panel scores for validation.

Additionally there are problems inherent to some of the constituent traits of MQ. For example, some MQ traits have been found to be optimum at intermediate levels, in certain species. The National Pork Producers Council, for instance, estimated intermediate optimum values for pork traits such as pH, intra-muscular fat (IMF) and colour in 1998 (Heuven *et al.*, 2003). With no linear relationship between these traits and MQ, their incorporation into breeding programmes is more complicated, which may have limited previous genetic selection for these traits. The improvement of a trait to its optimum and the reduction of the variance surrounding a trait are challenging breeding goals, but can be addressed using the correct design of breeding programme: for example, using techniques such as canalising selection (e.g. SanCristobal-Gaudy *et al.*, 1998).

The main barrier to the inclusion of MQ traits in livestock breeding programmes, however, is the lack of sufficient direct financial incentives to justify the costs of breeding to improve MQ. The price received by the farmer per animal, in the majority of payment systems for sheep, beef cattle and pigs across different countries, is based largely on weight, carcass composition, conformation, or a combination of these factors. This helps to explain the success and uptake of breeding schemes designed to improve characteristics related to growth, lean meat yield and fatness. With no direct reward to farmers for improved MEQ, however, there is no demand for breeders to supply breeding stock with higher genetic merit for these traits. Difficulties in achieving a consensus on the definition of quality, in addition to the lack of widely available measures of MQ merit on which to base such reward schemes, are amongst the main constraints.

Direct measurements of MQ, through taste panel or chemical testing for example, are usually expensive and time-consuming, requiring highly trained staff and expensive laboratory equipment. To obtain information about the genetic potential for improved MQ of animals which are selection candidates for breeding, direct measurements must be taken on relatives (for example, progeny or siblings) of the animal, rather than the animal itself, since these tests require animals to be slaughtered. This incurs a substantial cost, since a pedigreed sample of animals large enough to provide estimates for genetic parameters with sufficient statistical accuracy, must be reared to a commercially relevant slaughter age before undergoing these expensive tests. Most of these *post-mortem* tests require some level of invasion or destruction of the muscles and the most commercially relevant parts of the carcass (the high-priced cuts) need to be used, often resulting in the loss, or reduction in value, of these joints for potential sale. Any genetic improvements made as a result of including direct or indirect measurements of MQ or MEQ in a breeding scheme would have to result in appropriate financial rewards to justify the costs.

Predictors of MQ measured in the live animal would allow selection of animals using their own performance data, as well as that of their relatives, and would not require large-scale collection of slaughter data. Measurements that would allow selection at an early age would reduce the generation interval and genetic 'lag' (the

time taken for improved genes to be disseminated down through generations), compared to schemes based on *post-mortem* measurement methods that require relatives to reach slaughter age before data can be collected. Several *in vivo* methods of assessing MQ indirectly have been (or are currently being) developed, which are becoming commercially available to breeders and have the potential to be widely used in breeding programmes to select for improved MQ.

Non-genetic factors, especially those relating to processing, can have large effects on MQ (Fig. 12.2). Many of these are discussed in detail in Parts III and IV of this book. The ability to compare selection candidates for a particular MQ trait will also depend on the method of measurement, including the specific equipment and technique employed. Furthermore, MQ characteristics of one muscle cannot be assumed to be representative of other muscles, even within the same animal. Variances in MQ and MEQ traits (e.g. shear force, IMF, moisture, protein, colour, sensory attributes), and the correlations between these traits have been reported to differ between muscles and even between samples taken from different locations within the same muscle (e.g. in beef: Shackelford *et al.*, 1995; Burrow *et al.*, 2001; Maher *et al.*, 2004). This suggests that muscle-specific strategies may be required for improving the quality and value of muscles within breeding schemes (Rhee *et al.*, 2004), with prior knowledge of the relationships between prediction methods and MEQ within that muscle. Non-genetic factors will affect the environmental variance and therefore the heritability of the MQ traits being measured. Therefore, measurements of MQ or MEQ traits should be standardised as far as possible, and statistical analysis performed which takes account of these sources of variation. Similar complications have been overcome, through the design of breeding programmes or genetic evaluation models, to allow selective breeding to be widely and successfully used for many other traits of value in livestock production.

12.3 Breeding programme design to include meat quality (MQ) goals

Genetic improvement programmes involve selecting animals to use for breeding future generations, based on their own performance and/or that of their relatives. The traits to be improved are termed breeding goals and the measurements that are taken and selected on in order to improve these goals are termed selection criteria. Selection to improve MQ traits is likely to occur as part of a wider breeding programme, also including other traits of importance. Knowledge of the genetic control of MQ traits, their mode of inheritance, relationships with other traits of interest and their relative importance must be understood before an optimum breeding programme including MQ can be properly designed.

If a MQ or MEQ trait of overriding importance is primarily controlled by a single locus, and there is a reliable DNA-based (or other) test for assigning genotypes to animals, then it is relatively simple to increase the frequency of the favourable gene (or decrease the frequency of an unfavourable gene) in the population by breeding from animals of the desired genotype. Wider implications

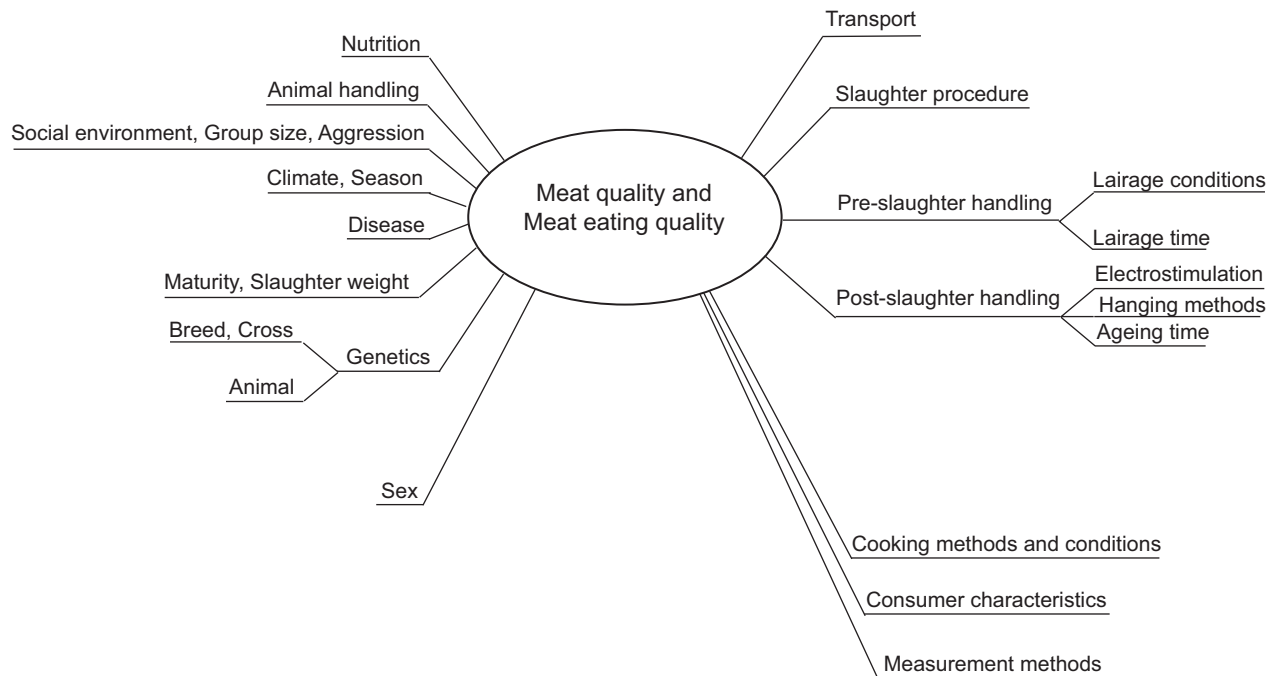


Fig. 12.2 Factors affecting MQ and MEQ.

of the use of molecular information in breeding programmes for MQ are discussed in Section 12.3.1. However, most MQ traits have a polygenic basis (controlled by several genes) and selection based on a small number of molecular markers can therefore exploit only part of the existing genetic variation. Even in cases where molecular genetic selection can be applied for MQ traits, this is often likely to take place within the context of a wider breeding programme. Therefore, molecular information should be integrated with existing quantitative genetics techniques for the most effective use of these new methods. Hence, and in view of upcoming *in vivo* techniques to measure MQ, genetic parameters and opportunities for conventional selection are of interest, and are discussed in Sections 12.3.2 to 12.3.8. Burgeoning information from genome mapping programmes in livestock is providing potential new tools to improve polygenic traits. In Section 12.5.2 we look at how such information may be used optimally to improve MQ and MEQ.

12.3.1 Single genes or quantitative trait loci affecting meat quality traits

Polymorphisms in some key genes and several quantitative trait loci (QTL) have been associated with MQ traits (mainly in pork and beef: Rothschild *et al.*, 2004; Jeon *et al.*, 2006; Hocquette *et al.*, 2007) and molecular genetic markers (polymorphic segments of DNA at a specific site on a chromosome that are associated with the trait of interest) have been identified. Some examples are given in Table 12.1. Markers associated with these genes of interest can either be direct markers (causative mutations), population-wide linkage disequilibrium markers (markers close to the functional mutation that are inherited with the gene), or linkage equilibrium markers (more loosely linked to the mutation and likely to undergo recombination). Some linked markers are in within-family linkage disequilibrium, rather than population-wide linkage disequilibrium, meaning that different marker alleles are associated with the gene in different families (Dekkers and Hospital, 2002; Navajas and Simm, 2004).

Marker Assisted Selection (MAS) of animals (selection using molecular information resulting from DNA analysis of a sample taken from a live animal) has great potential for use in selection programmes to increase MQ. MAS can be used to select animals for breeding that carry favourable alleles that already exist within a population, or to eliminate undesirable alleles from a population. MAS is especially advantageous for traits with low heritabilities (in which phenotypes are poor predictors of genotype) or for traits where phenotypic information is difficult or expensive to obtain, such as MQ traits.

Marker assisted introgression (MAI) can be used to introduce a single gene with a favourable effect from a different population. This is usually achieved by crossing the target population with a population carrying the desired gene, then repeatedly backcrossing to the original population, using molecular information to maintain the introduced gene but recover the majority of the genes from the original population over several generations.

One of the advantages of DNA information comes from the fact that samples of blood or tissue can be taken at a young age, allowing DNA analyses to be

Table 12.1 Examples of major genes or QTL in different species affecting meat quality

Species	Major gene/QTL	Effects on carcass and meat quality	Breeding implications
Pigs	<i>FABP</i> genes	Influences IMF content	Allows selection for IMF independent of backfat depth
	<i>RYR1</i> (Ryanodine receptor 1, 'halothane gene')	Reduces ultimate pH and water-holding capacity. Increases PSE and variation in MQ	Can be eliminated from the population with the help of molecular genetic selection
	<i>PRKAG3</i> (new alleles of the 'RN' gene)	Reduces processed meat yield, ultimate pH, lean colour intensity. Increases drip loss and variation in MQ	
Cattle	Polymorphisms in the myostatin gene (also in sheep)	Causes 'double muscling' (increased muscle growth). Increases muscle:bone, dressing %, water content. Reduces subcutaneous fat, marbling, collagen content, lean colour intensity, flavour.	Careful use in selection programs can improve MQ, but lowers also IMF and is associated with other negative side effects in cattle – increased dystocia, lower stress resistance
	QTL on BTA29 <i>CAPNI</i> (calpain 1)	Influences tenderness	Carriers of favourable markers for more tender meat can be selected at a young age
	QTL on BTA2, BTA3, BTA27	Influences marbling	Allows selection for marbling independent of overall fatness
Sheep	Callipyge	Reduces carcass fat, marbling, tenderness. Increases muscularity, lean yield, dressing %, connective tissue content	Can be eliminated from the population with the help of molecular genetic selection
	Carwell (Loin-Max)	Increases lean yield, eye muscle depth. Slightly reduces tenderness, but this effect can be eliminated by enhanced processing	Can be used to enhance muscle growth without substantial effects on MQ

performed to identify animals with the preferred genotypes at the markers associated with the gene of interest. It provides information on the genotypes of the selection candidates very soon in their lives, allowing earlier selection decisions, and is therefore advantageous for increasing the rate of genetic improvement.

Despite the potential of MAS, in particular, for use in selection programmes to increase MQ, the application of molecular information in commercial livestock breeding programmes has been limited to date. Markers are practical only to use

across a population if they are tightly linked to the gene. Otherwise the relationship between the marker and the trait of interest may change over time as a result of recombination. In such cases, the marker may no longer be inherited with the gene of interest, making it unreliable. If linked markers are used that are in within-family linkage disequilibrium, phenotypic data, as well as molecular information, is required to identify the preferred alleles within each family for the implementation of MAS. However, when selection is based on direct markers or markers that are tightly linked to QTL, the requirements for phenotypic recording are lower, as the same marker alleles should result in the preferred genotype across families (Navajas and Simm, 2004), so animals can be selected based on marker genotypes alone. Nevertheless, direct and indirect effects of genes can differ, depending on the genetic background of the population in which they are expressed and the production system, so prior testing is required to validate the markers against an appropriate background, as well as regular monitoring of the effect of each genotype on the trait of interest across generations, which is time-consuming and expensive. For some traits, the proportion of the genetic variance explained by the marker(s) might be too small to make efficient use of the molecular genetic information. Even when suitable markers have been found and validated, the cost of genotyping may be prohibitive and it is often unclear how molecular genetic information can be integrated with other estimates of genetic merit for all traits of economic importance. These concerns must be overcome to allow the promise of MAS to be fulfilled in breeding programmes.

Many chromosomal regions that have been associated with carcass and MQ traits show non-additive inheritance patterns (Heuven *et al.*, 2003). For example, the Carwell gene (Table 12.1) has a dominant mode of inheritance. No significant differences in eye muscle area were found between progeny that inherited the gene from their sire, dam or both parents, although non-carriers had significantly lower eye muscle areas (Jopson *et al.*, 2001). The Callipyge gene (Table 12.1) has a mode of inheritance termed polar over-dominance – only heterozygous animals inheriting the mutation from their sire show the phenotypic effect (Cockett *et al.*, 1999). Imprinted genes (only expressed in offspring if inherited from one parent) have also been linked to pork quality traits and imply that the expected phenotypic covariance is reduced between parents and offspring relative to that between sibs (Heuven *et al.*, 2003).

Furthermore, in MQ traits as in other traits, the effects of QTL or major genes frequently depend on the genetic background (strain or breed) in which they are found. The phenotype of a single gene mutation can be modulated, either moderately or severely, in certain genetic backgrounds, due to epistatic interactions, where so-called modifier genes act in combination with the causative gene (e.g. Montagutelli, 2000; Varga *et al.*, 2003; Jannink, 2007). An example is the mutation in the myostatin gene responsible for double-muscling of cattle breeds such as the Belgian Blue, which is also found in other breeds such as the South Devon, where the effect on muscling is less extreme than in the Belgian Blue (Wiener *et al.*, 2002), and in the Highland, where no double-muscling phenotype is observed (Williams, J.L. pers. communication).

Identified mutations, candidate genes or QTL for improving MQ in pork, beef and lamb have been identified (Table 12.1). However, the inheritance patterns, as well as direct and indirect effects of these genes or QTL, require thorough investigation against the relevant genetic background before the appropriate use of such genes or chromosomal segments in breeding programmes can be considered. Further information on progress with identifying genes or QTL responsible for MQ traits in beef, pigs and sheep is contained in previous chapters.

Once validated in the target population, molecular information for QTL or linked markers can be used to increase the precision of estimated breeding values for traits such as those involved in MQ, resulting in a greater response to selection within a breeding programme. However, the use of pedigree and performance records alongside molecular information would allow optimised selection, without unacceptable rates of inbreeding or loss of genetic variation (Simm *et al.*, 2005).

12.3.2 Estimating breeding values

Accurate identification of animals of high genetic merit is a key component of creating genetic improvement in practice. Over the last few decades, best linear unbiased prediction (BLUP) has become the most widely used method for predicting genetic merit of farm animals. BLUP is a statistical method that uses pedigree and performance records, as well as information on non-genetic factors likely to influence performance, to disentangle (additive) genetic and non-genetic (management and 'environmental') effects in the best possible way. BLUP can make optimal use of performance information from all classes of relatives, as well as the candidates for selection themselves. BLUP estimated breeding values (EBVs) can be produced simultaneously for a series of traits of economic importance, providing that estimates of the phenotypic and genetic (co)variances are available.

12.3.3 Selection indices

A selection index is a means of selecting animals on more than one trait simultaneously, based on the performance of the individual and its relatives. In terms of MQ traits, only indirect (predictor) traits can be measured on the selection candidates themselves, since direct measures of MQ require slaughter of the animal. Direct and indirect traits can be measured on relatives. BLUP provides the optimal way of combining information from these different sources. Index scores predicting genetic worth in overall economic merit can be derived by weighting the BLUP EBV for each breeding goal trait by its economic value and summing these across traits.

The weighting on each trait in a multi-trait index depends on the amount of additive genetic variation in each trait, the genetic relationships among the traits and their relative economic importance. A valuable property of BLUP EBVs, and index scores derived from them, is that they can be compared across different years, herds or flocks, or contemporary groups, provided that there are genetic

links among them (Simm, 1998). This greatly increases the pool of animals that can be fairly compared prior to selection, and allows genetic trends to be monitored over time.

The annual response to selection using a multi-trait index will be smaller in any component trait than could be achieved if selection was performed on that trait alone. However, to improve overall performance and achieve the highest rate of change in overall economic merit, it is important to consider all traits of major functional or economic value. This reduces the risk of selection on a given trait having a deleterious affect on another important trait that is not monitored.

Economic values represent the marginal profit that would result from one unit genetic change in a trait (Simm, 1998), and are often difficult to assign for MEQ traits. Whilst the value of a trait such as drip loss can be related to loss in saleable meat yield, this and other traits will also determine customer preference of meat, to which it is difficult to assign an economic value (Heuven *et al.*, 2003). Moreover, different levels of the supply chain (e.g. slaughterhouses, processors, and retailers) may have different economic drivers from consumers. Hovenier *et al.* (1993) developed a method to derive economic values for traits with an intermediate optimum, such as pH value. This approach was extended by von Rohr *et al.* (1999) in order to take several price levels into account. The price levels in both studies were derived from market surveys, firstly based on consumers' willingness to pay more for better quality, and secondly on a survey of the slaughter and retail industry. Otto *et al.* (2007) extended this method further to account for non-normally distributed MQ traits.

Desired-gains or restricted indices have been proposed by some authors to define the relative economic values for multi-trait selection involving breeding goals for which it is difficult to derive economic values. Using this method, the weightings on each trait depend on the preferred change in one trait relative to changes in other traits, rather than predicted economic benefits. An example might be an index that is intended to maintain IMF, for which no direct economic value is available, but which might have a positive effect on MEQ, while improving other components of growth and carcass composition, such as producing leaner carcasses.

12.3.4 Two-stage selection

Many of the measurement methods that give the most accurate predictions of MEQ are expensive and time-consuming, and are not feasible to record on a large number of animals. Two-stage selection allows the most effective use of such methods, across the population of interest. The two stages involved include:

- (i) an initial (pre-)selection of animals using a method that is feasible to record on a large number of animals, but may be a less accurate predictor of the trait of interest
- (ii) further selection of the best sub-set of animals from stage (i) using a measurement that is more accurate at predicting the trait of interest, but also more expensive.

The optimal proportion of animals included in the sub-set that goes on to the second stage of selection depends on: the correlations between the measurements taken in the first and second stages; the proportion of animals required for breeding; the heritability of the traits under selection; the cost of the second-stage measurements; and the economic benefit of improving the trait of interest (Jopson *et al.*, 2004).

This type of selection system would be of value in a breeding programme designed to improve MQ, since expensive methods that measure or predict MQ with high accuracy would be required only on a sub-set of the population under selection. Pre-selection of animals more likely to have improved MQ could be achieved using an indirect live animal predictor of MQ in the first stage of selection (e.g. ultrasound scanning). This could then be followed by a second stage of direct MQ measurements (e.g. mechanical, chemical or taste panel measurements) taken on slaughtered relatives of the best sub-set identified in the first stage, or one of the more accurate, but expensive, *in vivo* predictors of MQ described later in this chapter (e.g. CT scanning, histology from biopsies).

Two-stage selection can be used alongside selection indices. Data from individuals and their relatives, on one or more traits, can be measured in the first stage of selection and run through BLUP software to produce EBVs and index values for each animal. Those with the highest scores can then be selected for more accurate measurement in the second stage of selection, the data from which can then be incorporated with that from the first stage and another BLUP analysis run on all available data to select the final animals for breeding. A practical way to combine MQ data with performance records from other traits of interest may be to record MQ only in the second stage. For example, perform an initial selection based on traditional breeding goals, such as growth rate and lean meat proportion, from which the top-performing animals go forward to a second stage of selection based on MQ characteristics (measured using an accurate predictor trait, or directly on slaughtered relatives). In this way the animals with the best MQ can be selected from within those with the best genetic potential for growth and carcass composition.

12.3.5 Progeny/sib testing

BLUP EBVs make optimal use of information for collateral relatives, such as sibs, and from progeny. However, if direct measurements *post-mortem* are the only option, this needs to be reflected in the breeding programme design. Progeny testing is a breeding programme where the genetic information on a candidate for selection comes from the performance of its progeny. Similarly, sib testing uses performance records from the full or half siblings of the selection candidates. The accuracy of selection based on these performance records is increased as the proportion of shared genes increases between the selection candidate and the relatives being measured (progeny > full sibs > half sibs), and also as the number of relatives being recorded increases (Simm, 1998). Progeny or sib selection programmes are commonly used where the trait of interest cannot be measured on

the live candidate animals; for example, if the trait can be measured only on one sex (e.g. milk production), or *post-mortem* (e.g. MQ).

Progeny or sib testing, using direct measures of MQ, is likely to increase the accuracy of selection compared with selection on a less accurate predictor trait. However, the relatives must reach slaughter age before they are tested, and selection decisions cannot be made until results from the time-consuming lab-based tests are available. Although progeny testing is likely to lead to an increase in the accuracy of selection compared to sib testing (due to an increased proportion of shared genes), an extra generation may be required before performance records are available from this class of relatives, causing an increase in the generation interval.

Progeny or sib testing is expensive and requires records from a sufficiently large number of animals to make the results robust. To make the most cost-effective use of these methods, two-stage selection could be employed, where only animals ranking highest in an initial screening process using a predictor trait go on to be evaluated by progeny or sib testing.

While only *designed* progeny testing appears to be feasible currently, if unique animal identification and post-slaughter on-line prediction of MQ become common, then *opportunistic* progeny testing (i.e. predicting breeding values for all well-represented sires, using all available progeny records, and records from other relatives) could offer a much larger-scale and more economical alternative (Simm *et al.*, 2006).

12.3.6 Response to selection

Four main factors affect the response to selection, over a given time period, which must be considered in the design of a breeding programme (Simm *et al.*, 2006):

- (i) the average selection intensity, i ;
- (ii) the additive genetic standard deviation, or standard deviation of breeding values, sd_A – a function of the phenotypic variation in the trait and its heritability;
- (iii) the accuracy of selection, r – which is a function of the heritability of the trait and the number and nature of records (e.g. from sibs or progeny) used to predict breeding values; and
- (iv) the average generation interval, L .

Annual response to selection, R , is affected by these factors as follows (after Falconer and Mackay, 1996):

$$R = i sd_A r / L \quad [12.1]$$

Therefore, response will be maximised when: selection intensity is increased (selected animals are increasingly superior to the mean); heritability of the trait is higher and phenotypic variation greater; the trait can be measured on the individual itself, as well as on a large number of close relatives, and it can be measured on both males and females; the selection criteria are not age-limited and can be measured

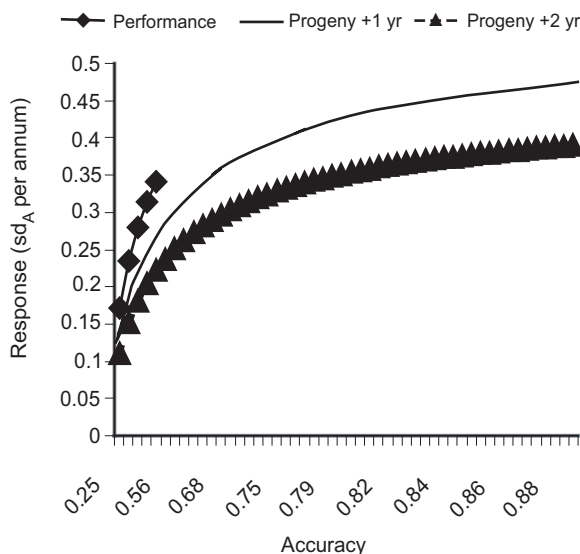


Fig. 12.3 Expected annual responses to selection (in additive genetic sd units) for a hypothetical meat quality trait.

Note: Heritability = 0.25, assuming that selection is based on (i) the animal's own performance using an *in vivo* predictor with accuracies ranging from low to perfect, (ii) progeny testing with results available a year later from up to 50 progeny, allowing the use of sires in their second year of life, and (iii) as for (ii) but with results available 2 years later than performance testing (from Simm *et al.*, 2006).

at a young age. Annual genetic changes in the range 1 to 3% of the mean in the trait (or multi-trait index) concerned are typical, and are cumulative across years with continuous selection (Simm *et al.*, 2005).

There are trade-offs between factors affecting response to selection, especially between accuracy and generation interval. For example, in progeny testing schemes, it is possible to achieve very high accuracies with sufficient progeny records. However, this takes a long time, and is costly. Often, annual response, and cost effectiveness, is maximised by using less accurate selection criteria earlier in the candidates' lives. Hence, in most meat-producing livestock, selection is usually based on performance measurements from selection candidates themselves, their collateral relatives and ancestors. (Though, usually, EBVs will be updated when records become available from descendants.) These measurements may be on indirect predictors for a trait such as MQ (possible live animal predictor traits are discussed below). Figure 12.3 shows the expected responses to selection for a hypothetical MEQ trait, for different performance and progeny testing scenarios. (In practice, the use of both methods, via two-stage selection, may be more cost effective.) (Simm *et al.*, 2006).

12.3.7 Genetic control of polygenic MQ traits

There are few studies on genetic variation of MQ and MEQ, because of the large sample sizes required for the estimation of the genetic parameters and consequently the high cost. Therefore, often variation between breeds is used as indication for genetic variance for these traits, as a breed comparison can be based on tens of animals whereas intra-breed estimation requires hundreds of pedigreed animals. The outcome of breed comparisons greatly depends on the breeds used and breed effects can be associated with variation in mature size/stage of maturity at slaughter, especially if double-muscled breeds are involved. Therefore, papers gathering evidence for additive genetic variance for MQ traits refer mostly to variance between breeds (e.g. [Thompson and Ball, 1997](#); [Newcom *et al.*, 2004](#); [Navajas *et al.*, 2007](#)) and intra-breed studies have been mainly restricted to species such as pigs (e.g. [Sellier, 1998](#), [Suzuki *et al.*, 2005a](#)), turkeys ([Bihan-Duval *et al.*, 2003](#)) and poultry in general ([Anthony, 1998](#)).

A higher rate of genetic improvement can be achieved by selecting on a trait with increased additive genetic variation. The heritability of a trait reflects the proportion of the observed (phenotypic) variation in the trait that is attributable to additive genetic variation, and therefore is controlled by an animal's additive genes, as opposed to environmental factors ([Fig. 12.2](#)). Estimates of heritabilities for MQ traits tend to fall within similar ranges across studies and species (e.g. pig review: [Sellier, 1998](#); beef review: [Marshall, 1999](#); general review: [Lambe and Simm, 2004](#)). Objective technological measures of MQ tend to have higher heritabilities (moderate–high for tenderness and IMF, low–moderate for colour or juiciness) than MEQ-related sensory traits (low heritabilities for subjective taste panel scores). Lower heritabilities may reflect difficulties in measuring these traits in a standardised, repeatable manner, rather than very low amounts of genetic variance ([Lambe and Simm, 2004](#)). Therefore, response to direct selection for sensory MEQ traits is expected to be lower than for objective measurements (especially of tenderness or IMF). Histological traits such as muscle fibre area and fibre type frequency, which are known to affect MQ traits, are also moderately heritable across species ([Rehfeldt *et al.*, 2004](#)).

12.3.8 Relationships between MQ and other important economic traits

To allow successful inclusion of MQ traits into a multi-trait selection programme, relevant genetic correlations for the species or genotype being used should contribute to its design. Most genetic associations that have been documented to date between MEQ and other traits have been derived from studies or breeding programmes designed to improve carcass and/or reproductive traits, where relationships with MQ traits have later been investigated on the resulting animals. Investigations of the effects of selection aimed at improving MEQ on other traits are rare.

Production traits

Most associations with MQ traits published in the literature are concerned with the

effect on MQ of selection for growth and carcass composition. Differences in MQ traits between breeds with different histories of genetic selection have been identified. *Bos taurus* breeds with high lean-to-fat ratios tend to have low levels of marbling fat and *vice versa*, whilst some other breeds (e.g. Japanese Black, Japanese Brown, Wagyu) have genetic potential for very high levels of marbling fat, but have low growth rates (Marshall, 1999). In sheep, specialist sire breeds grow more quickly and have been reported in some studies to have less tender meat compared to several other divergent breeds (general purpose, dam, sire, hair breeds) (e.g. Shackelford *et al.*, 2003, 2005). However, in cattle, results from studies investigating correlations between growth and tenderness (instrumental or sensory) have been mixed, mostly showing little association.

Estimates of genetic correlations between growth or carcass weight and other technological and sensory MQ traits are few in number, but tend to be low. In a review of the genetics of MQ in cattle, Marshall (1999) found little evidence of negative correlations between growth and MQ traits. In a later study, involving a larger number of MQ traits, Reverter *et al.* (2003) found evidence that selection for increased growth rate in beef cattle has positive effects on a range of MQ traits. In sheep studies, growth tends to show little genetic association with MQ traits (e.g. Hopkins *et al.*, 2005). In Duroc pigs, favourable genetic correlations with daily gain have been found for IMF and mechanical tenderness measurements (Suzuki *et al.*, 2005b), although these associations may differ in commercial white breeds.

Clear associations have been identified between MQ and major genes for muscling (Table 12.1); for example, the double-muscling gene in cattle, or the Callipyge gene in sheep (Cockett *et al.*, 1999). However, the relationship between MQ and muscling controlled by many genes (polygenic) is less clear. Some sheep studies report a negative genetic association between muscling (e.g. ultrasound muscle depth) and IMF or sensory scores for MEQ (e.g. Hopkins *et al.*, 2005) and similar trends have been observed in pigs (e.g. for eye muscle area versus IMF or mechanical tenderness in Duroc pigs, Suzuki *et al.*, 2005b). Conversely, little association has been found between lamb muscularity and MEQ traits by other researchers (e.g. Johnson *et al.*, 2005; Navajas *et al.*, 2007), suggesting that improvements in muscularity would not have unfavourable effects on MEQ. In a review of cattle studies to 1999, Marshall summarised that selection for leanness may have slight negative effects on water-holding capacity, and a negligible to moderately negative effect on IMF.

In general, increased carcass fatness is associated with improved MEQ. Therefore, selection for reduced carcass fat is likely to have a negative effect on MQ. Although genetic correlations between visual marbling and subcutaneous fat were found to be low in some cattle populations, suggesting that selection could act to decrease subcutaneous fat whilst maintaining marbling fat, genetic correlations between actual IMF and subcutaneous fat are stronger (Marshall, 1999). Moderate positive genetic correlations between fatness and tenderness have also been found in cattle, which is likely to be linked to IMF content (Reverter *et al.*, 2003). Lines or breeds of sheep selected for reduced carcass fat also tend to score lower for MEQ characteristics (e.g. Karamichou *et al.*, 2006; Navajas *et al.*, 2007). However, this

positive effect of carcass fatness is probably linked to IMF percentage and there may be an optimum level of fatness, above which MEQ is reduced.

Moderate genetic correlations between MQ and carcass traits suggest that unfavourable relationships could be selected against. For example, it would be possible to select animals with higher IMF, but lower carcass fat (moderately positively correlated). The use of molecular information may further distinguish genes associated with only IMF or both IMF and subcutaneous fat. For example, Duthie *et al.* (2007) found a QTL for IMF on chromosome 8, where no QTLs for subcutaneous fat have been found, whilst Mohrmann *et al.* (2006) detected a QTL for IMF on chromosome 6 which is closely associated with a QTL affecting subcutaneous loin fat. Some evidence for partially independent genetic regulation of fat aggregation in different fat depots has been reviewed by Bünger and Hill (2005).

In summary, although results are mixed, traditional breeding goals of increased muscle and reduced fat may be unfavourably genetically associated with some MQ traits in different species. However, genetic correlations are not too large to allow selection to improve these traits simultaneously. By including MQ as a breeding goal in a multi-trait selection index for these species, progress in the desired direction in MQ and carcass quality should be possible, albeit at a slower rate than selection on fewer goal traits.

Functional traits

Very few estimates of genetic (or indeed phenotypic) correlations have been reported between MQ and functional traits, such as fertility, reproductive capability, survival, behaviour, health or disease resistance.

Selection histories of different breeds for different purposes are known to influence carcass characteristics. For example, sheep breeds selected for maternal characteristics, such as prolificacy and milking ability, have larger internal fat depots in relation to carcass weight and total fat, and smaller eye muscle areas, than terminal sire breeds (Wood *et al.*, 1980; Wood and MacFie, 1980; Shackelford *et al.*, 2003, 2005). More tender, stronger flavoured meat is also produced from maternal breeds than those with a selection history for lean growth (e.g. Shackelford *et al.*, 2003; Shackelford *et al.*, 2005, 2007), which is probably linked to differences in fat content, especially IMF.

Relationships between reproductive traits and MQ traits in pigs are inconsistent across different traits and different studies, and no firm associations are evident (e.g. Hermes *et al.*, 2000; Serenius *et al.*, 2004). Unfavourable correlations were found between prolificacy traits in pigs and percentages of fat and lean in the carcass, with the exception of those with piglet mortality (Serenius *et al.*, 2004). In general, although reproductive or maternal traits may be unfavourably correlated with carcass composition, in cases where associations with MQ traits are observed they tend to be favourable, perhaps largely due to increased total fatness and IMF.

The possibility of including selection for disease resistance or improved health into breeding programmes has gained much attention in recent years. Studies investigating associations between MQ traits and the genetic potential for resist-

ance to different diseases or health problems of economic importance are not common. Genetic correlations have been estimated between osteochondrosis lesions in pigs and MQ traits and were generally unfavourable (Kadarmideen *et al.*, 2004). The authors of this study suggested that a selection programme to improve a health trait such as leg weakness should also include production and MQ breeding goals to reduce any negative effects on these traits.

Temperament of animals and their genetic susceptibility to stress can have significant impacts on MQ. Stress prior to slaughter can lead to poor quality dry, firm, dark (DFD) meat in beef cattle, and pale, soft, exudative (PSE) meat in pigs. Temperament of cattle, scored using various behavioural tests, is moderately heritable and calmer temperaments are phenotypically and genetically associated with improved MQ, in terms of shear force and tenderness (e.g. Kadel *et al.*, 2006; King *et al.*, 2006). Therefore, selection for either tenderness or temperament is likely to have a positive affect on the other. Genetic selection against susceptibility to Porcine Stress Syndrome will reduce variation in MQ and reduce the incidence of PSE.

Further relationships between MQ and functional traits should be investigated before inclusion of these traits into breeding programmes is recommended. In some breeds in particular (e.g. maternal breeds), the economic benefit from improving functional traits such as those associated with reproductive success, greatly outweighs the likely benefits for improvements in MQ under the majority of the present payment systems.

12.4 Techniques for measuring meat quality

Some of the current and emerging techniques to measure or predict MQ will be summarised briefly here. Although described in greater detail elsewhere (e.g. Chapter 22), it is important to outline these methods to allow understanding of how they could contribute to breeding schemes which include MQ information. [Table 12.2](#) summarises the general levels of accuracy reported in the literature for each method, as far as possible, as well as rating the methods in terms of relative cost. These accuracies relate different measurements to direct technological or sensory measurements of MQ or MEQ. However, differences will occur depending on which individual MQ or MEQ trait the measurement is being compared to.

12.4.1 *Post-mortem* measurements

Direct measurements of MQ can be taken only on slaughtered animals *post-mortem*, and many are destructive, leading to loss or reduced value of meat for sale. On the other hand, there are some physiological indicators, such as the rate and magnitude of changes in temperature and pH prior to the onset of *rigor mortis*, which are known to affect MQ traits such as tenderness, colour and drip loss, and which can easily be measured on the carcass using probes. Several other methods for *post-mortem* prediction of MQ from whole carcasses, joints or cuts of meat,

Table 12.2 Accuracy and relative cost of measuring MQ and MEQ traits using different methods (*=low, ***=moderate, *****=high)

Method	Accuracy of predicting MQ/MEQ Live animal	Post-slaughter	Relative cost
Marbling score		***	*
VIA	(sheep)*	(cattle)***	**
NIR		*****	**
Meat surface image analysis		*****	**
Fibre-optic probe		**	**
Technological measurements		*****	***
Taste panel		*****	*****
CT (sheep)	****		****
Ultrasound			
(beef)	****		**
(pigs)	***		
Surface electromyography (SEMG)	****		***
Histology (from biopsy or carcass sample)	***	***	****

varying in their sophistication and accuracy, have been developed, several of which are described below.

Marbling scores

Quartered beef carcasses are commonly visually assessed for marbling or IMF, using a subjective score awarded by trained assessors. Different scoring systems and anatomical assessment sites are used in various countries (e.g. USDA, AUS-MEAT, Ferguson, 2004). As well as predicting IMF content, marbling scores give information about distribution and size of IMF depots, which may affect the attractiveness of meat. Correlations between IMF and marbling score are generally moderate, but vary depending on the system used, amongst other factors. Nevertheless, EBVs for marbling in beef are available in USA and Australia for different breeds.

Video image analysis (VIA)

VIA systems have been developed for use in abattoirs that automatically assess chilled quartered sides of beef for marbling grade and colour of meat and fat. These systems use transverse images of the rib section, and results from various systems suggest that VIA can predict visual marbling score and IMF with fairly high accuracy and repeatability. VIA also allows the spatial characteristics of IMF to be objectively defined (Ferguson, 2004).

X-ray Computed Tomography (CT)

CT scanning facilitates the measurement of areas and average densities of fat, muscle and bone in cross-sectional scans through the body of an animal. Since commercially available CT scanning machines have been designed for use in

human medicine, only livestock species small enough to fit through the circular gantry can undergo whole-body scanning. CT scanning has been shown to be accurate at estimating composition in carcasses and can be used as a cost-effective alternative to dissection (for example, in pigs, Jopson *et al.*, 1995a; in sheep, Jopson *et al.*, 2004). CT is non-destructive and carcasses can be sold for human consumption following scanning.

As described in the following section, muscle density as measured by CT in live lambs can now be used as a predictor of IMF content and hence of MEQ. Therefore, similar measurements taken on scanned carcasses or joints are likely to be useful for predicting IMF in a non-destructive way *post-mortem*. This may be a valuable technique for beef cattle, where CT scanning of live animals is not an option, as they are too large to fit through the scanner. Trials are currently under way at SAC to investigate the possibility of CT scanning beef joints to predict MQ in a non-destructive manner.

Imaging analysis of cut meat surfaces

Information relating to characteristics of the surface of cut meat may be able to predict MQ in a non-invasive and non-destructive manner, allowing subsequent sale of the meat. For example, combinations of a number of geometric and texture characteristics of the surface of uncooked meat can be used to predict cooked meat tenderness with moderate to high accuracy (e.g. Chandraratne *et al.*, 2006). Similarly, parameters resulting from DEXA scans of whole steaks are capable of estimating tenderness with moderately high accuracy (Kroger *et al.*, 2006).

Histology/NIR

The metabolic and contractile properties of the muscle, determined by muscle fibre type distribution, influence variation in MQ traits (such as tenderness, colour, water-holding capacity and flavour) between animals or between muscles, in different livestock species (Klont *et al.*, 1998; Picard *et al.*, 2002). Characteristics such as frequencies and areas of different muscle fibre types, can be determined by performing laboratory-based histological and histochemical assays on small muscle samples removed from the carcass.

Visible and near infrared reflectance spectroscopy (Vis/NIR) has been found to predict fat and moisture content of beef, pork and lamb successfully (Ferguson, 2004; Andres *et al.*, 2007). Moderate to high correlations have been reported between Vis/NIR measurements and marbling in intact beef muscles (Ferguson, 2004), colour attributes, tenderness, sensory chewiness and juiciness in beef steaks (Liu *et al.*, 2003), sensory texture, juiciness, flavour, abnormal flavour and overall liking in lamb (Andres *et al.*, 2007).

The use of on-line systems for predicting MQ in a non-destructive way at the abattoir has been investigated. Several systems, including probes that use fibre-optic technology, have been developed to assess tenderness and other aspects of MQ, mainly for beef cattle (Koohmaraie *et al.*, 2005) and pigs. Many of those designed to predict tenderness are not sufficiently accurate (Koohmaraie *et al.*, 2005) and results from a study on pigs suggested that a fibre optic probe could

predict IMF with only moderate success and could not predict drip loss (Hoving-Bolink *et al.*, 2005).

Technological/ chemical/mechanical MQ measurements

Objective, laboratory-based measurements of MQ traits *post-mortem* are most commonly used for assessing MQ. These ‘technological’ traits, as they are often referred to, include measurements of tenderness (e.g. shear force, calpastatin activity, myofibrillar fragmentation index), IMF (chemical extraction), vitamin E content, water-holding capacity (e.g. conductivity) and colour (e.g. L^* , a^* , b^* parameters). These methods have been explained in detail in the literature.

Taste panel analysis

Taste panel scoring of meat samples is subjective, but most relevant for the assessment of MEQ. Panel members are chosen for their ability to distinguish flavours and textures and trained in how to score different characteristics. Meat samples are prepared and cooked under precise and uniform conditions, and then presented to panel members in specialised testing areas. Traits are mainly scored on scales of 1–5 or 1–8 and include tenderness, juiciness, flavour, presence of abnormal flavours and overall liking.

Consumer panels can also be used, where consumers are provided with meat to cook as they wish and must then fill in a questionnaire to rate taste, appearance, satisfaction and other characteristics of the meat they have eaten.

12.4.2 Live animal measurements

Ultrasound scanning

Using real-time ultrasound scanning it is possible to predict IMF with moderate to high accuracy in beef cattle (e.g. Izquierdo *et al.*, 1994; Hassen *et al.*, 2001) and pigs (e.g. Newcom *et al.*, 2002). Ultrasound predictors also correlate well with visual marbling scores and carcass quality grade in beef cattle (Williams, 2002). In beef cattle at least, accurate predictions of IMF from ultrasound have been made without including subcutaneous fat thickness in the prediction, which makes predictions of IMF and subcutaneous fat (and EBVs derived from these predictions) less dependent. This has potential benefits if both traits are to be included in the breeding goals of a scheme with a positive weighting in the index for IMF (with the intention of improving MQ), alongside subcutaneous fat with a negative weighting (to reduce fat in this depot and improve carcass fatness grade). This technique can be used on-farm and is relatively easy and inexpensive to use. Therefore, a large number of animals could be measured in this way and their data incorporated into a genetic evaluation.

No suitable method has been developed for using ultrasound to predict intramuscular fat in lambs. This may be due to the smaller size of the *M. longissimus dorsi* in this species and the difference in distribution of IMF through the muscle.

CT scanning

CT measurement of areas and average densities of fat, muscle and bone in cross-sectional scans through the body of a live animal can be used to predict total weights of each tissue. Sheep and pigs have been successfully CT scanned *in vivo* for the last few decades and the resulting data on carcass composition have been used in commercial breeding programmes, at least for sheep (e.g. Jopson *et al.*, 1995b; Jones *et al.*, 2002). Recent research in different sheep breeds has also shown strong negative genetic and phenotypic correlations between IMF and CT muscle density (Karamichou *et al.*, 2006; Navajas *et al.*, 2006). Since fat has a higher density than muscle fibre, a higher concentration of IMF will reduce the overall lean tissue density. Taste panel scores for flavour, juiciness and overall palatability have also shown strong negative correlations with CT muscle density, although no genetic association with toughness has been identified (Karamichou *et al.*, 2006).

Electromyography

Evoked surface electromyography (SEMG) records electrical signals in active muscle fibres through the skin surface. SEMG recordings have been related to muscle fibre composition and muscle fibre diameters in different species, which have important effects on MQ (Tygesen *et al.*, 2005). Work in pigs and lambs has also shown associations of SEMG with the glycogen content of muscle fibres, *post-mortem* rate of pH decline and shear force measurements of meat (Andersen and Harrison, unpublished data; Tygesen and Harrison, unpublished data). There is therefore potential to predict MQ and MEQ in livestock using SEMG analysis.

Infrared thermography

Thermographic images produced from live domestic animals, such as cattle or pigs, can be used to identify animals that have mean temperatures significantly above or below the normal surface temperatures for unstressed animals. This method has been suggested for detecting animals with a high probability of poor MQ in an *ante-mortem* environment (Tong *et al.*, 1997).

Histological or chemical tests of tissue samples obtained via biopsy

Histological properties of muscle (e.g. muscle fibre type proportions, fibre areas, glycolytic potential, etc.) can be studied on samples obtained from muscle biopsies taken from live animals, and could be used as genetic predictors of MEQ traits. Such information could be incorporated into breeding programmes, and there is evidence that these traits are sufficiently heritable (reviewed by Rehfeldt *et al.*, 2004). Although muscle samples can be taken from the live animal at a young age, the animal must be old enough for the muscle fibre types not to undergo metabolic transformation and change between biopsy and slaughter.

Biopsy samples of fat and muscle from live animals can also be used to test for traits such as intramuscular fat percentage, fatty acid composition, vitamin E levels (known to affect shelf life), β -carotene concentration (affects fat colour), pH and water-holding capacity.

12.5 Future trends

12.5.1 The use of breeding programmes to improve the consistency/uniformity of MQ

Although management, and especially processing methods, have major effects on MQ, the production of livestock with reduced genetic variation or increased genetic robustness may help to reduce inconsistencies in MQ. Reducing MQ variation through breeding has not been examined thoroughly, except to exclude deleterious single genes from populations (e.g. halothane, Callipyge). This is largely because there are few good MQ predictors and little financial incentive. There is also a difference between traits that have an intermediate optimum and those where continuous improvement is the goal. Sufficient genetic variation must remain in MQ traits within populations to enable producers to cater to variable market needs. However, there may be opportunities for larger breeding companies to select for reduced variation, within differentiated lines for specific markets (ideally through rapid molecular genetic selection), whilst maintaining genetic variation across the population as a whole. Furthermore, there may be a genetic component of the environmental residual variation among animals. San Christobal-Gauly *et al.* (1998) proposed a method of canalised selection, expected to result in a reduction in residual variation of MQ traits without reducing the genetic variation.

12.5.2 Application of advanced genomic methodology

Further advances in genomics, such as identification of further QTL for MQ traits, fine mapping of existing QTL and identification of single nucleotide polymorphisms markers (SNPs) for the chromosomal regions of interest for MQ, will greatly enhance the chances for successful selection of animals with improved MQ at a young age using a small DNA sample.

Genome-wide selection is potentially one of the most exciting new techniques for selection of animals, especially for traits that are difficult to measure, such as MQ (Meuwissen *et al.*, 2001). This form of marker-based selection uses SNPs spaced at frequent intervals along the genome to genotype animals for all markers at once. The effects on the trait of interest of the interval between each neighbouring pair of markers are estimated for all intervals simultaneously. The effects of each haplotype (linked alleles that are inherited as a unit from one parent) for every interval in the genome can be estimated. By summing these effects for the genotype of an animal, 'genomic' estimated breeding values can be calculated, which have estimated accuracies of around 80%, regardless of the size of the heritability of the trait (Schaeffer, 2006). To use such a strategy in a breeding programme, the animal effects in a genetic evaluation model would be replaced by random haplotype interval effects, and no relationship matrix would be required (Schaeffer, 2006). Compared to progeny testing, genetic change could be several times higher using genome-wide selection and the relative costs much lower. Benefits could be further increased if selection intensities or accuracies of genomic EBVs could be increased, or genotyping costs reduced.

12.6 Sources of further information and advice

A full discussion on the design of breeding programmes for genetic improvement of cattle and sheep is given by Simm (1998), including chapters on general areas such as response to selection and predicting breeding values, as well as chapters on species-specific breeding strategies. Chapters on the genetics of MQ in sheep, pigs and cattle were published between 1997 and 1999 by CAB International and provide valuable summaries of the genetic control of MQ in each species (Thompson and Ball, 1997; Sellier, 1998; Marshall, 1999). More recently, chapters discussing the use of carcass and MQ in breeding programmes, either using traditional breeding methods or incorporating molecular technologies were included in the *Encyclopedia of Meat Science* (Lambe and Simm, 2004; Navajas and Simm, 2004).

The majority of pig breeding programmes in different countries are run by private breeding companies. However, for beef cattle and sheep, national genetic evaluations are undertaken in several countries, the details of which (including breeding goal traits) can be found on the websites of the companies involved. For example, in the UK, evaluations for MLC's 'Sheepbreeder' and 'Beefbreeder' schemes (<http://www.signetfbc.co.uk/>) are run by EGENES (Edinburgh GENetic Evaluation Services, <http://www.sac.ac.uk/research/sls/about/services/edinburghgeneticevaluations/>), in association with Beef and Sheep Company (BASCO), to provide a service to UK breeders. Similar services are offered in other countries for these species, for example, Breedplan in Australia for beef cattle (<http://breedplan.une.edu.au/bplan.htm>) and Sheep Improvement Limited in New Zealand for sheep (<http://www.sil.co.nz/>). The inclusion of MQ traits into these national breeding programmes is gaining increasing interest.

12.7 References

- Andres S, Murray I, Navajas E A, Fisher A V, Lambe N R and Bünger L (2007), 'Prediction of sensory characteristics of lamb meat samples by near infrared reflectance spectroscopy', *Meat Science*, 76 (3), 509–516.
- Anthony N B (1998), 'A review of genetic practices in poultry: Efforts to improve meat quality', *Journal of Muscle Foods*, 9 (1), 25–33.
- Bihan-Duval E, Berri C, Baeza E, Sante V, Astruc T, Remignon H, Le Pottier G, Bentley J, Beaumont C and Fernandez X (2003), 'Genetic parameters of meat technological quality traits in a grand-parental commercial line of turkey', *Genetics Selection Evolution*, 35 (6), 623–635.
- Bray R W (1966), 'Pork Quality—Definition, Characteristics and Significance', *Journal of Animal Science*, 25 (3), 839–842.
- Bünger L and Hill W G (2005), 'Genetics of body composition and metabolic rate', in Eisen E J, *The Mouse in Animal Genetics and Breeding Research*, London, Imperial College Press, 131–160.
- Burrow H M, Moore S S, Johnston D J, Barendse W and Bindon B M (2001), 'Quantitative and molecular genetic influences on properties of beef: A review', *Australian Journal of Experimental Agriculture*, 41, 893–919.
- Cartwright T C (1970), 'Selection criteria for beef cattle for the future', *Journal of Animal Science*, 30, 706–711.

- Chandraratne M R, Samarasinghe S, Kulasiri D and Bickerstaffe R (2006), 'Prediction of lamb tenderness using image surface texture features', *Journal of Food Engineering*, 77 (3), 492–499.
- Cockett N E, Jackson S P, Snowden G D, Shay T L, Berghmans S, Beever J E, Carpenter C and Georges M (1999), 'The Callipyge Phenomenon: Evidence for Unusual Genetic Inheritance', *Journal of Animal Science*, 77 (Supplement 2), 221–227.
- Dekkers J C M and Hospital F (2002), 'The use of molecular genetics in the improvement of agricultural populations', *Nature Reviews Genetics*, 3 (1), 22–32.
- Duthie C, Simm G, Doeschl-Wilson A, Kalm E, Knap P W and Roehe R (2007), 'Novel quantitative trait loci for chemical body composition traits in pigs', *Proceedings of the British Society of Animal Science*, Southport, April 2007, 3.
- Falconer D S and Mackay T F C (1996), *Introduction to Quantitative Genetics*, Fourth edn, Harlow, Longman.
- Ferguson D M (2004), 'Objective on-line assessment of marbling: A brief review', *Australian Journal of Experimental Agriculture*, 44 (7), 681–685.
- Hassen A, Wilson D E, Amin V R, Rouse G H and Hays C L (2001), 'Predicting percentage of intramuscular fat using two types of real-time ultrasound equipment', *Journal of Animal Science*, 79 (1), 11–18.
- Hermesch S, Luxford B G and Graser H U (2000), 'Genetic parameters for lean meat yield, meat quality, reproduction and feed efficiency traits for Australian pigs. 3. Genetic parameters for reproduction traits and genetic correlations with production, carcass and meat quality traits', *Livestock Production Science*, 65 (3), 261–270.
- Heuven H C M, van Wijk H J and van Arendonk J A M (2003), 'Combining traditional breeding and genomics to improve pork quality', *Outlook on Agriculture*, 32 (4), 235–239.
- Hocquette J F, Lehnert S, Barendse W, Cassar-Malek I and Picard B (2007), 'Recent advances in cattle functional genomics and their application to beef quality', *Animal*, 1 (1), 159–173.
- Honikel K O (1991), 'Assessment of Meat Quality', in Fiems L O, Cottyn B G and Demeyer D I, *Animal Biotechnology and the Quality of Meat Production*, Amsterdam, Oxford, New York, Tokyo, Elsevier, 107–125.
- Hopkins D L, Hegarty R S A and Farrell T C (2005), 'Relationship between sire estimated breeding values and the meat and eating quality of meat from their progeny grown on two planes of nutrition', *Australian Journal of Experimental Agriculture*, 45, 525–533.
- Hovenier R, Brascamp E W, Kanis E, van der Werf J H J and Wassenberg A P A M (1993), 'Economic values of optimum traits: The example of meat quality in pigs', *Journal of Animal Science*, 71, 1429–1433.
- Hoving-Bolink A H, Vedder H W, Merks J W M, de Klein W J H, Reimert H G M, Frankhuizen R, van den Broek W H A M and Lambooij E E (2005), 'Perspective of NIRS measurements early post mortem for prediction of pork quality', *Meat Science*, 69 (3), 417–423.
- Izquierdo M M, Amin V R, Wilson D E and Rouse G H (1994), 'Models to predict intramuscular fat percentage in live beef animals using realtime ultrasound and image paramaters: Report on data from 1991–1994', *Unpublished*.
- Jannink J L (2007), 'Identifying quantitative trait locus by genetic background interactions in association studies', *Genetics*, 176 (1), 553–561.
- Jeon J T, Lee J H, Kim K S, Park C K and Oh S J (2006), 'Application of DNA markers in animal industries', *Australian Journal of Experimental Agriculture*, 46 (2), 173–182.
- Johnson P L, Purchas R W, McEwan J C and Blair H T (2005), 'Carcass composition and meat quality differences between pasture-reared ewe and ram lambs', *Meat Science*, 71 (2), 383–391.
- Jones H E, Lewis R M, Young M J and Simm G (2002), 'Incorporating CT measures of composition and muscularity into selection programs for Suffolk sheep', *Proceedings of the 7th World Congress of Genetics Applied to Livestock Production*, Montpellier, France, 29, 461–464.

- Jopson N B, Amer P and McEwan J C (2004), 'Comparison of two-stage selection breeding programmes for terminal sire sheep', *Proceedings of the NZ Society of Animal Production*, 64, 212–216.
- Jopson N B, Kolstad K, Sehested E and Vangen O (1995a), 'Computed tomography as an accurate and cost effective alternative to carcass dissection', *Proceedings of the Australian Association of Animal Breeding and Genetics*, 11.
- Jopson N B, McEwan J C, Dodds K G and Young M J (1995b), 'Economic benefits of including computed tomography measurements in sheep breeding programmes', *Proceedings of the Association for the Advancement of Animal Breeding and Genetics*, 11, 194–197.
- Jopson N B, Nicoll G B, Stevenson-Barry J M, Duncan S, Greer G J, Bain W E, Gerard E M, Glass B C, Broad T E and McEwan J C (2001), 'Mode of inheritance and effects on meat quality of the rib-eye muscling (REM) QTL in Sheep', *Proceedings of the Association for the Advancement of Animal Breeding and Genetics*, 14, 111–114.
- Kadarmideen H N, Schworer D, Ilahi H, Malek M and Hofer A (2004), 'Genetics of osteochondral disease and its relationship with meat quality and quantity, growth, and feed conversion traits in pigs', *Journal of Animal Science*, 82 (11), 3118–3127.
- Kadel M J, Johnston D J, Burrow H M, Graser H U and Ferguson D M (2006), 'Genetics of flight time and other measures of temperament and their value as selection criteria for improving meat quality traits in tropically adapted breeds of beef cattle', *Australian Journal of Agricultural Research*, 57 (9), 1029–1035.
- Karamichou E, Richardson R I, Nute G R, McLean K A and Bishop S C (2006), 'Genetic analyses of carcass composition, as assessed by X-ray computer tomography, and meat quality traits in Scottish Blackface sheep', *Animal Science*, 82 (2), 151–162.
- King D A, Pfeiffer C E S, Randel R D, Welsh T H, Oliphint R A, Baird B E, Curley K O, Vann R C, Hale D S and Savell J W (2006), 'Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle', *Meat Science*, 74 (3), 546–556.
- Klont R E, Brocks L and Eikelenboom G (1998), 'Muscle fibre type and meat quality', *Meat Science*, 49 (Supplement 1), S219–S229.
- Koohmaraie M, Shackelford S D and Wheeler T L (2005), 'Biological bases that determine beef tenderness', *The Science of Beef Quality. 8th Annual Langford Food Industry Conference. Proceedings of the British Society of Animal Science*, 13–19.
- Kroger C, Bartle C M, West J G, Purchas R W and Devine C E (2006), 'Meat tenderness evaluation using dual energy X-ray absorptiometry (DEXA)', *Computers and Electronics in Agriculture*, 54 (2), 93–100.
- Lambe N R and Simm G (2004), 'Animal breeding and genetics: Traditional animal breeding', in Jensen W K, Devine C and Dikeman M, *Encyclopaedia of Meat Science*, Oxford, Elsevier, 11–19.
- Liu Y, Lyon B G, Windham W R, Realini C E, Pringle T D and Duckett S (2003), 'Prediction of color, texture, and sensory characteristics of beef steaks by visible and near infrared reflectance spectroscopy. A feasibility study', *Meat Science*, 65 (3), 1107–1115.
- Maher S C, Mullen A M, Moloney A P, Buckley D J and Kerry J P (2004), 'Quantifying the extent of variation in the eating quality traits of the *M. longissimus dorsi* and *M. semimembranosus* of conventionally processed Irish beef', *Meat Science*, 66 (2), 351–360.
- Marshall D M (1999), 'Genetics of meat quality', in Fries R and Ruvinsky A, *The Genetics of Cattle*, Wallingford, CAB International, 605–636.
- Meuwissen T H, Hayes B J and Goddard M E (2001), 'Prediction of total genetic value using genome-wide dense marker maps', *Genetics*, 157, 1819–1829.
- Mohrmann M, Roehre R, Knap P W, Looft H, Plastow G S and Kalm E (2006), 'Quantitative trait loci associated with AutoFOM grading characteristics, carcass cuts and chemical body composition during growth of *Sus scrofa*', *Animal Genetics*, 37, 435–443.
- Montagutelli X A V I (2000), 'Effect of the Genetic Background on the Phenotype of Mouse

- Mutations', *Journal Of The American Society Of Nephrology*, 11 (90002), S101–S105.
- Navajas E A, Lambe N R, Bünger L, Glasbey C A, Fisher A V, Wood J D and Simm G (2006), 'Genetics of carcass shape and eating quality in sheep', *Proceedings of the 9th Langford Food Industry Conference: New Developments in Sheepmeat Quality*, University of Bristol, UK.
- Navajas E A, Lambe N R, Fisher A V, Nute G R, Bünger L and Simm G (2007), 'Muscularity and eating quality of lambs: effects of breed, sex, and selection of sires using muscularity measurements by CT', *Meat Science*, 79, 105–112.
- Navajas E A and Simm G (2004), 'Animal breeding and genetics: Molecular genetic markers and marker assisted selection', in Jensen W K, Devine C and Dikeman M, *Encyclopedia of Meat Science*, Oxford, Elsevier, 11–19.
- Newcom D W, Baas T J and Lampe J F (2002), 'Prediction of intramuscular fat percentage in live swine using real-time ultrasound', *Journal of Animal Science*, 80 (12), 3046–3052.
- Newcom D W, Stalder K J, Baas T J, Goodwin R N, Parrish F C and Wiegand B R (2004), 'Breed differences and genetic parameters of myoglobin concentration in porcine longissimus muscle', *Journal of Animal Science*, 82 (8), 2264–2268.
- Otto G, Knap P W, Roehe R, Looft H, Caverio D and Kalm E (2007), 'Different approaches of estimating economical values for drip loss as lognormally distributed trait', *Livestock Science*, 112, 43–5.
- Picard B, Lefaucheur L, Berri C and Duclos M J (2002), 'Muscle fibre ontogenesis in farm animal species', *Reproduction Nutrition Development*, 42, 415–431.
- Rehfeldt C, Fiedler I and Stickland N C (2004), 'Number and Size of Muscle Fibres in Relation to Meat Production', in Te Pas M F W, Everts M E and Haagsman H P, *Muscle Development of Livestock Animals: Physiology, Genetics, and Meat Quality*, Wallingford, Oxfordshire, UK, CABI Publishing, 1–38.
- Reverter A, Johnston D J, Ferguson D M, Perry D, Goddard M E, Burrow H M, Oddy V H, Thompson J M and Bindon B M (2003), 'Genetic and phenotypic characterisation of animal, carcass, and meat quality traits from temperate and tropically adapted beef breeds. 4. Correlations among animal, carcass, and meat quality traits', *Australian Journal of Agricultural Research*, 54 (2), 149–158.
- Rhee M S, Wheeler T L, Shackelford S D and Koohmaraie M (2004), 'Variation in palatability and biochemical traits within and among eleven beef muscles', *Journal of Animal Science*, 82 (2), 534–550.
- Rothschild M F, Bidanel J P and Ciobanu D C (2004), 'Genome Analysis of QTL for Muscle Tissue Development and Meat Quality', in te Pas M F, Everts M E and Haagsman H P, *Muscle Development of Livestock Animals: Physiology, Genetics and Meat Quality*, Cambridge, CABI Publishing, 247–266.
- Russell B C, McAlister G, Ross I S and Pethick D W (2005), 'Lamb and sheep meat eating quality – Industry and scientific issues and the need for integrated research', *Australian Journal of Experimental Agriculture*, 45 (5), 465–467.
- SanCristobal-Gaudy M, Elsen J M, Bodin L and Chevalier C (1998), 'Prediction of the response to a selection for canalisation of a continuous trait in animal breeding', *Genetic Selection Evolution*, 30, 423–451.
- Schaeffer L R (2006), 'Strategy for applying genome-wide selection in dairy cattle', *Journal of Animal Breeding and Genetics*, 123 (4), 218–223.
- Sellier P (1998), 'Genetics of Meat and Carcass Traits', in Rothschild M F and Ruvinsky A J, *The Genetics of the Pig*, Wallingford, Oxfordshire, UK, CAB International, 463–510.
- Serenius T, Sevon-Aimonen M L, Kauser A, Mantysaari E A and Maki-Tanila A (2004), 'Genetic associations of prolificacy with performance, carcass, meat quality, and leg conformation traits in the Finnish Landrace and Large White pig populations', *Journal of Animal Science*, 82 (8), 2301–2306.
- Shackelford S D, Leymaster K A, Wheeler T L and Koohmaraie M (2003), 'Lamb Meat Quality Progress Report Number 1. Preliminary Results of an Evaluation of Effects of

- Breed of Sire on Carcass Composition and Sensory Traits of Lamb', *U.S. Meat Animal Research Center : Sheep Publication Reprints*.
- Shackelford S D, Leymaster K A, Wheeler T L and Koohmaraie M (2005), 'Lamb Meat Quality Progress Report Number 2. Preliminary Results of an Evaluation of Effects of Breed of Sire on Carcass Composition and Sensory Traits of Lamb', *U.S. Meat Animal Research Center : Sheep Publication Reprints*.
- Shackelford S D, Wheeler T L and Koohmaraie M (1995), 'Relationship Between Shear Force and Trained Sensory Panel Tenderness Ratings of 10 Major Muscles from Bos-Indicus and Bos-Taurus Cattle', *Journal of Animal Science*, 73 (11), 3333–3340.
- Simm G (1998), *Genetic Improvement of Cattle and Sheep*, Tonbridge, Farming Press.
- Simm G, Bünger L, Navajas E A, Lambe N R and Villanueva B (2006), 'Including meat quality in sheep breeding programmes', *Proceedings of the Langford Conference*, University of Bristol, May 2006.
- Simm G, Bünger L, Villanueva B and Hill W G (2005), 'Limits to yield of farm species: Genetic improvement of livestock', in Sylvester-Bradley R and Wiseman J, *Yields of Farmed Species: Constraints and Opportunities in the 21st Century*, Nottingham, Nottingham University Press, 123–141.
- Suzuki K, Irie M, Kadowaki H, Shibata T, Kumagai M and Nishida A (2005a), 'Genetic parameter estimates of meat quality traits in Duroc pigs selected for average daily gain, longissimus muscle area, backfat thickness, and intramuscular fat content', *Journal of Animal Science*, 83 (9), 2058–2065.
- Suzuki K, Kadowaki H, Shibata T, Uchida H and Nishida A (2005b), 'Selection for daily gain, loin-eye area, backfat thickness and intramuscular fat based on desired gains over seven generations of Duroc pigs', *Livestock Production Science*, 97 (2–3), 193–202.
- Thompson J M and Ball A J (1997), 'Genetics of Meat Quality', in Piper L and Ruvinsky A, *The Genetics of Sheep*, Wallingford, Oxfordshire, UK, CAB International, 523–538.
- Tong A K W, Jones S D M and Schaefer A L (1997), *Method for detecting poor meat quality in groups of live animals*, United States Patent 5595444.
- Tygesen M P, Therkildsen M and Harrison A P (2005), 'Non-invasive measurement of muscle development in lambs postnatally – implications for meat quality', *Archiv für Tierzucht – Archives of Animal Breeding*, 48 (Special Issue), 56–62.
- Varga L, Müller G, Szabo G, Pinke O, Korom E, Kovacs B, Patthy L and Soller M (2003), 'Mapping modifiers affecting muscularity of the myostatin mutant (*Mstn^{Cmpt-dl1Abc}*) Compact mouse', *Genetics*, 165, 257–267.
- Von Rohr P, Hofer A and Kunzi N (1999), 'Economic values for meat quality traits in pigs', *Journal of Animal Science*, 77, 2633–2640.
- Wiener P, Smith J A, Lewis A M, Woolliams J A and Williams J L (2002), 'Muscle-related traits in cattle: The role of the myostatin gene in the South Devon breed', *Genetics Selection Evolution*, 34, 221–232.
- Williams A R (2002), 'Ultrasound applications in beef cattle carcass research and management', *Journal of Animal Science*, 80 (E. Suppl. 2), E183–E188.
- Wood J D and MacFie H J H (1980), 'The significance of breed in the prediction of lamb carcass composition from fat thickness measurements', *Animal Production*, 31, 315–319.
- Wood J D, MacFie H J H, Pomeroy R W and Twinn D J (1980), 'Carcass composition in four sheep breeds: The importance of type of breed and stage of maturity', *Animal Production*, 30, 135–152.

Genetic-based diagnostic tools for predicting meat quality

W. Barendse, CSIRO Livestock Industries, Australia

Abstract: This chapter reviews the current state of genetic-based diagnostic tools for predicting meat quality in livestock species. The need for better tools for predicting the genetic basis of meat quality is explored. The methods for developing genetic-based diagnostic tests for predicting meat quality are discussed. Then the current status of all genetic linkage and association studies into meat quality in livestock are listed, with a discussion of which ones are confirmed, and a listing of likely genes in all species is given. Finally, the future directions that genetic improvement might take are explored.

Key words: QTL, cattle, pig, sheep, chicken, goat, duck, aquaculture.

13.1 Introduction: the need for better methods to predict meat quality

In this review, the genetic improvement of the tastes and textures of meat will be considered. Meat quality is a broad category that includes the safety, the tastes and textures, and the effect on health of meat. The aspects of the control of hazards associated with meat safety are usually those associated with the control of specific pathogens or zoonotic agents, be they viral, bacterial, or parasitic, and are largely outside the scope of this chapter, since they are largely affected by the controls governments or the industry put into place for specific pathogens through microbiological and parasitological monitoring and control. Genetic control of host resistance to pathogens is unlikely to affect meat safety in the short term because: some pathogens can be infective at very low levels so host resistance would need to be complete; the infection can occur post-mortem; the meat will be inspected to determine whether it is fit for human consumption, irrespective of the perceived

Note: For the full text, the publisher owns the copyright on assignment from CSIRO on the condition that the publisher grants CSIRO an irrevocable, non-exclusive, royalty-free licence of copyright for CSIRO's internal purposes, further research and public speeches, and for research data including illustrations and tables, CSIRO grants the publisher a non-exclusive, royalty-free licence of copyright for the nominated publication and CSIRO and any relevant third parties retain ownership of all copyright to those research data.

genetic resistance of the animals. The effects on the health benefits of the products would need to consider the genetic variation not only within the meat species but also the genetic variation within humans, and that is also largely outside the scope of this chapter.

Meat quality varies for different species and each trait differs in the amount of genetic variation available for selection. Variation in meat quality, *viz.* textures, tastes and composition, has both a genetic as well as an environmental component operating during the development, growth, and slaughter of animals and the storage, packaging and preparation of meat. The amount of genetic variation varies substantially between traits. Table 13.1 shows the range of additive genetic variation for these traits – the narrow sense heritability, which represents what breeders can use to improve a population. It does not represent the full genetic contribution to these traits.

Nevertheless, it is also clear that what is meant by meat quality is different between species. For example, tenderness is of importance in some species, such as cattle, but in poultry, the main meat quality traits are colour and ultimate pH. Given the heritability measured in some populations of a species for some of these traits, this suggests that genetic approaches would be of value for some but not all meat quality traits in those populations, but in other species, improvement would be largely due to control of the management and processing of the trait. Occasionally, a trait with low heritability can be broken down into subordinate traits, each of which might be of higher heritability (e.g. fertility versus the components of fertility such as age at first corpus luteum). Or, a trait with low heritability might be affected by a rare allele with a large effect that could be analysed through molecular genetic methods. But in general, the lower the heritability the more difficult it will be to improve the trait through selective breeding. Care needs to be taken in interpreting low heritabilities to mean that there is no or little genetic variation for a trait. The heritability is simply the ratio of the additive genetic variation to the total variation. Importantly, a heritability applies to a particular population and environment, and does not apply generally to all populations of a species. So one population may have little additive genetic variation but another may have much more, depending on the ancestry. Or, the environmental or non-additive genetic variation may be relatively large in some cases, lowering the heritability, but where the environmental variability is more controlled, the heritability for the same sample of animals would be higher.

Table 13.1 does not contain any carcass quality traits, such as overall fat percentage or retail meat yield, or carcass weight: these may have an indirect effect on meat quality (such as where increased growth brings younger animals to market, with consequent improvements in meat quality) but here we will restrict ourselves to meat quality in the strict sense. For some livestock species, such as the goat, although there are heritabilities published and summarised (Shrestha and Fahmy, 2007), there are none for meat quality. Of course, fish species also show differences in carcass and flesh quality and heritabilities and these have been collected in a review of aquaculture heritabilities (Gjedrum, 2000).

In spite of the high heritability for some meat quality traits, improving meat

Table 13.1 Some heritabilities for meat quality traits in livestock and aquacultural species

Species	Type	Country	Trait	Heritability	Reference
<i>Bos taurus</i>	Taurine	USA	Marbling	0.57 ± 0.13	(Wheeler <i>et al.</i> , 2001a)
			LT SF ¹	0.22 ± 0.12	
			Juiciness	0.09 ± 0.11	
			Flavour	0.07 ± 0.11	
			IMF	0.55 ± 0.14	
	Taurine	Australia	LT SF	0.11 ± 0.06 ²	(Johnston <i>et al.</i> , 2003)
			Juiciness	0.15 ± 0.06	
			Flavour	0.05 ± 0.06	
			MQ4	0.13 ± 0.06	
	Zebu ³	Australia	LT SF	0.31 ± 0.09	
			Juiciness	0.20 ± 0.08 ⁴	
			Flavour	0.23 ± 0.08	
	Taurine	Australia	IMF	0.38 ± 0.04 ⁵	(Reverter <i>et al.</i> , 2003)
			IMF	0.39 ± 0.03 ⁵	
<i>Gallus gallus</i>	Broiler	France	Ultimate pH	0.49 ± 0.11	(Le Bihan-Duval <i>et al.</i> , 1999)
			Lightness	0.75 ± 0.08	
			redness	0.81 ± 0.04	
			yellowness	0.64 ± 0.06	
			IMF	0.08 ± 0.04	
<i>Ovis aries</i>	Merino	Australia	Meat pH	0.27 ± 0.09	(Fogarty <i>et al.</i> , 2003)
			Lightness	0.14 ± 0.07	
			redness	0.02 ± 0.06	
			yellowness	0.04 ± 0.06	
	Composite	France	IMF	0.22	(Moreno <i>et al.</i> , 2001)
<i>Sus scrofa</i>	Large White/Landrace	Australia	Meat pH	0.14 ± 0.04	(Hermesch <i>et al.</i> , 2000)
			Lightness	0.29 ± 0.06	
			Drip Loss	0.23 ± 0.05	
			IMF	0.35 ± 0.06	
	Duroc/Landrace	USA	Meat pH	0.14 ± 0.08	(Lo <i>et al.</i> , 1991)
			IMF	0.52 ± 0.13	
			Cooking loss	0.06 ± 0.06	
			Tenderness ⁶	0.17 ± 0.08	
			Off flavour	0.03 ± 0.06	
			Consumer acceptance	0.34 ± 0.11	

¹ Longissimus thoracis shear force; ² SE of heritability given as a range of 0.04–0.08 for the table see original reference; ³ Mixture of purebred zebu (e.g. Brahman) and breeds with some zebu ancestry; ⁴ SE of heritability given as a range of 0.07–0.09 for the table see original reference; ⁵ SE personal communication A. Reverter; ⁶ this is the objective measure of tenderness, for taste panel tenderness, $h^2 = 0.45 \pm 0.12$.

quality will always be difficult using conventional means. Firstly, traits affecting meat quality have important limitations (Thompson, 2002; Pethick *et al.*, 2004), such as that they may be measured in castrated animals but are bred using males

that cannot be measured, since measurement would involve killing the animal. Often the performance of the castrated animal is quite different to the uncastrated one, which means that the phenotype needs to be adjusted in some way. An example in cattle is that bulls show only trace amounts of intramuscular fat (Savell *et al.*, 1986), so measurements based on intramuscular fat in bulls would not be expected to be closely related to intramuscular fat in their steer offspring. In many species, the fastest genetic progress is made where elite sires are developed in a top tier of the industry, among the seedstock breeders (Robertson and Rendel, 1950), and, if the most promising animals are castrated before their performance can be measured, then this presents a significant impediment to genetic improvement. Secondly, the measurements may be time consuming or expensive, such as those for tenderness, intramuscular fat or retail beef yield; or they may be subjective, such as marbling. In some cases, regression equations linking observable attributes of the animals to the trait have been formulated, such as those for retail beef yield or tenderness. But in many cases the technology is either insufficiently accurate (such as retail beef yield using subcutaneous fat thickness), or the technology is expensive and has not been implemented widely (such as Viascan®, which is a video image analysis system using calibrated measurements of carcass dimensions and colour across the carcass to predict retail meat yield) (Hopkins *et al.*, 2004). Thirdly, many meat quality traits are negatively correlated to other important traits of the animal – for example, intramuscular fat and retail meat yield, which increases the difficulty of finding animals that are superior for both traits. These reasons show that there are limits to the extent to which conventional selection can be applied in populations and that significant improvement may come from the application of molecular genetic methods to the improvement of meat quality.

Molecular approaches will have varying success in a species, and apart from the traits that are considered to constitute meat quality, will depend upon the genetic resources available and the ease with which large databases can be accumulated for that species. At present the most important resources are: databases of DNA variants organized into maps, these maps consisting preferably of a genome sequence; repositories of DNA clones, such as BAC libraries; databases of large numbers of animals with many accurate trait measurements; and tissue samples from animals at a wide variety of developmental stages (Barendse, 2005). For some traits, more specific resources will be required, and if there are particular modes of inheritance for the traits, such as DNA imprinting, then the samples will need to be adequate to demonstrate the additional complexity.

13.2 Developing genetic-based diagnostic tests for predicting meat quality

Developing genetic-based diagnostic tests for predicting meat quality follows a well-understood pathway that applies to all traits. These steps are (i) determine whether there is a genetic contribution to the trait, (ii) map the genes affecting the

trait to a linkage map, (iii) identify the genetic subregion of the map that contains mutations affecting the trait, (iv) identify diagnostic and/or causative polymorphisms from within the subregion that can be used in any population. These steps form a hierarchy of increasing genetic resolution.

The first step is to determine whether there is sufficient genetic variability for the trait in the target population. Part of this first step is to determine whether there are any genes that can be identified using a segregation analysis, which would mean that there was a mutation of sufficiently large effect that its presence could be identified just by scoring the phenotype. Examples of this for meat quality would be mutations such as the double muscling phenotype in cattle (Hanset *et al.*, 1982) or pale soft exudative meat (O'Brien, 1987) in pigs. The heritability, or alternatively, the total genetic variation, should be estimated; the larger the genetic variability, the smaller the sample required to find genes associated with the trait.

The next step is to locate these genes, whether quantitative trait loci (QTL) or major genes, to linkage maps. In the initial phase this means assembling pedigrees that segregate the phenotype of the major gene or variable pedigrees for the trait of interest, to map QTL. These pedigrees, preferably spanning three generations, are then measured for a range of traits and genotyped, usually for a few hundred mapped microsatellite polymorphisms that cover as much of the genome as possible. Multi-locus interval mapping methods are generally used, implemented via a range of software, to locate genes affecting the trait (Lander and Botstein, 1989; Haley and Knott, 1992; Basten *et al.*, 1994; Georges *et al.*, 1995; Seaton *et al.*, 2002). Studies are often compared to determine whether there is sufficient evidence to continue to the next phase, that of identifying the genes affecting the trait. More recently, the prospect of high densities of SNP across a genome has meant the possibility of greater progress in identifying genes affecting complex traits (Collins, 1995; Ozaki *et al.*, 2002; Valdar *et al.*, 2006; Barendse *et al.*, 2007c), but high densities of SNP come with the attendant problem of a much higher false discovery rate (Kruglyak, 1999; Carlson *et al.*, 2004).

Once the genes are located to chromosomes, the next phase is to identify the subregion of the chromosome that affects the trait and to begin the analysis of positional candidate genes. This is a fairly labour-intensive process, walking toward a mutation, but the most efficient way is to use positional candidate genes; that is, candidate genes that are located to the interval in which the QTL or genetic effects have been located (Collins, 1995). With major genes, the effect of a particular mutation can be tested using non-genetic methods, because the effects are usually large, but for QTL, particularly QTL with effects less than 0.5 phenotypic standard deviations, the effects are generally too small to be confirmed by non-genetic methods. In addition, for major genes, the phenotype is usually sufficiently discrete that carriers of the mutation can be unambiguously identified, but for QTL, the confidence intervals are large (Visscher *et al.*, 1996) and QTL are abundant, and so it is more difficult to be sure that a particular SNP is responsible for the QTL, or that an individual's extreme trait value is due to any particular DNA variant.

Finding the causative mutations for QTL is difficult, and the next phase need

not proceed all the way to causative mutations, merely to diagnostic tests. Polymorphisms near to causative alleles will give similar levels of predictability, particularly where LD exceeds an r^2 of 0.8 – in that case the genotypes are so highly correlated that the genotypes at one polymorphism essentially track the genotypes at another polymorphism (Edwards, 1980; Chapman *et al.*, 2003; Terwilliger and Hiekkalinna, 2006). Although linkage disequilibrium makes it difficult to prove that a particular polymorphism is causative, it does mean that the causative mutation does not need to be found before a diagnostic test can be used. Greater certainty can be achieved by using haplotypes of SNP that bracket the QTL, because recombination may occur between the SNP and QTL, but in a haplotype of close markers, recombination can be detected. Furthermore, the haplotypes may explain more variation, partly due to the detection of recombination but also because they may report on several close QTL in the same region whose effects may be additive.

To be usable, a diagnostic test merely has to show that a particular allele has positive or neutral effects in all breeds or studies, although this does not mean it will be commercialised. However, if the effects are negative in some populations and positive in others, that is, in some populations there is a statistically significant effect but the ‘wrong’ genotype(s) are positively associated with the trait, then the marker is too far away from the causative allele and could be used only in a breed- or herd-specific way.

Every effort should be made to generate a useful set of statistics for these studies as it is in the long-term interest of the discipline to do so. The reporting of the results of these studies is not uniform, and often, important statistics are omitted that are required to use these diagnostic tests. Some methods of analysis do not allow the explicit estimation of means and standard errors for each genotype, but the following should be reported as a minimum:

- (i) The actual sample size of animals with joint genotypes and phenotypes, not the number of animals used in total with missing data.
- (ii) The additive and dominance effects and their standard errors, expressed as a proportion of the phenotypic standard deviation.
- (iii) The allele frequency found in the population, not merely that found in the limited number of ancestors in a particular experimental cross.
- (iv) The allele that increases the trait value.
- (v) The percentage of the variance explained by the polymorphism.

Placing them all into one table would be a huge convenience. One should note that the genotypic standard errors of the mean are not a substitute for the standard errors of the additive and dominance effects, which are the more important standard errors, and the standard errors of the additive and dominance effects can be estimated from the error variance or preferably directly by regression. For studies that validate or confirm a previously reported association, it is important to estimate the size of effect that would be significant given the size of the sample in the validation experiment. Then, if the study fails to discover an association, a correct interpretation of the failure is more likely – it is easy for a QTL validation

to fail, just use a sample size that is too small or use an inappropriate breed. Failure to report a full range of statistics is not only the fault of researchers; occasionally journals will request smaller tables in the interests of saving space, and researchers have to prioritise which statistics to report.

Many of the early tests are being evaluated for their impacts on other traits, even when there is no expectation that they should be associated. This is a good process, but failure to find an association to a new trait does not mean that the original association is suspect, although that assumption has occasionally been made.

13.3 Current status of development and future potential

At present, but only for the very near future, there are few DNA tests for meat quality traits in a small number of livestock species. Meat quality is actually one of the few areas that does have diagnostic tests in livestock; there are even fewer commercialised diagnostic tests available for other traits of cattle, to use one example (Hocquette *et al.*, 2007). More of these meat quality diagnostic tests are being reported, and the onset of genome wide association studies will result in large numbers of potential tests for meat quality traits (Barendse *et al.*, 2007c).

The lists of QTL studies reported here are strictly those in which a meat quality emphasis can be identified, even if it happened that the largest number of traits in the paper were carcass rather than meat quality. There are as many or even more studies, depending upon the species, on carcass quality that do not have an aspect of meat quality in them.

These studies can be divided not only by species but by stage of development. There are those studies in which the major gene or QTL have been located to a chromosome, then the exploration of possible candidate genes or diagnostic markers for those QTL, the evaluation of the candidate genes or potential diagnostic markers in a range of studies, and finally the evaluation of the diagnostic markers for effects on other traits. The studies below are organised by species and stage of development.

13.3.1 Cattle

In cattle, most of the studies into genes for meat quality have been for the traits of marbling or intra-muscular fat and meat tenderness. For marbling, only one major gene has been described, for double muscling (Hanset *et al.*, 1982), but its effects are negative and appear to be a pleiotropic effect of increased muscularity (Table 13.2). The effects of the myostatin gene causing the double muscling phenotype (Grobet *et al.*, 1997) have been shown to increase tenderness, decrease marbling and affect many other production traits (Uytterhaegen *et al.*, 1994; Casas *et al.*, 1998; Wheeler *et al.*, 2001b; Caballero *et al.*, 2007). Apart from DNA variation at myostatin, there are no studies reported for a major gene affecting differences in meat tenderness.

Several studies of carcass and meat quality identified chromosomes associated

Table 13.2 Mutations with major effects on meat quality traits

Species	Gene	Trait	Original trait ¹	Breed restriction	Commercialised
Pig	RYR1	Drip Loss ⁻	Growth	Pietrain, etc.	Yes
	PRKAG3	ultimate pH ⁻	Growth	Hampshire, etc.	?
Cattle	GDF8	Tenderness ⁺ & IMF ⁻	Growth	Belgian Blue, Charolais,	Yes
Sheep	CLPG	Tenderness ⁻ & IMF ⁻	Growth	Poll Dorset, etc.	?

¹ Trait originally selected for and for which the animals were originally noticed as having a superior phenotype.

Table 13.3 Genes with confirmed¹ QTL effects on meat quality

Species	Gene	Traits	Other traits ²	Commercialised
Cattle	TG	marbling	unconfirmed	yes
	Leptin	marbling	yes	yes
	GH1	marbling	yes	yes
	mtDNA	marbling	no ³	no
	CAST	tenderness	unconfirmed	yes
	CAPN1	tenderness	no	yes
Swine	FABP3	IMF	unknown	unknown
	FABP4	IMF	unknown	unknown
	LEPR	IMF	unknown	unknown

¹ More than one study has reported a significant association even if one or more studies have failed to find a significant association; ² Other traits are non-meat quality traits such as growth, or milk yield;

³ No means that none have been reported or even tested so far, that no association to other traits was found.

with marbling and tenderness. Almost all of these are studies consist of families of experimental cattle that were genotyped for DNA microsatellites covering more than 80–90 percent of the genome. These include studies of marbling, tenderness and carcass quality QTL (Barendse, 1997; Fitzsimmons *et al.*, 1998; Keele *et al.*, 1999; Casas *et al.*, 2000, 2001, 2003; Smith *et al.*, 2000; Rexroad *et al.*, 2001; MacNeil and Grosz, 2002; Kim *et al.*, 2003, 2004; Mizoshita *et al.*, 2004; Choi *et al.*, 2006; Drinkwater *et al.*, 2006; Mizoguchi *et al.*, 2006; Alexander *et al.*, 2007). Several of these have been refined further in chromosome specific studies (Imai *et al.*, 2007). They represent a large number of studies but few of these QTL have been turned into diagnostic tests.

Marbling has been shown to be associated with DNA variants at several genes, some of which have been confirmed in independent studies, and some of these variants have been tested against other traits (Table 13.3). The TG5 test for marbling (Barendse, 1997; Barendse *et al.*, 2004) has been tested in other studies (Thaller *et al.*, 2003; Rincker *et al.*, 2006; Wood *et al.*, 2006; Casas *et al.*, 2007;

Shin and Chung, 2007a; Van Eenennaam *et al.*, 2007) and the consensus is that it affects marbling, although it is unlikely to be the causative mutation – the studies in which it has not been found positive have generally been small samples and the frequency is very low in zebu breeds. It has been tested for backfat (Moore *et al.*, 2003) and milk fat (Khatib *et al.*, 2007) but no effect on these traits was found. The effect of leptin SNP (Buchanan *et al.*, 2002) on marbling or intra-muscular fat is also not always found, even in large samples, and alternative DNA variants have been shown to have stronger effects than the original SNP (Nkrumah *et al.*, 2004; Barendse *et al.*, 2005; Schenkel *et al.*, 2005; Di Stasio *et al.*, 2007). Effects on several traits have been reported, including milk production (Buchanan *et al.*, 2003) and overall body fatness. The growth hormone 1 SNP shows effects on several fat traits, with variable effects on growth and fatness (Taylor *et al.*, 1998; Chikuni and Mitsuhashi, 2002; Barendse *et al.*, 2006). It too shows effects on milk yield and composition. Finally, there are mitochondrial effects reported on beef quality (Mannen *et al.*, 1998; Mannen *et al.*, 2003; Jiang *et al.*, 2005).

Other genes have been associated with marbling but have not been confirmed by other researchers so far. SCD affects the level of saturation of fatty acids and its effect on marbling and intramuscular fat has been reviewed recently (Smith *et al.*, 2006). However, only one study so far shows an effect of DNA variation of SCD on intramuscular fat in cattle (Taniguchi *et al.*, 2004). Nevertheless, differences in activity of this gene affects fatness in several species, either through differential gene expression as in pigs (Doran *et al.*, 2006) or through genetic variation as in humans (Warensjo *et al.*, 2007). Effects on marbling of the GHR (Hale *et al.*, 2000), DGAT1, a gene affecting milk fat (Thaller *et al.*, 2003), RORC (Barendse, 2003; Barendse *et al.*, 2007a), FABP4 (Michal *et al.*, 2006), ADIPOQ (Morsci *et al.*, 2006), CPE (Shin and Chung, 2007b), CEBPA (Shin *et al.*, 2007), TCAP (Cheong *et al.*, 2007), have been reported. CRH has been associated with IMF (Wibowo *et al.*, 2007). IGF2 appears to affect IMF and eye muscle area (Goodall and Schmutz, 2007). A large number of genes and genetic regions have been identified for IMF using a genome-wide association study of 9260 single nucleotide polymorphisms (Barendse and Reverter-Gomez, 2007), but these associations will need to be confirmed. Finally, FASN affects the fatty acid composition of fat in beef cattle (Morris *et al.*, 2007), as does SREBP1 (Hoashi *et al.*, 2007), and although this is not a direct effect on marbling, the speed at which marbling appears in the chiller depends on the melting temperature of the fat, which is affected by the fatty acid composition. These associations need to be confirmed as well as evaluated in large samples in several breeds so that the effects can be accurately quantified.

There are several tests for meat tenderness in cattle (Table 13.3) and the two main genes, CAPN1 and CAST (Barendse, 2002; Page *et al.*, 2002) have been confirmed several times, so far without fail (White *et al.*, 2005; Casas *et al.*, 2006; Morris *et al.*, 2006; Schenkel *et al.*, 2006; Costello *et al.*, 2007; Van Eenennaam *et al.*, 2007), with some evidence that DNA variation at these two genes interact in affecting the amount of meat tenderness (Barendse *et al.*, 2007b). There are fewer associations between DNA variants for meat tenderness than tests for marbling,

and so far, DNA variants for only one other gene, CAPN3, have been associated with meat tenderness (Barendse, 2006). This has yet to be confirmed in independent tests. Although these particular DNA variants have not been examined for effects on other traits, the CAST gene has been associated with fertility traits in cattle (Garcia *et al.*, 2006). A large number of genes and genetic regions have been identified for meat tenderness using a genome-wide association study of 9260 single nucleotide polymorphisms (Barendse and Reverter-Gomez, 2007), but these associations need to be confirmed.

13.3.2 Pigs

In pigs, two genes of major effect on pork quality have been examined in great detail (Table 13.2). The first to be identified is that associated with mutations in the ryanodine gene (RYR1) gene (Fujii *et al.*, 1991; Otsu *et al.*, 1991), which increases growth significantly but increases halothane sensitivity and results in pale, soft exudative pork. Homozygous animals will often succumb to stress. Animals with this mutation have been examined in detail (Kuciel *et al.* 1995; Rempel *et al.*, 1995; Sather and Jones, 1996; Lahucky *et al.*, 1997; Larzul *et al.*, 1997; Urban *et al.*, 1998; Monin *et al.*, 1999; Gispert *et al.*, 2000; Thaller *et al.*, 2000; Tor *et al.*, 2001; Bridi *et al.*, 2003), and schemes have been proposed of how to incorporate the mutation into structured breeding programmes. The other major effect, the Rendemente Napole (RN) mutation at the PRKAG3 gene, also increases the growth rate and meat yield of the carcass but is associated with low ultimate pH and poor ham quality due to reduction in the glycogen content of the meat. It too has been examined exhaustively (Moeller *et al.*, 2003; Lindahl *et al.*, 2004; Stalder *et al.*, 2005; Carr *et al.*, 2006; Enfalt *et al.*, 2006), with one study examining both genes (Hamilton *et al.*, 2000).

QTL mapping studies have identified several chromosomes with linkage to meat quality traits. These include a genome-wide analysis of meat quality such as meat tenderness, cooking loss, colour and ultimate pH (De Koning *et al.*, 1999; Ovilo *et al.*, 2002; Geldermann *et al.*, 2003; Stearns *et al.*, 2005; Liu *et al.*, 2007; Sanchez *et al.*, 2007), with specific subsequent analyses of individual chromosomes (Ovilo *et al.*, 2000, 2005; Rothschild *et al.*, 2005; Kim *et al.* 2006; Demars *et al.*, 2007; Meyers *et al.*, 2007), fatty acid composition (Clop *et al.*, 2003), a survey of genes affecting ultimate pH and other meat quality traits (Evans *et al.*, 2003), X-linked DNA markers for several pig meat quality traits including IMF (Gaboreanu *et al.*, 2004), QTL for pork off flavours (Glenn *et al.*, 2007), confirmation of a QTL for meat colour (van Wijk *et al.*, 2007), and the evaluation of genes for drip loss (Jennen *et al.*, 2007; Otto *et al.*, 2007). Indirect measures of meat quality such as for muscle fibre type, and including comparisons to meat quality, have been performed (Wimmers *et al.*, 2007a). In one case, additional QTL were discovered serendipitously – a study of IGF2, a carcass composition QTL, showed other QTL affecting meat quality, including ultimate pH on the same chromosome as IGF2, but due to other genes (Estelle *et al.*, 2005).

Population association studies have shown the effects of several genes on

intramuscular fat (Table 13.3). These include FABP3, heart fatty acid binding protein, (Gerbens *et al.*, 1997) and FABP4, adipocyte fatty acid binding protein (Gerbens *et al.*, 1998) on intra-muscular fat, which has been confirmed in several studies (Chmurzynska *et al.*, 2004; Zeng *et al.*, 2005; Damon *et al.*, 2006; Uemoto *et al.*, 2007); and FABP3 on meat quality (Arnyasi *et al.*, 2006), although associations to these genes have not always been found (Gerbens *et al.*, 2001; Nechtelberger *et al.*, 2001) and some studies suggest that it is DNA variation near these genes that affects intramuscular fat (Ovilo *et al.*, 2002b). FABP4 is in the same interval as a QTL that has been associated with fatty acid composition (Perez-Enciso *et al.*, 2000). The leptin receptor (LEPR) has been associated with intramuscular fat but there have also been confirmations and lack of confirmation (Ovilo *et al.*, 2002b; Chmurzynska *et al.*, 2004; Mackowski *et al.*, 2005). Variation at LEPR has been confirmed to affect back fat thickness and overall fatness (Chen *et al.*, 2004; Ovilo *et al.*, 2005) with the suggestion that it may affect fecundity and other traits (Chen *et al.*, 2004), which would be consistent with the gene expression data for this gene (Guay *et al.*, 2001). The gene MC4R, originally implicated in fatness and feed intake (Kim *et al.*, 2000), was shown to be implicated in fatty acid profile and meat colour (Ovilo *et al.*, 2006).

There are other studies in which a gene has been associated with a meat quality trait. These include the influence of CAST on meat tenderness (Rothschild and Ciobanu, 2003), MYF5 on drip loss (Carmo *et al.*, 2005), the effects on IMF of members of the MYOD family (Verner *et al.*, 2007), GH1 on several pork growth and meat quality traits (De Faria *et al.*, 2006), CRH on pig meat quality including meat colour (Murani *et al.*, 2006), CSTF1 on meat colour, growth and leanness (Rothschild and Ramos, 2006), and LPL on several meat qualities including pH and drip loss, not carcass fatness (Zhang *et al.*, 2005). These are QTL effects, some of them with opposing effects on several traits, and they will need to be confirmed in independent studies to quantify their effects more broadly.

Finally, studies are now being performed where gene expression microarrays are identifying candidate genes for meat quality based on gene expression differences and these genes have been examined for genetic variation. Tests of these genes have led to associations between the DNA variants and the traits of interest, although the markers are not thought either to be causative or found to be associated with the trait in all breeds (Wimmers *et al.*, 2007b).

13.3.3 Sheep

There is not much published work on DNA tests for sheep meat quality. Three major genes affecting muscularity have been defined. The first is due to the Callipyge (CLPG) gene (Table 13.2), which causes an increase in the size of the muscles of the hindquarters of the sheep (Cockett *et al.*, 1994; Freking *et al.*, 2002). CLPG appears to have negative effects on sheep meat tenderness (Koohmaraie *et al.*, 1995), apparently due to changes in the effects of calpastatin and calpain. A second genetic variant near to CLPG, the *Carwell* allele, appears to have smaller effects on growth of the sheep and slight negative effects on meat tenderness

(Jopson *et al.*, 2001). Mutations at the third gene affecting lean yield in sheep, in the myostatin gene GDF8 (Clop *et al.*, 2006), showed no effect on several meat quality traits in a moderately sized study (Johnson *et al.*, 2005) but a larger, more recent study has shown a negative effect on flavour, tenderness and overall satisfaction in consumer tests (Kijas *et al.*, 2007). Other QTL that have been mapped appear to affect sheep fatty acid composition (Karamichou *et al.*, 2006a) and meat and carcass quality (Karamichou *et al.*, 2006b).

13.3.4 Chicken and other birds

So far, there have been no reports of a major gene affecting a meat quality trait in chickens. Most of the mapping work for QTL in chicken are for traits affecting growth, carcass, disease and egg-laying traits, and these studies have been reviewed recently (Abasht *et al.*, 2006). For meat quality, several studies have now been reported, these include QTL for breast meat colour (Van Kaam *et al.*, 1999), meat colour and other meat quality traits as well as egg quality (Wright *et al.*, 2006; Le Bihan-Duval *et al.*, 2007) and intra-muscular fat (Jennen *et al.*, 2005). The amount of breast meat is a carcass trait, but obviously the amount of a carcass that consists of this prime cut affects meat quality and several studies with some common chromosomal regions have been reported (De Koning *et al.*, 2004; Lagarrigue *et al.*, 2006; McElroy *et al.*, 2006). Although most of the work has been performed in the chicken, and this will only increase due to the genome sequence of the chicken being known, one study of ultimate pH and drip loss has been reported in duck (Huang *et al.*, 2007).

13.4 Future trends

The survey of the literature above suggests that there is a wealth of raw material of QTL effects located to chromosomes in many species that have not yet been transformed into diagnostic tests (Fig. 13.1). In closed breeding systems or in families, haplotypes of DNA markers may be able to use such QTL information for improving livestock, since the haplotypes will act as a bracket to ensure that the QTL is transferred from one generation to the next. However, to be able to use these QTL in any population or breed, diagnostic markers that are in strong allelic association with the QTL, or preferably the QTL themselves, will need to be identified. Finding such diagnostic tests is difficult and costly, and the lack of progress in turning QTL to diagnostic tests represents to some extent the difficulty of the process. Improved tools may help in identifying diagnostic markers or the actual genetic variation for the QTL.

The most important immediate future direction will be identifying the genes or genetic regions that correspond to the QTL that have already been identified. The most important tool will be a high density of single nucleotide polymorphisms, preferably mapped to a genome sequence. If the density is sufficiently high, that is, of the order of $2\text{--}3 \times 10^5$ single nucleotide polymorphisms for a livestock species,

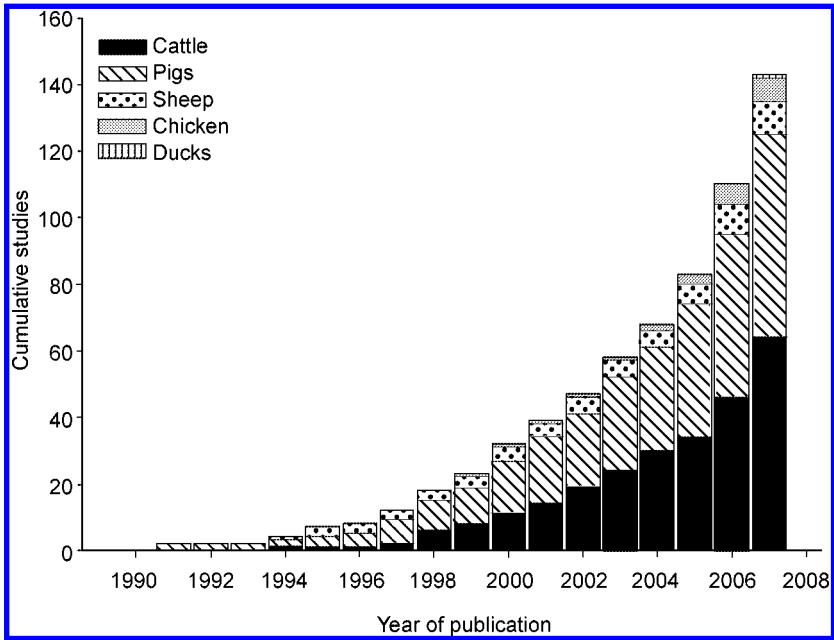


Fig. 13.1 The cumulative number of papers linking chromosomal regions or genes to meat quality in different species plotted over time. Note the steadily increasing size of the field and the spread of the field outside of pigs and cattle.

then diagnostic markers for traits will be identified quite easily. Recent work has shown that even 9000 SNP spread more or less randomly across the bovine genome can be used to identify many diagnostic markers or show the locations of diagnostic markers to within a QTL detection distance of 30 kb in the bovine genome (Barendse *et al.* 2007c).

What appears to be an alternative strategy, but actually converges in practice, is the notion of genome selection. Genome selection (Meuwissen *et al.*, 2001) is the use of high densities of anonymous SNP to predict the genetic merit of animals. This has been elaborated (Gianola *et al.*, 2006; Schaeffer, 2006) using other models or the exploration of the consequences, to determine how influential such methods would be. Obviously, a large number of SNP associated with a trait begins to resemble whole genome selection once the panel of SNP becomes large enough, and these approaches converge. How much effort should be invested to do genome selection versus large panels of SNP is still an area of vigorous debate, partly because not all animals are sufficiently valuable that genome selection would be cost-effective to implement as a routine tool, and partly because genome selection depends upon linkage disequilibrium to be effective and linkage disequilibrium breaks down over time. Genome selection using causative mutations for QTL may thus be the long-term convergence of these two ways of thinking.

Panels of SNP will not only be used for breeding but will also be used for

predicting the phenotype. Phenotype prediction is important where the underlying gene frequencies in the population do not need to be changed, but the genetic variability in a particular part of the supply chain needs to be reduced. Then animals that will perform more consistently will be required. In that case it will not only be the additive effects of each chromosomal segment or diagnostic test that is important, but also the dominance deviation, epistasis and genotype \times environmental interactions that will be important. For meat quality, this means that these other genetic effects need to be estimated and reported, not just the additive effects. How cost effective this will be will depend on the accuracy of these tests, their costs, and the increased profit that they provide. These panels of SNP could be integrated into national evaluation systems such as Meat Standards Australia, where the genetic component of meat quality is indicated by the percentage of zebu ancestry in the cattle. Specifying zebu ancestry is a crude way of delimiting genetic components to meat quality, and over time more refined or precise methods could be incorporated.

13.5 Sources of further information and advice

Further information on the genetics of meat quality can be found in journals such as *Animal Genetics*, *Australian Journal of Agricultural Research*, *Australian Journal of Experimental Agriculture*, *BMC Genetics*, *BMC Genomics*, *Canadian Journal of Animal Science*, *Genetic Selection Evolution*, *Genetica*, *Genetics*, *Genome*, *Genome Research*, *Genomics*, *Journal of Animal Breeding and Genetics*, *Journal of Animal Science*, *Mammalian Genome* and *Meat Science*. Occasionally this research will be found in the multidisciplinary journals, *Science*, *Nature*, *Proceedings of National Academy of Science (USA)* as well as in journals of medical genetics such as *Nature Genetics*.

Information on genetic testing can be obtained from such companies as Bovigen, Catapult Genetics (previously Genetic Solutions P/L and Catapult), Merial, Prescribe Genomics CO, and industry bodies in many countries. There are also breed societies in many countries that take an informed interest in the performance of their breed.

13.6 Acknowledgements

I thank Mike Goddard for discussing the estimation of standard errors for the additive and dominance effects.

13.7 References

- Abasht, B., Dekkers, J. C. M. and Lamont, S. J. (2006), Review of quantitative trait loci identified in the chicken. *Poultry Science*, **85**, 2079–2096.
- Alexander, L. J., MacNeil, M. D., Geary, T. W., Snelling, W. M., Rule, D. C. and Scanga,

- J. A. (2007), Quantitative trait loci with additive effects on palatability and fatty acid composition of meat in a Wagyu–Limousin F-2 population. *Animal Genetics*, **38**, 506–513.
- Aryasi, M., Grindflek, E., Javor, A. and Lien, S. (2006), Investigation of two candidate genes for meat quality traits in a quantitative trait locus region on SSC6: The porcine short heterodimer partner and heart fatty acid binding protein genes. *Journal of Animal Breeding and Genetics*, **123**, 198–203.
- Barendse, W. (1997), *Assessing lipid metabolism*. WO9923248 US 6383751.
- Barendse, W. (2002), *DNA markers for meat tenderness*. WO02064820.
- Barendse, W. (2003), *DNA markers for marbling*. WO2004070055.
- Barendse, W. (2005), The transition from quantitative trait loci to diagnostic test in cattle and other livestock. *Australian Journal of Experimental Agriculture*, **45**, 831–836.
- Barendse, W. (2006), *Assessing the tenderness of meat from an animal by testing the animal for a genetic marker in the calpain3 (CAPN3) gene associated with Warner–Bratzler peak force variation or for a genetic marker located other than in CAPN3*. WO2007053891.
- Barendse, W., Bunch, R., Thomas, M., Armitage, S., Baud, S. and Donaldson, N. (2004), The TG5 thyroglobulin gene test for a marbling quantitative trait loci evaluated in feedlot cattle. *Australian Journal of Experimental Agriculture*, **44**, 669–674.
- Barendse, W., Bunch, R. J. and Harrison, B. E. (2005), The leptin C73T missense mutation is not associated with marbling and fatness traits in a large gene mapping experiment in Australian cattle. *Animal Genetics*, **36**, 86–88.
- Barendse, W., Bunch, R. J., Harrison, B. E. and Thomas, M. B. (2006), The growth hormone 1 GH1:c.457C>G mutation is associated with relative fat distribution in intra-muscular and rump fat in a large sample of Australian feedlot cattle. *Animal Genetics*, **37**, 211–214.
- Barendse, W., Bunch, R. J., Kijas, J. W. and Thomas, M. B. (2007a), The effect of genetic variation of the retinoic acid receptor-related orphan receptor C gene on fatness in cattle. *Genetics*, **175**, 843–853.
- Barendse, W., Harrison, B. E., Hawken, R. J., Ferguson, D. M., Thompson, J. M., Thomas, M. B. and Bunch, R. J. (2007b), Epistasis between calpain 1 and its inhibitor calpastatin within breeds of cattle. *Genetics*, **176**, 2601–2610.
- Barendse, W., Reverter, A., Bunch, R. J., Harrison, B. E., Barris, W. and Thomas, M. B. (2007c), A validated whole genome association study of efficient food conversion. *Genetics*, **176**, 1893–1905.
- Barendse, W. and Reverter-Gomez, A. (2007), *A method for assessing traits selected from longissimus dorsi peak force, intramuscular fat, retail beef yield and net feed intake in bovine animals*. WO2007012119. Australia.
- Basten, C. J., Weir, B. S. and Zeng, Z. B. (1994), Zmap – a QTL cartographer. In: *5th World Congress on Genetics Applied to Livestock Production* (ed. by Smith, C., Gavora, J. S., Benkel, B., Chesnais, J., Fairfull, W., Gibson, J. P., Kennedy, B. W. and Burnside, E. B.) Vol 22, 65–66. Guelph, Ontario, Canada.
- Bridi, A. M., Rubensam, J. M., Nicolaiewsky, S., Lopes, R. F. F. and Lobato, J. F. P. (2003), Effect of the halothane genes and rearing systems on meat quality of pork. *Revista Brasileira De Zootecnia–Brazilian Journal of Animal Science*, **32**, 1362–1370.
- Buchanan, F. C., Fitzsimmons, C. J., van Kessel, A. G., Thue, T. D., Winkelman-Sim, D. C. and Schmutz, S. M. (2002), Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. *Genetics Selection Evolution*, **34**, 105–116.
- Buchanan, F. C., van Kessel, A. G., Waldner, C., Christensen, D. A., Laarveld, B. and Schmutz, S. M. (2003), Hot topic: An association between a leptin single nucleotide polymorphism and milk and protein yield. *Journal of Dairy Science*, **86**, 3164–3166.
- Caballero, B., Sierra, V., Olivan, M., Vega-Naredo, I., Tomas-Zapico, C., Alvarez-Garcia, O., Tolivia, D., Hardeland, R., Rodriguez-Colunga, M. J. and Coto-Montes, A. (2007), Activity of cathepsins during beef aging related to mutations in the myostatin gene. *Journal of the Science of Food and Agriculture*, **87**, 192–199.

- Carlson, C. S., Eberle, M. A., Kruglyak, L. and Nickerson, D. A. (2004), Mapping complex disease loci in whole-genome association studies. *Nature*, **429**, 446–452.
- Carmo, F. M. D., Guimaraes, S. E. F., Lopes, P. S., Pires, A. V., Guimaraes, M. F. M., da Silva, M., Schierholt, A. S., Silva, K. D. E. and Gomide, L. A. D. (2005), Association of MYF5 gene allelic variants with production traits in pigs. *Genetics and Molecular Biology*, **28**, 363–369.
- Carr, C. C., Morgan, J. B., Berg, E. P., Carter, S. D. and Ray, F. K. (2006), Growth performance, carcass composition, quality, and enhancement treatment of fresh pork identified through deoxyribonucleic acid marker-assisted selection for the Rendement Napole gene. *Journal of Animal Science*, **84**, 910–917.
- Casas, E., Keele, J. W., Shackelford, S. D., Koohmaraie, M., Sonstegard, T. S., Smith, T. P. L., Kappes, S. M. and Stone, R. T. (1998), Association of the muscle hypertrophy locus with carcass traits in beef cattle. *Journal of Animal Science*, **76**, 468–473.
- Casas, E., Shackelford, S. D., Keele, J. W., Koohmaraie, M., Smith, T. P. L. and Stone, R. T. (2003), Detection of quantitative trait loci for growth and carcass composition in cattle. *Journal of Animal Science*, **81**, 2976–2983.
- Casas, E., Shackelford, S. D., Keele, J. W., Stone, R. T., Kappes, S. M. and Koohmaraie, M. (2000), Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. *Journal of Animal Science*, **78**, 560–569.
- Casas, E., Stone, R. T., Keele, J. W., Shackelford, S. D., Kappes, S. M. and Koohmaraie, M. (2001), A comprehensive search for quantitative trait loci affecting growth and carcass composition of cattle segregating alternative forms of the myostatin gene. *Journal of Animal Science*, **79**, 854–860.
- Casas, E., White, S. N., Shackelford, S. D., Wheeler, T. L., Koohmaraie, M., Bennett, G. L. and Smith, T. P. L. (2007), Assessing the association of single nucleotide polymorphisms at the thyroglobulin gene with carcass traits in beef cattle. *Journal of Animal Science*, **85**, 2807–2814.
- Casas, E., White, S. N., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., Riley, D. G., Chase, C. C., Johnson, D. D. and Smith, T. P. L. (2006), Effects of calpastatin and mu-calpain markers in beef cattle on tenderness traits. *Journal of Animal Science*, **84**, 520–525.
- Chapman, J. M., Cooper, J. D., Todd, J. A. and Clayton, D. G. (2003), Detecting disease associations due to linkage disequilibrium using haplotype tags: A class of tests and the determinants of statistical power. *Human Heredity*, **56**, 18–31.
- Chen, C. C., Chang, T. and Su, H. Y. (2004), Characterization of porcine leptin receptor polymorphisms and their association with reproduction and production traits. *Animal Biotechnology*, **15**, 89–102.
- Cheong, H. S., Yoon, D., Kim, L. H., Park, B. L., Lee, H. W., Han, C. S., Kim, E. M., Cho, H., Chung, E. R., Cheong, I. *et al.* (2007), Titin-cap (TCAP) polymorphisms associated with marbling score of beef. *Meat Science*, **77**, 257–263.
- Chikuni, K. and Mitsuhashi, T. (2002), *Method of evaluating useful cattle*. WO02077279 CA 2441938.
- Chmurzynska, A., Mackowski, M., Szydlowski, M., Melonek, J., Kamyczek, M., Eckert, R., Rozycki, M. and Switonski, M. (2004), Polymorphism of intronic microsatellites in the A-FABP and LEPR genes and its association with productive traits in the pig. *Journal of Animal and Feed Sciences*, **13**, 615–624.
- Choi, I. S., Kong, H. S., Oh, J. D., Yoon, D. H., Cho, B. W., Choi, Y. H., Kim, K. S., Choi, K. D., Lee, H. K. and Jeon, G. J. (2006), Analysis of microsatellite markers on bovine chromosomes 1 and 14 for potential allelic association with carcass traits in Hanwoo (Korean cattle). *Asian–Australasian Journal of Animal Sciences*, **19**, 927–930.
- Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibe, B., Bouix, J., Caiment, F., Elsen, J.-M., Eychenne, F. *et al.* (2006), A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nat. Genet.*, **38**, 813–818.

- Clop, A., Ovilo, C., Perez-Enciso, M., Cercos, A., Tomas, A., Fernandez, A., Coll, A., Folch, J. M., Barragan, C., Diaz, I. *et al.* (2003) Detection of QTL affecting fatty acid composition in the pig. *Mammalian Genome*, **14**, 650–656.
- Cockett, N. E., Jackson, S. P., Shay, T. L., Nielsen, D., Moore, S. S., Steele, M. R., Barendse, W., Green, R. D. and Georges, M. (1994), Chromosomal localization of the callipyge gene in sheep (*Ovis aries*) using bovine DNA markers. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 3019–3023.
- Collins, F. S. (1995), Positional cloning moves from perditional to traditional. *Nature Genetics*, **9**, 347–350.
- Costello, S., O'Doherty, E., Troy, D. J., Ernst, C. W., Kim, K. S., Stapleton, P., Sweeney, T. and Mullen, A. M. (2007), Association of polymorphisms in the calpain I, calpain II and growth hormone genes with tenderness in bovine *M-longissimus dorsi*. *Meat Science*, **75**, 551–557.
- Damon, M., Louveau, I., Lefaucheur, L., Lebret, B., Vincent, A., Leroy, P., Sanchez, M. P., Herpin, P. and Gondret, F. (2006), Number of intramuscular adipocytes and fatty acid binding protein-4 content are significant indicators of intramuscular fat level in crossbred Large White × Duroc pigs. *Journal of Animal Science*, **84**, 1083–1092.
- De Faria, D. A., Guimaraes, S. E. F., Lopes, P. S., Pires, A. V., Paiva, S. R., Sollero, B. P. and Wenceslau, A. A. (2006), Association between G316A growth hormone polymorphism and economic traits in pigs. *Genetics and Molecular Biology*, **29**, 634–640.
- De Koning, D. J., Haley, C. S., Windsor, D., Hocking, P. M., Griffin, H., Morris, A., Vincent, J. and Burt, D. W. (2004), Segregation of QTL for production traits in commercial meat-type chickens. *Genetical Research*, **83**, 211–220.
- De Koning, D. J., Janss, L. L. G., Rattink, A. P., van Oers, P. A. M., de Vries, B. J., Groenen, M. A. M., van der Poel, J. J., de Groot, P. N., Brascamp, E. W. and van Arendonk, J. A. M. (1999), Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs (*Sus scrofa*). *Genetics*, **152**, 1679–1690.
- Demars, J., Riquet, J., Sanchez, M. P., Billon, Y., Hocquette, J. F., Lebret, B., Iannuccelli, N., Bidanel, J. P., Milan, D. and Gondret, F. (2007), Metabolic and histochemical characteristics of fat and muscle tissues in homozygous or heterozygous pigs for the body composition QTL located on chromosome 7. *Physiological Genomics*, **30**, 232–241.
- Di Stasio, L., Brugiapaglia, A., Galloni, M., Destefanis, G. and Lisa, C. (2007), Effect of the *leptin* c.73T>C mutation on carcass traits in beef cattle. *Animal Genetics*, doi:10.1111/j.1365-2052.2007.01595.x.
- Doran, O., Moule, S. K., Teye, G. A., Whittington, F. M., Hallett, K. G. and Wood, J. D. (2006), A reduced protein diet induces stearoyl-CoA desaturase protein expression in pig muscle but not in subcutaneous adipose tissue: Relationship with intramuscular lipid formation. *British Journal of Nutrition*, **95**, 609–617.
- Drinkwater, R. D., Li, Y., Lenane, I., Davis, G. P., Shorthose, R., Harrison, B. E., Richardson, K., Ferguson, D., Stevenson, R., Renaud, J. *et al.* (2006), Detecting quantitative trait loci affecting beef tenderness on bovine chromosome 7 near calpastatin and lysyl oxidase. *Australian Journal of Experimental Agriculture*, **46**, 159–164.
- Edwards, J. H. (1980), Allelic association in man. In: *Population Structure and Genetic Disorders* (ed. by Eriksson, A. W., Forsius, H. R., Nevanlinna, H. R., Workman, P. L. and Norio, R. K.) 239–255. London, Academic Press.
- Enfalt, A. C., von Seth, G., Josell, A., Lindahl, G., Hedebro-Velander, I., Braunschweig, M., Andersson, L. and Lundstrom, K. (2006), Effects of a second mutant allele (V199I) at the PRKAG3 (RN) locus on carcass composition in pigs. *Livestock Science*, **99**, 131–139.
- Estelle, J., Mercade, J., Noguera, J. L., Perez-Enciso, M., Ovilo, C., Sanchez, A. and Folch, J. M. (2005), Effect of the porcine IGF2-intron3-G3072A substitution in an outbred Large White population and in an Iberian × Landrace cross. *Journal of Animal Science*, **83**, 2723–2728.
- Evans, G. J., Giuffra, E., Sanchez, A., Kerje, S., Davalos, G., Vidal, O., Illan, S., Noguera,

- J. L., Varona, L., Velander, I. *et al.* (2003), Identification of quantitative trait loci for production traits in commercial pig populations. *Genetics*, **164**, 621–627.
- Fitzsimmons, C. J., Schmutz, S. M., Bergen, R. D. and McKinnon, J. J. (1998), A potential association between the BM1500 microsatellite and fat deposition in beef cattle. *Mammalian Genome*, **9**, 432–434.
- Fogarty, N. M., Safari, E., Taylor, P. J. and Murray, W. (2003), Genetic parameters for meat quality and carcass traits and their correlation with wool traits in Australian Merino sheep. *Australian Journal of Agricultural Research*, **54**, 715–722.
- Freking, B. A., Murphy, S. K., Wylie, A. A., Rhodes, S. J., Keele, J. W., Leymaster, K. A., Jirtle, R. L. and Smith, T. P. L. (2002), Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals. *Genome Research*, **12**, 1496–1506.
- Fujii, J., Otsu, K., Zorzato, F., Deleon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J. and MacLennan, D. H. (1991), Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science*, **253**, 448–451.
- Gaboreanu, A. M., Grapes, L., Ramos, A. M., Kim, J. J. and Rothschild, M. F. (2004), Characterization of an X-chromosome PCR-RFLP marker associated with fat deposition and growth in the pig. *Animal Genetics*, **35**, 401–403.
- Garcia, M. D., Michal, J. J., Gaskins, C. T., Reeves, J. J., Ott, T. L., Liu, Y. and Jiang, Z. (2006), Significant association of the calpastatin gene with fertility and longevity in dairy cattle. *Animal Genetics*, **37**, 304–305.
- Geldermann, H., Muller, E., Moser, G., Reiner, G., Bartenschlager, H., Cepica, S., Stratil, A., Kuryl, J., Moran, C., Davoli, R. *et al.* (2003), Genome-wide linkage and QTL mapping in porcine F-2 families generated from Pietrain, Meishan and Wild Boar crosses. *Journal of Animal Breeding and Genetics*, **120**, 363–393.
- Georges, M., Nielsen, D., Mackinnon, M., Mishra, A., Okimoto, R., Pasquino, A. T., Sargeant, L. S., Sorensen, A., Steele, M. R., Zhao, X. Y. *et al.* (1995), Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics*, **139**, 907–920.
- Gerbens, F., Jansen, A., van Erp, A. J. M., Harders, F., Meuwissen, T. H. E., Rettenberger, G., Veerkamp, J. H. and te Pas, M. F. W. (1998), The adipocyte fatty acid-binding protein locus: Characterization and association with intramuscular fat content in pigs. *Mammalian Genome*, **9**, 1022–1026.
- Gerbens, F., Rettenberger, G., Lenstra, J. A., Veerkamp, J. H. and tePas, M. F. W. (1997), Characterization, chromosomal localization, and genetic variation of the porcine heart fatty acid-binding protein gene. *Mammalian Genome*, **8**, 328–332.
- Gerbens, F., Verburg, F. J., Van Moerkerk, H. T. B., Engel, B., Buist, W., Veerkamp, J. H. and te Pas, M. (2001), Associations of heart and adipocyte fatty acid-binding protein gene expression with intramuscular fat content in pigs. *Journal of Animal Science*, **79**, 347–354.
- Gianola, D., Fernando, R. L. and Stella, A. (2006), Genetic-assisted prediction of genetic value with semiparametric procedures. *Genetics*, **173**, 1761–1776.
- Gispert, M., Faucitano, L., Oliver, M. A., Guardia, M. D., Coll, C., Siggins, K., Harvey, K. and Diestre, A. (2000), A survey of pre-slaughter conditions, halothane gene frequency, and carcass and meat quality in five Spanish pig commercial abattoirs. *Meat Science*, **55**, 97–106.
- Gjedrum, T. (2000), Genetic improvement of cold-water fish species. *Aquaculture Research*, **31**, 25–33.
- Glenn, K. L., Ramos, A. M. and Rothschild, M. F. (2007), Analysis of FMO genes and off flavour in pork. *Journal of Animal Breeding and Genetics*, **124**, 35–38.
- Goodall, J. J. and Schmutz, S. M. (2007), IGF2 gene characterization and association with rib eye area in beef cattle. *Animal Genetics*, **38**, 154–161.
- Grobet, L., Martin, L. J. R., Poncelet, D., Pirotin, D., Brouwers, B., Riquet, J., Schoeberlein, A., Dunner, S., Menissier, F., Massabanda, J. *et al.* (1997), A deletion in the bovine myostatin gene causes the double-muscling phenotype in cattle. *Nature Genetics*, **17**, 71–74.

- Guay, F., Palin, M. F., Matte, J. J. and Laforest, L. P. (2001), Effects of breed, parity, and folic acid supplement on the expression of leptin and its receptors' genes in embryonic and endometrial tissues from pigs at day 25 of gestation. *Biology of Reproduction*, **65**, 921–927.
- Hale, C. S., Herring, W. O., Shibuya, H., Lucy, M. C., Lubahn, D. B., Keisler, D. H. and Johnson, G. S. (2000), Decreased growth in Angus steers with a short TG-microsatellite allele in the P1 promoter of the growth hormone receptor gene. *Journal of Animal Science*, **78**, 2099–2104.
- Haley, C. S. and Knott, S. A. (1992), A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, **69**, 315–324.
- Hamilton, D. N., Ellis, M., Miller, K. D., McKeith, F. K. and Parrett, D. F. (2000), The effect of the Halothane and Rendement Napole genes on carcass and meat quality characteristics of pigs. *Journal of Animal Science*, **78**, 2862–2867.
- Hanset, R., Michaux, C., Dessy-Doize, C. and Burtonboy, G. (1982), Studies on the 7th rib cut in double muscled and conventional cattle. Anatomical, histological and biochemical aspects. In: *Muscle Hypertrophy of Genetic Origin and its Use to Improve Beef Production* (ed. by King, J. W. B. and Menissier, F.) 341–349. The Hague, Martinus Nijhoff Publishers.
- Hermesch, S., Luxford, B. G. and Graser, H.-U. (2000), Genetic parameters for lean meat yield, meat quality, reproduction and feed efficiency traits for Australian pigs 1. Description of traits and heritability estimates. *Livestock Production Science*, **65**, 239–248.
- Hoashi, S., Ashida, N., Ohsaki, H., Utsugi, T., Sasazaki, S., Taniguchi, M., Oyama, K., Mukai, F. and Mannen, H. (2007), Genotype of bovine sterol regulatory element binding protein-1 (SREBP-1) is associated with fatty acid composition in Japanese Black cattle. *Mammalian Genome*, **18**, 880–886.
- Hocquette, J. F., Lehnert, S. A., Barendse, W., Casser-Malek, I. and Picard, B. (2007), Recent advances in cattle functional genomics and their application to beef quality. *Animal*, **1**, 159–173.
- Hopkins, D. L., Safari, E., Thompson, J. M. and Smith, C. R. (2004), Video image analysis in the Australian meat industry – precision and accuracy of predicting lean meat yield in lamb carcasses. *Meat Science*, **67**, 269–274.
- Huang, Y., Haley, C. S., Wu, F., Hu, S., Hao, J., Wu, C. and Li, N. (2007), Genetic mapping of quantitative trait loci affecting carcass and meat quality traits in Beijing ducks (*Anas platyrhynchos*). *Animal Genetics*, **38**, 114–119.
- Imai, K., Matsughige, T., Watanabe, T., Sugimoto, Y. and Ihara, N. (2007), Mapping of a quantitative trait locus for beef marbling on bovine chromosome 9 in purebred Japanese Black cattle. *Animal Biotechnology*, **18**, 75–80.
- Jennen, D. G. J., Brings, A. D., Liu, G., Jungst, H., Tholen, E., Jonas, E., Tesfaye, D., Schellander, K. and Phatsara, C. (2007), Genetic aspects concerning drip loss and water-holding capacity of porcine meat. *Journal of Animal Breeding and Genetics*, **124 Suppl.1**, 2–11.
- Jennen, D. G. J., Vereijken, A. L. J., Bovenhuis, H., Crooijmans, R., van der Poel, J. J. and Groenen, M. A. M. (2005), Confirmation of quantitative trait loci affecting fatness in chickens. *Genetics Selection Evolution*, **37**, 215–228.
- Jiang, Z. H., Kunej, T., Michal, J. J., Gaskins, C. T., Reeves, J. J., Busboom, J. R., Dovc, P. and Wright, R. W. (2005), Significant associations of the mitochondrial transcription factor A promoter polymorphisms with marbling and subcutaneous fat depth in Wagyu × Limousin F-2 crosses. *Biochemical and Biophysical Research Communications*, **334**, 516–523.
- Johnson, P. L., McEwan, J. C., Dodds, K. G., Purchas, R. W. and Blair, H. T. (2005), Meat quality traits were unaffected by a quantitative trait locus affecting leg composition traits in Texel sheep. *Journal of Animal Science*, **83**, 2729–2735.
- Johnston, D. J., Reverter, A., Ferguson, D. M., Thompson, J. M. and Burrow, H. M. (2003),

- Genetic and phenotypic characterisation of animal, carcass, and meat quality traits from temperate and tropically adapted beef breeds. 3. Meat quality traits. *Australian Journal of Agricultural Research*, **54**, 135–147.
- Jopson, N. B., Nicoll, G. B., Stevenson-Barry, J. M., Duncan, S., Greer, G. J., Bain, W. E., Gerard, E. M., Glass, B. C., Broad, T. E. and McEwan, J. C. (2001), Mode of inheritance and effects on meat quality of the rib-eye muscling (REM) QTL in sheep. *Proceeding of the Association for the Advancement of Animal Breeding and Genetics*, **14**, 111–114.
- Karamichou, E., Richardson, R. I., Nute, G. R., Gibson, K. P. and Bishop, S. C. (2006a), Genetic analyses and quantitative trait loci detection, using a partial genome scan, for intramuscular fatty acid composition in Scottish Blackface sheep. *Journal of Animal Science*, **84**, 3228–3238.
- Karamichou, E., Richardson, R. I., Nute, G. R., McLean, K. A. and Bishop, S. C. (2006b), A partial genome scan to map quantitative trait loci for carcass composition, as assessed by X-ray computer tomography, and meat quality traits in Scottish Blackface Sheep. *Animal Science*, **82**, 301–309.
- Keele, J. W., Shackelford, S. D., Kappes, S. M., Koohmaraie, M. and Stone, R. T. (1999), A region on bovine chromosome 15 influences beef *longissimus* tenderness in steers. *Journal of Animal Science*, **77**, 1364–1371.
- Khatib, H., Zaitoun, I., Chang, Y. M., Maltecca, C. and Boettcher, P. (2007), Evaluation of association between polymorphism within the thyroglobulin gene and milk production traits in dairy cattle. *Journal of Animal Breeding and Genetics*, **124**, 26–28.
- Kijas, J. W., McCulloch, R., Hocking Edwards, J. E., Oddy, V. H., Lee, S. H. and Van Der Werf, J. (2007), Evidence for multiple alleles effecting muscling and fatness at the ovine GDF8 locus. *BMC Genetics*, **8**, 80.
- Kim, J. H., Lim, H. T., Park, E. W., Rodriguez, C., Silio, L., Varona, L., Mercade, A., Jeon, J. T. and Ovilo, C. (2006), Polymorphisms in the promoter region of the porcine acyl-coA dehydrogenase, medium-chain (ACADM) gene have no effect on fat deposition traits in a pig Iberian × Landrace cross. *Animal Genetics*, **37**, 430–431.
- Kim, J. W., Hong, J. M., Lee, Y. S., Chae, S. H., Choi, C. B., Choi, I. H. and Yeo, J. S. (2004), Identification of new microsatellite DNAs in the chromosomal DNA of the 44 Korean cattle (Hanwoo). *Asian–Australasian Journal of Animal Sciences*, **17**, 1329–1333.
- Kim, J. W., Park, S. I. and Yeo, J. S. (2003), Linkage mapping and QTL on chromosome 6 in Hanwoo (Korean cattle). *Asian–Australasian Journal of Animal Sciences*, **16**, 1402–1405.
- Kim, K. S., Larsen, N., Short, T., Plastow, G. and Rothschild, M. F. (2000), A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. *Mammalian Genome*, **11**, 131–135.
- Koohmaraie, M., Shackelford, S. D., Wheeler, T. L., Lonergan, S. M. and Doumit, M. E. (1995), A muscle hypertrophy condition in lamb (callipyge): Characterization of effects on muscle growth and meat quality traits. *Journal of Animal Science*, **73**, 3596–3607.
- Kruglyak, L. (1999), Prospects for whole-genome linkage disequilibrium mapping of common disease genes. *Nature Genetics*, **22**, 139–144.
- Kuciel, J., Dvorak, J., Nespor, F., Nebola, M. and Hartl, J. (1995), pH(1) values of meat of hybrid pigs with heterozygous genotypes of Hal locus. *Zivocisna Vyroba*, **40**, 417–420.
- Lagarrigue, S., Pitel, F., Carre, W., Abasht, B., Le Roy, P., Neau, A., Amigues, Y., Sourdoux, M., Simon, J., Cogburn, L. *et al.* (2006), Mapping quantitative trait loci affecting fatness and breast muscle weight in meat-type chicken lines divergently selected on abdominal fatness. *Genetics Selection Evolution*, **38**, 85–97.
- Lahucky, R., Christian, L. L., Kovac, L., Stalder, K. J. and Bauerova, M. (1997), Meat quality assessed ante- and post mortem by different ryanodine receptor gene status of pigs. *Meat Science*, **47**, 277–285.
- Lander, E. S. and Botstein, D. (1989), Mapping Mendelian factors underlying quantitative traits using RFLP maps. *Genetics*, **121**, 185–199.
- Larzul, C., LeRoy, P., Gueblez, R., Talmant, A., Gogue, J., Sellier, P. and Monin, G. (1997),

- Effect of halothane genotype (NN, Nn, nn) on growth, carcass and meat quality traits of pigs slaughtered at 95 kg or 125 kg live weight. *Journal of Animal Breeding and Genetics – Zeitschrift Fur Tierzucht Und Zuchtungsbiologie*, **114**, 309–320.
- Le Bihan-Duval, E., Berri, C., Pitel, F., Nadaf, J., Sibut, V., Jenkins, C. and Duclos, M. J. (2007), A general approach combining QTL research and gene expression profiling to identify genes controlling chicken meat quality. *Sciences Des Aliments*, **27**, 143–152.
- Le Bihan-Duval, E., Millet, N. and Remignon, H. (1999), Broiler meat quality: Effect of selection for increased carcass quality and estimates of genetic parameters. *Poultry Science*, **78**, 822–826.
- Lindahl, G., Enfalt, A. C., von Seth, G., Josell, A., Hedebo-Velander, I., Andersen, H. J., Braunschweig, M., Andersson, L. and Lundstrom, K. (2004), A second mutant allele (V199I) at the PRKAG3 (RN) locus – I. Effect on technological meat quality of pork loin. *Meat Science*, **66**, 609–619.
- Liu, G., Jennen, D. G. J., Tholen, E., Juengst, H., Kleinwachter, T., Holker, M., Tesfaye, D., Un, G., Schreinemachers, H. J., Murani, E. *et al.* (2007), A genome scan reveals QTL for growth, fatness, leanness and meat quality in a Duroc–Pietrain resource population. *Animal Genetics*, **38**, 241–252.
- Lo, L. L., McLaren, D. G., McKeith, F. K., Fernando, R. L. and Novakofski, J. (1991), Genetic analyses of growth, real-time ultrasounds, carcass, and pork quality traits in Duroc and Landrace pigs: II. Heritabilities and correlations. *Journal of Animal Science*, **70**, 2387–2396.
- Mackowski, M., Szymoniak, K., Szydlowski, M., Kamyczek, M., Eckert, R., Rozycki, M. and Switonski, M. (2005), Missense mutations in exon 4 of the porcine LEPR gene encoding extracellular domain and their association with fatness traits. *Animal Genetics*, **36**, 135–137.
- MacNeil, M. D. and Grosz, M. D. (2002), Genome-wide scans for QTL affecting carcass traits in Hereford × composite double backcross populations. *Journal of Animal Science*, **80**, 2316–2324.
- Mannen, H., Kojima, T., Oyama, K., Mukai, F., Ishida, T. and Tsuji, S. (1998), Effect of mitochondrial DNA variation on carcass traits of Japanese Black cattle. *Journal of Animal Science*, **76**, 36–41.
- Mannen, H., Morimoto, M., Oyama, K., Mukai, F. and Tsuji, S. (2003), Identification of mitochondrial DNA substitutions related to meat quality in Japanese Black cattle. *Journal of Animal Science*, **81**, 68–73.
- McElroy, J. P., Kim, J. J., Harry, D. E., Brown, S. R., Dekkers, J. C. M. and Lamont, S. J. (2006), Identification of trait loci affecting white meat percentage and other growth and carcass traits in commercial broiler chickens. *Poultry Science*, **85**, 593–605.
- Meuwissen, T. H. E., Hayes, B. J. and Goddard, M. E. (2001), Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, **157**, 1819–1829.
- Meyers, S. N., Rodriguez-Zas, S. L. and Beever, J. E. (2007), Fine-mapping of a QTL influencing pork tenderness on porcine chromosome 2. *BMC Genetics*, **8**, 69.
- Michal, J. J., Zhang, Z. W., Gaskins, C. T. and Jiang, Z. (2006), The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu × Limousin F2 crosses. *Animal Genetics*, **37**, 400–402.
- Mizoguchi, Y., Watanabe, T., Fujinaka, K., Iwamoto, E. and Sugimoto, Y. (2006), Mapping of quantitative trait loci for carcass traits in a Japanese Black (Wagyu) cattle population. *Animal Genetics*, **37**, 51–54.
- Mizoshita, K., Watanabe, T., Hayashi, H., Kubota, C., Yamakuchi, H., Todoroki, J. and Sugimoto, Y. (2004), Quantitative trait loci analysis for growth and carcass traits in a half-sib family of purebred Japanese Black (Wagyu) cattle. *Journal of Animal Science*, **82**, 3415–3420.
- Moeller, S. J., Baas, T. J., Leeds, T. D., Emmett, R. S. and Irvin, K. M. (2003), Rendement Napole gene effects and a comparison of glycolytic potential and DNA genotyping for

- classification of Rendement Napole status in Hampshire-sired pigs. *Journal of Animal Science*, **81**, 402–410.
- Monin, G., Larzul, C., Le Roy, P., Culioli, J., Mourot, J., Rousset-Akrim, S., Talmant, A., Touraille, C. and Sellier, P. (1999), Effects of the halothane genotype and slaughter weight on texture of pork. *Journal of Animal Science*, **77**, 408–415.
- Moore, S. S., Li, C., Basarab, J., Snelling, W. M., Kneeland, J., Murdoch, B., Hansen, C. and Benkel, B. (2003), Fine mapping of quantitative trait loci and assessment of positional candidate genes for backfat on bovine chromosome 14 in a commercial line of *Bos taurus*. *Journal of Animal Science*, **81**, 1919–1925.
- Moreno, C., Bouix, J., Brunel, J. C., Weisbecker, J. L., Francois, D., Lantier, F. and Elsen, J. M. (2001), Genetic parameter estimates for carcass traits in the inra401 composite sheep strain. *Livestock Production Science*, **69**, 227–232.
- Morris, C. A., Cullen, N. G., Glass, B. C., Hyndman, D. L., Manley, T. R., Hickey, S. M., McEwan, J. C., Pitchford, W. S., Bottema, C. D. K. and Lee, M. A. H. (2007), Fatty acid synthase effects on bovine adipose fat and milk fat. *Mammalian Genome*, **18**, 64–74.
- Morris, C. A., Cullen, N. G., Hickey, S. M., Dobbie, P. M., Veenvliet, B. A., Manley, T. R., Pitchford, W. S., Kruk, Z. A., Bottema, C. D. K. and Wilson, T. (2006), Genotypic effects of calpain 1 and calpastatin on the tenderness of cooked *M. longissimus dorsi* steaks from Jersey × Limousin, Angus and Hereford-cross cattle. *Animal Genetics*, **37**, 411–414.
- Morsci, N. S., Schnabel, R. D. and Taylor, J. F. (2006), Association analysis of adiponectin and somatostatin polymorphisms on BTA1 with growth and carcass traits in Angus cattle. *Animal Genetics*, **37**, 554–562.
- Murani, E., Ponsuksili, S., Schellander, K. and Wimmers, K. (2006), Association of corticotropin-releasing hormone gene variation with performance and meat quality traits in commercial pig lines. *Animal Genetics*, **37**, 509–512.
- Nechtelberger, D., Pires, V., Solkner, J., Stur, I., Brem, G., Mueller, M. and Mueller, S. (2001), Intramuscular fat content and genetic variants at fatty acid-binding protein loci in Austrian pigs. *Journal of Animal Science*, **79**, 2798–2804.
- Nkrumah, J. D., Li, C., Basarab, J. B., Guercio, S., Meng, Y., Murdoch, B., Hansen, C. and Moore, S. S. (2004), Association of a single nucleotide polymorphism in the bovine leptin gene with feed intake, feed efficiency, growth, feeding behaviour, carcass quality and body composition. *Canadian Journal of Animal Science*, **84**, 211–219.
- O'Brien, P. J. (1987), Etiopathogenetic defect of malignant hyperthermia – hypersensitive calcium-release channel of skeletal-muscle sarcoplasmic-reticulum. *Veterinary Research Communications*, **11**, 527–559.
- Otsu, K., Khanna, V. K., Archibald, A. L. and Maclellan, D. H. (1991), Cosegregation of porcine malignant hyperthermia and a probable causal mutation in the skeletal-muscle ryanodine receptor gene in backcross families. *Genomics*, **11**, 744–750.
- Otto, G., Roehe, R., Looft, H., Thielking, L., Knap, P. W., Rothschild, M. F., Plastow, G. S. and Kalm, E. (2007), Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. *Meat Science*, **75**, 185–195.
- Ovilo, C., Clap, A., Noguera, J. L., Oliver, M. A., Barragan, C., Rodriguez, C., Silo, L., Toro, M. A., Coll, A., Folch, J. M. *et al.* (2002a), Quantitative trait locus mapping for meat quality traits in an Iberian × Landrace F-2 pig population. *Journal of Animal Science*, **80**, 2801–2808.
- Ovilo, C., Fernandez, A., Noguera, J. L., Barragan, C., Leton, R., Rodriguez, C., Mercade, A., Alves, E., Folch, J. M., Varona, L. *et al.* (2005), Fine mapping of porcine chromosome 6 QTL and LEPR effects on body composition in multiple generations of an Iberian by Landrace intercross. *Genetical Research*, **85**, 57–67.
- Ovilo, C., Fernandez, A., Rodriguez, M. C., Nieto, M. and Silio, L. (2006), Association of MC4R gene variants with growth, fatness, carcass composition and meat and fat quality traits in heavy pigs. *Meat Science*, **73**, 42–47.

- Ovilo, C., Oliver, A., Noguera, J. L., Clop, A., Barragan, C., Varona, L., Rodriguez, C., Toro, M., Sanchez, A., Perez-Enciso, M. *et al.* (2002b), Test for positional candidate genes for body composition on pig chromosome 6. *Genetics Selection Evolution*, **34**, 465–479.
- Ovilo, C., Perez-Enciso, M., Barragan, C., Clop, A., Rodriguez, C., Oliver, M. A., Toro, M. A. and Noguera, J. L. (2000), A QTL for intramuscular fat and backfat thickness is located on porcine Chromosome 6. *Mammalian Genome*, **11**, 344–346.
- Ozaki, K., Ohnishi, Y., Iida, A., Sekine, A., Yamada, R., Tsunoda, T., Sato, H., Sato, H., Hori, M., Nakamura, Y. *et al.* (2002), Functional SNPs in the lymphotoxin- α gene that are associated with susceptibility to myocardial infarction. *Nature Genetics*, **32**, 650–654.
- Page, B. T., Casas, E., Heaton, M. P., Cullen, N. G., Hyndman, D. L., Morris, C. A., Crawford, A. M., Wheeler, T. L., Koohmaraie, M., Keele, J. W. *et al.* (2002), Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *Journal of Animal Science*, **80**, 3077–3085.
- Perez-Enciso, M., Clop, A., Noguera, J. L., Ovilo, C., Coll, A., Folch, J. M., Babot, D., Estany, J., Oliver, M. A., Diaz, I. *et al.* (2000), A QTL on pig chromosome 4 affects fatty acid metabolism: Evidence from an Iberian by Landrace intercross. *Journal of Animal Science*, **78**, 2525–2531.
- Pethick, D. W., Harper, G. S. and Oddy, V. H. (2004), Growth, development and nutritional manipulation of marbling in cattle: A review. *Australian Journal of Experimental Agriculture*, **44**, 705–715.
- Rempel, W. E., Lu, M. Y., Mickelson, J. R. and Louis, C. F. (1995), The effect of skeletal-muscle ryanodine receptor genotype on pig performance and carcass quality traits. *Animal Science*, **60**, 249–257.
- Reverter, A., Johnston, D. J., Ferguson, D. M., Perry, D., Goddard, M. E., Burrow, H. M., Oddy, V. H., Thompson, J. M. and Bindon, B. M. (2003), Genetic and phenotypic characterisation of animal, carcass, and meat quality traits from temperate and tropically adapted beef breeds. 4. Correlations among animal, carcass, and meat quality traits. *Australian Journal of Agricultural Research*, **54**, 149–158.
- Rexroad, C. E., Bennett, G. L., Stone, R. T., Keele, J. W., Fahrenkrug, S. C., Freking, B. A., Kappes, S. M. and Smith, T. P. L. (2001), Comparative mapping of BTA15 and HSA11 including a region containing a QTL for meat tenderness. *Mammalian Genome*, **12**, 561–565.
- Rincker, C. B., Pyatt, N. A., Berger, L. L. and Faulkner, D. B. (2006), Relationship among GeneSTAR marbling marker, intramuscular fat deposition, and expected progeny differences in early weaned Simmental steers. *Journal of Animal Science*, **84**, 686–693.
- Robertson, A. and Rendel, J. M. (1950), The use of progeny testing with artificial insemination in dairy cattle. *Journal of Genetics*, **50**, 21–31.
- Rothschild, M. F. and Ciobanu, D. C. (2003), *Novel alleles characterized by polymorphism in calpastatin gene, which are useful to genetically type animals*. Patent WO2003060151.
- Rothschild, M. F., Ramos, A., Kim, K. S., Costa Do Amaral Ramos, A. M., Ramos, A. M. C. D. and Kim, K. (2005), *Selecting a first pig by marker assisted selection of a quantitative trait loci associated with, e.g. meat or growth traits or fatness comprises determining the presence of a locus located on chromosome 17 in the first pig*. Patent WO2005001032.
- Rothschild, M. F. and Ramos, A. M. C. D. (2006), *Selecting first pig by marker assisted selection of quantitative trait locus associated with meat quality, fat and/or growth traits, by determining presence of locus on chromosome 17 in pig and selecting first pig comprising marker*. Patent WO2006101623–A2.
- Sanchez, M. P., Iannuccelli, N., Basso, B., Bidanel, J. P., Billon, Y., Gandemer, G., Gilbert, H., Larzul, C., Legault, C., Riquet, J. *et al.* (2007), Identification of QTL with effects on intramuscular fat content and fatty acid composition in a Duroc \times Large White cross. *BMC Genetics*, **8**, 55.
- Sather, A. P. and Jones, S. D. M. (1996), The effect of genotype on feedlot performance,

- carcass composition, and lean meat quality from commercial pigs. *Canadian Journal of Animal Science*, **76**, 507–516.
- Savell, J. W., Cross, H. R. and Smith, G. C. (1986), Percent ether extractable fat and moisture content of beef *longissimus* muscle as related to USDA marbling score. *Journal of Food Science*, **51**, 838, 840.
- Schaeffer, L. R. (2006), Strategy for applying genome-wide selection in dairy cattle. *Journal of Animal Breeding and Genetics*, **123**, 218–223.
- Schenkel, F. S., Miller, J. R., Jiang, Z., Mandell, I. B., Ye, X., Li, H. and Wilton, J. W. (2006), Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science*, **84**, 291–299.
- Schenkel, F. S., Miller, S. P., Ye, X., Moore, S. S., Nkrumah, J. D., Li, C., Yu, J., Mandell, I. B., Wilton, J. W. and Williams, J. L. (2005), Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science*, **83**, 2009–2020.
- Seaton, G., Haley, C. S., Knott, S. A., Kearsey, M. and Visscher, P. M. (2002), QTL Express: Mapping quantitative trait loci in of simple and complex pedigrees. *Bioinformatics*, **18**, 339–340.
- Shin, S. C. and Chung, E. R. (2007a) Association of SNP marker in the thyroglobulin gene with carcass and meat quality traits in Korean cattle. *Asian–Australasian Journal of Animal Sciences* **20**, 172–177.
- Shin, S. C. and Chung, E. R. (2007b), SNP detection of carboxypeptidase E gene and its association with meat quality and carcass traits in Korean cattle. *Asian–Australasian Journal of Animal Sciences*, **20**, 328–333.
- Shin, S. C., Kang, M. J. and Chung, E. R. (2007), Identification of a novel SNP associated with meat quality in C/EBP alpha gene of Korean cattle. *Asian–Australasian Journal of Animal Sciences*, **20**, 466–470.
- Shrestha, J. N. B. and Fahmy, M. H. (2007), Breeding goats for meat production. 3. Selection and breeding strategies. *Small Ruminant Research*, **67**, 113–125.
- Smith, S. B., Lunt, D. K., Chung, K. Y., Choi, C. B., Tume, R. K. and Zembayashi, M. (2006), Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. *Animal Science Journal*, **77**, 478–486.
- Smith, T. P., Casas, E., Rexroad, C. E., 3rd, Kappes, S. M. and Keele, J. W. (2000), Bovine CAPN1 maps to a region of BTA29 containing a quantitative trait locus for meat tenderness. *Journal of Animal Science*, **78**, 2589–2594.
- Stalder, K. J., Rothschild, M. F. and Lonergan, S. M. (2005), Associations between two gene markers and indicator traits affecting fresh and dry-cured ham processing quality. *Meat Science*, **69**, 451–457.
- Stearns, T. M., Beever, J. E., Southey, B. R., Ellis, M., McKeith, F. K. and Rodriguez-Zas, S. L. (2005), Evaluation of approaches to detect quantitative trait loci for growth, carcass, and meat quality on swine chromosomes 2, 6, 13, and 18. I. Univariate outbred F2 and sib-pair analyses. *Journal of Animal Science*, **83**, 1481–1493.
- Taniguchi, M., Utsugi, T., Oyama, K., Mannen, H., Kobayashi, M., Tanabe, Y., Ogino, A. and Tsuji, S. (2004), Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. *Mammalian Genome*, **15**, 142–148.
- Taylor, J. F., Coutinho, L. L., Herring, K. L., Gallagher, D. S., Breneman, R. A., Burney, N., Sanders, J. O., Turner, R. V., Smith, S. B., Miller, R. K. *et al.* (1998), Candidate gene analysis of GH1 for effects on growth and carcass composition of cattle. *Animal Genetics*, **29**, 194–201.
- Terwilliger, J. D. and Hiekkalinna, T. (2006), An utter refutation of the ‘Fundamental Theorem of the HapMap’. *European Journal of Human Genetics*, **14**, 426–437.
- Thaller, G., Dempfle, L., Schlecht, A., Wiedemann, S., Eichinger, H. and Fries, R. (2000), Effects of the MHS locus on growth, carcass and meat quality traits in F-2 crosses between Mangalitza and Pietrain breeds. *Archiv Fur Tierzucht – Archives of Animal Breeding*, **43**, 263–275.

- Thaller, G., Kuhn, C., Winter, A., Ewald, G., Bellmann, O., Wegner, J., Zuhlke, H. and Fries, R. (2003), DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. *Animal Genetics*, **34**, 354–357.
- Thompson, J. (2002), Managing meat tenderness. *Meat Science*, **62**, 295–308.
- Tor, M., Estany, J., Villalba, D., Cubilo, D., Tibau, J., Soler, J., Sanchez, A. and Noguera, J. L. (2001), A within-breed comparison of RYR1 pig genotypes for performance, feeding behaviour, and carcass, meat and fat quality traits. *Journal of Animal Breeding and Genetics*, **118**, 417–427.
- Uemoto, Y., Suzuki, K., Kobayashi, E., Sato, S., Shibata, T., Kadowaki, H. and Nishida, A. (2007), Effects of heart fatty acid-binding protein genotype on intramuscular fat content in Duroc pigs selected for meat production and meat quality traits. *Asian–Australasian Journal of Animal Sciences*, **20**, 622–626.
- Urban, T., Krenkova, L. and Kuciel, J. (1998), Variability of pork meat quality determined by biopsy method in live pigs in various genotypes of RYR1 gene. *Czech Journal of Animal Science*, **43**, 209–213.
- Uytterhaegen, L., Claeys, E., Demeyer, D., Lippens, M., Fiems, L. O., Boucque, C. Y., Vandevoorde, G. and Bastiaens, A. (1994), Effects of double-muscling on carcass quality, beef tenderness and myofibrillar protein-degradation in Belgian Blue white bulls. *Meat Science*, **38**, 255–267.
- Valdar, W., Solberg, L. C., Gauguier, D., Burnett, S., Klenerman, P., Cookson, W. O., Taylor, M. S., Rawlins, J. N. P., Mott, R. and Flint, J. (2006), Genome-wide genetic association of complex traits in heterogeneous stock mice. *Nature Genetics*, **38**, 879–887.
- Van Eenennaam, A. L., Li, J., Thallman, R. M., Quaas, R. L., Dikeman, M. E., Gill, C. A., Franke, D. E. and Thomas, M. G. (2007), Validation of commercial DNA tests for quantitative beef quality traits. *Journal of Animal Science*, **85**, 891–900.
- Van Kaam, J., Groenen, M. A. M., Bovenhuis, H., Veenendaal, A., Vereijken, A. L. J. and van Arendonk, J. A. M. (1999), Whole genome scan in chickens for quantitative trait loci affecting carcass traits. *Poultry Science*, **78**, 1091–1099.
- van Wijk, H. J., Buschbell, H., Dibbitts, B., Liefers, S. C., Harlizius, B., Heuven, H. C. M., Knol, E. F., Bovenhuis, H. and Groenen, M. A. M. (2007), Variance component analysis of quantitative trait loci for pork carcass composition and meat quality on SSC4 and SSC11. *Journal of Animal Science*, **85**, 22–30.
- Verner, J., Humpolicek, P. and Knoll, A. (2007), Impact of MYOD family genes on pork traits in Large White and Landrace pigs. *Journal of Animal Breeding and Genetics*, **124**, 81–85.
- Visscher, P. M., Thompson, R. and Haley, C. S. (1996), Confidence intervals in QTL mapping by bootstrapping. *Genetics*, **143**, 1013–1020.
- Warenso, E., Ingelsson, E., Lundmark, P., Lannfelt, L., Syvanen, A. C., Vessby, B. and Riserus, U. (2007), Polymorphisms in the SCD1 gene: Associations with body fat distribution and insulin sensitivity. *Obesity*, **15**, 1732–1740.
- Wheeler, T. L., Cundiff, L. V., Shackelford, S. D. and Koohmaraie, M. (2001a), Characterization of biological types of cattle (Cycle V): Carcass traits and *longissimus* palatability. *Journal of Animal Science*, **79**, 1209–1222.
- Wheeler, T. L., Shackelford, S. D., Casas, E., Cundiff, L. V. and Koohmaraie, M. (2001b), The effects of Piedmontese inheritance and myostatin genotype on the palatability of *longissimus thoracis, gluteus medius, semimembranosus*, and *biceps femoris*. *Journal of Animal Science*, **79**, 3069–3074.
- White, S. N., Casas, E., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., Riley, D. G., Chase Jr, C. C., Johnson, D. D., Keele, J. W. and Smith, T. P. L. (2005), A new single nucleotide polymorphism in CAPN1 extends the current tenderness marker test to include cattle of *Bos indicus*, *Bos taurus*, and crossbred descent. *Journal of Animal Science*, **83**, 2001–2008.
- Wibowo, T. A., Michal, J. J. and Jiang, Z. (2007), Corticotropin releasing hormone is a

- promising candidate gene for marbling and subcutaneous fat depth in beef cattle. *Genome*, **50**, 939–945.
- Wimmers, K., Murani, E., Ngu, N. T., Schellander, K. and Ponsuksili, S. (2007a), Structural and functional genomics to elucidate the genetic background of microstructural and biophysical muscle properties in the pig. *Journal of Animal Breeding and Genetics*, **124** Suppl.1, 27–34.
- Wimmers, K., Murani, E., Pas, M., Chang, K. C., Davoli, R., Merks, J. W. M., Henne, H., Muraniova, M., da Costa, N., Harlizius, B. *et al.* (2007b), Associations of functional candidate genes derived from gene-expression profiles of prenatal porcine muscle tissue with meat quality and muscle deposition. *Animal Genetics*, **38**, 474–484.
- Wood, I. A., Moser, G., Burrell, D. L., Mengersen, K. L. and Hetzel, D. J. S. (2006), A meta-analytic assessment of a thyroglobulin marker for marbling in beef cattle. *Genetics Selection Evolution*, **38**, 479–494.
- Wright, D., Kerje, S., Lundstrom, K., Babol, J., Schutz, K., Jensen, P. and Andersson, L. (2006), Quantitative trait loci analysis of egg and meat production traits in a red junglefowl × White Leghorn cross. *Animal Genetics*, **37**, 529–534.
- Zeng, Y. Q., Wang, G. L., Wang, C. F., Wei, S. D., Wu, Y., Wang, L. Y., Wang, H. and Yang, H. L. (2005), Genetic variation of H-FABP gene and association with intramuscular fat content in Laiwu Black and four western pig breeds. *Asian–Australasian Journal of Animal Sciences*, **18**, 13–16.
- Zerehdaran, S., Vereijken, A. L. J., Van Arendonk, J. A. M. and Van der Waaij, E. H. (2004), Estimation of genetic parameters for fat deposition and carcass traits in broilers. *Poultry Science*, **83**, 521–525.
- Zhang, B. Z., Lei, M. C., Deng, C. Y., Xiong, Y. H., Zuo, B. and Li, F. E. (2005), Association between PCR-RFLP polymorphism of the fifth intron in lipoprotein lipase gene and productive traits in pig resource family. *Asian–Australasian Journal of Animal Sciences*, **18**, 458–462.

Part III

Improving the quality of fresh meat: production strategies

Optimising the nutritional profile of beef

K. Nuernberg, Research Institute for the Biology of Farm Animals, Germany

Abstract: Beef is an excellent source of high biological value protein, a significant source of long-chain *n*-3 fatty acids, of vitamins (A, B₆, B₁₂, D, E) and of highly bioavailable forms of essential minerals and trace elements such as zinc, copper and iron. The relative proportion of nutrients and the fatty acid composition of adipose and muscle tissues can be affected by factors such as diet, species, fatness, age/weight, depot site, gender, breed, season and hormones. This chapter describes various nutritional approaches to enhance mainly the beneficial fatty acids in beef.

Key words: fatty acids, conjugated linoleic acids, minerals, vitamins.

14.1 Introduction: the potential to improve the nutritional profile of beef

Beef is an excellent source of high biological value protein, a significant source of long-chain *n*-3 fatty acids, of vitamins (A, B₆, B₁₂, D, E) and of highly bioavailable forms of essential minerals and trace elements such as zinc, copper and iron (Biesalski, 2005; Howe *et al.*, 2006; Scollan *et al.*, 2006a,b). In red meat, and especially beef, the essential amino acids are well balanced (in the ratio needed by humans). Therefore, meat is considered a source of high-quality protein (Pensel, 1997). Lysine and leucine are the most abundant essential amino acids found in beef, whereas the non-essential amino acids are glutamic acid and aspartic acid (Franco *et al.*, 2006). The concentration of individual amino acids is independent of breed, with a few exceptions (Hollo *et al.*, 2007). The cysteine and leucine content is positively correlated to the intramuscular fat content in bulls (Hollo *et al.*, 2007). However, over the last 10–15 years, these positive attributes have often been overshadowed due to the prominence given to several negative attributes (associations between red meat and cancer, and non-nutritional issues such as

animal health scares, e.g. BSE). Beef is seen to be a major source of saturated fat, which has been implicated in many modern lifestyle diseases, particularly coronary heart disease. In general, there is a greater demand by consumers for foods perceived as natural, fresh tasting, healthy and nutritious (Morrissey and Kerry, 2004).

Lean meat has a low intramuscular fat content, typically 1–5% and is accepted as being 'low in fat'. Marbling fat is an important meat quality trait in relation to juiciness, aroma and tenderness. This fat depot is of most interest in relation to fatty acid composition and human health. The relationships between dietary fat and incidence of lifestyle diseases, particularly coronary heart disease, are well established and this has contributed towards the development of specific guidelines from various nutrition and health organisations in relation to fat in the diet (WHO, 2003; DGE, 2004). The recommendation for fat intake is 15–30% of the total daily energy intake. Reducing the intake of saturated fatty acids, SFA (which is known to raise total and low-density lipoprotein cholesterol) to <10%, and increasing the intake of *n*-3 polyunsaturated fatty acids (PUFA) to <1–2% of total daily energy intake, is particularly encouraged. Among the *n*-3 PUFA, eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3) are the most protective for cardiovascular health (Warburg, 2004; Mozaffarian and Rimm, 2006; Wang *et al.*, 2006). Meat, fish, fish oils and eggs are important sources of these *n*-3 PUFA for man. Beef and lamb contributed 28% of the total long-chain *n*-3 fatty acid intake of adults in Australia (Howe *et al.*, 2006). A similar recent investigation in the UK estimated the potential mean intake of LC*n*-3PUFA (EPA, DHA) as 105.4 mg/day/person from meat and meat products and 231 mg/day in total from enriched animal-derived foods including milk (Givens and Gibbs, 2006).

This has resulted in increased attention being devoted to increasing these fatty acids in other important food sources. Important also is the balance between *n*-6 and *n*-3 fatty acids in the diet because α -linolenic acid (ALA) can provide sufficient amounts of tissue EPA and DHA through the *n*-3 PUFA elongation-desaturation pathway (Williams and Burdge, 2006). Another PUFA group of interest is conjugated linoleic acid (CLA) isomers. Many beneficial effects have been attributed to CLA in prevention of atherosclerosis, different types of cancer, and hypertension, and in improving immune function (Bhattacharya *et al.*, 2006; Park and Pariza, 2007) but further investigations are needed to clarify results according to the impact of individual CLA isomers. Beef and other ruminant products such as milk are natural dietary sources of CLA.

While animal production factors such as nutrition, genetics and environment have little influence on protein content and amino acid profiles, fat content and fatty acid composition in cattle may be altered and there are many papers reporting the effects of feeding on lipid composition of the meat (Nuernberg *et al.*, 2005; Mir *et al.*, 2006; Smith *et al.*, 2006; Sami *et al.*, 2004, 2006; Dannenberger *et al.*, 2007; Noci *et al.*, 2007a; Moloney, 2007). Hence, this chapter is primarily concerned with progress in altering the fatty acid composition of beef.

14.2 Optimising the nutritional profile of beef

The relative proportion of nutrients and the fatty acid composition of adipose and muscle tissues can be affected by factors such as diet, species, fatness, age/weight, depot site, gender, breed, season and hormones (see reviews [Nuernberg and Ender, 1998](#); [Wood et al., 2004](#); [DeSmet et al., 2004](#)). The fatty acid distribution differs between various tissues, including intra- and intermuscular, as well as abdominal (e.g. perirenal, omental) and subcutaneous fat. This chapter will be focused mainly on intramuscular fat and less on subcutaneous fat.

14.2.1 Effect of genetic factors and growth (age/weight)

Genetic variability consists of differences between breeds, lines/crosses or individuals. Genetic factors affect beef fatty acid profile to a lower extent than diet. The effects of age and weight of animals on fatty acid contribution are related to body fatness ([Nuernberg et al., 1999](#); [DeSmet et al., 2004](#); [Scollan et al., 2006a](#)). The main change with ageing and growth of animals is an increase in total body fat and in adipocyte diameter ([Wegner et al., 1998](#); [Albrecht et al., 2006](#)). Growth from birth to slaughter at 24 months was accompanied by an increase in fat deposition, first in subcutaneous fat and later in intramuscular fat, and a continuous rise in the relative amount of SFA ([Enser, 1991](#); [Nuernberg et al., 1999](#)). [Nuernberg et al. \(1999\)](#) studied the intramuscular fatty acid composition in three cattle breeds (German Holstein, Galloway, Belgian Blue) during growth. At 18 months of age, the Belgian Blue contained the lowest intramuscular fat with highest PUFA proportion and the lowest SFA content. The variation in fat content affects the fatty acid profile. The result of *de novo* fatty acid synthesis is lauric (C12:0), myristic (C14:0) and palmitic acids (C16:0). With increasing fatness during growth, the deposition of SFA into adipocytes increases, whilst the relative proportion of phospholipids decreases, leading to an alteration of the PUFA/SFA ratio (P/S ratio). The amount of phospholipids is relatively constant between 0.4 and 0.6% in muscle ([Wood et al., 1999](#); [Lorenz et al., 2002](#); [Dannenberger et al., 2007](#)). The phospholipids, as the structural components of cellular membranes, have a high PUFA content, whereas triacylglycerols have a lipid storage function and low PUFA concentration ([Dannenberger et al., 2007](#)). The relationship between P/S ratio and the intramuscular fat of *longissimus* muscle in cattle is shown in [Fig. 14.1](#) (taken from [DeSmet et al., 2004](#)). The low muscle fat level (< 1%) of double-muscled White Blue Belgian bulls explains the high P/S ratio of 0.5–0.6 ([Raes et al., 2003](#)).

[DeSmet et al. \(2004\)](#) used a set of literature data from different breeds and feeding regimes ($n = 113$), and the intramuscular fat content accounted for 85% of the variation in the P/S ratio.

Breed differences in intramuscular fat and subcutaneous fat are generally small at normal slaughter weights and are often associated with the fatness of animals ([Table 14.1](#)). The deposition of fat reflects differences in the balance between uptake of exogenous fatty acids, *de novo* synthesis and degradation of

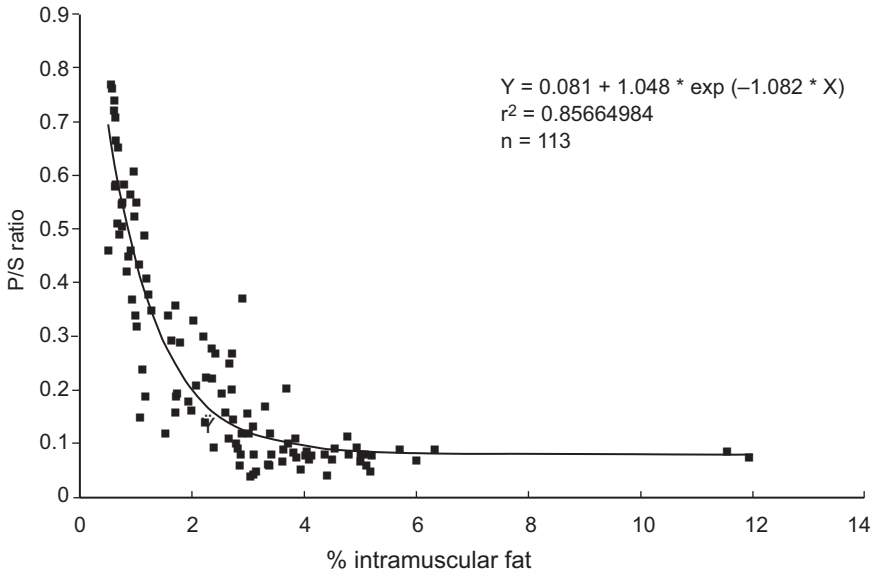


Fig. 14.1 Relationship between P/S ratio and intramuscular fat (%) in beef *longissimus* muscle (DeSmet *et al.*, 2004).

triacylglycerols. The Japanese Black Wagyu breed is known for its extensive marbling and comparatively small amount of external fat. Subcutaneous and intramuscular fat of purebred Black Wagyu steers (Sturdivant *et al.*, 1992; Oka *et al.*, 2002; Chung *et al.*, 2006) or crossbred cattle (Kazala *et al.*, 1999; Elias Calles *et al.*, 2000; Mir *et al.*, 2003, 2004) is enriched in monounsaturated fatty acids (MUFA) and has a higher MUFA/SFA ratio compared to Angus or European crossbred beef cattle. High oleic acid is desirable for human health (Wahle *et al.*, 2004a) and some studies have reported a positive association between desirable flavour and oleic acid proportion (Melton *et al.*, 1982; Mandell *et al.*, 1997). However, the high intramuscular fat content, the low P/S ratio and low *n*-3 fatty acid concentration of beef from Wagyu cattle can negatively affect the nutritional value.

The effect of breed and growth potential on the fatty acid profile of subcutaneous and intramuscular fat were reported by Flachowsky *et al.* (1995), Laborde *et al.* (2001), Pitchford *et al.* (2002); Huerta-Leidenz *et al.* (1993), Nuernberg *et al.* (1999), Moreno *et al.* (2008) and Indurain *et al.* (2006). Jersey cattle deposited higher C18:0, SFA, MUFA and C16:1 concentrations compared to Limousin cattle (Siebert *et al.*, 2003). The authors demonstrated that the Δ^9 desaturase activity was higher in Jersey-sired cattle. Direct comparison of results from different studies is difficult because the diet, as the main effect on fat composition, is frequently different.

Steers and heifers are fatter than bulls (Enser, 1991). Malau-Aduli *et al.* (1998,

Table 14.1 Effect of breed on the fatty acid composition of intramuscular fat in *longissimus* muscle in cattle

	Simmental ¹ steers	Red Angus ¹ steers	HF of New Zealand ² steers	European Descent ² steers	Belgian Blue × ² HF steers	German HF ³ bulls	German Simmental ³ bulls
SFA	44.13	46.22	44.70	46.31	48.81	43.61	44.49
MUFA	48.53	46.11	46.73	45.01	43.22	43.00	37.28
PUFA	5.51	6.20	3.31	3.27	4.18	7.47	9.07
IMF	5.73	4.37	7.25	6.58	3.07	2.7	2.6

¹ Laborede *et al.*, 2001; ² Moreno *et al.*, 2008; ³ Nuernberg *et al.*, 2005.

2000) reported differences in muscle phospholipid and triacylglycerol composition between heifers and steers. Steers accumulated more palmitic acid and MUFA and less PUFA in muscle compared to heifers. Breed and sex differences in muscle *n*-6 PUFA or *n*-3 PUFA concentrations are usually small (Mir *et al.*, 2000; Labord *et al.*, 2001; Knight *et al.*, 2003; Dannenberger *et al.*, 2006).

14.2.2 Effect of feeding on fat content and composition (except CLA and TVA)

Consumer requirements for food that is safe, healthy and of consistent eating quality are increasing. For beef, much attention has been given to lipids. The most effective means of manipulating the lipid and its fatty acid composition is through nutrition, by strategic use of forages and dietary lipids. Dietary recommendations are to reduce consumption of fat, SFA and *trans* fatty acids and to increase consumption of *n*-3 fatty acids to give a more favourable balance between *n*-6 and *n*-3 fatty acids (see introduction). The beneficial effects of *n*-3 fatty acids, particular the long-chain *n*-3 fatty acids (LC*n*-3PUFA) are well documented and include anti-atherogenic, anti-arrhythmic, anti-thrombotic and anti-inflammatory effects (Chong *et al.*, 2006; Givens *et al.*, 2006). Beside seafood, meat is a major source of LC*n*-3PUFA for Australians (43%, Howe *et al.*, 2006).

There are many nutritional approaches to enhance beneficial fatty acids in ruminant fat (see reviews Wood *et al.*, 2003; Scollan *et al.*, 2006a; Givens *et al.*, 2006). Dietary sources of *n*-3 fatty acids include fish oil (EPA and DHA), microalgae (EPA, DHA), plant seed and oils (walnut, rapeseed, linseed, olive; ALA) and forages (ALA). While most of the linoleic acid (C18:2*n*-6, LA) and ALA in feed is hydrogenated in the rumen, a small proportion can escape and, once absorbed, is available for incorporation in tissues. The SFA are the result of the biohydrogenation by rumen bacteria of dietary unsaturated fat. Biohydrogenation of PUFA in the rumen is reduced with high concentrate diets (Doreau and Ferlay, 1994). Loor *et al.* (2005a) reported 92%, 85% and 75% hydrogenation of LA in cows fed 5% sunflower oil, linseed oil and fish oil, respectively, for 4 weeks. Cows

fed linseed oil had 95% hydrogenation of ALA compared to 84% for fish or sunflower oil. Biohydrogenation of EPA and DHA in fish oil averaged 92 and 89%. Despite this biohydrogenation, a proportion of dietary PUFA bypasses the rumen intact and can be absorbed and deposited.

Plants have the unique ability to synthesise *de novo* ALA, which is the substrate for the synthesis of EPA and DHA by elongation and desaturation. The formation of LCn-3PUFA by marine algae and their transfer through the food chain to fish causes the high content of these important fatty acids in fish oil. Inclusion of fish oil or micro-algae in the diet of steers increases the EPA and DHA content in muscle lipids (Scollan *et al.*, 2001, Kook *et al.*, 2002). Increasing fish oil (0, 10, 20 or 40 g/kg concentrate) had no impact on SFA, MUFA or PUFA of intramuscular and adipose tissue, EPA and DHA were increased (Noci *et al.*, 2007a). These results are consistent with Choi *et al.* (2000) and Scollan *et al.* (2001). Both fish oil and micro-algae are effective in enhancing the concentration of LCn-3PUFA; however, there are concerns that the oxidative stability is reduced and there are negative effects on flavour. Peroxidation can be prevented by antioxidant inclusion.

In northern Europe, an important feed for beef cattle is fresh grass (Givens, 2005). Forages such as grass or clover contain a high proportion of ALA and 10–16% LA (Nuernberg *et al.*, 2005; Elgersma *et al.*, 2003). The fatty acid content of fresh grass is very low on a dry matter basis (2–2.5%) and fatty acids are mainly present in an esterified form (98%). In silages, 27–73% of total fatty acids are present as free fatty acids (Elgersma *et al.*, 2003). It has been found that the amount of ALA in forages varies with species, cutting date, cutting interval, growth stage, fertilisation and conservation (Boufaied *et al.*, 2003; Clapham *et al.*, 2005; Dewhurst *et al.*, 2006). Forages are a good source of ALA to enhance *n*-3 fatty acids in beef but there are effects on the biohydrogenation process in the rumen (Palmquist *et al.*, 2005; Dewhurst *et al.*, 2006). Studies in dairy cows (Dewhurst *et al.*, 2003) and in steers (Lee *et al.*, 2003) identified more extensive ruminal biohydrogenation of ALA in grass silage compared to white clover silage because of the higher ruminal passage kinetics of white clover.

Several studies (Table 14.2) have shown an increase in *n*-3 fatty acid proportions and a reduced ratio of *n*-6/*n*-3 PUFA in muscle from bulls, steers or heifers consuming grass or grass silage compared to those consuming concentrate diets. The proportion of grass in the diet (French *et al.*, 2000), the type of grass/grass silage (Noci *et al.*, 2007a) and the length of time on grass before slaughter (Noci *et al.*, 2005a) affect the total fatty acid composition of muscle. Decreasing concentrate in the diet of grazing cattle and effectively increasing grass intake caused a decrease in SFA proportion and an increase of *n*-3 fatty acids in muscle fat of steers (French *et al.*, 2000, Table 14.2). Wilting of grass prior to ensiling (unwilted v wilted) did not affect the *n*-3 fatty acids or the *n*-6/*n*-3 PUFA ratio in muscle and adipose tissue of steers (Noci *et al.*, 2007a). Increasing the duration of grazing before slaughter of heifers led to a significant decrease in SFA in muscle and subcutaneous fat, a simultaneous increase in the *n*-3 fatty acid proportion and a decrease in *n*-6/*n*-3 PUFA ratio in both tissues, from 2.21 to 1.46 and from 2.64

Table 14.2 Influence of different diets on *n*-3 fatty acids, the ratio between the sum of *n*-6/*n*-3 fatty acids, CLA*cis*-9,*trans*-11 and C18:1*trans*-11 in beef muscle (g/100 g total fatty acids)

	Animal	C18:3 <i>n</i> -3 concentrate	Grass/ GS ⁵	<i>n</i> -6/ <i>n</i> -3 ratio concentrate	Grass/ GS ⁵	CLA <i>cis</i> -9, <i>trans</i> -11 concentrate	Grass/ GS ⁵	C18:1, <i>trans</i> -11 concentrate	Grass/ GS ⁵
Nuernberg <i>et al.</i> , 2005	German Simmental bulls	0.46a	2.22b	8.34a	2.04b	0.72a	0.87b	3.19a ¹	4.28b
Nuernberg <i>et al.</i> , 2005	German Holstein bulls	0.34a	1.67b	6.49a	1.94b	0.75a	0.84b	2.83a ¹	4.37b
Noci <i>et al.</i> , 2005a	Charolais crossbred heifers	1.03a	1.29b ²	2.21a	1.46b	0.50a	0.71b	1.35a	3.01b
French <i>et al.</i> , 2000	Crossbred steers	0.71	1.13	3.61	2.33	0.47	1.08	No values	
Realini <i>et al.</i> , 2004	Hereford steers	0.35a	1.34b	3.00a	1.44b	0.23a	0.41b	No values	
Hollo <i>et al.</i> , 2005	Hungarian Grey bulls	0.61a	3.21b	6.24a	2.86b	0.36	0.81b	0.57	0.81
Laborde <i>et al.</i> , 2002	Crossbred steers	0.35a	0.47b ³	4.53a	3.10b	0.32a	0.35b	No values	
Steen <i>et al.</i> , 2003	Heifers	0.70a ⁴	2.30b	5.90a	1.70b	No values			
Steen <i>et al.</i> , 2003	Steers	0.80a	3.50b	6.10a	1.50b	No values			

¹ Sum of the isomers C18:1*trans*-6-*trans*-11.

² 158 days on grass before slaughter.

³ Steers were fed 112 days 100% alfalfa silage followed by high grain to slaughter.

⁴ Calculated ALA = total *n*-3 fatty acid – LC*n*-3PUFA.

a,b Significant differences between feeding groups at $p \leq 0.05$.

⁵ GS = grass silage.

to 1.65, respectively (Noci *et al.*, 2005b; Table 14.2). Feeding fresh grass or grass silage/concentrate supplemented with crushed linseed compared to maize silage/concentrate resulted in a higher *n*-3 fatty acid concentration in total fat and in both triacylglycerols and polar fraction of muscle, in liver, heart and subcutaneous fat (Nuernberg *et al.*, 2005, Dannenberger *et al.*, 2004, 2005, Table 14.2). Higher proportions of all individual *n*-3 fatty acids in muscle phospholipids were also reported by Warren *et al.* (2002). Forage-fed Limousin-cross steers incorporated higher ALA and lower oleic acid in beef compared to grain-fed steers (Mandell *et al.*, 1997). Retail beef from two different grass-based feeding systems contained higher concentrations of *n*-3 fatty acids than beef from an intensive production system (Razminowicz *et al.*, 2006).

Feeding mixtures of grass and red clover relative to grass alone increased the incorporation of ALA in the muscle of finishing Charolais steers and resulted in a decrease in the *n*-6/*n*-3 PUFA ratio (Scollan *et al.*, 2006b). Companion investigations showed that red clover reduced the ruminal biohydrogenation of PUFA, which is possibly related to its polyphenol oxidase activity (Lee *et al.*, 2004). The effects of polyphenol oxidase involve protection of plant lipids from lipolysis as well as denaturation of plant lipases (Dewhurst *et al.*, 2006).

Feeding plant oils and oilseeds is a common nutritional means to manipulate the fatty acid composition of beef (Enser *et al.*, 1999; Mir *et al.*, 2003; Khanal and Olsen, 2004; Noci *et al.*, 2007b). Concentrates used in cattle feeding contain less than 5% and oilseeds 20–50% lipids on a dry matter basis. Including sunflower oil in the concentrate led to an increase in *n*-6 fatty acids in muscle fat, mainly because of the higher LA proportion (Noci *et al.*, 2005b; Mir *et al.*, 2003). Linseed/linseed oil (Scollan *et al.*, 2001; Raes *et al.*, 2003; Nuernberg *et al.*, 2005, Mach *et al.*, 2006) and rapeseed/rapeseed oil (Scheeder *et al.*, 2001; Komprda *et al.*, 2002; Mach *et al.*, 2006) increased the *n*-3 fatty acids and lowered the *n*-6/*n*-3 ratio. The infusion of sunflower or linseed oil into the duodenum of crossbred Salers × Charolais fattening steers altered the composition and distribution of plasma lipids and increased the concentration of PUFA (Scislowski *et al.*, 2005).

There are opportunities to accumulate ALA and LC*n*-3PUFA in beef by feeding forages, plant seeds or oils that contain ALA (linseed, rapeseed). Ruminants will desaturate and elongate ALA *in vivo* (albeit to a small extent) to increase the concentration of the long chain *n*-3 fatty acids and thus enrich the meat. The human intake of ALA and LC*n*-3 PUFA can be increased by consuming the enriched beef. If the beef is modified by dietary manipulation to higher PUFA concentrations, protection of these lipids against peroxidation is necessary.

14.2.3 Factors influencing the conjugated linoleic acid and C18:1*trans* isomers in beef

In recent years, CLA isomers have received much attention due to their potential beneficial effects on human health (Wahle *et al.*, 2004b; Schmid *et al.*, 2006; Tricon and Yaqoob, 2006). Many beneficial effects have been attributed to CLA in the prevention of different types of cancer, atherosclerosis, hypertension, obesity

Table 14.3 Concentration (mg/100 g fresh muscle) of CLA cis-9 , trans-11 and C18:1 trans-11 in *longissimus* muscle of different beef genotypes

Breed/sex	Diet	CLA cis-9 , trans-11 (mg/100 g)	Intram. fat (%)	References
Wagye, steers	6% Sunflower oil	134	10.4	Mir <i>et al.</i> , 2004
Wagye \times Limousin, steers	6% Sunflower oil	76	6.4	Mir <i>et al.</i> , 2004
Limousin, steers	6% Sunflower oil	59	4.8	Mir <i>et al.</i> , 2004
German Simmental, bulls	Grass/grass silage	12	1.5	Dannenberger <i>et al.</i> , 2006
German Holstein, bulls	Grass/grass silage	17	2.3	Dannenberger <i>et al.</i> , 2006
Crossbred steers,	Soyoil	43.1	4.6	Lorenzen <i>et al.</i> , 2007
Crossbred steers,	Pasture	19.4	3.7	Lorenzen <i>et al.</i> , 2007
Belgian Blue, bulls	Crushed linseed	4.3	0.98	Raes <i>et al.</i> , 2004
Belgian Blue, bulls	Extruded linseed	4.2	1.01	Raes <i>et al.</i> , 2004
Crossbred steers	High forage	35	3.4	Steen and Porter, 2003
Crossbred steers	High concentrate	15	4.4	Steen and Porter, 2003

and enhancement of the immune system in both animals and humans (see review [Bhattacharya et al.](#), 2006). The main dietary source of CLA is ruminant meat, milk and their products. It is known that CLA cis-9 , trans-11 is formed from two sources. One source is from ruminal biohydrogenation of LA to stearic acid in the rumen by *Butyrivibrio fibrisolvens* and other bacteria (Kepler *et al.*, 1966; Jenkins, 1993). Very recently, Fukuda *et al.* (2006a,b) found two new strains of *Butyrivibrio fibrosolvens*. One strain (MDT-10) has great ability to hydrogenate LA to C18:1 trans-11 (TVA), and the other strain (MDT-5) rapidly isomerises LA and ALA to CLA cis-9 , trans-11 and to C18:3 cis-9 , trans-11 , cis-15 . The authors hypothesised that the introduction of MDT-10 to the rumen might increase the amount of absorbed TVA and therefore increase the conversion of TVA to CLA cis-9 , trans-11 in tissues (Fukuda *et al.* 2006a,b). The second source is the endogenous conversion of TVA by Δ^9 -desaturase in the mammary gland of dairy cows and ruminant adipose tissue (Griinari *et al.*, 2000; Kay *et al.*, 2004). TVA is a common intermediate produced during ruminal biohydrogenation of ALA and LA (Destailats *et al.*, 2005; Bessa *et al.*, 2000). Griinari *et al.* (2000) demonstrated that endogenous synthesis of CLA cis-9 , trans-11 from TVA represented the primary source in the milk fat of lactating cows. More recently, Mosley *et al.* (2006) confirmed the conversion of dietary TVA to CLA cis-9 , trans-11 in the mammary gland (catalysed by Δ^9 -desaturase) using ^{13}C -labeled TVA. There appears to be a relationship between TVA and CLA in different tissues (Enser *et al.*, 1999; Shen *et al.*, 2007).

Tables 14.2 and 14.3 reflect the high variation of the CLA concentration in beef due to variation in diet, breed and gender. However, the amount of CLA in beef is small. Breeds with high intramuscular fat content will deliver a greater concentra-

tion (Wagye, 134 mg/100 g muscle) compared to breeds with low muscle fat (Belgian Blue, 4 mg/100 g muscle).

The CLA and TVA content in beef can be increased by different dietary strategies (Khanal and Dhiman, 2004; Collomb *et al.*, 2006; De La Torre *et al.*, 2006; Schmid *et al.*, 2006; Lorenzen *et al.*, 2007). Diets containing high levels of ALA (forages) and LA (grains, plant oils) cause an increased deposition of CLA in beef (Enser *et al.*, 1999; French *et al.*, 2000; Mir *et al.*, 2003; Aharoni *et al.*, 2004; Nuernberg *et al.*, 2005; Scollan *et al.*, 2006a).

The effect of fish oil on the lipid metabolism of steers is to inhibit the transition of TVA or vaccenic acid to stearic acid in the rumen resulting in a build-up of this intermediate in the biohydrogenation pathway of C18 PUFA (Lee *et al.*, 2005).

Very recently Griinari and Shingfield (2006) established that the CLAcis-9, *trans*-11 accumulation in ruminant lipids can be increased up to ten fold by feeding diets that promote the production of TVA in the rumen. The focus should be on ruminal formation of TVA rather than CLAcis-9, *trans*-11. The amount and composition of lipid supplements, as well as the composition of the basal diet and the concentrate/forage ratio, are important factors regulating the extent of biohydrogenation and the formation of different intermediates for further biosynthesis of CLA in beef and milk (Dewhurst *et al.*, 2006). Increasing the concentrate:forage ratio in the diet resulted in a higher concentration of C18:1*trans*-10 in rumen bacteria (Piperova *et al.*, 2000, 2002; Vlaeminck *et al.*, 2006). A shift to C18:1*trans*-10 is related to a decrease in milk fat synthesis (Griinari *et al.*, 1998). High forage and the addition of 3% linseed oil increased the TVA yield in milk (Loor *et al.*, 2005b). The TVA proportion in total *longissimus* muscle lipids of crossbred Charolais steers increased from 1.35% (without grazing before slaughter) to 3.01% (158 days on pasture before slaughter; Noci *et al.*, 2005a). Feeding strategies with high forage:concentrate ratios or exclusively grass-based diets could induce changes in ruminal conditions, such as pH, available carbohydrates, and ruminal flow, which enhance the C18:1*trans*-11 production as a precursor of *de novo* synthesis of CLAcis-9, *trans*-11. Increasing inclusion of sunflower oil in concentrates led to a linear enhanced incorporation of CLAcis-9, *trans*-11 in the intramuscular fat of muscle (Noci *et al.*, 2005b). Beef from steers fed increasing fish oil supplement (0, 10, 20, 30 g/kg) accumulated increasing TVA and CLAcis-9, *trans*-11 in the total lipids of the *longissimus* muscle (Noci *et al.*, 2007b). Heifers offered pasture supplemented with sunflower oil had a greater proportion of TVA and CLAcis-9, *trans*-11 in total muscle fat compared to those on unsupplemented grazing or grazing with linseed oil supplement (Noci *et al.*, 2007a).

In general, *trans* fatty acids are thought to be detrimental to health, particularly to cardiovascular function (Stender and Dyerberg, 2003; Mozaffarian *et al.*, 2006). *Trans* fatty acids include a wide range of fatty acids, with isomers of C18:1 and both conjugated and non-conjugated C18:2 as major components. A reduction in the daily consumption of *trans* fatty acids to below 1% is recommended (Willet, 2006). The main C18:1*trans* isomer in ruminant meat is C18:1*trans*-11. Corl *et al.* (2003) and Lock *et al.* (2004) reported an anti-carcinogenic effect of TVA in rats.

More research is needed to differentiate between the metabolic and health effects of different C18:1 *trans* isomers of industrial and ruminant origin and the impact of different CLA isomers.

14.3 Optimising the quantity of vitamins and micronutrients in beef

The main factors influencing the acceptability and eating quality of meat are tenderness, colour, freshness, water-holding capacity, oxidative stability and flavour. Intramuscular fat and the desaturation of lipids have a major influence on the quality of beef. Modifying the fatty acid composition of beef has consequences for anti-oxidative capacity. One of the main factors limiting the quality and acceptability of beef and beef products is lipid oxidation. In raw beef, this process results in the formation of brown pigments (myoglobin oxidation), increased drip loss and the development of rancid odours and flavours (Morrissey and Kerry, 2004). Feeding cattle with lipid supplements rich in PUFA led to higher levels of PUFA in blood which are the preferential targets of free radicals and may activate peroxidation processes (Durand *et al.*, 2005). The sensitivity of PUFA to peroxidation depends on the level of antioxidants, especially vitamin E, a nutrient important both for the health of animals and for the stability of the lipids. To enhance meat quality, vitamin E has been incorporated in cattle diets at levels ranging from 1000 to 2500 IU/head/day of *all-rac*-tocopheryl acetate (Sanders *et al.*, 1997; Schwarz *et al.*, 1998; Yang *et al.*, 2002). The level of consumption and the duration of supplementation increased the tissue concentration of vitamin E in cattle (Liu *et al.*, 1995). These authors suggest that a daily allowance of vitamin E of 500 IU/steer for 126 days has benefits for the colour display life of beef. Pasture-fed beef, while having higher PUFA concentrations also contains more α -tocopherol, carotenoids and flavonoids which stabilise the PUFA (Scollan *et al.*, 2006a). Beef from cattle finished on grass had higher vitamin E contents and lower lipid oxidation when compared to beef from cattle finished on concentrate (Mercier *et al.*, 2004; Descalzo *et al.*, 2000, 2007; Realini *et al.*, 2004). Grazing cattle showed increased concentrations of α -tocopherol in liver, muscles, and fatty tissue, similar to those obtained when grain-fed cattle were supplemented with supra-nutritional (2500 IU/head/day for 132 days prior slaughter) doses of vitamin E (Yang *et al.*, 2002). The objective of supplementation with supra-nutritional levels of vitamin E is to achieve sufficient tissue concentration of α -tocopherol to maximise the anti-oxidative efficiency and to protect not only against lipid peroxidation but also myoglobin oxidation (Morrissey and Kerry, 2004). Feeding ground rape seed to bulls enhanced significantly the vitamin E content in muscle, blood and fatty tissue (Flachowsky *et al.*, 1994). The target level of vitamin E for extended colour and lipid stability in raw muscle should be 3.3 mg α -tocopherol/kg (Arnold *et al.*, 1993). The improvements in meat quality include reduction of thiobarbituric acid reactive substances (TBARS, an indicator of rancidity and off-flavours) scores to below 0.5 mg malondialdehyde equivalents, which is the

borderline level for detection of off-flavours by trained sensory panellists (Dunshea *et al.*, 2005).

Further anti-oxidative substances are ascorbic acid and carotenoids. Ascorbic acid regenerates vitamin E in living animals. Because of its ability to promote lipid oxidation by reducing transition metal ions *in vitro*, dietary supplementation with ascorbate is of questionable value (Morrissey and Kerry, 2004). Radical quenching and anti-oxidative activity of carotenoids have been reported (Paiva and Russell, 1999) but more research is needed to clarify the anti-oxidative/pro-oxidative phenomenon reported by Krinsky and Johnson (2005). In cattle, β -carotene is essentially the only carotenoid absorbed from the intestine (Yang *et al.*, 1992). Grazing of cattle increased the β -carotene concentration in different tissues compared to grain-fed animals (Yang *et al.*, 2002). Supplementation of supra-nutritional doses of vitamin E decreased the β -carotene content in pasture-fed steers. The authors concluded that high vitamin E levels may interfere with the absorption of β -carotene.

Meat is an excellent source of some micronutrients such as iron, zinc, selenium, vitamin A, vitamin B₁₂ and folic acid. Vitamin A and B₁₂ cannot be compensated by plant-derived pro-vitamins. Iron as heme iron in meat (especially liver) has a much higher bioavailability than iron contained in plants (Biesalski, 2005). Meat and liver (100 g/day) can supply up to 50% of the recommended daily intake (DGE, 2004) for iron, zinc, selenium, vitamins B₁₂, B₁, B₂, B₆, and 100% of vitamin A (Biesalski, 2005). Kirchgessner *et al.* (1995) investigated the B vitamin concentrations of different carcass cuts in bulls, heifers and steers. The authors reported an average vitamin B₆ content of 2.6 mg (bulls), 3.1 mg (heifers) and 3.0 mg (steers) per kg fresh lean tissue of the foreloin. In Italy, meat and meat products supply 20, 47, 48, and 28% of selenium, zinc, niacin and thiamin daily requirements, respectively (Lombardi-Boccia *et al.*, 2004).

Dietary selenium affects the selenium concentration in the muscle of beef. When provided as organically bound selenium for 126 days, a high selenium concentration in the diet increased the selenium content in the muscle of steers (1.33 v 3.32–4.41 ppm), and did not negatively affect the performance of finishing beef steers (Lawler *et al.*, 2004). The concentration of selenium in beef is related to the selenium content of soil ($r = 0.53$, $p < 0.01$, Hintze *et al.*, 2002). Under special circumstances (low concentration of minerals in the feed), supplementation of trace minerals is required to meet the daily allowance for cattle feeding. Supplementation of trace minerals is essential to ensure optimal growth, health and reproduction of the animals.

11.4.4 Future trends and conclusions

Different feeding strategies (grass-based diets, oil seeds or free oil supplements, rumen protected lipids) can improve the nutritional value of beef. Advances in genomic technologies are likely to provide future opportunities in the quest for achieving marbling deposition and will supply knowledge on the control of muscle

and adipose tissue fat composition. Polymorphisms in some key genes have been related to beef quality variables (Kühn *et al.*, 2005; Hocquette *et al.*, 2007). If the concentration of SFA can be reduced and replaced with fatty acids with proven health benefits, it could be expected that consumers would look more favourably on animal products (Ponnampalam *et al.*, 2006).

Ruminant meat production systems are often based on intensive use of high concentrate rations. Inclusion of greater amounts of forage is needed to increase beneficial fatty acids in beef.

Whilst there is now much evidence to show that it is possible to enhance the beneficial fatty acids in fresh and processed beef, there are some serious concerns about human health attributes (milk and CVD, beef and cancer, etc.). Research is urgently required to fully characterise the benefits associated with consumption of fatty acid enriched beef. In future, the role of animal feed composition in producing beef and beef products closer to the optimum composition for human health will become increasingly important.

14.5 References

- Aharoni Y., Orlov A., Brosh A. (2004), Effects of high-forage content and oilseed supplementation of fattening diets on conjugated linoleic acid (CLA) and *trans* fatty acids profiles of beef lipid fractions. *Anim. Feed Sci. Technol.*, 117, 43–60.
- Albrecht E., Teuscher F., Ender K., Wegner J. (2006), Growth- and breed-related changes of marbling characteristics in cattle. *J. Anim. Sci.*, 84, 1067–1075.
- Arnold R.N., Arp S.C., Scheller K.K., Williams S.N., Schaeffer D.M. (1993), Tissue equilibrium and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *J. Anim. Sci.*, 71, 105–118.
- Bessa R.J.B., Santos-Silva J., Ribeiro J.M.R., Portugal A.V. (2000), Reticulo-rumen biohydrogenation and the enrichment of ruminant edible products with linoleic acid conjugated isomers. *Livest. Prod. Sci.*, 63, 201–211.
- Bhattacharya A., Banu J., Rahman M., Causey J., Fernandes, G. (2006), Biological effects of conjugated linoleic acids in health and disease. *J. Nutritional Biochemistry*, 17, 789–810.
- Biesalski, H.-K. (2005), Meat as a component of a healthy diet – Are there risks or benefits if meat is avoided in the diet? *Meat Sci.*, 70 (3), 509–524.
- Boufaied H., Chouinard P.Y., Tremblay G.F., Petit H.V., Michaud R., Belanger G. (2003), Fatty acids in forages. 1. Factors affecting concentrations. *Can. J. Anim. Sci.*, 83, 501–511.
- Choi N.J., Enser M., Wood J.D., Scollan N.D. (2000), Effect of breed on the deposition in beef muscle and adipose tissue of dietary *n*-3 polyunsaturated fatty acids. *Anim. Sci.*, 71, 509–519.
- Chong E.W.-T., Sinclair A.J., Guymer R.H. (2006), Facts on fat. *Clinical and Experimental Ophthalmology*, 34, 464–471.
- Chung K.Y., Lunt D.K., Choi C.B., Chae S.H., Rhoades R.D., Adams, T.H., Booren B., Smith S.B. (2006), Lipid characteristics of subcutaneous adipose tissue and *M. longissimus thoracis* of Angus and Wagyu steers fed to US and Japanese endpoints. *Meat Sci.*, 73, 432–441.
- Clapham W.M., Foster J.G., Neel J.P.S., Fedders J.M. (2005), Fatty acid composition of traditional and novel forages. *J. Agric. Food Chem.*, 53, 10068–10073.
- Collomb M., Schmid A., Sieber R., Wechsler D., Ryhanen E.L. (2006), Conjugated linoleic acids in milk fat: Variation and physiological effects. Review. *Internat. Dairy J.*, (16) 1347–1361.

- Corl B.A., Barbano D.M., Bauman D.E., Ip C. (2003), *Cis*-9, *trans*-11 CLA derived endogenously from *trans*-11 18:1 reduces cancer risk in rats. *J. Nutr.*, 133, 2893–2900.
- Dannenberger D., Nuernberg G., Scollan N., Ender K., Nuernberg K. (2004), Effect of diet on the deposition of n-3 fatty acids, conjugated linoleic and C18:1 *trans* fatty acid isomers in muscle lipids of German Holstein bulls. *J. Agric. Food Chem.*, 52, 6607–6615.
- Dannenberger D., Nuernberg G., Scollan N., Ender K., Nuernberg K. (2007), Diet alters the fatty acid composition of individual phospholipids classes in beef muscle. *J. Agric. Food Chem.*, 55, 452–460.
- Dannenberger D., Nuernberg K., Nuernberg G., Scollan N., Steinhart H., Ender K. (2005), Effect of pasture vs. concentrate diet on CLA isomer distribution in different tissue lipids of beef cattle. *Lipids*, 40 (6), 589–598.
- Dannenberger D., Nuernberg K., Nuernberg G., Ender K. (2006), Carcass- and meat quality of pasture v concentrate fed German Simmental and German Holstein bulls. *Arch. Anim. Breeding, Dummerstorf*, 49, 315–328.
- De La Torre A., Gruffat D., Durand D., Micol D., Peyron A., Scislawski V., Bauchart D. (2006), Factors influencing proportion and composition of CLA in beef. *Meat Sci.*, 73, 258–268.
- Descalzo A.M., Insani E.M., Margaria C.A., Garcia P.T., Josifovich J., Pensel N.A. (2000), Antioxidant status and lipid oxidation in fresh Argentine beef from pasture- and grain-fed steers with vitamin E supra-nutritional supplementation. In *Proc 46th International Congress of Meat Science and Technology*, 562–563.
- Descalzo A.M., Rossetti L., Grigioni G., Irurueta M., Sancho A.M., Carrete J., Pensel N.A. (2007), Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. *Meat Sci.*, 75, 299–307.
- DeSmet S., Raes K., Demeyer, D. (2004), Meat fatty acid composition as affected by genetic factors. *Animal Research*, 53, 81–88.
- Destailhats F., Trotter J.P., Galvez J.M., Angers P. (2005), Analysis of alpha-linolenic acid biohydrogenation intermediates in milk fat with emphasis on conjugated linolenic acids. *J. Dairy Sci.*, 88, 3231–3239.
- Dewhurst R.J., Evans R.T., Scollan N.D., Moorby J.M., Merry R.J., Wilkins R.J. (2003), Comparison of grass and legume silages for milk production. 2. In vivo and in sacco evaluations of rumen function. *J. Dairy Sci.*, 86, 2612–2621.
- Dewhurst R.J., Shingfield K.J., Lee M.R.F., Scollan N.D. (2006), Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Anim. Feed Sci. Technol.*, 131, 168–206.
- DGE (2004), *Nutrition report of the German Society of Human Nutrition (DGE)*. 2004, Ed. DGE, Bonn, p. 63
- Doreau M., Ferlay A. (1994), Digestion and utilization of fatty acids by ruminants. *Anim. Feed Sci. Technol.*, 45, 379–396.
- Dunshea F.R., D'Souza D.N., Pethick D.W., Harper G.S., Warner R.D. (2005), Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. *Meat Sci.*, 71, 8–38.
- Durand D., Scislawski V., Gruffat D., Chillard Y., Bauchart D. (2005), High-fat rations and lipid peroxidation in ruminants: Consequences on the health of animals and quality of their products. In: *Indicators of milk and beef quality*, EAAP Publication, No 112, Eds. Hocquette J.F., Gigli S., Wageningen Academic Publishers, 137–150.
- Elgersma A., Ellen G., van der Horst H., Muuse B.G., Boer H., Tamminga S. (2003), Comparison of fatty acid composition of fresh and ensiled perennial ryegrass (*Lolium perenne* L.), affected by cultivar and regrowth interval. *Anim. Feed Sci. Technol.*, 108, 191–205.
- Elias Calles J.A., Gaskins C.T., Busboom J.R., Duckett S.K., Cronrath J.D., Reeves J.J. (2000), Sire variation in fatty acid composition of crossbred Wagyu steers and heifers. *Meat Sci.*, 56, 23–29.
- Enser M., Scollan N.D., Choi N.J., Kurt E., Hallett K., Wood J.D. (1999), Effect of dietary

- lipid on the content of conjugated linoleic acid (CLA) in beef muscle. *Anim. Sci.*, 69, 143–146.
- Enser M.B. (1991), Animal carcass fats and fish oils. In: J.B. Rossel and J.L.R. Pritchard eds. *Analysis of Oilseeds, Fats and Fatty Foods*. Elsevier Appl. Sci., London and New York, 329–394.
- Flachowsky G., Richter G.H., Wendemuth M., Möckel P., Graf H., Jahreis G., Lübke F. (1994), Influence of rape seed in beef cattle feeding on fatty acid composition, vitamin E concentration and oxidative stability of body fat. *Zeitschrift für Ernährungswissenschaften (European Journal of Nutrition)*, 33, 277–285. (German)
- Flachowsky G., Sander-Hertsch L., Augustini C., Richter G.H., Möckel P. (1995), Fatty acid pattern and characteristic criteria of meat quality in fattening bulls of the crosses Limousin × Black-and-White dairy cattle and Fleckvieh × Black-and-White dairy cattle and of the Yellow cattle breed. *Züchtungskunde*, 67, 220–229. (German)
- Franco, D., Moreno, T., Bispo, E., Pérez Seijas, N., Monserrat, L. (2006), Free amino acids and constituted amino acids of protein of four muscles and liver from *Rubia gallega*. *Proc. of 52nd ICoMST*, 727–728.
- French P., Stanton C., Lawless F., O’Riordan E.G., Monahan F.J., Caffey P.J., Moloney A.P. (2000), Fatty acid composition, including conjugated fatty acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *J. Anim. Sci.*, 78, 2849–2855.
- Fukuda S., Suzuki Y., Murai M., Asanuma N., Hino T. (2006a), Isolation of a Novel Strain of *Butyrivibrio fibrisolvens* that Isomerizes Linoleic Acid to Conjugated Linoleic Acid without Hydrogenation, and its Utilization as a Probiotic for Animals. *J. Appl. Microbiol.*, 100, 787–794.
- Fukuda S., Suzuki Y., Murai M., Asanuma N., Hino T. (2006b), Augmentation of Vaccenate Production and Suppression of Vaccenate Biohydrogenation in Cultures of Mixed Ruminal Microbes. *J. Dairy Sci.*, 89, 1043–1051.
- Givens D.I. (2005), The role of animal nutrition in improving the nutritive value of animal-derived foods in relation to chronic disease. *Proc. Nutri. Soc.*, 64, 395–402.
- Givens D.I., Gibbs R.A. (2006), Very long chain *n*-3 polyunsaturated fatty acids in the food chain in the UK and the potential of animal-derived foods to increase the intake. *Nutrition Bulletin*, 31, 104–110.
- Givens D.I., Kliem K.E., Gibbs R.A. (2006), The role of meat as a source of *n*-3 polyunsaturated fatty acids in human diet. *Meat Sci.*, 74, 209–218.
- Griinari J.M., Dwyer D.A., McGuire M.A., Bauman D.E., Palmquist D.L., Nurmela K.V. (1998), Trans-octadecenoic acids and milk fat depression in lactating cows. *J. Dairy Sci.*, 81, 1251–1261.
- Griinari J.M., Corl B.A., Lacy S.H., Chouinard P.Y., Nurmela K.V., Bauman D.E. (2000), Conjugated Linoleic Acid Is Synthesized Endogenously in Lactating Dairy Cows by Delta(9)-desaturase. *J. Nutr.*, 130, 2285–2291.
- Griinari J.M., Shingfield K. (2006), Metabolism of conjugated linoleic acid in ruminants. *Proc. German Society of Nutrition Conference ‘Fats and Health’*, 19–20 October 2006, Frankfurt, Germany.
- Hintze K.J., Lardy G.P., Marchello M.J., Finley J.W. (2002), Selenium accumulation in beef: Effect of dietary selenium and geographical area of animal origin. *J. Agric. Food Chem.*, 50 (14), 3938–3942.
- Hocquette J.F., Lehnert S., Barendse W., Cassar-Malek I., Picard B. (2007), Recent advances in cattle functional genomics and their application to beef quality. *Animal*, 1, 159–173.
- Hollo G., Nuernberg K., Hollo I., Csapo J., Seregi J., Repa I., Ender K. (2007), Effect of feeding on the composition *longissimus* muscle of Hungarian Grey and Holstein Friesian bulls. III. Amino acid composition and mineral content. *Arch Anim Breeding*, 50, 575–586.
- Hollo G., Nuernberg K., Repa I., Hollo I., Seregi J., Pohn G., Ender K. (2005), Effect of

- feeding on the composition of intramuscular fat in *longissimus* muscle and different fatty tissues of Hungarian Grey and Holstein Friesian bulls. 1. Fatty acid profile. *Arch. Anim. Breeding*, 48 (6), 537–546. (in German)
- Howe, P., Meyer, B., Record, S., Baghurst, K. (2006), Dietary intake of long-chain ω -3 polyunsaturated fatty acids: Contribution of meat sources. *Nutrition*, 22, 47–53.
- Huerta-Leidenz N.O., Cross H.R., Savell J.W., Lunt D.K., Baker J.F., Pelton L.S., Smith S.B. (1993), Comparison of the fatty acid composition of subcutaneous adipose tissue from mature Brahman and Hereford cows. *J. Anim. Sci.*, 71 (3), 625–30.
- Indurain G., Beriain M.J., Goni M.V., Arana A., Purroy A. (2006), Composition and estimation of intramuscular and subcutaneous fatty acid composition in Spanish young bulls. *Meat Sci.*, 73, 326–334.
- Jenkins T.C. (1993), Lipid metabolism in the rumen. Review. *J. Dairy Sci.*, 76, 3851–3863.
- Kay J.K., Mackle T.R., Auldist M.J., Thomson N.A., Bauman D.E. (2004), Endogenous synthesis of cis-9, trans-11 conjugated linoleic acid in dairy cows fed fresh pasture. *J. Dairy Sci.*, 87, 369–378
- Kazala E.C., Lozeman F.J., Mir P.S., Laroche A., Bailey D.R.C., Weselake R.J. (1999), Relationship of fatty acid composition to intramuscular fat content in beef from crossbred Wagye cattle. *J. Anim. Sci.*, 77, 1717–1725.
- Kepler C.R., Hirons K.P., McNeill J.J., Tove S.B. (1966), Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. *J. Biol. Chem.*, 241, 1350–1354.
- Khanal R.C. and Dhiman T.R. (2004), Biosynthesis of Conjugated Linoleic Acid (CLA): A Review. *Pakistan J. Nutr.*, 3 (2), 72–81.
- Khanal R.C. and Olson K.C. (2004), Factors affecting conjugated linoleic acid (CLA) content in milk, meat, and egg: A review. *Pakistan J. Nutr.*, 3 (2), 82–98.
- Kirchgessner M., Roth-Maier D.A., Heindl U., Schwarz F. (1995), B-vitamins (thiamin, vitamin B6, panthothenic acid) in lean tissue of growing cattle of the German Simmental breed under different feeding intensities. *Z. Lebensm. Unters. Forsch.*, 201, 20–24. (German)
- Knight T.W., Knowles S., Death A.F., West J., Agnew M., Morris C.A., Purchas R.W. (2003), Factors affecting the variation in fatty acid compositions in lean beef from grass-fed cattle in New Zealand and the implications for human health. *New Zealand J. Agric. Res.*, 46, 83–95.
- Komprda T., Dvorak R., Fialova M., Dvorak P. (2002), Effect of heat-treated rapeseed cake on fatty acid pattern in meat of fattened bulls. *Czech. J. Anim. Sci.*, 47 (2), 64–71.
- Kook K., Choi B.H., Sun S.S., Garcia F., Myung K.H. (2002), Effect of fish oil supplement on growth performance, ruminal metabolism and fatty acid composition of *longissimus* muscle in Korean cattle. *Asian-Australasian J. Anim. Sci.*, 15 (1), 66–71.
- Krinsky N.I., Johnson E.J. (2005), Carotenoid action and their relation to health and disease. *Molecular Aspects of Medicine*, 26 (6), 459–516.
- Kühn Ch., Levezuel H., Renand G., Goldammer T., Schwerin M., Williams J. (2005), Genetic markers for beef quality. In: Hocquette J.F. and Gigli (eds.) *Indicators of milk and beef quality*. EAAP publication, No. 112, 23–32.
- Laborde F.L., Mandell I.B., Tosh J.J., Buchanan-Smith J.G., Wilton J.W. (2002), Effect of management strategy on growth performance, carcass characteristics, fatty acid composition, and palatability attributes in crossbred steers. *Can. J. Anim. Sci.*, 82, 49–57.
- Laborde F.L., Mandell I.B., Tosh J.J., Wilton J.W., Buchanan-Smith J.G. (2001), Breed effects on growth performance, carcass characteristics, fatty acid composition, and palatability attributes in finishing steers. *J. Anim. Sci.*, 79, 355–365.
- Lawler T.L., Taylor J.B., Finley J.W., Caton J.S. (2004), Effect of supranutritional and organically bound selenium on performance, carcass characteristics, and selenium distribution in finishing beef steers. *J. Anim. Sci.*, 82, 1488–1493.
- Lee M.R.F., Harris L.J., Dewhurst R.J., Merry R.J., Scollan N.D. (2003), The effect of clover

- silages on long chain fatty acid transformations and digestions in beef steers. *Anim. Sci.*, 76, 491–501.
- Lee M.R.F., Tweed J.K.S., Moloney A.P., Scollan N.D. (2005), Effects of fish oil supplementation on rumen metabolism and the biohydrogenation of unsaturated fatty acids in beef steers given diets containing sunflower oil. *Anim. Sci.*, 80 (3), 361–367.
- Lee M.R.F., Winters A.L., Scollan N.D., Dewhurst R.J., Theodorou M.K., Minchen F.R. (2004), Plant-mediated lipolysis and proteolysis in red clover with different polyphenol oxidase activity. *J. Sci. Food Agric.*, 84 (13), 1639–1645.
- Liu Q., Lanari M.C., Schaefer D.M. (1995), A review of dietary vitamin E supplementation for improvement of beef quality. *J. Anim. Sci.*, 73, 3131–3140.
- Lock A.L., Corl B.A., Barbano D.M., Bauman D.E., Ip C. (2004), The anticarcinogenic effect of *trans*-11 18:1 is dependent on its conversion to *cis*-9, *trans*-11 CLA by Delta 9-desaturase in rats. *J. Nutr.*, 134, 2698–2704.
- Lombardi-Boccia G., Lanzi S., Lucarini M., Di Lullo G. (2004), Meat and meat products consumption in Italy: Contribution to trace elements, heme iron and selected B vitamins supply. *Int. J. Vitam. Nutr. Res.*, 74 (4), 247–251.
- Loor J.J., Ueda K., Ferlay A., Chillard Y., Doreau M. (2005a), Intestinal flow and digestibility of *trans* fatty acids and conjugated linoleic acids (CLA) in dairy cows fed high-concentrated diet supplemented with fish oil, linseed oil, or sunflower oil. *Anim. Feed Sci. Technol.*, 119, 203–225.
- Loor J.J., Ferlay A., Ollier M., Doreau M., Chillard Y. (2005b), Relationship among *trans* and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. *J. Dairy Sci.*, 88, 726–740.
- Lorenz S., Buettner A., Ender K., Nuernberg G., Papstein H.-J., Schieberle P., Nuernberg K. (2002), Influence of keeping system on the fatty acid composition in the *longissimus* muscle of bulls and odorants formed after pressure-cooking. *Eur. Food Res. Technol.*, 214, 112–118.
- Lorenzen C.L., Golden J.W., Martz F.A., Grün I.U., Ellersieck M.R., Gerrish J.R., Moore K.C. (2007), Conjugated linoleic acid content of beef differs by feeding regime and muscle. *Meat Sci.*, 75, 159–167.
- Mach N., Devant M., Diaz I., Font-Furnols M., Oliver M.A., Garcia J.A., Bach A. (2006), Increasing the amount of *n*-3 fatty acid in meat from young Holstein bulls through nutrition. *J. Anim. Sci.*, 84 (11), 3039–3048.
- Malau-Aduli A.E.O., Edriss M.A., Siebert B.D., Bottema C.D.K., Deland M.P.B., Pitchford W.S. (2000), Estimates of genetic parameters for triacylglycerol fatty acids in beef cattle at weaning and slaughter. *J. Anim. Physiol. Anim. Nutr.*, 83 (4–5), 169–180.
- Malau-Aduli A.E.O., Siebert B.D., Bottema C.D.K., Pitchford W.S. (1998), Breed comparison of the fatty acid composition of muscle phospholipids in Jersey and Limousin cattle. *J. Anim. Sci.*, 76, 766–773.
- Mandell I.B., Gullett E.A., Buchanan-Smith J.G., Campbell C.P. (1997), Effects of diet and slaughter endpoint on carcass composition and beef quality in Charolais cross steers. *Can. J. Anim. Sci.*, 77 (3), 403–414.
- Melton S.L., Amiri M., Davis G.W., Backus W.R. (1982), Flavour and chemical characteristics of ground beef from grass-, forage-grain- and grain-finished steers. *J. Anim. Sci.*, 55, 77–87.
- Mercier Y., Gatellier P., Renner M. (2004), Lipid and protein oxidation in vitro, and antioxidant potential in meat from Charolais cows finished on pasture or mixed diet. *Meat Sci.*, 66, 476–473.
- Mir P.S., Ivan M., He M.L., Pink B., Okine E., Goonewardene L., McAllister T.A., Weslake R., Mir Z. (2003), Dietary manipulation to increase conjugated linoleic acids and other desirable fatty acids in beef: A review. *Can. J. Anim. Sci.*, 83, 673–685.
- Mir P.S., McAllister T.A., Scott S., Aalhus J., Baron V., McCartney D., Charmley E., Goonewardene L., Basarab J., Okine E., Weslake R., Mir Z. (2004), Conjugated linoleic acid-enriched beef production. *Am. J. Clin. Nutr.*, 79 (suppl), 1207S–1211S.

- Mir P.S., McAllister T.A., Gibb D.J., Okine E.K. (2006), Dietary oil rich in polyunsaturated fatty acids for ruminants: Post-ruminal digesta characteristics and their implications on production. *Can. J. Anim. Sci.*, 86 (2), 159–170.
- Mir Z., Paterson L.J., Mir P.S. (2000), Fatty acid composition and conjugated linoleic acid content of intramuscular fat in crossbred cattle with and without Wagyu genetics fed a barley-based diet. *Can. J. Anim. Sci.*, 80, 195–197.
- Moloney A.P. (2007), Enrichment of omega-3 fatty acids and CLA in beef by diet modification. *Irish Veterinary J.*, 60 (3), 180
- Moreno T., Keane M.G., Noci F., Moloney A.P. (2008), Fatty acid composition of muscle from Holstein–Friesian steers of New Zealand and European/American descent and from Belgian Blue × Holstein–Friesian steers, slaughtered at two weights. *Meat Sci.*, 80 (3), 157–169.
- Morrisey P.A., Kerry J.P. (2004), Lipid oxidation and the shelf-life of muscle foods. In Ed. Steele R. *Understanding and measuring the shelf-life of food*. CRC Press, Woodhead Publishing Limited, Cambridge England, 2004. 357–395.
- Mosley E.E., Shafii B., Moate P.J., McGuire M.A. (2006), Cis-9, trans-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. *J. Nutr.*, 136, 570–575.
- Mozaffarian D., Katan M.B., Ascherio A., Stampfer M.J., Willett W.C. (2006), Trans fatty acids and cardiovascular disease. *New England J. Med.*, 354 (15), 1601–1613.
- Noci F., Monahan F.J., French P., Moloney A.P. (2005a), The fatty acid composition of muscle fat and subcutaneous adipose tissue of pasture-fed beef heifers: Influence of the duration of grazing. *J. Anim. Sci.*, 83, 1167–1178.
- Noci F., O’Kiely P.O., Monahan F.J., Stanton C., Moloney A.P. (2005b), Conjugated linoleic acid concentration in *M. Longissimus dorsi* from heifers offered sunflower oil-based concentrates and conserved forages. *Meat Sci.*, 69, 509–518.
- Noci F., Monahan F.J., Scollan N.D., Moloney A.P. (2007a), The fatty acid composition of muscle and adipose tissue of steers offered unwilted or wilted grass silage supplemented with sunflower oil and fish oil. *Brit. J. Nutr.*, 97, 502–513.
- Noci F., French P., Monahan F.J., Moloney A.P. (2007b), The fatty acid composition of muscle fat and subcutaneous adipose tissue of grazing heifers supplemented with plant oil-enriched concentrates. *J. Anim. Sci.*, 85, 1–12.
- Nuernberg K., Ender K. (1998), Influences on lipid composition in muscle and fatty tissues of farm animals and its relation to human nutrition. *Livestock Production Sci.*, 45, 145–156.
- Nuernberg, K., Dannenberger, D., Nuernberg, G., Ender, K., Voigt, J., Scollan, N.D., Wood, J.D., Nute, G.R., Richardson, I. (2005), Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of *longissimus* muscle in different cattle breeds. *Livestock Prod. Sci.*, 94, 137–147.
- Nuernberg K., Ender B., Papstein H.-J., Wegner J., Ender K., Nuernberg G. (1999), Effects of growth and breed on the fatty acid composition of the muscle lipids in cattle. *European Food Res. Technol.*, 208 (5–6), 332–335.
- Oka A., Iwaki, F., Dohgo T., Ohtagaki S., Noda M., Shiozaki T., Endoh O., Ozaki M. (2002), Genetic effects on fatty acid composition of carcass fat of Japanese Black Wagyu steers. *J. Anim. Sci.*, 80, 1005–1011.
- Paiva S.A.R., Russell R.M. (1999), β -Carotene and other carotenoids as antioxidants. *J. Amer. College Nutrition*, 18, 426–433.
- Palmquist D.L., Lock A.L., Shingfield K.J., Bauman D.E. (2005), Biosynthesis of conjugated linoleic acid in ruminants and humans. In: Taylor S.L. (Ed.), *Adv. Food Nutr. Res.*, Vol. 50, Elsevier Academic Press, San Diego, CA, 179–217.
- Park Y., Pariza M.W. (2007), Mechanisms of body fat modulation by conjugated linoleic acid (CLA). *Food Research International*, 40 (3), 311–323.
- Pensel, N. (1997), The future of red meat in human diet. *Int. Cent. Agric. Biosci.*, 26, 159–164.

- Piperova L.S., Teter B.B., Bruckental I., Sampugna J., Mills S.E., Yurawecz M.P., Fritsche J., Ku K., Erdman R.A. (2000), Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-drepressing diet. *J. Nutr.*, 130, 2568–2574.
- Piperova L.S., Sampugna J., Teter B.B., Kalscheur K.F., Yurawecz M.P., Ku Y., Morehouse K.M., Erdman R.A. (2002), Duodenal and milk *trans* octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that post absorptive synthesis is the predominant source of cis-9-containing CLA in lactating dairy cows. *J. Nutr.*, 132, 1235–1241.
- Pitchford W.S., Deland M.P.B., Siebert B.D., Malau-Aduli A.E., Bottema C. D. K. (2002), Genetic variation in fatness and fatty acid composition of crossbred cattle. *J. Anim. Sci.*, 80, 2825–2832.
- Ponnampalam E.N., Mann N.J., Sinclair A.J. (2006), Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and trans fatty acids in Australian beef cuts: Potential impact on human health. *Asia Pac. J. Clin. Nutr.*, 15 (1), 1–8.
- Raes K., DeSmet S., Balcaen A., Claeys E., Demeyer D. (2003), Effect of diets rich in *n*-3 polyunsaturated fatty acids on muscle lipids and fatty acids in Belgian Blue double-muscled young bulls. *Reproduction Nutr. Dev.*, 4, 331–345.
- Raes K., Fievez V., Chow T.T., Ansorena D., Demeyer D., DeSmet S. (2004), Effect of diet and dietary fatty acids on the transformation and incorporation of C18 fatty acids in double-muscled Belgian Blue young bulls. *J. Agric. Food Chem.*, 52, 6035–6041.
- Razminowicz R.H., Kreuzer M., Scheeder M.R.L. (2006), Quality of retail beef from two grass-based production systems in comparison with conventional beef. *Meat Sci.*, 73, 351–361.
- Realini C.E., Duckett S.K., Brito Q.W., Dalla Rizza M., De Mattos D. (2004), Effect of pasture vs concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Sci.*, 73, 351–361.
- Sami A.S., Augustini C., Schwarz F.J. (2004), Effect of feeding intensity and time on feed on intramuscular fatty acid composition of Simmental bulls. *J. Anim. Physiol. Anim. Nutr.*, 88, 179–187.
- Sami A.S., Koegel J., Eichinger H., Freudenreich P., Schwarz F. (2006), Effects of the dietary energy source on meat quality and eating quality attributes and fatty acid profile of Simmental bulls. *Anim. Res.*, 55, 287–299.
- Sanders S.K., Morgan J.B., Wulf D.M., Tatum J.D., Williams S.N., Smith G.C. (1997), Vitamin E supplementation of cattle and shelf-life of beef for the Japanese market. *J. Anim. Sci.*, 75, 2634–2640.
- Scheeder M.R.L., Casutt M.M., Roulin M., Escher F., Dufey P.A., Kreuzer M. (2001), Fatty acid composition, cooking loss and texture of beef patties from meat of bulls fed different fats. *Meat Sci.*, 58 (3), 321–328.
- Schmid A., Collomb M., Sieber R., Bee G. (2006), Conjugated linoleic acid in meat and meat products: A review. *Meat Sci.*, 73 (1), 29–41.
- Schwarz F.J., Augustini C., Timm M., Kirchgessner M., Steinhart H. (1998), Effect of vitamin E on α -tocopherol concentration in different tissues and oxidative stability of bull beef. *Livest. Prod. Sci.*, 56, 165–171.
- Scislowski V., Bauchart D., Gruffat D., Laplaud P.M., Durand D. (2005), Effects of dietary *n*-6 or *n*-3 polyunsaturated fatty acids protected or not against ruminal hydrogenation on plasma lipids and their susceptibility to peroxidation in fattening steers. *J. Anim. Sci.*, 83 (9), 2162–2174.
- Scollan N.D., Choi N.J., Kurt E., Fisher A.V., Enser M., Wood J.D. (2001), Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *Brit. J. Nutr.*, 85 (1), 115–124.
- Scollan N., Hocquette J.-F., Nuernberg K., Dannenberger D., Richardson I., Moloney A. (2006a), Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Sci.*, 74, 17–33.

- Scollan N.D., Costa P., Hallett K.G., Nute G.R., Word J.D., Richardson R.I. (2006b), The fatty acid composition of muscle fat and relationships to meat quality in Charolais steers: Influence of level of red clover in the diet. *Proc. Brit. Soc. Anim. Sci.*, York, 27–29 March, 23.
- Shen X., Nuernberg G., Nuernberg K., Zhao R., Scollan N., Ender K., Dannenberger D. (2007), *Trans* vaccenic acid and CLA $c_{18:1}$ in rumen and different tissues of pasture- and concentrate-fed beef cattle. *Lipids*, 42, 1093–1103.
- Siebert B.D., Pitchford W.S., Kruk Z.A., Kuchel H., Deland M.P., Bottema C.D. (2003), Differences in delta-9 desaturase activity between Jersey- and Limousin-sired cattle. *Lipids*, 38 (5), 539–543.
- Smith S.B., Lunt D.K., Chung K.Y., Choi C.B., Tume R.K., Zembayashi M. (2006), Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. *Anim. Sci. J.*, 77 (5), 478–486.
- Steen R.W.J., Porter M.G. (2003), The effects of high-concentrate diets and pasture on the concentration of conjugated linoleic acid in beef muscle and subcutaneous fat. *Grass and Forage Science*, 58, 50–57.
- Steen R.W.J., Lavery N.P., Kilpatrick D.J., Porter, M.G. (2003), Effects of pasture and high-concentrate diets on the performance of beef cattle, carcass composition at equal growth rates, and the fatty acid composition of beef. *New Zealand J. Agric. Res.*, 46 (2), 69–81.
- Stender S., Dyerberg J. (2003), The influence of *trans* fatty acids on health. *A Report of the Danish Nutrition Council, Fourth Ed.*, 34, 1–84.
- Sturdivant C.A., Lunt D.K., Smith G.C., Smith S.B. (1992), Fatty acid composition of subcutaneous and intramuscular adipose tissues and *M. longissimus dorsi* of Wagyu cattle. *Meat Sci.*, 32, 449–458.
- Tricon S. and Yaqoob P. (2006), Conjugated linoleic acid and human health: A critical evaluation of the evidence. *Curr. Opinion Clin. Nutr. Metab. Care*, 9 (2), 105–110.
- Vlaeminck B., Fievez V., Demeyer D., Dewhurst R.J. (2006), Effect of Forage : Concentrate Ratio on Fatty Acid Composition of Rumen Bacteria Isolated From Ruminant and Duodenal Digesta. *J. Dairy Sci.*, 89, 2668–2678.
- Wahle K.W., Caruso D., Ochoa J.J., Quiles J.L. (2004a), Olive oil and modulation of cell signaling in disease prevention. *Lipids*, 39 (12), 1223–31.
- Wahle K.W., Heys S.D., Rotondo D. (2004b), Conjugated linoleic acids: Are they beneficial or detrimental to health? *Prog. Lipid Res.*, 43, 553–587.
- Wang C., Harris W.S., Chung M. (2006), *n*-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: A systematic review. *Am. J. Clin. Nutr.*, 84, 5–17.
- Warburg U. (2004), What are the health effects of fat? *Eur. J. Nutr.*, 43, Suppl., I6–I11.
- Warren H., Scollan N.D., Hallett K., Enser M., Richardson I., Nute G., Wood, J.D. (2002), The effects of breed and diet on the lipid composition and quality on the lipid composition and meat quality of bovine muscle. *Proc. 48th Internat. Congress Meat Sci. Technol.*, Rome, 1, 370–371.
- Wegner J., Albrecht E., Ender K. (1998) Morphologic aspects of subcutaneous and intramuscular fatty tissue growth in cattle. *Arch. Tierz.*, 41, 313–320. (German)
- WHO (2003), *Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO Expert Consultation*. WHO Technical Report Series 916, Geneva.
- Willet W.C. (2006), The scientific basis for TFA regulation – is it sufficient? Comments from the USA. *Atherosclerosis Supplements*, 7, 69–71.
- Williams C.M., Burdge G. (2006), Long-chain *n*-3 PUFA: Plant v marine sources. *Proceedings of the Nutrition Society*, 65, 42–50.
- Wood J.D., Enser M., Fisher A.V., Nute G.R., Richardson I.R., Sheard P.R. (1999), Manipulating meat quality and composition. *Proc. Nutr. Soc.*, 58, 363–370.
- Wood J.D., Nute G.R., Richardson I.R., Whittington F.M., Southwood O., Plastow G., Mansbridge R., da Costa N., Chang K.C. (2004), Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Sci.*, 67 (4), 651–667.

- Wood J.D., Richardson I.R., Nute G.R., Fisher A.V., Campo M.M., Kasapidou E., Sheard P.R., Enser M. (2003), Effects of fatty acids on meat quality: A review. *Meat Sci.*, 66, 21–32.
- Yang A., Brewster M.J., Lanari M.C., Tume R.K. (2002), Effect of vitamin E supplementation on α -tocopherol and β -carotene concentration in tissues from pasture- and grain-fed cattle. *Meat Sci.*, 60 (1), 35–40.
- Yang A., Larsen T.W., Tume R.K. (1992), Carotenoid and retinol concentrations in serum, adipose tissue and liver and carotenoid transport in sheep, goats and cattle. *Australian Journal of Agricultural Research*, 43, 1807–1809.

Optimising the nutritional and sensorial profile of pork

J. Mourot, INRA, France

Abstract: The rearing factors influencing pork meat sensorial and nutritional qualities are considered in this chapter. The selection of animals according to growth performance modifies these qualities: however, lipid content may be decreased and the meat considered to be dry by the consumer. The factor that most influences pork nutritional quality is the diet. A direct relationship exists between the dietary fatty acids and those deposited in the meat. Fatty acids beneficial to human health such as *n*-3 fatty acids can be introduced into the feed. When supplied as linen seeds into the pig's feed, they appear in higher quantities on the consumer's plate.

Key words: rearing factors, lipids, fatty acids, linolenic acid, linen seed.

15.1 Introduction

Pork is the meat most frequently eaten in Europe (approximately 42 kg per year per person). This consumption represents approximately 48% of all meat products. Depending on the country, 1/3 to 1/4 of this meat is consumed fresh and the rest is processed. Due to the extent of this consumption the meat produced should be of good sensorial and nutritional quality.

The pork meat of today differs from that produced by pigs 50 years ago. The selection of animals according to growth performance has modified the sensorial and nutritional qualities of the meat. The carcass is less fat and the animals are slaughtered younger at the same weight. The lipid content of the meat is thus decreased. However, in the medical world, pork still has a negative image of being a fat meat, although this is no longer the case.

The decreased lipid content affects both the taste and nutritional composition of pork. In this chapter, we will examine the current nutritional composition, the factors that can influence meat sensorial and nutritional qualities, and finally the

Table 15.1 Nutritional composition of pig meat (per 100 g) (from Culioli *et al.*, 2003)

	Fillet		Fillet		Fillet
Energy (kJ)	475	Iron (mg)	1.2	Vitamin B6 (mg)	0.45
Water (g)	74.4	Niacin (mg)	4.3	Vitamin B12 (µg)	0.7
Proteins (g)	21	Vitamin E (mg)	0.1	Folate (µg)	4
Lipids (g)	3.2	Thiamin (mg)	1	Sodium (mg)	125
Cholesterol (mg)	65				

approaches being developed to improve these qualities in accordance with the desires of the nutritionists, consumer expectations and animal welfare.

15.2 Pork composition

The composition of pork meat, as in all animal species, varies according to its anatomical location. In contrast to bovines, the fat tissue in numerous pieces of meat is easily removed. The resulting pork is a lean meat with a lipid content of 2 to 3% depending on the muscles, i.e. less than that of bovines.

The protein content is approximately 18% in most cuts and the amino acid composition is well balanced and corresponds to human requirements (Culioli *et al.*, 2003).

The cholesterol content of the fresh meat is approximately the same as in other species, i.e. 50 to 80 mg per 100 g depending on the piece. The mineral composition and vitamin content is comparable to that of other species with the exception of iron which is present in lower amounts than in ruminants (Table 15.1).

The fatty acid composition varies according to the anatomical location of the tissues. Table 15.2 shows the results of a recent laboratory study in which pigs received the same standard feed. The external, internal and intramuscular adipose tissues are compared. Two muscles, one from the loin and the other from the ham, are also compared, together with different cuts of meat (whole chop or belly). The liver composition is also reported. Oleic acid is the most important fatty acid in muscles and fat tissues, representing between 35 and 45% of the total fatty acids. The muscles are richer in unsaturated fatty acids than the fat tissues. The sum of the unsaturated fatty acids in the backfat is less than 12 or 15% of the total fatty acids. If this value of 15% is exceeded for polyunsaturated fatty acids, problems may occur during processing due to their possible peroxidation (Wood *et al.*, 2008).

The intermuscular adipose tissue in the ham is the one with the highest C18:2 *n-6* fatty acid content. This difference in fatty acids composition is thus indicative of a different potential for fatty acid synthesis between different adipose tissues (Mourot and Kouba, 1998).

Table 15.2 Average composition in fatty acids of various tissues of pigs receiving the same standard diet (as percentage of identified fatty acids)

	Loin	Ham	Backfat	Inter- muscular fat	Leaf fat	Chop	Fillet	Chest	Liver
C14:0	1.31	1.08	1.16	1.40	1.33	1.28	1.18	1.34	0.40
C14:1	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C16:0	24.38	22.89	24.67	25.09	27.80	25.39	24.34	25.61	15.95
C16:1 (<i>n</i> -7)	3.38	3.39	2.10	2.60	1.80	2.59	2.56	2.93	1.11
C18:0	11.45	10.17	14.27	12.19	18.73	13.68	12.53	12.65	25.26
C18:1 (<i>n</i> -9)	40.83	41.23	41.15	36.57	36.22	42.07	43.81	43.77	16.97
C18:2 (<i>n</i> -6)	12.73	14.74	13.21	18.29	11.38	11.58	12.03	10.83	15.66
C20:0	0.26	0.23	0.31	0.20	0.29	0.26	0.26	0.22	0.41
C18:3 (<i>n</i> -3)	0.42	0.49	0.81	1.15	0.72	0.65	0.68	0.67	0.45
C20:1 (<i>n</i> -9)	0.65	0.63	1.05	0.72	0.72	0.92	0.96	0.82	0.28
C20:2	0.36	0.40	0.67	0.65	0.44	0.49	0.58	0.48	0.41
C20:3 (<i>n</i> -6)	0.32	0.32	0.08	0.12	0.06	0.10	0.10	0.05	0.51
C20:4 (<i>n</i> -6)	2.67	3.06	0.31	0.46	0.27	0.61	0.61	0.34	17.53
C20:5 (<i>n</i> -3)	0.16	0.23	0.01	0.03	0.02	0.04	0.04	0.03	0.54
C24:0	0.04	0.02	0.00	0.00	0.00	0.01	0.01	0.03	0.03
C24:1	0.45	0.47	0.09	0.19	0.08	0.15	0.15	0.10	1.01
C22:5 (<i>n</i> -3)	0.39	0.43	0.08	0.17	0.08	0.12	0.11	0.08	2.06
C22:6 (<i>n</i> -3)	0.18	0.20	0.03	0.15	0.04	0.06	0.06	0.03	1.42
SFA	37.45	34.39	40.41	38.88	48.14	40.62	38.32	39.85	42.05
MUFA	45.34	45.75	44.40	40.09	38.84	45.74	47.48	47.63	19.38
PUFA	17.22	19.86	15.19	21.03	13.02	13.64	14.20	12.53	38.57
<i>n</i> -6 total	15.72	18.12	13.60	18.87	11.71	12.29	12.74	11.23	33.70
<i>n</i> -3 total	1.15	1.35	0.93	1.50	0.87	0.87	0.88	0.82	4.46
<i>n</i> -6/ <i>n</i> -3	13.77	10.71	13.77	11.89	13.77	13.77	13.77	13.77	13.77
18:2/18:3	30.58	30.35	16.38	15.89	15.69	17.80	17.82	16.07	34.83
SFA/MUFA	0.83	0.75	0.91	0.97	1.24	0.89	0.81	0.84	2.17

15.3 The sensorial qualities of pork

Pork does not present any major defects. Meat tenderness is not a problem, whereas it can be in bovines. The meat is often considered dry by the consumer. A relationship exists between meat flavour and lipid content. As the animals have been selected for growth performance, they are slaughtered younger at the same weight as previously and, as the intramuscular adipose tissue is laid down at a late stage in the life of the animal, are less fat.

The lipid content of the meat is thus less developed in animals that are slaughtered younger. The thickness of the covering fat and meat colour are important meat appreciation criteria. However, comparative studies of consumers in various countries have revealed differences for other appreciation criteria. The system of production greatly influences the consumer's choice (Ngapo *et al.*, 2003).

Taints associated with sexual odours affect boars that are slaughtered after the onset of puberty. These are due to the accumulation of malodorous compounds, such as androstenone and skatol (product of tryptophan degradation in the bowel) in the fat tissue. Approximately 20% of boars will exhibit such defects. The way to avoid such taint is to castrate the animals before they are 7 days old. Most male pigs in the United Kingdom, Ireland and Spain are not castrated. This is still a practice in many other European countries, but the legislative changes in 2009 will make it obligatory to find other solutions.

15.4 Effects of breeding factors on meat sensorial and nutritional qualities

The sensorial and nutritional qualities of pork are influenced by genetics. At equivalent weight, the animals of local breeds or of breeds with a slow growth rate are slaughtered when older than those selected for rapid growth (Serra *et al.*, 1998). The covering fat is more developed and the lipid content higher. Approximately 82 to 85% of the lipids were in the backfat tissue of local breeds vs. 70% for the selected breeds. The same occurs in muscle where the intramuscular lipids content is higher (3.9% vs. 1.8%, respectively in the *longissimus dorsi*). Taste trials have underlined the fatter and less firm texture of roast pork from local breeds, which could explain why consumers prefer these (Labroue *et al.*, 2001).

The factor that most influences pork nutritional quality is the diet composition. There is a direct relationship between the fatty acids supplied by the diet and those deposited in the meat (Mourot and Hermier, 2001). This also affects the technological quality of the fat tissues (Wood *et al.*, 2008). This characteristic is used to modify the nutritional quality of the meat. The protein fraction of the diet has no effect on the amino acid composition of pork.

Tests have been carried out with supplements of Fe, Cu or Se, the main objective being to improve growth performance, the health of the animal or to protect against stress oxidisers. These supplements can affect the mineral content of the meat. However, these changes are usually more apparent in the offal and the muscle tends to be little affected (Table 15.3). The first two elements most affect the liver and kidney. Supplementary Fe will have no effect on meat flavour or on peroxidation measured as TBARS (Miller *et al.*, 1994). The Se content may be increased in the muscle and fat tissue. This could protect the action of vitamin E and thus be of interest in reducing lipid peroxidation. However, certain doses of Se must not be exceeded.

The vitamin content of the meat, like the mineral content, is related to dietary supply. This is particularly apparent with vitamin E, the deposition in the meat increasing with the quantity ingested. This permits the reduction of lipid oxidation in standard pigs and in heavy pigs (Guo *et al.*, 2006; Corino *et al.*, 1999). An additional supply of 150 mg of α tocopherol in the diet of growing pigs increased the vitamin E content in muscle from 2.9 to 4.2 μg per g of muscle (Phillips *et al.*, 2001). This would also have an effect on human nutrition.

Table 15.3 Effect of supplementation of minerals in the diet on the contents in the meat

Minerals	Dose in diet (mg/kg)	Liver content (mg/kg)	Muscle content (mg/kg)
Fe ¹	60	100	9
	200	150	11
Cu ²	50	50	1.5
	250	500	5.5
Se ³	+ 0		0.06
	+ 0.5 ⁴		0.25

¹ from Miller *et al.*, 1994; ² from Bradley *et al.*, 1983; ³ from Mahan and Parett, 1996; ⁴ + means additional quantity to amount already in diet.

The combined supply of tocopherol with vitamin C also intensifies the colour of the meat and gives a better visual impression (Geesink *et al.*, 2004). However, the vitamin C content in fresh meat is difficult to determine and, what is more, will be partially destroyed during cooking.

The deposition of fat tissue and the fatty acids composition are influenced by the rearing temperature. At low temperatures, outdoor animals protect themselves against the cold by developing more fat tissue, in which the fatty acid composition is modified. The amount of polyunsaturated fatty acids is increased to permit an increase in membrane permeability. The proportions of saturated C16:0 and C18:0 fatty acids and polyunsaturated C18:2 and C18:3 fatty acids are reduced and the proportions of monounsaturated C16:1 and especially C18:1 fatty acids are increased (Fuller *et al.*, 1974; Kouba *et al.*, 1999).

15.5 Orientation of pig production

Pig production has to adapt to consumer expectations. Environmental concerns must be respected and animal welfare taken into consideration. The consumer also wishes the sensorial and nutritional qualities of pork to be improved.

15.5.1 Sensorial quality: the consumers' desire

The consumer tends to consider pork as dry and rather tasteless. This is one consequence of the reduced lipid content of the meat. Thus one aim would be to increase the intramuscular lipids content . . . but to what value?

Fernandez *et al.* (1999) examined the influence of intramuscular fat content on pork sensorial attributes and consumer acceptability. Two experiments were conducted to evaluate the effect of intramuscular fat content on the composition of the lipid fraction and on the sensorial qualities of the *longissimus lumborum* muscle. Each experiment involved 32 castrated male pigs, selected after slaughter from either 125 Duroc × Landrace or 102 Tia Meslan × Landrace crossbred animals, and showing considerable variability in intramuscular fat content, i.e.

from <1.25 to >3.5%. In both experiments, the lipid analyses indicated that increases in intramuscular fat content were almost entirely linked to increases in the triglyceride content of the muscle. In Experiment 2, a higher intramuscular fat content was associated with a higher free fatty acid content. The marbling score was significantly affected by the intramuscular fat level. In Experiment 1, a trend towards a favourable effect of high intramuscular fat levels on flavour ($p = 0.09$) and tenderness ($p = 0.055$) was observed. In Experiment 2, an increased intramuscular fat level was associated with significantly higher juiciness and flavour scores. The results from this study indicated that the variability in intramuscular fat levels of the muscle was almost entirely due to the variability in triglyceride contents. Favourable effects of increased intramuscular fat levels on the sensorial attributes of pork were demonstrated in both experiments using different types of animals, but the nature and the magnitude of these effects depended on the experiment. The optimal value seems to be an intramuscular lipid content between 2.5 and 3%.

At least two approaches have been envisaged in the studies being carried out to attain this objective. First, a lipid supplement in the gestation–lactation diet of the sow leads to an increased number of fat cells in the piglet at birth and at weaning. The effect of this is to increase the lipid content of the muscle in 100 kg pigs, the subcutaneous fat tissue being little affected (Quiniou *et al.*, 2008). These initial studies now need to be confirmed.

Another approach consists of making crosses between pigs from non-selected breeds and present-day pigs. This is currently the aim of the European program Q Porkchain.

Consumer expectations also include the visual aspect, i.e. thickness of the covering fat and meat colour, not forgetting the price, which remains a determining factor as regards purchasing intentions (Ngapo *et al.*, 2003). The reintroduction of too much external fat will therefore need to be avoided in the various research programs currently in progress.

As regards sexual odours of boars, and in response to directives which will perhaps recommend banning the castration of young piglets, research is under way to investigate immunochemical castration by vaccinating animals against androstenone production or by destroying the testicular tissue with mineral salts (Prunier *et al.*, 2006). For the first approach, it will be necessary to ensure that no antibody remains in the meat because of possible effects on humans. Consumers will also have to be willing to consume such meat. As for the second approach, a testicular oedema has been observed, which is certainly a cause of pain and it is difficult to demonstrate its advantage, as regards welfare, over surgical castration.

Odour due to the presence of skatol can be decreased by modifying the diet. A feed rich in fermentable carbohydrates (inulin, potato starch) is very effective in reducing the skatol content of fat tissue (Lösle and Claus, 2005).

15.5.2 Nutritional quality: the desire of the nutritionists

Human nutritionists would like, on the one hand, to see a decrease in the lipid and

saturated fatty acid content of our food and, on the other hand, an increase in the proportion of unsaturated *n*-3 fatty acids. Because of its extensive consumption in most European countries, pork could help bring this about. Due to the relationship existing between the fatty acids in the pig's diet and those deposited in the meat, fatty acids beneficial to human health can be introduced into the feed and will eventually end up on the consumer's plate.

In past years, numerous trials have been carried out on two main families of fatty acids. One is the conjugated linoleic acids and the other the *n*-3 fatty acids.

The term conjugated linoleic acid (CLA) describes a mixture of many geometric and positional diene isomers of linoleic acid. CLA isomers can differ in the positions and configuration of the double bond pairs (7,9 – 8,10 – 9,11 – 10,12 – 11,13 and 12,14 have been identified in milk fat) (Pariza *et al.*, 2001). Interest in CLA is based on a number of their health-related biological properties, e.g. their antioxidant and anti-obesity activity (Lin *et al.*, 1995) and more particularly their anticarcinogenic activity, as demonstrated in a wide range of animal models (Banni and Martin, 1998). This nutrient partitioning effect may be related to a reduction in skeletal muscle catabolism induced by immune stimulation (Corino *et al.*, 2002). CLA can offer nutritional advantages to both man and pig (Schmid *et al.*, 2006).

Studies were carried out in growing pigs and in heavy pigs. In heavy pigs (Corino *et al.*, 2003), the treatment was begun at 96 kg BW and continued up to about 170 kg BW. Different CLA levels (0%, 0.25% and 0.5% of the CLA preparation) were added to the so-called iso-energetic diets. The amount of CLA added to the diet of growing finishing pigs (50 to 105 kg) was 2% (Bee, 2001). The CLA preparation contained 65% of CLA isomers (half *cis* 9 *trans* 11 and half *trans* 10 *cis* 12) as the free fatty acids. Dietary CLA supplementation reduced lipid synthesis in the adipose tissue. It significantly increased the saturated fatty acid (SFA) content and decreased the monounsaturated fatty acid (MUFA) content. The concentrations of polyunsaturated fatty acid (PUFA) were similar in all three dietary groups of heavy pigs. The CLA contents in the adipose tissue of pigs fed diets supplemented with 0.25 and 0.5% CLA were similar (Table 15.4). In finishing pigs, the CLA content was increased, but the amount in the diet was also greater. No differences in texture, flavour, salt or overall acceptability were observed nor any statistical differences between colour values in heavy pigs.

Table 15.4 Fatty acid composition in adipose tissue of pigs fed diets containing 0%, 0.25% 0.5% or 2 % CLA

Dietary CLA added %	0 ¹	0.25 ¹	0.5 ¹	2.0 ²
Total CLA	traces	0.92	0.85	4.2
Total SFA	38.8	43.7	41.8	44.6
Total MUFA	47.2	42.1	43.6	37.7
Total PUFA	13.8	12.8	13.3	17.7(a)

¹ Heavy pig (160 kg), from Corino *et al.*, 2003; ² Pig in finishing (105 kg), from Bee, 2001.

Table 15.5 Comparison of diets enriched with sunflower oil or extruded linen seeds on the fatty acid composition, as percentage of identified fatty acids, in muscle and adipose tissue of pigs at 100 and 160 kg (from Corino *et al.*, 2008)

Liveweight Diet	110 kg				160 kg			
	Sunflower		Linseed		Sunflower		Linseed	
	Muscle	Backfat	Muscle	Backfat	Muscle	Backfat	Muscle	Backfat
SFA	37.82	40.62	37.75	40.89	38.64	40.37	38.83	41.38
MUFA	44.78	42.35	44.28	41.59	48.07	43.13	46.64	42.76
PUFA	17.40	17.03	17.97	17.51	13.29	16.49	14.54	15.86
C18:2 <i>n</i> -6	13.24	15.00	12.33	12.74	10.10	14.52	9.61	10.45
C18:3 <i>n</i> -3	0.48	0.90	1.48	3.29	0.35	0.78	1.49	3.82
C20:5 <i>n</i> -3	0.12	0.02	0.36	0.03	0.07	0.01	0.39	0.03
C22:5 <i>n</i> -3	0.36	0.04	0.59	0.11	0.25	0.06	0.57	0.12
C22:6 <i>n</i> -3	0.07	0.02	0.14	0.05	0.06	0.03	0.09	0.04
SFA	37.82	40.62	37.75	40.89	38.64	40.37	38.83	41.38
MUFA	44.78	42.35	44.28	41.59	48.07	43.13	46.64	42.76
PUFA	17.40	17.03	17.97	17.51	13.29	16.49	14.54	15.86
Σ <i>n</i> -6	15.99	15.38	15.04	13.43	12.26	14.87	11.70	11.30
Σ <i>n</i> -3	1.02	0.98	2.57	3.48	0.72	0.88	2.53	4.01
C18:2/C18:3	27.55	17.33	8.34	3.88	29.43	18.78	6.48	2.73

The nutritional interest of CLA for man, which seemed promising a few years ago, is now questioned. At present, the natural CLA in milk seems to have a beneficial effect. However, the other forms of CLA, especially those supplied in industrial CLA as the *cis* 10 *trans* 12 C18:2 *n*-6 do not seem to have the hoped for effects on human health. For this reason, and until the roles of the various CLA are better known, the addition of industrial CLA to pig diets no longer seems desirable.

Another group of fatty acids, the *n*-3 fatty acids, is of interest in human health. The preventive role of *n*-3 fatty acids in cardiovascular diseases is now well known. The objective in man is to decrease the intake of *n*-6 fatty acids and increase that of *n*-3 fatty acids. The desirable ratio for *n*-6/*n*-3 would be around 5. Although this was the case in the past, the current human diet often yields a value of 15 to 30. This change is due to modifications in pig breeding practices and human eating habits, which has led to an excessive consumption of *n*-6 fatty acids (Ailhaud *et al.*, 2006). By changing the quality of the diet fed to pigs, more *n*-3 fatty acids could be made available in human nutrition. Numerous studies have been set up with this in mind. They mainly involve supplementing the diet with flax oil but also colza and more recently, hemp. Contradictory results have been obtained with flax, depending on the flax variety used. Some flax seeds are low in fat (10%) and *n*-3 fatty acids (10 to 15% of the total fatty acids), whereas others have been selected for their high fat content (50 %) and richness in *n*-3 fatty acids (about 60% of the total fatty acids).

These latter seeds will thus be more effective because they supply more *n*-3 fatty acids. The addition of extruded flax seeds to the pig's diet, rather than linseed oil, seems to produce more effective deposition of *n*-3 fatty acids in the meat. Studies in the growing pig and in heavy pigs showed that the C18:3 *n*-3 content of

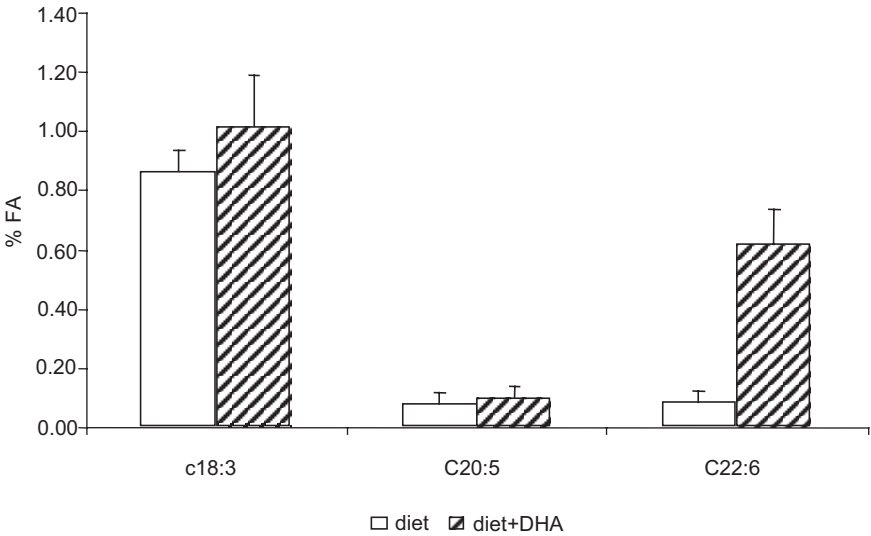


Fig. 15.1 Effect of DHA provided in the diet on the *n*-3 fatty acids content in the backfat (% of the identified fatty acids). The standard diet supplied 0.16% EPA and 0.15% DHA (17 mg EPA and 28 mg DHA/kg diet) and the diet enriched with DHA supplied 0.3% EPA and 4.5% DHA (66 mg EPA and 1.24 g DHA/kg) (Mourrot, unpublished data).

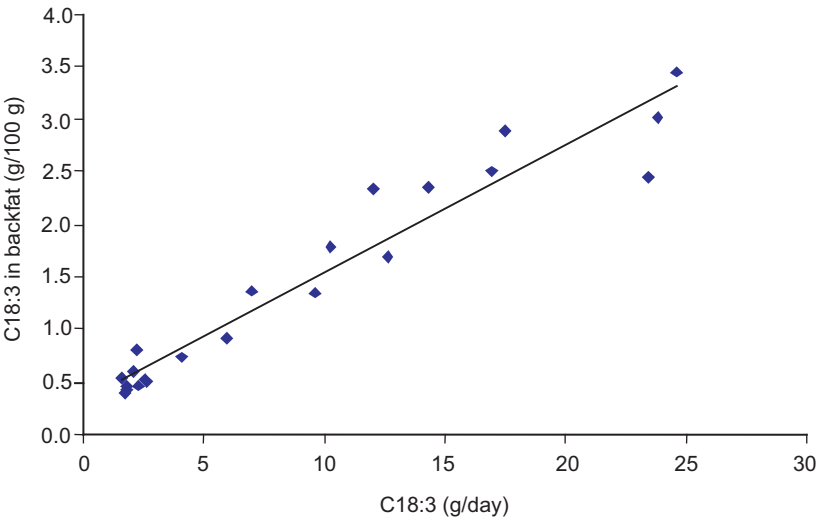


Fig. 15.2 Relation between the ingested quantity of C18:3 (g/day) and the deposited quantity of C18:3 in backfat (g FA per 100 g of adipose tissue). Each point represents an experimental lot, with an average of 10 to 16 pigs/lot (Mourrot, unpublished data).

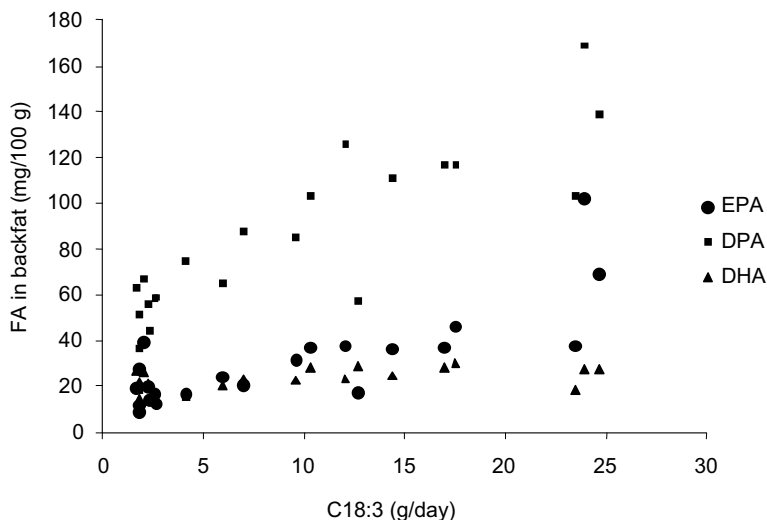


Fig. 15.3 Relation between the ingested quantity of C18:3 (g/day) and the deposited quantity of PUFA LC in backfat (mg FA per 100 g of adipose tissue). Each point represents an experimental lot, with an average of 10 to 16 pigs/lot (Mourot, unpublished data).

the meat can be strongly increased by feeding the animals extruded flax seeds rich in C18:3 (Table 15.5) (Corino *et al.*, 2008). However, the long chain fatty acids derived from the C18:3 *n*-3 precursor, such as C20:5 *n*-3 or C22:6 *n*-3, are little increased, due to the weak activity of the delta 5 and 6 desaturase observed in most animals and humans.

Introduction of large quantities of C20:6 (DHA) into the pig's diet has not given the expected results. The meat is enriched in this fatty acid, but not to the extent that had been hoped for (Fig. 15.5). This fatty acid is probably used for peroxisomal oxidation and is thus less available for storage. Linear regression shows the relationship between the amount of C18:3 (g/day) ingested and the quantity of C18:3 (Fig. 15.2) or long chain fatty acids deposited in the backfat (Fig. 15.3). The r^2 values are high and significant for C18:3 ($r^2 = 0.92$), C20:5 ($r^2 = 0.61$) and C22:5 ($r^2 = 0.74$) but lower and at the limit of significance for C22:6 ($r^2 = 0.28$). No plateau effect was demonstrated for C18:3 with the amounts used, indicating a close relationship between the quantities ingested and those deposited in the meat. The most efficient deposition of this fatty acid should thus be obtained by using flax seed with a high C18:3 content.

The sensorial quality of meats enriched with *n*-3 fatty acids may be altered. When *n*-3 fatty acids are supplied in small amounts and as flax seeds (3% of flax seeds rich in *n*-3, that is 1.5% fats with 50% of *n*-3 fatty acids), the meat products are considered good by consumers, and are often considered improved. However, if the fatty acids are added in higher amounts and as linseed oil, then the meat may have an unpleasant smell (Kouba *et al.*, 2003).

The differences between the observed effects of flax seeds and linseed oils on

the meat are due to the presence of tannins in the seed which protect the fatty acids against oxidation, not only before consumption by the animal but also inside the animal. Linseed oil may also add peroxidised acids to the diet.

15.5.3 Rearing system

Other approaches to improve pork sensorial and nutritional qualities are also being investigated, such as modifications of the breeding system. Comparisons between animals raised outdoors and animals raised in farm buildings or between animals in a piggery and those in semi-open air with access to an outdoor area are in progress.

For example, in a recent study conducted by Lebret *et al.* (2007), the influence of rearing conditions on growth performance, carcass, stress reactions at slaughter, and pork meat quality was evaluated in growing-finishing pigs. At 35 kg live weight, pigs were allocated to either a conventional system (fully slatted floor, 0.65 m²/pig, considered as control) or to an alternative system (sawdust bedding with free access to an outdoor area, 2.4 m²/pig), until slaughter at approximately 110 kg LW. The pigs had free access to standard growing and finishing diets. The trials were conducted in spring, summer, and winter. Compared with the conventionally reared pigs, those in the alternative system exhibited a higher growth rate (+10%, $P < 0.001$) due to their larger feed intake (+0.23 kg/d, $P < 0.01$), resulting in a heavier body weight at slaughter (+7 kg, $P < 0.001$). The alternative pigs had thicker backfat (+2.4 mm, $P < 0.01$) and lower lean meat content (−2.0% points, $P < 0.001$) than the conventionally reared pigs. The alternative system slightly increased meat yellowness (b^* value) in the muscles (+ 0.5 to 0.7 unit, $P < 0.001$) but did not affect redness (a^*) or lightness (L^*). The intramuscular fat content in the *longissimus muscle* was increased (+17%, $P < 0.001$). This value is relatively close to that recommended (2.3 v 3 respectively) in the study report by Fernandez *et al.* (1999). Outdoor rearing during summer and winter improved meat juiciness, whereas odour, flavour, and tenderness were unaffected. The influence of rearing conditions on all the other traits studied was not dependent on the season.

Diet strategies to modify the animal's body composition are also being tested. Compensatory growth affects performance and meat quality traits. The aim is to produce pigs with a higher age at slaughter and modified rates of lipid and protein deposition as a result of compensatory growth, so that the meat has better sensorial qualities (Heyer *et al.*, 2007). At an average live weight of 30 kg, pigs either received feed *ad libitum* during growing and finishing or restricted feeding, i.e. 65% *ad libitum* during the growing phase and *ad libitum* during finishing. At 110 kg the pigs subjected to restricted feeding were 19 d older than the *ad libitum* fed pigs and their intramuscular fat levels were lower. Thus the initial objective to increase the age at slaughter was attained, but the intramuscular lipid content remained the same, meaning that this approach is of little value in improving meat sensorial quality. The production costs would also be higher.

Outdoor animals are reared in pastures. Apart from the feed, they eat grass which increases the *n-3* fatty acids content (+0.5 to 0.7% of *n-3* fatty acids). This

Table 15.6 Effect of standard diet on fatty acid composition in outdoor pigs on pasture and indoor pigs (% identified of fatty acids in muscle or in backfat)

	Muscle		Backfat	
	Outdoor	Indoor	Outdoor	Indoor
SFA	40.66	38.96	43.65	45.08
MUFA	46.17	42.99	43.79	39.96
PUFA	13.18	18.06	12.57	14.96
C18:3 <i>n</i> -3	1.17	0.62	1.56	0.82
C20:5 <i>n</i> -3	0.07	0.10	0.04	0.04
C22:5 <i>n</i> -3	0.11	0.16	0.08	0.10
C22:6 <i>n</i> -3	0.07	0.06	0.04	0.04
Total <i>n</i> -3	1.42	0.94	1.71	1.00
C18:2/C18:3	10.23	21.74	7.85	16.31

will have a positive effect on the nutritional quality of the meat (Table 15.6, Mourot, unpublished data).

Other outdoor systems are also being tested, involving access to acorns (Rey *et al.*, 2006) or sweet chestnuts, when the meat is destined for specific types of processing (e.g. Spain, Corsica. . .) The sensorial quality of the meat from such pigs is often better. Nevertheless, the consumer's perception may be influenced by positive images of this type of production or even by holiday memories. The consumer's judgement tends to be highly favourable but is not always justified.

15.6 Conclusions

Globally, the sensorial and nutritional quality of standard pork does not present any major defects but could still be improved. However, pig production must also take into account consumer concerns for animal welfare and the environment, not forgetting the need to keep production costs attractive.

Pork nutritional quality can easily be improved to satisfy the desires of both human nutritionists and the consumers. Rearing pigs on a diet enriched with *n*-3 fatty acids will increase the content of these fatty acids in the meat. This will be beneficial to human health. Odour defects due to peroxidation will need to be avoided by controlling the supply source (extruded flax seed being preferable to linseed oil) and by making sure that a suitable quantity of antioxidant is present in the feed. Research with plant antioxidants should be encouraged as the investigations currently in progress are producing interesting results.

Studies on changes in rearing systems to ensure animal welfare and improve meat quality must be continued. Until now, the effects of such changes on meat quality have been apparently limited and are more dependent on genetics and the nature of the diet than in the standard production systems. However, in the mind of the consumer, the positive image of these new pig production systems is highly beneficial and conducive to pork consumption.

15.7 References

- Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM and Guesnet P (2006), Temporal changes in dietary fats: Role of *n*-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity, *Prog. Lipid Res.*, 45, 203–236.
- Banni S and Martin JC (1998), Conjugated linoleic acid and metabolites In *Trans Fatty Acids in Human Nutrition* edited by JL Sebedio and WW Christie, The Oily Press Ltd, Dundee, Scotland.
- Bee G (2001), Dietary conjugated linoleic acids affect tissue lipid composition but not *de novo* lipogenesis in finishing pigs, *Anim. Res.*, 50, 383–399.
- Bradley BL, Graber G, Condon RJ and Frobish LT (1983), Effects of graded levels of dietary copper on copper and iron concentrations in swine tissues, *J. Anim. Sci.*, 56, 625–630.
- Corino C, Oriani G, Pantaleo L, Pastorelli G and Salvatori G (1999), Influence of dietary vitamin E supplementation on 'heavy' pig carcass characteristics, meat quality, and vitamin E status, *J. Anim. Sci.*, 77, 1755–61.
- Corino C, Bontempo V and Sciannimanico D (2002), Effects of dietary conjugated linoleic acid on some aspecific immune parameters and acute phase protein in weaned piglets, *Can. J. Anim. Sci.*, 82, 115–117.
- Corino C, Magni S, Pastorelli G, Rossi R and Mourot J (2003), CLA in heavy pig nutrition: Influence on growth, meat quality and dry-cured ham sensory characteristics, *J. Anim. Sci.*, 81, 2219–2229.
- Corino C, Musella M and Mourot J (2008), Influences of extruded linseed on growth, carcass composition and meat quality of pigs slaughtered at 110 and 160 kg liveweight, *J. Anim. Sci.*, 1850–1860.
- Culioli J, Berri C and Mourot J (2003), Muscle foods: Consumption, composition and quality, *Sciences des Aliments*, 23, 13–34.
- Fernandez X, Monin G, Talmant A, Mourot J and Lebret B (1999), Influence of intramuscular fat content on the quality of pig meat. 1 – Composition of the lipidic fraction and sensory characteristics of muscle *Longissimus lumborum*, *Meat Sci.*, 53, 59–65.
- Fuller M F, Duncan W R H, and Boyne A W (1974), Effect of environmental temperature on the degree of unsaturation of depot fats of pigs given different amounts of food, *J. Sci. Food Agric.*, 25, 210–217.
- Geesink GH, van Buren RGC, Savenije B, Verstegen MWA, Ducro BJ, van der Palen JPG and Hemke G (2004), Short-term feeding strategies and pork quality, *Meat Science*, 67, 1–6.
- Guo Q, Richert BT, Burgess JR, Webel DM, Orr DE, Blair M, Grant AL and Gerrard DE (2006), Effect of dietary vitamin E supplementation and feeding period on pork quality, *J. Anim. Sci.*, 84, 3071–3078.
- Heyer A and Lebret B (2007), Compensatory growth response in pigs: Effects on growth performance, composition of weight gain at carcass and muscle levels, and meat quality, *J. Anim. Sci.*, 85, 769–778.
- Kouba M, Hermier D and Le Dividich J (1999), Influence of a high ambient temperature on stearoyl-CoA-desaturase activity in the growing pig, *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 124, 7–13.
- Kouba M, Enser M, Whittington FM, Nute GR and Wood JD (2003), Effect of a high linoleic acid diet on lipogenic enzyme activities, fatty acid composition and meat quality in the growing pig, *J. Anim. Sci.*, 81, 1967–1979.
- Labroue F, Marsac H, Luquet M, Gruand J, Mourot J, Neelz V, Legault C and Ollivier L (2001), Performances of French local breeds In *Pig Genetic Resources in Europe* EAAP publication No 104, Wageningen Press, p 51–57.
- Lebret B, Meunier-Salaün MC, Foury A, Mormède P, Dransfield E and Dourmad JY (2006), Influence of rearing conditions on performance, behavioral, and physiological responses of pigs to preslaughter handling, carcass traits, and meat quality, *J. Anim. Sci.*, 84, 2436–2447.

- Lin H, Boylston TD, Chang MJ, Luedecke LO and Shultz TD (1995), Survey of the conjugated linoleic acid contents of dairy products, *J. Dairy Sci.*, 78, 2358–2365.
- Lösel D and Claus R (2005), Dose-dependent effects of resistant potato starch in the diet on intestinal skatol formation and adipose tissue accumulation in the pig, *J. Vet. Med. A.*, 52, 209–212.
- Mahan DC and Parrett NA (1996), Evaluating the efficacy of selenium-enriched yeast and sodium selenite on tissue selenium retention and serum glutathione peroxidase activity in grower and finisher swine, *J. Anim. Sci.*, 74, 2967–74.
- Miller MA, Shaw A and Kraut J (1994), A structure of oxy-peroxidase as a model for the transient enzyme: peroxide complex, *Nat. Struct. Biol.*, 1, 524–31.
- Mourot J and Hermier D (2001), Lipids in monogastric animal meat. *Reprod. Nut. Dev.*, 41, 109–118.
- Mourot J and Kouba M (1998), Lipogenesis in Muscles of Growing Large White and Meishan. Pigs, *Lives. Prod. Sci.*, 55, 127–133.
- Ngapo TM, Dransfield E, Martin JF, Magnusson M, Bredahl L and Nute GR (2003), Consumer perceptions: Pork and pig production. Insights from France, England, Sweden and Denmark, *Meat Science*, 66, 125–134.
- Pariza MW, Park Y and Cook M (2001), The biologically active isomers of conjugated linoleic acid, *Progr. Lipid Res.*, 40, 283–298.
- Phillips AL, Faustman C, Lynch MP, Govoni KE, Hoagland TA and Zinn SA (2001), Effect of dietary α -tocopherol supplementation on color and lipid stability in pork, *Meat Science*, 58, 389–393.
- Prunier A, Bonneau M, Von Borell E, Cinotti S, Gunn M, Fredriksen B, Giersing M, Morton DB, Tuytens F and Velarde A (2006), A review of the welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods, *Animal Welfare*, 15, 277–289.
- Quiniou N, Richard S, Mourot J and Etienne M (2008), Effect of dietary fat or starch addition during gestation and/or lactation on lactating performance of sows, piglets' survival and progeny's performance after weaning, *Animal*, doi: 10.1017/S1751731108002991, published online by Cambridge University Press, 11 August 2008.
- Rey AI, Daza A, López-Carrasco C and López-Bote CJ (2006), Feeding Iberian pigs with acorns and grass in either free-range or confinement affects the carcass characteristics and fatty acids and tocopherols accumulation in *Longissimus dorsi* muscle and backfat, *Meat Science*, 73, 66–74.
- Schmid A, Collomb M, Sieber R and Bee G (2006), Conjugated linoleic acid in meat and meat products: A review, *Meat Science*, 73, 29–41.
- Serra X, Gil F, Pérez-Enciso M, Oliver MA, Vázquez JM, Gispert M, Díaz I, Moreno F, Latorre R and Noguera JL (1998), A comparison of carcass, meat quality and histochemical characteristics of Iberian (Guadyrbas line) and Landrace pigs, *Livestock Production Science*, 56, 215–223.
- Wood JF, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI and Whittington FM (2008), Fat deposition, fatty acid composition and meat quality: A review, *Meat Science*, 78, 343–358.

16

Using antioxidants and nutraceuticals as dietary supplements to improve the quality and shelf-life of fresh meat

M. N. O'Grady and J. P. Kerry, University College Cork, Ireland

Abstract: A number of factors are responsible for maintaining acceptable fresh meat quality. Colour is an important quality attribute influencing the consumer's purchase decision. Oxidation of muscle lipids ultimately leads to off-odours and off-flavours, thereby reducing fresh meat quality. Microbial growth also has a negative impact on the quality of fresh meat. This chapter examines the effects of vitamin E supplementation (α -tocopheryl acetate) in the diets of meat-producing animals and poultry. The influence of dietary plant extracts/nutraceuticals (green tea catechins, grape seed extract, bearberry, oregano and rosemary) containing compounds that exhibit antioxidant/antimicrobial activity, on the quality of fresh meat and poultry are also evaluated.

Key words: green tea catechins, grape seed extract, bearberry, oregano, rosemary.

16.1 Introduction

Meat is considered to be a vital component of a healthy diet, an excellent source of protein, essential minerals, trace elements and vitamins and is strongly associated with strength and virility in our culture (Tarrant, 1998). Consumption of meat is influenced by factors such as product characteristics, consumer and environmental issues. In recent times, a lack of consumer confidence in meat has been triggered by the link between meat consumption and human illnesses such as cardiovascular disease, cancer, hypertension and obesity (Jiménez-Colmenero *et al.*, 2001). The meat industry has also been undermined by food scares relating to outbreaks of *Salmonella*, pathogenic strains of *E. coli*, antibiotic and growth promoting substances abuse and BSE.

The three important properties by which consumers judge meat are appearance, texture and flavour. Appearance, specifically colour, is an important quality attribute influencing the consumer's decision to purchase. The muscle protein myoglobin is primarily responsible for the colour of fresh meat (Faustman and Cassens, 1990). Lipid oxidation is a major quality deteriorative process in muscle foods resulting in a variety of breakdown products which produce off-odours and flavours, thereby lowering meat quality. Concerns regarding the safety and toxicity of synthetic antioxidants prompted research into the use of antioxidant compounds from natural sources. Vitamin E (α -tocopherol), a lipid-soluble antioxidant, has been recognised as an essential nutrient for the growth and health of all species of animals. Since the early 1990s, the major thrust of research involving supplementation of cattle, pig and poultry diets with increased levels of vitamin E (as α -tocopheryl acetate), which resulted in elevated tissue vitamin E levels, was concerned with its ability to delay lipid oxidation in muscle foods. In fresh beef, several researchers found that vitamin E not only delayed lipid oxidation but also delayed myoglobin oxidation (Faustman *et al.*, 1989; Arnold *et al.*, 1992). Post-mortem addition of vitamin E was not as effective a means of reducing lipid oxidation, when compared to dietary supplementation, where vitamin E is incorporated directly into the membrane where lipid oxidation is initiated (Mitsumoto *et al.*, 1993).

In recent times, as the link between diet and chronic disease prevention continues to grow, much interest exists in developing healthier meats and meat products, which would serve to increase consumer confidence in meat whilst conferring potential health benefits to the consumer. The terms 'functional food' and 'nutraceutical' are used interchangeably and are usually defined as any substance that may be considered a food or part of a food which provides medical or health benefits, including the prevention and treatment of disease (DeFelice, 1992). The functional properties of many plant extracts have been investigated for their potential use as novel nutraceuticals. In addition to antioxidant and antimicrobial activity, nutraceuticals are believed to modulate the aetiology of many chronic diseases such as coronary heart disease (Somova *et al.*, 2003), cancer (Higdon and Frei, 2003), hypertension, osteoporosis and diabetes (Maghrani *et al.*, 2004). With specific reference to meat and meat products, exogenous addition of natural extracts from plants such as rosemary (Formanek *et al.*, 2001) and oregano (Sánchez-Escalante *et al.*, 2003) delayed lipid oxidation in muscle foods, thereby enhancing meat quality and shelf-life. The antioxidant potential of such plant extracts in meat and meat products may be increased or enhanced if administered at increased levels in animal and poultry diets.

This review aims to examine the use of vitamin E and alternatives such as plant extracts/nutraceuticals containing antioxidant compounds, in the diets of meat-producing animals and poultry. The effects of such extracts on fresh meat quality parameters and shelf-life will also be evaluated.

16.2 Factors affecting fresh meat quality and shelf-life: appearance (colour), lipid oxidation and microbiology

16.2.1 Fresh meat colour

Meat colour is dependent on the concentration and chemical state of the meat pigments, primarily myoglobin and haemoglobin, and on the physical characteristics of meat, such as its light scattering and absorbing properties (Kropf, 1993). The myoglobin concentration of muscle varies between and within animal species and is affected by factors such as age, exercise, and diet of the animal, as well as by genetic and environmental factors (Livingston and Brown, 1981).

Myoglobin can exist in one of three forms: deoxymyoglobin, oxymyoglobin or metmyoglobin. Interconversion of the three pigment states is possible and the dominant pigment form depends on localised conditions (Kropf, 1993). Deoxymyoglobin, frequently referred to as myoglobin or reduced myoglobin, contains iron in the ferrous (Fe^{2+}) state and is characterised by the absence of a ligand at the sixth coordinate position of the haem group. It is purplish-red in colour and is responsible for the colour of meat immediately after cutting into a deep muscle, or of meat stored under a vacuum (Renerre, 1990). Oxymyoglobin, a cherry-red form of the pigment, forms very quickly after exposure of deoxymyoglobin to oxygen. The pigment must be in the ferrous state for oxygenation to occur and oxygen occupies the sixth binding site of the ferrous haem iron (Livingston and Brown, 1981). In red meats, oxymyoglobin imparts the colour that consumers associate with freshness (Faustman and Cassens, 1990).

The colour of fresh red meats is relatively short-lived and both deoxymyoglobin and oxymyoglobin readily oxidise to metmyoglobin, in which the haem iron has been oxidised to the ferric (Fe^{3+}) state and water occupies the sixth coordinate position. Oxymyoglobin is more stable towards oxidation than deoxymyoglobin, partly due to hydrogen bonding between the bound oxygen and a distal histidine residue of the globin moiety (Giddings, 1977). Metmyoglobin is incapable of binding oxygen and is thus physiologically inactive (Faustman and Cassens, 1990). Metmyoglobin gives meat a brown colour which consumers associate with a lack of freshness, and unacceptability (Hood and Riordan, 1973). Once formed, metmyoglobin remains the predominate form of myoglobin in muscle unless reducing systems such as metmyoglobin reductase are active (Livingston and Brown, 1981; Ledward, 1985).

A number of factors contribute individually to discoloration in fresh meats during storage. In the complex meat system, interactions between factors contribute significantly to the difficulty of identifying a solution to the problem of discoloration (Faustman and Cassens, 1990). During storage, the rate of metmyoglobin accumulation is related to intrinsic factors such as muscle pH, muscle fibre type and the age, breed, sex and diet of animals, as well as extrinsic factors such as pre-slaughter treatment of animals and hot-boning, electrical stimulation and chilling of carcasses. In addition, during retail display, environmental factors such as temperature, oxygen availability, type of lighting, microbial

growth and storage atmosphere (air, vacuum, modified atmosphere packaging) influence the saleable shelf-life of fresh meats.

16.2.2 Lipid oxidation in post-slaughter muscle

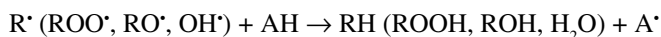
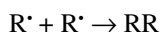
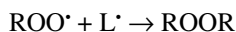
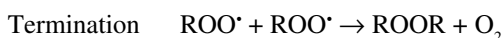
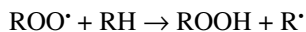
Post-slaughter biochemical changes which accompany the conversion of muscle to meat give rise to conditions where the process of lipid oxidation is no longer tightly controlled, and the balance between pro-oxidative and antioxidative capacity favours oxidation. The rate and extent of subsequent lipid oxidation in muscle foods is influenced by both pre-slaughter factors, such as stress and diet, and post-slaughter factors, such as postmortem pH decline, carcass temperature and electrical stimulation (Morrissey *et al.*, 1994). In addition, post-slaughter processes such as grinding, mincing, chopping, flaking, deboning, restructuring or cooking lead to disruption of cell membranes (Gray *et al.*, 1994). The resultant break-up of cell compartments permits the interaction of pro-oxidants with unsaturated fatty acids and oxygen, the generation of free radicals and propagation of the oxidative reaction (Asghar *et al.*, 1988). Tims and Watts (1958) used the term 'warmed-over flavour' to describe the rancid or stale off-flavour that develops as a result of lipid oxidation in cooked meat in refrigerated storage. Warmed-over flavour has also been reported in raw meat that is ground and exposed to air (Sato and Hegarty, 1971) and in unheated products, such as mechanically separated or restructured meats, in which the muscle structure is disrupted and air is incorporated (Gray and Pearson, 1987).

The susceptibility of meats to oxidise depends on factors such as muscle fatty acid composition and the level of muscle pro- and antioxidants (Gray *et al.*, 1994). Susceptibility to oxidation increases with increasing unsaturation and, because membranal phospholipids are highly unsaturated, it is generally believed that lipid oxidation in muscle foods is initiated in this fraction (Gray and Pearson, 1987). Although phospholipids make up only 0.43–1.0% of muscle weight (Hornstein *et al.*, 1961), their susceptibility to oxidation makes them important contributors to deterioration in flavour, colour and nutritive value (Igene *et al.*, 1980; Gatellier *et al.*, 1995).

Mechanism of lipid oxidation

Lipid oxidation is the process by which molecular oxygen reacts with unsaturated lipids to form lipid peroxides. The process involves the formation of lipid radicals from unsaturated lipids, the uptake of oxygen, rearrangement of double bonds, and the eventual breakdown of lipid peroxides to produce a variety of products including alcohols, aldehydes and ketones (Gardner, 1975). The direct reaction of lipids with oxygen is spin-forbidden because the ground state of lipids is of singlet multiplicity whereas oxygen is of triplet multiplicity. However, the spin restriction can be overcome by initiators or initiating variables such as temperature, physiological reduction of oxygen to water, photosensitisers, radiation, singlet oxygen, oxygen–transition metal complexes, or by enzymic (lipoxygenase-like) catalysis. Lipid oxidation is known to proceed by a free radical chain reaction mechanism

involving initiation, propagation/branching and termination stages (Hamilton *et al.*, 1997).



Initiation occurs when a hydrogen atom is abstracted from a methylene group on an unsaturated fatty acid molecule (RH) to form a lipid radical (R[•]). Propagation involves the reaction of the lipid radical (R[•]) with molecular oxygen to form a lipid peroxy radical (ROO[•]). Since ROO[•] is more highly oxidised than R[•] or RH, it preferentially oxidises other unsaturated fatty acids and propagates the chain reaction by abstracting a hydrogen atom from another unsaturated fatty acid. Lipid hydroperoxides (ROOH) formed in the propagation reaction are both products of oxidation and substrates for further reaction. The lipid hydroperoxides may undergo homolytic scission to form alkoxy (RO[•]) and hydroxyl radicals (OH[•]), which are capable of propagating further oxidation and lead to chain branching. Termination involves the reaction of free radicals to form non-initiating and non-propagating products. Chain-breaking antioxidants (AH) terminate the free radical chain reaction by donating hydrogen atoms to free radical species and forming less reactive products.

The mechanism of initiation of lipid oxidation in muscle has been the subject of much research and a number of review articles have been written on the subject (Kanner *et al.*, 1987; Asghar *et al.*, 1988; Gutteridge and Halliwell, 1990). Potential initiators include oxygen derivatives (superoxide, hydroxyl radicals, singlet oxygen) as well as oxygen-transition metal complexes. Among the latter, the ferryl porphyrin cation radical, a H₂O₂-activated form of metmyoglobin, was proposed as an initiator of lipid oxidation (Kanner and Harel, 1985). In addition to the non-enzymic pathways, lipid oxidation may be initiated by lipoxxygenase (Kanner *et al.*, 1987) or NADH/NADPH-dependent enzymic lipid oxidation systems associated with muscle membrane fractions (Lin and Hultin, 1976).

16.2.3 Microbiology of meat

Fresh meat is a highly perishable food product which, unless correctly stored, processed, packaged and distributed, spoils quickly and becomes hazardous due to microbial growth (McDonald and Sun, 1999). The potential for microbial contamination is influenced by factors such as animal condition prior to slaughter, slaughter-plant practices, extent of handling and subsequent storage conditions (Jackson *et al.*, 1996). Growth of spoilage organisms such as *Brochothrix thermosphacta*, *Pseudomonas* spp. and lactic acid bacteria to high numbers results in meat unfit for human consumption. Pathogens, depending on the species and whether they are present, such as *Listeria monocytogenes*, *Salmonella* spp. and *E. coli* 0157:H7 can grow and cause illness by ingestion of the bacterial cells themselves or from toxins they produce. A combination of intrinsic (for example, pH, acidity, presence of antimicrobials, nutrient availability) and extrinsic factors (temperature, relative humidity, packaging characteristics and interactions) determine the microbiology of meat (McDonald and Sun, 1999). The intrinsic nature of most raw meats with high water activities (> 0.98), moderate pH (5.5–6.5) and readily available sources of energy, carbon and other nutrients, makes them ideal for most microbial growth (Varnam and Sutherland, 1995). Meat spoilage is not always evident and consumers indicate that gross discoloration, strong off-odours and slime development constitute the main qualitative criteria for the rejection of fresh meat (Nychas *et al.*, 2008).

Many plant extracts/nutraceuticals, such as rosemary oleoresin and green tea extract display potent antimicrobial activity in fresh meat (Ahn *et al.*, 2004; Kumudavally *et al.*, 2008), thereby improving safety and shelf-life characteristics.

16.3 Chemistry and structure of vitamin E

‘Vitamin E’ is the generic name for a group of lipid-soluble compounds known as tocopherols and tocotrienols (Rice and Kennedy, 1986). Structurally, vitamin E is composed of a chromanol nucleus (2 rings; one phenolic and one heterocyclic) and a phytol side chain (Fig. 16.1). Eight isomers of vitamin E exist namely α -, β -, γ -, and δ -tocopherol and α -, β -, γ -, and δ -tocotrienol. The four tocopherols have saturated phytol side chains and vary only in the number and patterns of methyl substitution on the phenolic ring. The four tocotrienols are similar to their corresponding tocopherols except that they contain three isolated double bonds on their phytol side chains (Kamal-Eldin and Appelqvist, 1996). α -Tocopherol is the most potent form of vitamin E and the main vitamin E isomer found in meat and meat products (Piironen *et al.*, 1985).

The antioxidant activity of α -tocopherol can be attributed to the ability of the hydroxyl group on the chromanol ring to quench highly reactive free radicals by donating a hydrogen atom to the free radical and yielding a stable chromanoxyl radical of the antioxidant (Kagan *et al.*, 1990). In animal tissues α -tocopherol is located in the cell membrane, closely associated with the membranal phospholipids (Kagan and Quinn, 1988). The phytol side chain of vitamin E is considered to be

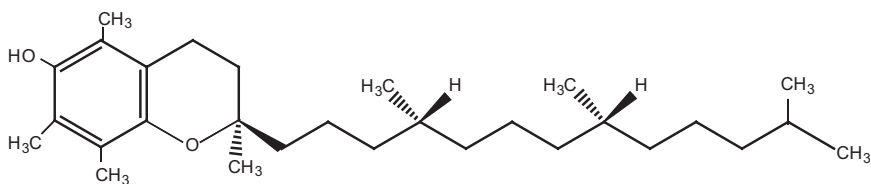


Fig. 16.1 Chemical structure of α -tocopherol.

important for the proper orientation of the molecule in the membrane but it is not directly involved in the antioxidant function.

Tocopherols are present naturally in oil seeds, leaves and other green parts of higher plants, and the protective effect of tocopherols in vegetable oils against lipid oxidation is well established (Jung and Min, 1990). Since animals are unable to synthesise vitamin E, they are therefore dependent on dietary sources to meet their nutritional requirements.

16.3.1 Dietary vitamin E supplementation

At supranutritional levels, the protective effect of α -tocopherol against lipid oxidation in biological systems is well established. Supplemental α -tocopherol is usually added to animal or poultry feed in the form of synthetic all-*rac*- α -tocopheryl acetate, adsorbed onto the surface of solid carriers such as silica or rice hulls, since acetylation protects α -tocopherol from pro-oxidative factors such as heat, light, alkali and metals such as Cu and Fe (MacPherson, 1994). α -Tocopheryl acetate is subsequently de-esterified in the gastrointestinal tract resulting in biologically active free tocopherol (Buckley *et al.*, 1995). Following de-esterification and absorption, α -tocopherol isomers are secreted into the lymph within chylomicrons (Gallo-Torres, 1980). The liver receives α -tocopherol from chylomicron remnants and resecretates it into plasma bound to very low density lipoproteins, which participate in exchange of α -tocopherol with low- and high-density lipoproteins (Behrens and Madere, 1991). Dietary vitamin E supplementation has been shown to result in elevated muscle α -tocopherol in chickens (Lauridsen *et al.*, 1997; Maraschiello *et al.*, 1999), turkeys (Mercier *et al.*, 1998; Renner *et al.*, 1999), beef cattle (Faustman *et al.*, 1989; O'Grady *et al.*, 2001), pigs (Buckley *et al.*, 1989; Hasty *et al.*, 2002) and lambs (Guidera *et al.*, 1997; Lauzurica *et al.*, 2005).

Lower levels of lipid oxidation in fresh meat from poultry or animals fed supplemental α -tocopheryl acetate is well documented. The supplementation of broiler chick diets (containing 5.5% partially hydrogenated soyabean oil) with 100 mg α -tocopheryl acetate/kg feed, for 49 days pre-slaughter, reduced lipid oxidation in leg and breast meat stored for up to 6 days at 4 °C (Lin *et al.*, 1989). Similarly, *ad libitum* supplementation of broiler diets with 100 I.U. α -tocopheryl acetate/kg feed for 42 days pre-slaughter reduced lipid oxidation in breast and thigh muscles stored for up to 12 days at 4 °C. Surface colour (CIE 'L' lightness,

'a' redness and 'b' yellowness values) and metmyoglobin accumulation were unaffected by dietary α -tocopheryl acetate (Ryu *et al.*, 2005). Guo *et al.* (2001) reported that, after 4 days of storage at 4 °C, lipid oxidation in chicken thigh meat significantly decreased with increasing level of dietary α -tocopheryl acetate (*ad libitum* supplementation of 5, 10, 20, 50 and 100 mg α -tocopheryl acetate for 42 days pre-slaughter). Sheldon *et al.* (1997) reported that *ad libitum* supplementation of turkey diets with increasing levels of α -tocopheryl acetate (max. 250 I.U./kg feed) for up to 18 weeks reduced lipid oxidation in turkey breast meat stored for up to 7 days at 4 °C. The growth performance of broiler chicks was not influenced by dietary vitamin E supplementation (Guo *et al.*, 2001; Ryu *et al.*, 2005).

In fresh beef, several researchers found that dietary α -tocopheryl acetate not only delayed lipid oxidation but also delayed oxymyoglobin oxidation (Arnold *et al.*, 1992, 1993a,b; Faustman *et al.*, 1989; O'Grady *et al.*, 1998). Supplementation of steer diets with 370 I.U. α -tocopheryl acetate/animal/day, for approximately 10 months pre-slaughter, resulted in lower levels of lipid oxidation and metmyoglobin formation, compared to controls, in *M. gluteus medius* patties stored aerobically at 4 °C for up to 6 days (Faustman *et al.*, 1989). Arnold *et al.* (1992) reported a study where beef animals were fed supplemental vitamin E at either 300 I.U. α -tocopheryl acetate/animal/day for 266 days (Diet 1), 1140 I.U. α -tocopheryl acetate/animal/day for 67 days (Diet 2), or 1200 I.U. α -tocopheryl acetate/animal/day for 38 days (Diet 3). All three dietary feeding regimes were effective in delaying meat discoloration. The colour stability of *M. longissimus lumborum* from animals fed Diets 1, 2 or 3 was extended by 2.5 days, 2.5 days and 4.8 days, respectively. The colour stability of *M. gluteus medius*, which discoloured more rapidly than *M. longissimus lumborum*, was extended by 1.6 days (Diet 3) or 3.8 days (Diet 2) as a result of dietary vitamin E supplementation. A marked increase in lipid oxidation occurred after 6 days of simulated retail display in *M. longissimus lumborum* obtained from animals that were not fed supplemental vitamin E. However, *M. longissimus lumborum* obtained from animals fed Diet 2 or 3 exhibited a small increase in lipid oxidation during 18 days of display.

The scientific literature contains many further studies reporting a colour-stabilising effect of dietary α -tocopheryl acetate supplementation on beef steaks stored under simulated retail or refrigerated display conditions. α -Tocopheryl acetate supplementation levels and duration of feeding were reported to result in an improvement in fresh beef colour stability included the following: 380 or 1447 I.U./animal/122 days (Chan *et al.*, 1995); 1204 I.U./animal/122 days (Chan *et al.*, 1996); 231, 486 or 2109 I.U./animal/42 or 126 days (Lanari *et al.*, 1996; Liu *et al.*, 1996a); 4036 I.U./animal/90 days (Chan *et al.*, 1998). Under similar storage conditions and in addition to colour stability, other studies report a positive effect of α -tocopheryl acetate on lipid stability in beef steaks as follows: 231 486 or 2109 I.U./animal/42 or 126 days (Liu *et al.*, 1996b); 820 or 1600 I.U./animal/100 days (Sanders *et al.*, 1997); 5500 I.U./animal/7 days (Mitsumoto *et al.*, 1998).

The extent to which an effect of dietary vitamin E supplementation is observed depends on the α -tocopherol status of cattle upon initiation of supplementation (Faustman *et al.*, 1998). Grazing before confinement of finishing cattle is common

practice in the beef cattle industry. Green forage is a good dietary source of α -tocopherol (Faustman *et al.*, 1998). Lanari *et al.* (2002) reported higher muscle α -tocopherol concentrations and greater colour stability in fresh *M. gluteus medius* and *M. semimembranosus* from pasture-fed cattle compared to grain-fed animals with no vitamin E supplementation. Delmore *et al.* (1998) fed cull cows either a basal α -tocopherol diet or supplemented diets giving a total of 50 400 I.U. vitamin E over a period of 14 days (3600 I.U./animal/day), 28 days (1800 I.U./animal/day) or 56 days (900 I.U./animal/day). They reported muscle α -tocopherol levels ranging from 2.39 mg/kg (basal diet) up to 4.21–5.44 mg/kg for cows fed from 14 up to 56 days. In contrast, Arnold *et al.* (1992) fed 79 800 I.U. vitamin E over 266 days (300 I.U./animal/day) and reported muscle α -tocopherol levels ranging from 0.9 mg/kg (basal) up to 3.8 mg/kg. Delmore *et al.* (1998) concluded that the higher vitamin E levels in the cull cow study could be attributed to the intake of forage for long periods prior to the trial, which led to an accumulation of vitamin E in muscle and a high initial basal vitamin E level.

Generally, dietary vitamin E supplementation does not affect feedlot performance characteristics such as average daily gain or carcass characteristics such as marbling score or quality grade (Arnold *et al.*, 1992, 1993a,b; Sanders *et al.*, 1997). Microbial load in intact (Arnold *et al.*, 1993b; Chan *et al.*, 1995, 1996; Sanders *et al.*, 1997) or ground beef (Cabedo *et al.*, 1998) was unaffected by dietary vitamin E supplementation.

Supplementation of porcine diets with vitamin E (200 mg α -tocopheryl acetate/kg feed for either 4 or 10 weeks pre-slaughter) reduced lipid oxidation in *M. longissimus dorsi* and pork patties during refrigerated storage (Buckley *et al.*, 1989). Similarly, lipid antioxidant activity has been reported in fresh pork, where pigs were fed 200 mg α -tocopheryl acetate/kg feed for 2 weeks pre-slaughter (Monahan *et al.*, 1990) or from 30–95 kg slaughter weight (Pfalzgraf *et al.*, 1995).

Although clear positive effects of dietary vitamin E supplementation on the colour stability of fresh beef have been reported in the scientific literature, similar effects have not consistently been observed in fresh pork. Monahan *et al.* (1994) reported that supplementation of pig diets (containing 3% oxidised corn oil), fed *ad libitum*, with 100 or 200 mg α -tocopheryl acetate/kg feed (30–98 kg slaughter weight) resulted in lower levels of lipid oxidation and improved colour stability (Hunter a value) in fresh *M. longissimus dorsi* stored for up to 8 days at 4 °C, compared to pork from pigs fed 10 mg α -tocopheryl acetate/kg feed. Lanari *et al.* (1995) reported positive effects of dietary vitamin E (198 or 207 mg α -tocopheryl acetate/kg feed for 105 days pre-slaughter) on the colour and lipid stability of fresh pork stored aerobically or in modified atmosphere packs (80% O₂ : 20% CO₂). However, the beneficial effects of dietary vitamin E on pork colour stability were detected only in samples stored under illuminated lighting conditions. Hoving-Bolink *et al.* (1998) reported that supplementation of pig diets with α -tocopheryl acetate (208 mg/kg) for 84 days pre-slaughter reduced lipid oxidation in *M. longissimus lumborum* and *M. psoas major*. A minor improvement in the colour stability of *M. longissimus lumborum* only was observed on day 6 of storage at 7 °C.

By contrast, a number of studies reported no beneficial effects of dietary vitamin E on fresh pork colour stability. Supplementation of pig diets (containing 6% sunflower oil) with 100 or 200 mg α -tocopheryl acetate/kg feed for approximately 6 months pre-slaughter did not significantly affect the surface colour (CIE L, a b values) of fresh *M. longissimus dorsi* (Zanardi *et al.*, 1999). Results from sensory evaluation of the colour stability of *M. longissimus dorsi*, stored in modified atmosphere packs (80% O₂ : 20% CO₂), indicated that the higher level of vitamin E supplementation (200 mg α -tocopheryl acetate/kg feed) may result in slower rates of myoglobin oxidation (metmyoglobin accumulation) (Zanardi *et al.*, 1999). Jensen *et al.* (1997) reported that supplementation of pig diets, fed *ad libitum*, with 100, 200 or 700 mg α -tocopheryl acetate (50–90 kg slaughter weight) did not improve the colour stability (Hunter L, a b values) of fresh *M. longissimus dorsi* or *M. psoas major* stored aerobically for up to 6 days at 4 °C. Significant reductions in lipid oxidation were observed. Guo *et al.* (2006) reported that supplementation of pig diets (containing normal corn, high oil corn or high oil corn plus 4% choice white grease) with 200 I.U. α -tocopheryl acetate/kg feed for 63 days pre-slaughter did not enhance the colour stability (CIE L, a b values) of *M. longissimus dorsi*. Lipid stability in fresh ground pork patties, stored for up to 6 days at 4 °C, increased as a result of dietary vitamin E supplementation. In pigs fed *ad libitum* diets containing 100 mg α -tocopheryl acetate for 84 days pre-slaughter, the surface colour (Hunter L, a b values) of *M. longissimus dorsi* stored aerobically was unaffected by dietary vitamin E supplementation (Cannon *et al.*, 1996). After 3 and 5 days of simulated retail display, lower levels of lipid oxidation in fresh *M. longissimus dorsi* from pigs fed supplemental vitamin E, compared to controls, were observed.

In general, the growth performance of pigs was not influenced by dietary vitamin E supplementation (Cannon *et al.*, 1996; Guo *et al.*, 2006). Additional fresh pork quality parameters such as pH, water-holding capacity, drip loss and microbiological status were unaffected by supplemental vitamin E (Cannon *et al.*, 1996; Guo *et al.*, 2006; Hoving-Bolink *et al.*, 1998; Jensen *et al.*, 1997).

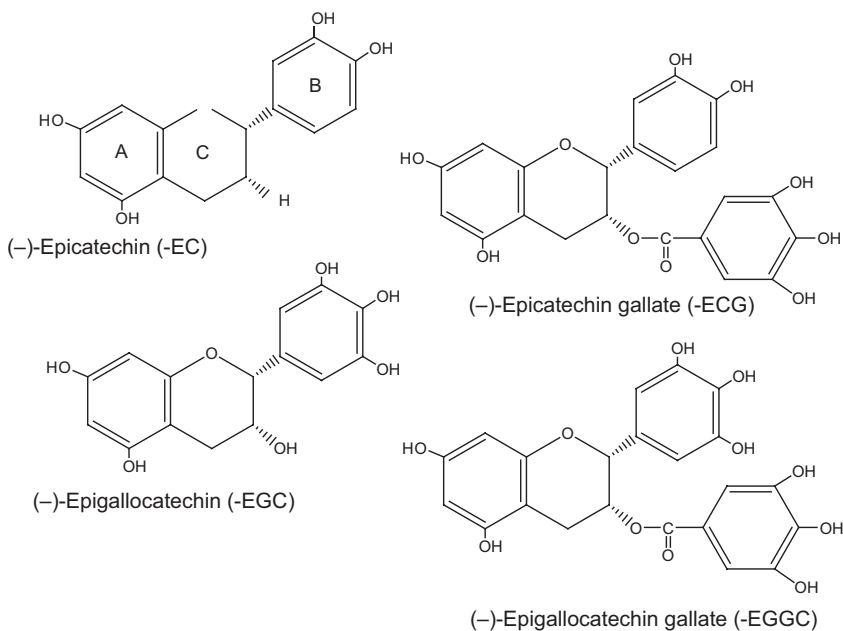
Positive effects of dietary vitamin E on fresh lamb meat quality have been reported. Guidera *et al.* (1997) reported a study where lamb diets were supplemented with 1000 mg α -tocopheryl acetate/kg feed for 10 weeks pre-slaughter. Lower levels of lipid oxidation in fresh *M. longissimus dorsi* and *M. Psoas major* stored aerobically for up to 7 days at 4 °C were observed, compared to meat from lambs fed the basal diet (20 mg α -tocopheryl acetate/kg feed). Positive effects on fresh lamb meat colour stability (Hunter L, a value) were also observed. Supplementation of lamb diets, fed *ad libitum*, with 250, 500 or 100 mg α -tocopheryl acetate/kg feed, for approximately 37 days pre-slaughter, delayed lipid oxidation and improved the colour stability of *M. longissimus dorsi* stored in modified atmosphere packs (70% O₂ : 30% CO₂) for up to 28 days at 2 ± 1 °C. The growth performance of lambs and microbial load in lamb meat, which limited the shelf-life of *M. longissimus dorsi* in the study, were unaffected by dietary vitamin E supplementation (Lauzurica *et al.*, 2005).

16.4 Chemistry and structure of green tea catechins

Tea (theaceae family) is one of the most widely consumed beverages in the world. Three types of tea products are made from the tea plant (*Camellia sinensis* L.), namely green tea (non-fermented), oolong tea (semi-fermented) and black tea (fermented). There are two main varieties of tea plants namely, *C. sinensis* var. *sinensis* (China tea) grown extensively in China, Japan and Taiwan and *C. sinensis* var. *assamica* (Assam tea) which predominates in south and southeast Asia including Malaysia and, more recently Australia (Chan *et al.*, 2007). An aqueous infusion of dried tea leaves contains catechins, caffeine, amino acids, carbohydrates, protein, chlorophyll, volatile compounds, fluoride, minerals and additional undefined compounds (Graham, 1992). Catechins are the major group of polyphenolic flavonoids found in tea representing 60–90% and 6–24% of the flavonoids in green and black teas, respectively (Higdon and Frei, 2003). During the black tea fermentation process, catechins degrade as a function of the fermentation temperature (Obanda *et al.*, 2001). Fermentation of green tea lowers the catechin content of tea from approximately 14 to 4% (w/w dry weight basis) (Peterson *et al.*, 2005). Auger *et al.* (2004) reported total catechin concentrations for green and black teas of 420 mg/l and 250 mg/l, respectively.

In recent years, the health promoting properties of green tea have received much attention. Potential health benefits of green tea, attributed to the polyphenolic catechin components present, include anti-cancer activity, treatment of cardiovascular disease and anti-inflammatory properties (Pastore and Fratellone, 2006; Yang *et al.*, 2007). Tea catechins are potent antioxidants and activity has been reported in a variety of test systems (Unno *et al.*, 2000; Yen and Chen, 1995; Yen *et al.*, 1997) including liposomes (Huang and Frankel, 1997) and an oil-in-water emulsion (Roedig-Penman and Gordon, 1997). He and Shahidi (1997) demonstrated the antioxidant activities of ground green tea, commercial tea extracts, green tea extracts and pure tea catechin isomers in a fish meat model system. The principal catechins found in green tea are (-)-epicatechin (-EC), (-)-epigallocatechin (-EGC), (-)-epicatechin gallate (-ECG), and (-)-epigallocatechin gallate (-EGCG) (Yilmaz, 2006) (Fig. 16.2a). Structural differences between tea catechin isomers result in varying degrees of antioxidant potency. -EC has an ortho-dihydroxyl group on the B-ring at carbons 3 and 4 and a hydroxyl group at carbon 3 on the C ring. -EGC differs from -EC in that it has three hydroxyl groups, at carbons 3, 4, and 5 on the B ring. -ECG has a gallate moiety esterified at carbon 3 on the C ring. -EGCG has both three hydroxyl groups, at carbons 3, 4, and 5 on the B ring and a gallate moiety esterified at carbon 3 on the C ring (Higdon and Frei, 2003). Catechins function as antioxidants due to both iron-chelating and free radical scavenging activity (Guo *et al.*, 1996). Tea catechins also exert antimicrobial activity (Almajano *et al.*, 2008; Yilmaz, 2006). Exogenous addition of green tea catechins (200–1000 mg/kg) exhibits potent antioxidant activity in raw minced beef (O'Grady *et al.*, 2006a; Tang *et al.*, 2006). Similarly, antioxidant activity of added green tea catechins (300 mg/kg) has been demonstrated in raw minced pork and poultry (chicken, duck and ostrich meat) (Tang *et al.*, 2001a). A

(a)



(b)

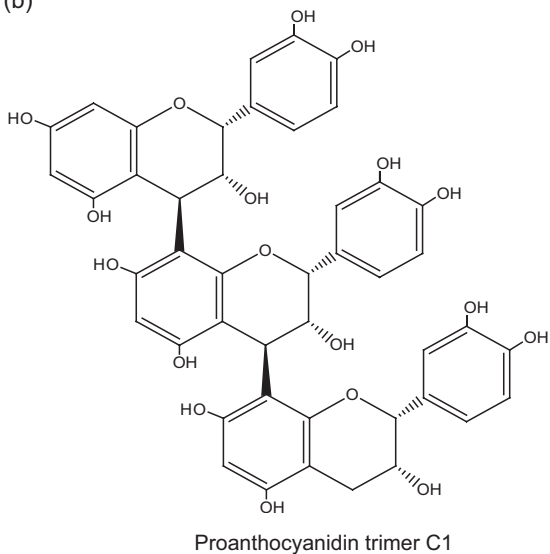


Fig. 16.2 Chemical structures of the main catechin isomers present in (a) green tea and (b) grape seed proanthocyanidin trimer C1.

preservative antimicrobial effect of green tea extract (100 ml extract/kg meat – sprayed twice) has been reported in sheep meat (mutton) (Kumudavally *et al.*, 2008).

16.4.1 Dietary supplementation with green tea catechins

Dietary feeding trials where poultry, pig and beef cattle diets are supplemented with green tea catechins have been reported in the scientific literature. Tang *et al.* (2001b) reported a study where poultry diets were supplemented with green tea catechins (50, 100, 200 or 300 mg/kg feed), and for comparative purposes α -tocopheryl acetate (200 mg/kg feed), for 6 weeks pre-slaughter. Following storage for 1, 3, 6 and 9 months at -20°C , chicken breast and thigh muscles were thawed, minced and stored for up to 10 days at 4°C . Lipid oxidation decreased with increasing dietary green tea catechin concentration in minced breast and thigh muscles. The antioxidant potency, as indicated by low levels of lipid oxidation, of dietary green tea catechins at concentrations of 200 and 300 mg/kg feed was similar to 200 mg α -tocopheryl acetate/feed.

In pigs, Mason *et al.* (2005) conducted a study where porcine diets were supplemented with green tea catechins (200 mg/kg), as part of the finisher diet, for approximately 33 days pre-slaughter. In raw *M. longissimus dorsi* slices stored in modified atmosphere packs (60% O_2 : 40% CO_2) for up to 10 days at 4°C , significantly lower levels of lipid oxidation were observed in pigs fed green tea catechins compared to the control. Overall, levels of lipid oxidation observed were low (< 0.2 mg malondialdehyde (MDA)/kg meat), implying that the magnitude of the effect of dietary green tea catechins on lipid oxidation in pork was small, or trends observed may have been due to inter-animal variation.

O'Grady *et al.* (2006a) reported a study where beef cattle were fed a concentrate-based diet supplemented with green tea catechins (81.43%) (1000 mg/animal/day) for 103 days before slaughter. In *M. Longissimus dorsi* stored aerobically or in modified atmosphere packs (80% O_2 : 20% CO_2) for up to 8 days at 4°C , colour (CIE a value) and lipid stability were not significantly improved as a result of dietary green tea catechin supplementation. In addition, the sensory properties of cooked beef slices were unaffected. These findings were in contrast to the positive effects of dietary green tea catechins on the oxidative stability of fresh poultry observed by Tang *et al.* (2001b). A number of factors were proposed as potential reasons for the lack of an effect of dietary green tea catechins on the oxidative stability of fresh beef. The pH range of the bovine rumen typically ranges from 5.5–7.0 (Van Soest, 1994). The abomasum accounts for gastric digestion where the pH is maintained in a fairly constant pH range of 1.6–2.5 (Merchen, 1988). Individual catechin isomers have varying degrees of pH sensitivity (Record and Lane, 2001; Zhu *et al.*, 1997). Zhu *et al.* (1997) reported that -EGC and -EGCG were unstable in alkaline solutions and almost completely degraded when incubated for 3 hours at pH 7.4. Under the same incubation conditions -EC remained unchanged while -ECG stability decreased by 20%. Similar findings were reported by Record and Lane (2001). Catechins -EGC and -EGCG repre-

sented >50% of the total catechin concentration of the dietary supplement used in the study. Therefore, the lack of a positive effect of dietary green tea catechins may have been due to degradation of catechin isomers in the alkaline conditions of the bovine rumen or intestine. Zhu *et al.* (1997) found that a mixture of catechin isomers was stable at acidic, < 4 pH values. Record and Lane (2001) reported a 10% loss in the antioxidant activity of catechins incubated at pH 2. Therefore, it is unlikely that catechin concentrations were reduced in the acidic conditions of the abomasum. An *in vitro* fermentation study also indicated that green tea catechins were not fermented in simulated rumen conditions indicating their 'potential availability' for absorption in the bovine intestine (O'Grady *et al.*, 2006a).

An additional study was carried out to determine if feeding green tea catechins at high levels would elicit an antioxidant response in bovine muscle (O'Grady *et al.*, 2006b). Beef cattle diets were supplemented with green tea catechins (1000, 4000 or 10 000 mg/animal/day) for 90 days pre-slaughter. Increasing dietary green tea catechin concentrations did not significantly improve the colour or lipid stability of *M. Longissimus dorsi* stored in modified atmosphere packs (80% O₂ : 20% CO₂) for up to 8 days at 4 °C (Table 16.1). Also, following HPLC analysis, green tea catechin isomers were not detected in *M. longissimus dorsi* samples indicating that green tea catechin isomers were not deposited in the muscle. An additional experiment was conducted to estimate the pH sensitivity of the green tea catechin supplement used in the dietary trials. Green tea catechin was pH adjusted (5.5–8.0) and subsequently added to minced beef. The antioxidant activity of green tea catechin decreased above pH 7.0. It was concluded that supplementation of beef cattle diets with green tea catechins, at levels up to 10 000 mg/animal/day, did not improve fresh beef quality, which may be attributed to pH sensitivity of catechin isomers (O'Grady *et al.*, 2006b).

16.5 Chemistry and structure of grape seed extract and bearberry compounds

Grape (*Vitis vinifera*) seeds from grape juice and wine processing can be separated, extracted, dried and purified into grape seed extract, a natural extract containing phenolic compounds (Lau and King, 2003). Grape seed proanthocyanidins (condensed tannins), which refers to procyanidin mixtures, are the most abundant flavonoids found in grape seed extract. Procyanidins are dimers, trimers and oligomers of the monomeric flavan-3-ols (+)-catechin, (–)-epicatechin and (–)-epicatechin-3-O-gallate (Yilmaz and Toledo, 2004) (Fig. 16.2a and b). From a health perspective, grape seed extract has been shown to act as an anticarcinogenic (Roy *et al.*, 2002) and cardio-protective agent (Shafiee *et al.*, 2003).

Bearberry, also known as *Uva ursi*, is a member of the evergreen heath (Ericaceae) family. Traditionally, the astringent leaves have been used in the treatment of bladder infections and afflictions of the urinary tract. Bearberry is reported to contain mainly hydroquinone derivatives (arbutin, methylarbutin), tannins, flavonoids, triterpenes and phenolcarboxylic acids (Annuk *et al.*, 1999).

Table 16.1 Effect of dietary green tea catechins (GTC) (0, 1000, 4000 and 10 000 mg GTC/animal/day) on surface redness ('a' value) and lipid oxidation (TBARS) in *M longissimus dorsi* stored in modified atmosphere packs (80% O₂ : 20% CO₂) (from O'Grady *et al.*, 2006b)

Group	Parameter	Storage time at 4 °C, days				
		0	2	4	6	8
	'a' redness value*					
Control		20.86 ± 0.70 ¹	18.03 ± 0.67 ¹	17.17 ± 0.65 ¹	15.31 ± 0.90 ¹	12.68 ± 1.75 ¹
+ GTC 1000		21.41 ± 0.71	18.08 ± 0.88	16.82 ± 0.88	13.77 ± 1.48	12.26 ± 1.39
+ GTC 4000		21.59 ± 0.77	18.23 ± 0.79	16.16 ± 0.76	15.56 ± 0.51	11.60 ± 0.70
+ GTC 10 000		21.67 ± 0.74	18.07 ± 0.85	15.96 ± 0.75	14.44 ± 0.97	12.09 ± 1.10
	TBARS**					
Control		0.07 ± 0.01 ¹	0.43 ± 0.10 ¹	0.43 ± 0.07 ¹	1.26 ± 0.38 ¹	1.80 ± 0.31 ¹
+ GTC 1000		0.07 ± 0.01	0.43 ± 0.06	0.74 ± 0.15	1.10 ± 0.17	2.16 ± 0.42
+ GTC 4000		0.07 ± 0.01	0.33 ± 0.05	0.52 ± 0.09	1.17 ± 0.19	2.07 ± 0.56
+ GTC 10 000		0.07 ± 0.01	0.35 ± 0.07	0.65 ± 0.14	1.35 ± 0.23	2.14 ± 0.64

¹ Within each day, no significance effects were observed for dietary treatment, $P > 0.05$. *CIE 'a' redness value, mean ± SEM. **TBARS, mg malondialdehyde (MDA)/kg muscle, mean ± SEM.

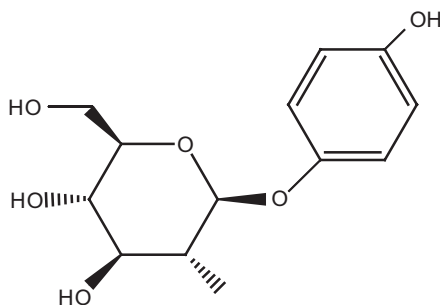


Fig. 16.3 Chemical structure of arbutin.

The antioxidant properties of grape seed extract (Jayaprakasha *et al.*, 2001; Lau and King, 2003) and bearberry (Amarowicz *et al.*, 2004; Ioku *et al.*, 1992) have been reported in a variety of test systems, including cell culture (Carpenter *et al.*, 2006). The antioxidant activity of bearberry is most likely attributed to the glycoside arbutin (Fig. 16.3) fraction present (Annuk *et al.*, 1999). Antimicrobial properties of grape seed extract against Gram-positive and Gram-negative bacteria have been reported previously (Jayaprakasha *et al.*, 2003). Ahn *et al.* (2004) demonstrated both antioxidant and antimicrobial activity of grape seed extract in raw ground beef. Bearberry extracts display potential antimicrobial benefits with respect to food-associated bacteria when used in combination with nisin (Dykes *et al.*, 2003). Furthermore, aqueous extracts of bearberry have been shown to alter the ability of *E. coli* (Türi *et al.*, 1999) and *H. pylori* (Annuk *et al.*, 1999) to cause infection. By contrast, Carpenter *et al.* (2007) reported that mesophilic plate counts in raw *M. longissimus dorsi* pork patties, stored in modified atmosphere packs (75% O₂ : 25% CO₂) for up to 12 days at 4 °C, were unaffected by the addition of grape seed extract (50–1000 mg/kg) and bearberry (10–1000 mg/kg). However, a positive lipid antioxidant effect was observed in raw pork patties as a result of grape seed extract and bearberry addition, thus demonstrating their potential for use as natural antioxidants in meat and meat products.

16.5.1 Dietary supplementation with grape seed extract and bearberry

To date, the scientific literature contains no information on the supplementation of meat producing animal diets with grape seed extract or bearberry. Lau and King (2003) reported a study where poultry diets were supplemented with two levels of grape seed extract (2.59% and 5.18% of diets fed *ad libitum*), which proved detrimental to the growth of chicks in the feeding trial. O'Grady *et al.* (2008) conducted a study where porcine diets were supplemented with grape seed extract or bearberry (100, 300 and 700 mg/kg feed) for 56 days pre-slaughter. In raw *M. Longissimus dorsi* stored in modified atmosphere packs (75% O₂ : 25% CO₂) for up to 16 days at 4 °C, colour (CIE L and a values) and lipid stability were not

significantly improved as a result of dietary grape seed extract or bearberry supplementation. Also, the sensory properties of cooked *M. Longissimus dorsi* stored in modified atmosphere packs (70% N₂ : 30% CO₂) for up to 28 days at 4 °C were unaffected by the dietary antioxidants. Results obtained were in contrast to previous dietary studies, where supplementation of porcine diets with antioxidant compounds, for example, vitamin E (α -tocopheryl acetate) improved the colour and lipid stability of fresh pork (Monahan *et al.*, 1994).

O'Grady *et al.* (2008) discussed a number of factors which may have limited the ability of dietary grape seed extract and bearberry to show an antioxidant effect in fresh pork meat. The levels of dietary antioxidants and duration of feeding were considered adequate when compared with previously published pig feeding trials. Factors such as chemical nature and composition, size and molecular weight of compounds present in grape seed extract (Donovan *et al.*, 2002; Manach *et al.*, 2004) and bearberry, interaction of compounds with proteins (Laurent *et al.*, 2007) or pH sensitivity (Spencer *et al.*, 2000; Zhu *et al.*, 2002) may have contributed to the lack of an antioxidant effect of dietary grape seed extract and bearberry in porcine muscle. Excretion of antioxidant compounds present in grape seed extract and bearberry (Deisinger *et al.*, 1996; English and Deisinger, 2005) in the animal urine or faeces was also possible.

16.6 Chemistry and structure of oregano and rosemary compounds

Oregano (*Origanum vulgare* L.) and rosemary (*Rosmarinus officinalis* L.) are aromatic herbs of the Labiatae (Lamiaceae) family and plants are highly distributed throughout the Mediterranean area (Mahmoud *et al.*, 2005). The essential oil extracted from oregano is known to possess antimicrobial (Sivropoulou *et al.*, 1996) and antioxidant activities (Economou *et al.*, 1991; Papageorgiou *et al.*, 2003) which are mainly attributed to carvacrol and thymol (Yanishlieva *et al.*, 1999). Structurally carvacrol and thymol are isomeric monoterpene phenols (Fig. 16.4) and constitute ~ 78% of the essential oil obtained from *O. vulgare* subsp. *hirtum* plants (commercially known as greek oregano). Other main oregano essential oil constituents are two monoterpene hydrocarbons (Fig. 16.4), γ -terpinene and *p*-cymene, which represent ~ 5 and 7% of the oregano essential oil phenol content, respectively (Adam *et al.*, 1998).

Rosemary, in addition to its use as a food flavouring agent, is also a well-valued medicinal herb, widely used in pharmaceutical products. Furthermore, extracts, essential oils and chemical constituents isolated from rosemary have demonstrated a number of interesting biological activities such as antioxidant (Del Baño *et al.*, 2003; Cuvelier *et al.*, 1996), antiulcerogenic (Dias *et al.*, 2000), anticarcinogenic (Offord *et al.*, 1995) and antimicrobial (Collins and Charles, 1987; Sacchetti *et al.*, 2005) properties. The antioxidant activity of rosemary is related to the presence of phenolic diterpenes such as carnosic acid and carnosol, which represent the major phenolics present (Fig. 16.5), methyl carnosate, rosmanol, epirsomanol and

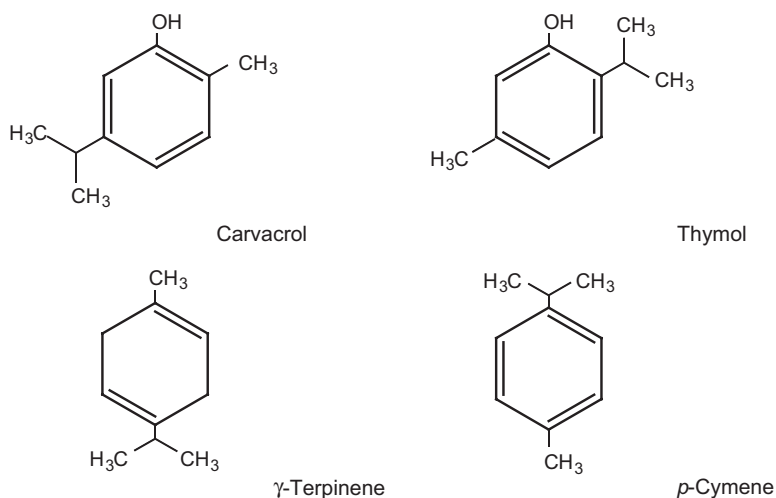


Fig. 16.4 Chemical structures of compounds present in oregano.

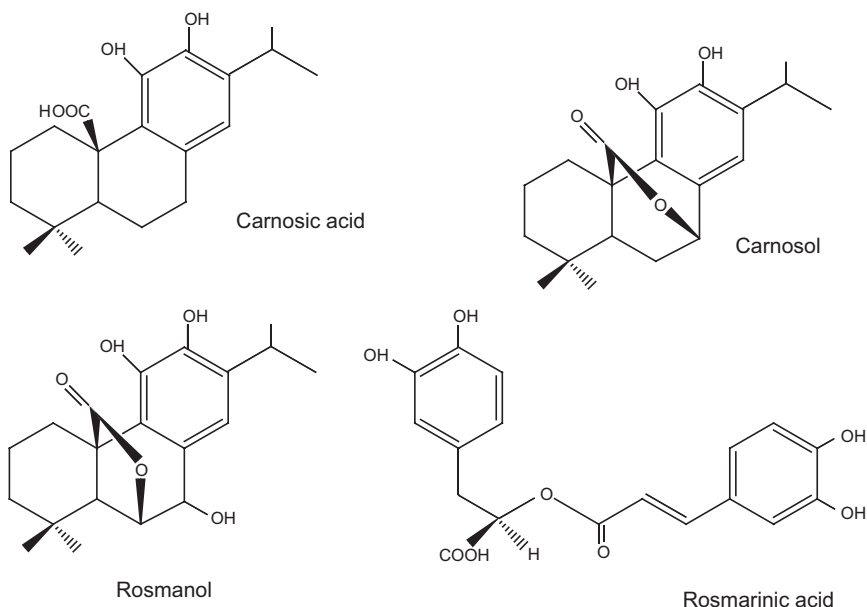


Fig. 16.5 Chemical structures of compounds present in rosemary.

7-methyl-epirosmanol (Ibañez *et al.*, 2003). Additional compounds such as rosmarinic acid, caffeic acid and flavonoids have also been associated with the antioxidant activity of rosemary (Del Baño *et al.*, 2003).

The antioxidant activity of oregano and rosemary has been shown in meat and meat products. Exogenous addition of oregano essential oil or extract

demonstrated antioxidant activity in ground raw pork and beef (Fasseas *et al.*, 2008; McGovern *et al.*, 2007; Sánchez-Escalante *et al.*, 2003). Beneficial antioxidant effects of rosemary extract or oleoresin, as a result of direct addition, have been reported in fresh ground beef (Balentine *et al.*, 2006; Formanek *et al.*, 2001; Han and Rhee, 2005; McGovern *et al.*, 2007; Sánchez-Escalante *et al.*, 2003), beef steaks (Djenane *et al.*, 2002) and pork (McCarthy *et al.*, 2001). Rosemary oleoresin (1%) exerted both antimicrobial and antioxidant activity in raw ground beef (Ahn *et al.*, 2004).

16.6.1 Dietary oregano supplementation

The antioxidant effect of dietary oregano supplementation has been demonstrated in poultry and lamb meat. Botsoglou *et al.* (2002) reported a study where chicks were fed supplemental α -tocopheryl acetate (200 mg/kg feed) or oregano essential oil (50 or 100 mg/kg feed) for 38 days pre-slaughter. The antioxidant potency of dietary antioxidants in raw chicken breast and thigh muscles in refrigerated storage for 9 days followed the order: 200 mg α -tocopheryl acetate/kg feed > 100 mg oregano essential oil/kg feed > 50 mg oregano essential oil/kg feed > control. Similar findings were reported in long-term frozen chicken meat (Botsoglou *et al.*, 2003a).

Supplementation of turkey diets with oregano essential oil (100 or 200 mg oregano essential oil/kg feed) for 4 weeks before slaughter, significantly reduced lipid oxidation in raw turkey breast and thigh muscle, in a dose-dependent manner (Botsoglou *et al.*, 2003c). Lowest levels of lipid oxidation were observed in meat from turkeys fed 100 mg oregano essential oil in combination with 100 mg α -tocopheryl acetate/kg feed, indicating synergistic effects of the dietary antioxidants examined. Similar findings were reported in long-term frozen turkey meat (Botsoglou *et al.*, 2003b) and in turkey breast, thigh, liver and heart tissue homogenates subjected to iron-induced lipid oxidation (Papageorgiou *et al.*, 2003). Govaris *et al.* (2004) reported that dietary oregano essential oil or α -tocopheryl acetate, both fed at a level of 200 mg/kg feed for 4 weeks pre-slaughter, reduced lipid oxidation in raw minced turkey breast and thigh muscle, thereby exerting a protective effect against pro-oxidative processing conditions such as mincing.

In contrast to the beneficial antioxidant effects of dietary oregano supplementation in fresh poultry meat, Janz *et al.* (2007) reported that supplementation of porcine diets, fed *ad libitum*, with rosemary essential oil (0.05%) and oregano oleoresin (0.05%) for 41 days pre-slaughter did not significantly improve fresh pork quality parameters such as colour (L value), lipid stability or sensorial properties compared to controls.

Simitzis *et al.* (2008) demonstrated an antioxidant effect of dietary oregano essential oil in fresh lamb meat. Lambs were twice daily fed 750 g and 600 g of a concentrate based feed and 600 g and 500 g of alfalfa hay, respectively, for two months before slaughter. The oregano supplemented group were fed concentrate sprayed with oregano essential oil (1 ml/kg feed). The inclusion of oregano

essential oil in lamb diets significantly reduced levels of lipid oxidation in *M. longissimus thoracis* during refrigerated and long-term frozen storage.

16.6.2 Dietary rosemary supplementation

The antioxidant activity of dietary rosemary supplementation has been demonstrated in poultry. Supplementation of poultry diets with rosemary oleoresin (500 mg/kg feed) for 6 weeks pre-slaughter resulted in reduced levels of lipid oxidation, compared to the control, in chicken leg and breast meat stored at 4 °C for up to 9 days (López-Bote *et al.*, 1998).

Basmacioğlu *et al.* (2004) conducted a study where poultry were fed, *ad libitum*, diets supplemented with 1.5% fish oil and α -tocopheryl acetate (200 mg/kg feed), oregano essential oil (150 or 300 mg/kg feed), rosemary essential oil (150 or 300 mg/kg feed) or a combination of oregano essential oil and rosemary essential oil (75 or 150 mg/kg feed) for 42 days pre-slaughter. Chicken meat (breast and thigh muscles) was minced and stored for up to 15 days at 4 °C. All dietary antioxidant treatments significantly reduced lipid oxidation (< 1 mg MDA/kg meat) compared to controls. After 15 days of storage, meat from chickens fed diets containing both oregano and rosemary essential oils (75 or 150 mg/kg feed) had the lowest levels of lipid oxidation compared to other dietary treatments. It was concluded that a possible synergistic effect may exist between rosemary and oregano essential oils in preventing lipid oxidation in meat enriched with *n*-3 polyunsaturated fatty acids.

Govarís *et al.* (2007) reported a study where turkeys were fed, *ad libitum*, a diet containing rosemary (ground dried leaves and flowers) (5 and 10 g rosemary/kg feed) and α -tocopheryl acetate (300 mg/kg feed) for 4 weeks pre-slaughter. In raw turkey breast fillets, lipid oxidation decreased with increasing concentration of rosemary, relative to the control, over the 12-day storage period. Lowest levels of lipid oxidation were observed in fillets from turkeys fed 300 mg α -tocopheryl acetate/kg feed). Dietary rosemary also reduced microbial growth in turkey breast fillets stored in the dark for 12 days at 4 °C. *In vitro* antimicrobial activity of the essential oil of rosemary against several foodborne pathogens such as *S. enteritidis*, *S. typhimurium*, *C. jejuni*, *E. coli* 0157:H7, *S. aureus*, *B. subtilis*, *P. aeruginosa* and *A. hydrophila* has been reported previously (Ahn *et al.*, 2004; Baratta *et al.*, 1998; Hammer *et al.*, 1999; Smith-Palmer *et al.*, 1998). Dietary α -tocopheryl acetate did not display antimicrobial activity. Similar findings were reported in raw pork by Cannon *et al.* (1995).

O'Grady *et al.* (2006b) reported a study where beef cattle were fed a concentrate-based diet supplemented with rosemary extract (40.07% diterpenes) (1000 mg/animal/day) for 103 days before slaughter. In *M. Longissimus dorsi* stored aerobically or in modified atmosphere packs (80% O₂ : 20% CO₂) for up to 8 days at 4 °C, colour (CIE *a* value) and lipid stability (TBARS, mg MDA/kg muscle) were not significantly improved as a result of dietary rosemary supplementation. In addition, the sensory properties of cooked beef slices were unaffected. An *in vitro* fermentation study also indicated that rosemary compounds were not fermented in

simulated rumen conditions indicating their 'potential availability' for absorption in the bovine intestine. Rosemary compounds were perhaps excreted in the animal urine or bio-transformed in the ruminant digestive system into unavailable forms.

In a recent study, pigs were fed, *ad libitum*, diets containing 2% oxidised linseed oil and supplemented with 40 mg α -tocopheryl acetate/kg feed, 40 mg rosemary extract (\pm 2 mg gallic acid)/kg feed or 40 mg α -tocopheryl acetate in combination with 40 mg rosemary extract for 115 or 122 days pre-slaughter (Haak *et al.*, 2008). The colour and lipid stability of raw *M. longissimus thoracis*, stored for up to 8 days at 4 °C, was unaffected by dietary rosemary supplementation. Lipid oxidation was reduced in the α -tocopheryl acetate supplemented groups only and no synergistic effects between dietary α -tocopheryl acetate and rosemary were observed. Similar findings were reported by Haak *et al.* (2006) where pigs were fed diets containing 2% linseed or soyabean oil plus 40 mg α -tocopheryl acetate/kg feed or 200 mg of an antioxidant complex (40 mg α -tocopheryl acetate, 40 mg citric acid, 64 mg rosemary and 56 mg gallic acid/kg feed). Cullen *et al.* (2005) reported that supplementation of pig diets, fed *ad libitum*, with 1 or 10 g of freeze dried rosemary/kg feed for 56 days pre-slaughter did not impact on the sensory characteristics of cooked pork patties.

16.7 Conclusions

Supplementation of poultry, beef cattle, pig and lamb diets with vitamin E elevates tissue α -tocopherol levels and enhances fresh meat quality. Lower levels of lipid oxidation in poultry and pig meat coupled with enhanced colour and lipid stability in fresh beef, as a result of supplemental vitamin E, have prompted much research in the use of dietary antioxidants as a means of enhancing the oxidative stability of fresh meats. Addition of vitamin E via dietary intervention results in greater antioxidant activity in meat when compared with exogenous or direct addition of vitamin E to meat and meat products. This was attributed to fact that dietary vitamin E results in effective deposition of the antioxidant in the cell membrane where lipid oxidation is initiated. Vitamin E is effectively absorbed in a monogastric or ruminant digestive system and distributed in a variety of muscle tissues.

Research into the use of natural antioxidants and health-promoting compounds from plant sources has resulted in experimental feeding trials, which examine the effects of plant extracts/nutraceuticals in the diets of meat-producing animals and poultry. Supplementation of monogastric poultry diets with green tea catechins, oregano or rosemary reduced levels of lipid oxidation in fresh chicken and turkey meat; therefore such compounds exhibit antioxidant properties similar to dietary vitamin E. Dietary rosemary supplementation also exhibited antimicrobial activity in fresh turkey meat. By contrast, dietary green tea catechins, oregano, rosemary, grape seed extract or bearberry did not enhance fresh pork quality. In ruminant animals, such as beef cattle, dietary green tea catechins and rosemary did not enhance fresh beef quality probably due to conditions in the bovine rumen. Dietary

oregano supplementation demonstrated lipid antioxidant activity in fresh lamb meat. Further research is necessary to examine the effects of dietary oregano supplementation in other ruminant animals, e.g. beef cattle.

The exact fate and metabolism in the porcine monogastric or ruminant digestive system of compounds present in the plant extracts reviewed is unknown, and merits further investigation. In addition, it is necessary to develop analytical methodologies to detect, in muscle tissues, active compounds present in dietary plant extracts. In the future, plant extracts alternative to those examined in this review need to be examined and evaluated for their potential as dietary supplements, to enhance the quality, safety and shelf-life of fresh meats.

16.8 References

- Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T. and Arsenakis, M. (1998), Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. *Journal of Agricultural and Food Chemistry*, 46, 1739–1745.
- Ahn, J., Grün, I. and Mustapha, A. (2004), Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. *Journal of Food Protection*, 67, 148–155.
- Almajano, M.P., Carbó, R., López Jiménez, J.A. and Gordon, M.H. (2008), Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry*, 108, 55–63.
- Amarowicz, R., Pegg, R.B., Rahimi-Moghaddam, P., Barl, B. and Weil, J.A. (2004), Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, 84, 551–562.
- Annuk, H., Hirno, S., Turi, E., Mikelsaar, M., Arak, E. and Wadstrom, T. (1999), Effect on cell surface hydrophobicity and susceptibility of *Helicobacter pylori* to medicinal plant extracts. *FEMS Microbiology Letters*, 172, 41–45.
- Arnold, R.N., Arp, S.C., Scheller, K.K., Williams, S.N. and Schaefer, D.M. (1993a), Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *Journal of Animal Science*, 71, 105–118.
- Arnold, R.N., Scheller, K.K., Arp, S.C., Williams, S.N., Buege, D.R. and Schaefer, D.M. (1992), Effect of long or short term feeding of α -tocopheryl acetate to holstein and crossbred beef steers on performance, carcass characteristics, and beef colour stability. *Journal of Animal Science*, 70, 3055–3065.
- Arnold, R.N., Scheller, K.K., Arp, S.C., Williams, S.N. and Schaefer, D.M. (1993b), Dietary α -tocopheryl acetate enhances beef quality in holstein and beef breed steers. *Journal of Food Science*, 58, 28–33.
- Asghar, A., Gray, J.I., Buckley, D.J., Pearson, A.M. and Booren, A.M. (1988), Perspectives on warmed-over flavour. *Food Technology*, 42, 102–108.
- Auger, C., Al-Awwadi, N., Bornet, A., Rouanet, J.-M., Gasc, F., Cros, G. and Teissedre, P.-L. (2004), Catechins and procyanidins in mediterranean diets. *Food Research International*, 37, 233–245.
- Balentine, C.W., Crandall, P.G., O'Bryan, C.A., Duong, D.Q. and Pohlman, F.W. (2006), The pre- and post-grinding application of rosemary and its effects on lipid oxidation and colour during storage of ground beef. *Meat Science*, 73, 413–421.
- Baratta, M.T., Dorman, H.J.D., Deans, S.G., Figueiredo, A.C., Barroso, J.G. and Ruberto, G. (1998), Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour and Fragrance Journal*, 13, 235–244.
- Basmacioğlu, H., Tokusoglu, Ö. and Ergül, M. (2004), The effect of oregano and rosemary essential oils or alpha-tocopheryl acetate on performance and lipid oxidation of meat

- enriched with n-3 PUFAs in broilers. *South African Journal of Animal Science*, 34, 197–210.
- Behrens, W.A. and Madere, R. (1991), Tissue discrimination between dietary RRR- α - and all-rac- α -tocopherols in rats. *Journal of Nutrition*, 121, 454–459.
- Botsoglou, N.A., Christaki, E., Fletouris, D.J., Florou-Paneri, P. and Spais, A.B. (2002), The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. *Meat Science*, 62, 259–265.
- Botsoglou, N.A., Fletouris, D.J., Florou-Paneri, P., Christaki, E. and Spais, A.B. (2003a), Inhibition of lipid oxidation in long-term frozen stored chicken meat by dietary oregano essential oil and α -tocopheryl acetate supplementation. *Food Research International*, 36, 207–213.
- Botsoglou, N.A., Govaris, A., Botsoglou, E.N., Grigoropoulou, S.H. and Papageorgiou, G. (2003b), Antioxidant activity of dietary oregano essential oil and α -tocopheryl acetate supplementation in long-term frozen stored turkey meat. *Journal of Agricultural and Food Chemistry*, 51, 2930–2936.
- Botsoglou, N.A., Grigoropoulou, S.H., Botsoglou, E., Govaris, A. and Papageorgiou, G. (2003c), The effects of dietary oregano essential oil and α -tocopheryl acetate on lipid oxidation in raw and cooked turkey during refrigerated storage. *Meat Science*, 65, 1193–1200.
- Buckley, D.J., Gray, J.I., Asghar, J.F., Price, J.F., Crackel, R.L., Booren, A.M., Pearson, A.M. and Miller, E.R. (1989), Effects of dietary antioxidants and oxidised oil on membranous lipid stability and pork product quality. *Journal of Food Science*, 54, 1193–1197.
- Buckley, D.J., Morrissey, P.A. and Gray, J.I. (1995), Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science*, 73, 3122–3130.
- Cabedo, L., Sofos, J.N. and Smith, G.C. (1998), Bacterial growth in ground beef patties made with meat from animals fed diets without or with supplemental vitamin E. *Journal of Food Protection*, 61, 36–40.
- Cannon, J.E., Morgan, J.B., Schmidt, G.R., Delmore, R.J., Sofos, J.N., Smith, G.C. and Williams, S.N. (1995), Vacuum-packaged precooked pork from hogs fed supplemental vitamin E: Chemical, shelf-life and sensory properties. *Journal of Food Science*, 60, 1179–1182.
- Cannon, J.E., Morgan, J.B., Schmidt, G.R., Tatum, J.D., Sofos, J.N., Smith, G.C., Delmore, R.J. and Williams, S.N. (1996), Growth and fresh meat quality characteristics of pigs supplemented with vitamin E. *Journal of Animal Science*, 74, 98–105.
- Carpenter, R., O'Callaghan, Y.C., O'Grady, M.N., Kerry, J.P. and O'Brien, N.M. (2006), Modulatory effects of resveratrol, citroflavan-3-ol and plant derived extracts on oxidative stress in U937 cells. *Journal of Medicinal Food*, 9, 187–195.
- Carpenter, R., O'Grady, M.N., O'Callaghan, Y.C., O'Brien, N.M. and Kerry, J.P. (2007), Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Science*, 76, 604–610.
- Chan, E.W.C., Lim, Y.Y. and Chew, Y.L. (2007), Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. *Food Chemistry*, 102, 1214–1222.
- Chan, W.K.M., Faustman, C., Velasquez-Pereira, J., McDowell, L.R. and Batra, T.R. (1998), Effects of α -tocopherol on metmyoglobin formation and reduction in beef from cattle fed soyabean or cottonseed meal diets. *Journal of Animal Science*, 76, 1421–1426.
- Chan, W.K.M., Hakkarainen, K., Faustman, C., Schaefer, D.M., Scheller, K.K. and Liu, Q. (1995), Colour stability and microbial growth relationships in beef as affected by endogenous α -tocopherol. *Journal of Food Science*, 60, 966–971.
- Chan, W.K.M., Hakkarainen, K., Faustman, C., Schaefer, D.M., Scheller, K.K. and Liu, Q. (1996), Dietary vitamin E effect on colour stability and sensory assessment of spoilage in three beef muscles. *Meat Science*, 42, 387–399.
- Collins, M.A. and Charles, H.P. (1987), Antimicrobial activity of carnosol and ursolic acid:

- Two anti-oxidant constituents of *Rosmarinus officinalis* L. *Food Microbiology*, 4, 311–315.
- Cullen, S.P., Monahan, F.J., Callan, J.J. and O'Doherty, J.V. (2005), The effect of dietary garlic and rosemary on grower-finisher pig performance and sensory characteristics of pork. *Irish Journal of Agricultural and Food Research*, 44, 57–67.
- Cuvelier, M.E., Richard, H. and Berset, C. (1996), Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *Journal of the American Oil Chemists' Society*, 73, 645–652.
- DeFelice, S.L. (1992), The nutraceutical initiative: A recommendation for U.S. economic and regulatory reforms. *Genetic Engineering News*, 12, 13–15.
- Deisinger, P.J., Hill, T.S. and English, J.C. (1996), Human exposure to naturally occurring hydroquinone. *Journal of Toxicology and Environmental Health*, 47, 31–46.
- Del Baño, M.J., Lorente, J., Castillo, J., Benavente-García, O., Del Río, J.A., Ortuño, A., Quirin, K.-W. and Gerard, D. (2003), Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinus officinalis*. Antioxidant activity. *Journal of Agricultural and Food Chemistry*, 51, 4247–4253.
- Delmore, R.J., Schmidt, G.R., Tatum, J.D., Sofos, J.N. and Smith, G.C. (1998), Supplementation of vitamin E to bovine females in 'White Cow', mature beef, upgrading programs. *Journal of Muscle Foods*, 9, 153–171.
- Dias, P.C., Foglio, M.A., Possenti, A. and de Carvalho, J.E. (2000), Antiulcerogenic activity of crude hydroalcoholic extract of *Rosmarinus officinalis* L. *Journal of Ethnopharmacology*, 69, 57–62.
- Djenane, D., Sánchez-Escalante, A., Beltrán, J.A. and Roncalés, P. (2002), Ability of α -tocopherol, taurine and rosemary, in combination with vitamin C, to increase the oxidative stability of beef steaks packaged in modified atmosphere. *Food Chemistry*, 76, 407–415.
- Donovan, J.L., Manach, C., Rios, L., Morand, C., Scalbert, A. and Rémésy, C. (2002), Procyanidins are not bioavailable in rats fed a single meal containing a grapeseed extract or the procyanidin dimer B3. *British Journal of Nutrition*, 87, 299–306.
- Dykes, G.A., Amarowicz, R. and Pegg, R.B. (2003), Enhancement of nisin antibacterial activity by a bearberry (*Arctostaphylos uva-ursi*) leaf extract. *Food Microbiology*, 20, 211–216.
- Economou, K.D., Oreopoulou, V. and Thomopoulos, C.D. (1991), Antioxidant activity of some plant extracts of the family labiatae. *Journal of the American Oil Chemists' Society*, 68, 109–113.
- English, J.C. and Deisinger, P.J. (2005), Metabolism and disposition of hydroquinone in Fischer 344 rats after oral or dermal administration. *Food and Chemical Toxicology*, 43, 483–493.
- Fasseas, M.K., Mountzouris, K.C., Tarantilis, P.A., Polissiou, M. and Zervas, G. (2008), Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chemistry*, 106, 1188–1194.
- Faustman, C. and Cassens, R.G. (1990), The biochemical basis for discoloration in fresh meat: A review. *Journal of Muscle Foods*, 1, 217–243.
- Faustman, C., Cassens, R.G., Schaefer, D.M., Buege, D.R., Williams, S.N. and Scheller, K.K. (1989), Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. *Journal of Food Science*, 54, 858–862.
- Faustman, C., Chan, W.K.M., Schaefer, D.M. and Havens, A. (1998), Beef colour update: The role for vitamin E. *Journal of Animal Science*, 76, 1019–1026.
- Formanek, Z., Kerry, J.P., Higgins, F.M., Buckley, D.J., Morrissey, P.A. and Farkas, J. (2001), Addition of synthetic and natural antioxidants to α -tocopheryl acetate supplemented beef patties: Effects of antioxidants and packaging on lipid oxidation. *Meat Science*, 58, 337–341.
- Gallo-Torres, H.E. (1980), Absorption. In *Vitamin E. A Comprehensive Treatise*, Machlin, L.J., ed., pp 170–192. Marcel Dekker Inc., New York.

- Gardner, H.W. (1975), Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with nonenzymic. *Journal of Agricultural and Food Chemistry*, 23, 129–136.
- Gatellier, P., Anton, M. and Renerre, M. (1995), Lipid peroxidation induced by H_2O_2 -activated metmyoglobin and detection of a myoglobin-derived radical. *Journal of Agricultural and Food Chemistry*, 43, 651–656.
- Giddings, G.G. (1977), The basis of colour in muscle foods. *CRC Critical Reviews in Food Science and Nutrition*, 9, 81–114.
- Govaris, A., Botsoglou, N., Papageorgiou, G., Botsoglou, E. and Ambrosiadis, I. (2004), Dietary versus post-mortem use of oregano oil and/or α -tocopherol in turkeys to inhibit development of lipid oxidation in meat during refrigerated storage. *International Journal of Food Sciences and Nutrition*, 55, 115–123.
- Govaris, A., Florou-Paneri, P., Botsoglou, E., Giannenas, I., Amvrosiadis, I. and Botsoglou, N. (2007), The inhibitory potential of feed supplementation with rosemary and/or α -tocopheryl acetate on microbial growth and lipid oxidation of turkey breast during refrigerated storage. *LWT Food Science and Technology*, 40, 331–337.
- Graham, H.N. (1992), Green tea composition, consumption, and polyphenol chemistry. *Preventive Medicine*, 21, 334–350.
- Gray, J.I. and Pearson, A.M. (1987), Rancidity and warmed-over flavour. In *Restructured Meat and Poultry Products, Advances in Meat Research Series*, Vol. 3, Pearson, A.M. and Dutson, T.R., ed., pp 221–269. Van Nostrand Reinhold Company Inc., New York.
- Gray, J.I., Pearson, A.M. and Monahan, F.J. (1994), Flavour and aroma problems and their measurement in meat, poultry and fish products. In *Quality Attributes and their Measurement in Meat, Poultry and Fish Products, Advances in Meat Research Series*, Vol. 9, Pearson, A.M. and Dutson, T.R., ed., pp 250–288. Blackie Academic and Professional (Chapman and Hall), Glasgow.
- Guidera, J., Kerry, J.P., Buckley, D.J., Lynch, P.B. and Morrissey, P.A. (1997), The effect of dietary vitamin E supplementation on the quality of fresh and frozen lamb meat. *Meat Science*, 45, 33–43.
- Guo, Q., Richert, B.T., Burgess, J.R., Webel, D.M., Orr, D.E., Blair, M., Fitzner, G.E., Hall, D.D., Grant, A.L. and Gerrard, D.E. (2006), Effects of dietary vitamin E and fat supplementation on pork quality. *Journal of Animal Science*, 84, 3089–3099.
- Guo, Q., Zhao, B., Li, M., Shen, S. and Xin, W. (1996), Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. *Biochimica et Biophysica Acta*, 1304, 210–222.
- Guo, Y., Tang, Q., Yuan, J. and Jiang, Z. (2001), Effects of supplementation with vitamin E on the performance and the tissue peroxidation of broiler chicks and the stability of thigh meat against oxidative deterioration. *Animal Feed Science and Technology*, 89, 165–173.
- Gutteridge, J.M.C. and Halliwell, B. (1990), The measurement and mechanism of lipid oxidation in biological systems. *Trends in Biochemical Sciences*, 15, 129–135.
- Haak, L., Raes, K., Smet, K., Claeys, E., Paelinck, H. and De Smet, S. (2006), Effect of dietary antioxidant and fatty acid supply on the oxidative stability of fresh and cooked pork. *Meat Science*, 74, 476–486.
- Haak, L., Raes, K., Van Dyck, S. and De Smet, S. (2008), Effect of dietary rosemary and α -tocopheryl acetate on the oxidative stability of raw and cooked pork following oxidised linseed oil administration. *Meat Science*, 78, 239–247.
- Hamilton, R.J., Kalu, C., Prisk, E., Padley, F.B. and Pierce, H. (1997), Chemistry of free radicals in lipids. *Food Chemistry*, 60, 193–199.
- Hammer, K.A., Carson, C.F. and Riley, T.V. (1999), Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*, 86, 985–990.
- Han, J. and Rhee, K.S. (2005), Antioxidant properties of selected oriental non-culinary/nutraceutical herb extracts as evaluated in raw and cooked meat. *Meat Science*, 70, 25–33.
- Hasty, J.L., van Heugten, E., See, M.T. and Larick, D.K. (2002), Effect of vitamin E on

- improving fresh pork quality in Berkshire- and Hampshire-sired pigs. *Journal of Animal Science*, 80, 3230–3237.
- He, Y. and Shahidi, F. (1997), Antioxidant activity of green tea and its catechins in a fish meat model system. *Journal of Agricultural and Food Chemistry*, 45, 4262–4266.
- Higdon, J.V. and Frei, B. (2003), Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Critical Reviews in Food Science and Nutrition*, 43, 89–143.
- Hood, D.E. and Riordan, E.B. (1973), Discoloration in pre-packaged beef: Measurement by reflectance spectrophotometry and shopper discrimination. *Journal of Food Technology*, 8, 333–343.
- Hornstein, I., Crowe, P.F. and Heimberg, M.J. (1961), Fatty acid composition of meat tissue lipids. *Journal of Food Science*, 26, 581–586.
- Hoving-Bolink, A.H., Eikelenboom, G., van Diepen, J. Th. M., Jongbloed, A.W. and Houben, J.H. (1998), Effect of dietary vitamin E supplementation on pork quality. *Meat Science*, 49, 205–212.
- Huang, S.W. and Frankel, E.N. (1997), Antioxidant activity of tea catechins in different lipid systems. *Journal of Agricultural and Food Chemistry*, 45, 3033–3038.
- Ibañez, E., Kubátová, A., Señoráns, F.J., Caverio, S., Reglero, G. and Hawthorne, S.B. (2003), Subcritical water extraction of antioxidant compounds from rosemary plants. *Journal of Agricultural and Food Chemistry*, 51, 375–382.
- Igene, J.O., Pearson, A.M., Dugan, L.R. Jr. and Price, J.F. (1980), Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. *Food Chemistry*, 5, 263–276.
- Ioku, K., Terao, J. and Nakatani, N. (1992), Antioxidative activity of arbutin in a solution and liposomal suspension. *Bioscience, Biotechnology and Biochemistry*, 56, 1658–1659.
- Jackson, T.C., Hardin, M.D. and Acuff, G.R. (1996), Heat resistance of *Escherichia coli* 0157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. *Journal of Food Protection*, 59, 230–237.
- Janz, J.A.M., Morel, P.C.H., Wilkinson, B.H.P. and Purchas, R.W. (2007), Preliminary investigation of the effects of low-level dietary inclusion of fragrant essential oils and oleoresins on pig performance and pork quality. *Meat Science*, 75, 350–355.
- Jayaprakasha, G.K., Singh, R.P. and Sakariah, K.K. (2001), Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chemistry*, 73, 285–290.
- Jayaprakasha, G.K., Selvi, T. and Sakariah, K.K. (2003), Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Research International*, 36, 117–122.
- Jensen, C., Guidera, J., Skovgaard, I.M., Staun, H., Skibsted, L.H., Jensen, S.K., Møller, A.J., Buckley, J. and Bertelsen, G. (1997), Effects of dietary α -tocopheryl acetate supplementation on α -tocopherol deposition in porcine *m. psoas major* and *m. longissimus dorsi* and on drip loss, colour stability and oxidative stability of pork meat. *Meat Science*, 45, 491–500.
- Jiménez-Colmenero, F., Carballo, J. and Cofrades, S. (2001), Healthier meat and meat products: Their role as functional foods. *Meat Science*, 59, 5–13.
- Jung, M.Y. and Min, D.B. (1990), Effects of α -, γ -, and δ -tocopherols on oxidative stability of soyabean oil. *Journal of Food Science*, 55, 1464–1465.
- Kagan, V.E. and Quinn, P.J. (1988), The interaction of α -tocopherol and homologues with shorter hydrocarbon chains with phospholipid bilayer dispersions. A fluorescence probe study. *European Journal of Biochemistry*, 171, 661–667.
- Kagan, V.E., Serbinova, E.A. and Packer, L. (1990), Recycling and antioxidant activity of tocopherol homologs of differing hydrocarbon chain lengths in liver microsomes. *Archives of Biochemistry and Biophysics*, 282, 221–225.
- Kamal-Eldin, A. and Appelqvist, L.-Å. (1996), The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids*, 31, 671–701.

- Kanner, J., German, J.B. and Kinsella, J.E. (1987), Initiation of lipid peroxidation in biological systems. *CRC Critical Reviews in Food Science and Nutrition*, 25, 317–364.
- Kanner, J. and Harel, S. (1985), Initiation of membranal lipid peroxidation by activated metmyoglobin and methaemoglobin. *Archives of Biochemistry and Biophysics*, 237, 314–321.
- Kropf, D.H. (1993), Colour stability: Factors affecting the colour of fresh meat. *Meat Focus International*, 1, 269–275.
- Kumudavally, K.V., Phanindrakumar, H.S., Tabassum, A., Radhakrishna, K. and Bawa, A.S. (2008), Green tea – A potential preservative for extending the shelf-life of fresh mutton at ambient temperature (25 ± 2 °C). *Food Chemistry*, 107, 426–433.
- Lanari, M.C., Brewster, M., Yang, A. and Tume, R.K. (2002), Pasture and grain finishing affect the colour stability of beef. *Journal of Food Science*, 67, 2467–2473.
- Lanari, M.C., Schaefer, D.M., Liu, Q. and Cassens, R.G. (1996), Kinetics of pigment oxidation in beef from steers supplemented with vitamin E. *Journal of Food Science*, 61, 884–889.
- Lanari, M.C., Schaefer, D.M. and Scheller, K.K. (1995), Dietary vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. *Meat Science*, 41, 237–250.
- Lau, D.W. and King, A.J. (2003), Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. *Journal of Agricultural and Food Chemistry*, 51, 1602–1607.
- Laurent, C., Besancon, P. and Caporiccio, B. (2007), Flavonoids from a grape seed extract interact with digestive secretions and intestinal cells as assessed in an *in vitro* digestion/caco-2 cell culture model. *Food Chemistry*, 100, 1704–1712.
- Lauridsen, C., Buckley, D.J. and Morrissey, P.A. (1997), Influence of dietary fat and vitamin E supplementation on α -tocopherol levels and fatty acid profiles in chicken muscle membranal fractions and on susceptibility to lipid peroxidation. *Meat Science*, 46, 9–22.
- Lauzurica, S., de la Fuente, J., Díaz, M.T., Álvarez, I., Pérez, C. and Cañeque, V. (2005), Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. *Meat Science*, 70, 639–646.
- Ledward, D.A. (1985), Post-slaughter influences on the formation of metmyoglobin in beef muscles. *Meat Science*, 15, 149–171.
- Lin, C.F., Gray, J.I., Asghar, A., Buckley, D.J., Booren, A.M. and Flegal, C.J. (1989), Effects of dietary oils and α -tocopherol supplementation on lipid composition and stability of broiler meat. *Journal of Food Science*, 54, 1457–1460, 1484.
- Lin, T.S. and Hultin, H.O. (1976), Enzymic lipid peroxidation in microsomes of chicken skeletal muscle. *Journal of Food Science*, 41, 1488–1489.
- Liu, Q., Scheller, K.K., Arp, S.C., Schaefer, D.M. and Frigg, M. (1996a), Colour coordinates for assessment of dietary vitamin E effects on beef colour stability. *Journal of Animal Science*, 74, 106–116.
- Liu, Q., Scheller, K.K., Arp, S.C., Schaefer, D.M. and Williams, S.N. (1996b), Titration of fresh meat colour stability and malondialdehyde development with Holstein steers fed vitamin E-supplemented diets. *Journal of Animal Science*, 74, 117–126.
- Livingston, D.J. and Brown, W.D. (1981), The chemistry of myoglobin and its reactions. *Food Technology*, 35, 244–252.
- López-Bote, C.J., Gray, J.I., Gomaa, E.A. and Flegal, C.J. (1998), Effect of dietary administration of oil extracts from rosemary and sage on lipid oxidation in broiler meat. *British Poultry Science*, 39, 235–240.
- MacPherson, A. (1994), Selenium, vitamin E and biological oxidation. In *Recent Advances in Animal Nutrition 1994*, Garnsworthy, P.C. and Cole, D.J.A., ed., pp 3–30. Nottingham University Press, Nottingham.
- Maghrani, M., Lemhadri, A., Zeggwagh, N.A., El Amraoui, M., Haloui, M., Jouad, H. and Eddouks, M. (2004), Effects of an aqueous extract of triticum repens on lipid metabolism in normal and recent-onset diabetic rats. *Journal of Ethnopharmacology*, 90, 331–337.

- Mahmoud, A.A., Al-Shihry, S.S. and Son, B.W. (2005), Diterpenoid quinines from rosemary (*Rosmarinus officinalis* L.). *Phytochemistry*, 66, 1685–1690.
- Manach, C., Scalbert, A., Morand, C., Rémésy, C. and Jiménez, L. (2004), Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition*, 79, 727–747.
- Maraschiello, C., Sárraga, C. and García Regueiro, J.A. (1999), Glutathione peroxidase activity, TBARS, and α -tocopherol in meat from chickens fed different diets. *Journal of Agricultural and Food Chemistry*, 47, 867–872.
- Mason, L.M., Hogan, S.A., Lynch, A., O'Sullivan, K., Lawlor, P.G. and Kerry, J.P. (2005), Effects of restricted feeding and antioxidant supplementation on pig performance and quality characteristics of *longissimus dorsi* muscle from Landrace and Duroc pigs. *Meat Science*, 70, 307–317.
- McCarthy, T.L., Kerry, J.P., Kerry, J.F., Lynch, P.B. and Buckley, D.J. (2001), Assessment of the antioxidant potential of natural food and plant extracts in fresh and previously frozen pork patties. *Meat Science*, 57, 177–184.
- McDonald, K. and Sun, D.-W. (1999), Predictive food microbiology for the meat industry: A review. *International Journal of Food Microbiology*, 52, 1–27.
- McGovern, L., O'Grady, M.N., Ryan, E., O'Brien, N.M. and Kerry, J.P. (2007), The effect of plant extracts on colour and lipid oxidation in raw and cooked beef patties stored in modified atmosphere packs. In *Proceedings 53rd International Congress of Meat Science and Technology*, (pp. 393–394), 5–10 August 2007, Beijing, China.
- Merchen, N.R. (1988), Digestion, absorption and excretion in ruminants. In *The Ruminant Animal – Digestive Physiology and Nutrition*, Church, D. C., ed., (pp 172–201). Prentice Hall, Englewood Cliffs, NJ.
- Mercier, Y., Gatellier, P., Viau, M., Remignon, H. and Renner, M. (1998), Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. *Meat Science*, 48, 301–318.
- Mitsumoto, M., Arnold, R.N., Schaefer, D.M. and Cassens, R.G. (1993), Dietary versus postmortem supplementation of vitamin E on pigment and lipid stability in ground beef. *Journal of Animal Science*, 71, 1812–1816.
- Mitsumoto, M., Ozawa, S., Mitsuhashi, T. and Koide, K. (1998), Effect of dietary vitamin E supplementation for one week before slaughter on drip, colour and lipid stability during display in Japanese black steer beef. *Meat Science*, 49, 165–174.
- Monahan, F.J., Asghar, A., Gray, J.I. and Buckley, D.J. (1994), Effect of oxidized dietary lipid and vitamin E on the colour stability of pork chops. *Meat Science*, 37, 205–215.
- Monahan, F.J., Buckley, D.J., Gray, J.I., Morrissey, P.A., Asghar, A., Hanrahan, T.J. and Lynch, P.B. (1990), Effect of dietary vitamin E on the stability of raw and cooked pork. *Meat Science*, 27, 99–108.
- Morrissey, P.A., Buckley, D.J., Sheehy, P.J.A. and Monahan, F.J. (1994), Vitamin E and meat quality. *Proceedings of the Nutrition Society*, 53, 289–295.
- Nychas, G.-J.E., Skandamis, P.N., Tassou, C.C. and Koutsoumanis, K.P. (2008), Meat spoilage during distribution. *Meat Science*, 78, 77–89.
- Obanda, M., Owuor, P.O. and Mang'oka, R. (2001), Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature. *Food Chemistry*, 75, 395–404.
- Offord, E.A., Macé, K., Ruffieux, C., Malnoë, A. and Pfeifer, A.M.A. (1995), Rosemary components inhibit benzo[a]pyrene-induced genotoxicity in human bronchial cells. *Carcinogenesis*, 16, 2057–2062.
- O'Grady, M.N., Carpenter, R., Lynch, P.B., O'Brien, N.M. and Kerry, J.P. (2008), Addition of grape seed extract and bearberry to porcine diets: Influence on quality attributes of raw and cooked pork. *Meat Science*, 78, 438–446.
- O'Grady, M.N., Maher, M., Troy, D.J., Moloney, A.P. and Kerry, J.P. (2006a), An assessment of dietary supplementation with tea catechins and rosemary extract on the quality of fresh beef. *Meat Science*, 73, 132–143.
- O'Grady, M.N., Maher, M., Troy, D.J., Moloney, A.P. and Kerry, J.P. (2006b), Dietary

- supplementation and addition of tea catechins: Assessment of the effects of catechin level and pH on antioxidant activity in fresh beef. In *Proceedings 52nd International Congress of Meat Science and Technology*, (pp. 735–736), 13–18 August 2006, Dublin, Ireland.
- O'Grady, M.N., Monahan, F.J., Bailey, J., Allen, P., Buckley, D.J. and Keane, M.G. (1998), Colour-stabilising effect of muscle vitamin E in minced beef stored in high oxygen packs. *Meat Science*, 50, 73–80.
- O'Grady, M.N., Monahan, F.J., Fallon, R.J. and Allen, P. (2001), Effect of dietary supplementation with vitamin E and organic selenium on the oxidative stability of beef. *Journal of Animal Science*, 79, 2827–2834.
- Papageorgiou, G., Botsoglou, N., Govaris, A., Giannenas, I., Iliadis, S. and Botsoglou, E. (2003), Effect of dietary oregano oil and α -tocopheryl acetate supplementation on iron-induced lipid oxidation of turkey breast, thigh, liver and heart tissues. *Journal of Animal Physiology and Animal Nutrition*, 87, 324–335.
- Pastore, R.L. and Fratellone, P. (2006), Potential health benefits of green tea (*Camellia sinensis*): A narrative review. *Explore: The Journal of Science and Healing*, 2, 531–539.
- Peterson, J., Dwyer, J., Bhagwat, S., Haytowitz, D., Holden, J., Eldridge, A.L., Beecher, G. and Aladesanmi, J. (2005), Major flavonoids in dry tea. *Journal of Food Composition and Analysis*, 18, 487–501.
- Pfalzgraf, A., Frigg, M. and Steinhart, H. (1995), α -Tocopherol contents and lipid oxidation in pork muscle and adipose tissue during storage. *Journal of Agricultural and Food Chemistry*, 43, 1339–1342.
- Piironen, V., Syvaöja, E.L., Varo, P., Salminen, K. and Koivistoinen, P. (1985), Tocopherols and tocotrienols in Finnish foods: Meat and meat products. *Journal of Agricultural and Food Chemistry*, 33, 1215–1218.
- Record, I.R. and Lane, J.M. (2001), Simulated intestinal digestion of green and black teas. *Food Chemistry*, 73, 481–486.
- Renerre, M. (1990), Review: Factors involved in the discoloration of beef meat. *International Journal of Food Science and Technology*, 25, 613–630.
- Renerre, M., Poncet, K., Mercier, Y., Gatellier, P. and Métro, B. (1999), Influence of dietary fat and vitamin E on antioxidant status of muscles of turkey. *Journal of Agricultural and Food Chemistry*, 47, 237–244.
- Rice, D. and Kennedy, S. (1986), Vitamin E – function and effects of deficiency. In *Animal Nutrition Events: Roche Symposium, The Value of Vitamins in Animal Nutrition*, pp 5–23, London.
- Roedig-Penman, A. and Gordon, M.H. (1997), Antioxidant properties of catechins and green tea extracts in model food emulsions. *Journal of Agricultural and Food Chemistry*, 45, 4267–4270.
- Roy, S., Khanna, S., Alessio, H.M., Vider, J., Bagchi, D., Bagchi, M. and Sen, C.K. (2002), Anti-angiogenic property of edible berries. *Free Radical Research*, 36, 1023–1031.
- Ryu, Y.-C., Rhee, M.-S., Lee, K.-M. and Kim, B.-C. (2005), Effects of different levels of dietary supplemental selenium on performance, lipid oxidation, and colour stability of broiler chicks. *Poultry Science*, 84, 809–815.
- Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M. and Bruni, R. (2005), Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry*, 91, 621–632.
- Sánchez-Escalante, A., Djenane, D., Torrescano, G., Beltrán, J.A. and Roncales, P. (2003), Antioxidant action of borage, rosemary, oregano, and ascorbic acid in beef patties packaged in modified atmosphere. *Journal of Food Science*, 68, 339–344.
- Sanders, S.K., Morgan, J.B., Wulf, D.M., Tatum, J.D., Williams, S.N. and Smith, G.C. (1997), Vitamin E supplementation of cattle and shelf-life of beef for the Japanese market. *Journal of Animal Science*, 75, 2634–2640.
- Sato, K. and Hegarty, G.R. (1971), Warmed-over flavour in cooked meats. *Journal of Food Science*, 36, 1098–1102.

- Shafiee, M., Carboneau, M.A., Urban, N., Descomps, B. and Leger, C.L. (2003), Grape and grape seed extract capacities at protecting LDL against oxidation generated by Cu^{2+} , AAPH or SIN-1 and at decreasing superoxide THP-1 cell production. A comparison to other extracts or compounds. *Free Radical Research*, 37, 573–584.
- Sheldon, B.W., Curtis, P.A., Dawson, P.L. and Ferket, P.R. (1997), Effect of dietary vitamin E on the oxidative stability, flavour, colour, and volatile profiles of refrigerated and frozen turkey breast meat. *Poultry Science*, 76, 634–641.
- Simitzis, P.E., Deligeorgis, S.G., Bizelis, J.A., Dardamani, A., Theodosiou, I. and Fegeros, K. (2008), Effect of dietary oregano oil supplementation on lamb meat characteristics. *Meat Science*, 79, 217–223.
- Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T. and Arsenakis, M. (1996), Antimicrobial and cytotoxic activities of origanum essential oils. *Journal of Agricultural and Food Chemistry*, 44, 1202–1205.
- Smith-Palmer, A., Stewart, J. and Fyfe, L. (1998), Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Applied Microbiology*, 26, 118–122.
- Somova, L.I., Shode, F.O., Ramnanan, P. and Nadar A. (2003), Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europea*, subspecies *Africana* leaves. *Journal of Ethnopharmacology*, 84, 299–305.
- Spencer, J.P.E., Chaudry, F., Pannala, A.S., Srai, S.K., Debnam, E. and Rice-Evans, C. (2000), Decomposition of cocoa procyanidins in the gastric milieu. *Biochemical and Biophysical Research Communications*, 272, 236–241.
- Tang, S., Sheehan, D., Buckley, D.J., Morrissey, P.A. and Kerry, J.P. (2001a), Anti-oxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle. *International Journal of Food Science and Technology*, 36, 685–692.
- Tang, S.Z., Kerry, J.P., Sheehan, D., Buckley, D.J. and Morrissey, P.A. (2001b), Antioxidative effect of dietary tea catechins on lipid oxidation of long-term frozen stored chicken meat. *Meat Science*, 57, 331–336.
- Tang, S.Z., Ou, S.Y., Huang, X.S., Li, W., Kerry, J.P. and Buckley, D.J. (2006), Effects of added tea catechins on colour stability and lipid oxidation in minced beef patties held under aerobic and modified atmospheric packaging conditions. *Journal of Food Engineering*, 77, 248–253.
- Tarrant, P.V. (1998), Some recent advances and future priorities in research for the meat industry. *Meat Science*, 49, Suppl. 1, S1–S16.
- Tims, M.J. and Watts, B.M. (1958), Protection of cooked meats with phosphates. *Food Technology*, 12, 240–243.
- Türi, E., Türi, M., Anuuk, H. and Arak, E. (1999), Action of aqueous extracts of bearberry and cowberry leaves and wild camomile and pineapple-weed flowers on *Escherichia coli* surface structures. *Pharmaceutical Biology*, 37, 127–133.
- Unno, T., Sugimoto, A. and Kakuda, T. (2000), Scavenging effect of tea catechins and their epimers on superoxide anion radicals generated by a hypoxanthine and xanthine oxidase system. *Journal of the Science of Food and Agriculture*, 80, 601–606.
- Van Soest, P.J. (1994), *Nutritional Ecology of the Ruminant* (2nd edition). New York: Comstock Publishing Associates – Cornell University Press.
- Varnam, A.H. and Sutherland, J.P. (1995), *Meat and Meat Products: Technology, Chemistry and Microbiology*, pp 46–120. Chapman and Hall, London.
- Yang, C.S., Lambert, J.D., Ju, J., Lu, G. and Sang, S. (2007), Tea and cancer prevention: Molecular mechanisms and human relevance. *Toxicology and Applied Pharmacology*, 224, 265–273.
- Yanishlieva, N.V., Marinova, E.M., Gordon, M.H. and Raneva, V.G. (1999), Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chemistry*, 64, 59–66.
- Yen, G.-C. and Chen, H.-Y. (1995), Antioxidant activity of various tea extracts in relation to their antitumagenicity. *Journal of Agricultural and Food Chemistry*, 43, 27–32.

- Yen, G.-C., Chen, H.-Y. and Peng, H.-H. (1997), Antioxidant and pro-oxidant effects of various tea extracts. *Journal of Agricultural and Food Chemistry*, 45, 30–34.
- Yilmaz, Y. (2006), Novel uses of catechins in foods. *Trends in Food Science and Technology*, 17, 64–71.
- Yilmaz, Y. and Toledo, R.T. (2004), Health aspects of functional grape seed constituents. *Trends in Food Science and Technology*, 15, 422–433.
- Zanardi, E., Novelli, E., Ghiretti, G.P., Dorigoni, V. and Chizzolini, R. (1999), Colour stability and vitamin E content of fresh and processed pork. *Food Chemistry*, 67, 163–171.
- Zhu, Q.Y., Holt, R.R., Lazarus, S.A., Ensuna, J.L., Hammerstone, J.F., Schmitz, H.H. and Keen, C.L. (2002), Stability of the flavan-3-ols epicatechin and catechin and related dimeric procyanidins derived from cocoa. *Journal of Agricultural and Food Chemistry*, 50, 1700–1705.
- Zhu, Q.Y., Zhang, A., Tsang, D., Huang, Y. and Chen, Z.-Y. (1997), Stability of green tea catechins. *Journal of Agricultural and Food Chemistry*, 45, 4624–4628.

Organic meat quality

A. Braghieri and F. Napolitano, Università degli Studi della Basilicata, Italy

Abstract: The present chapter briefly describes the international basic standards in use for the organic farming of meat animals. The situation of the two main organic meat markets (viz. oversupplies in EU and fast growth in USA), where demand and supply are both developed, and the potential development in other countries, where the internal demand is limited and the production is export-oriented, is then examined in terms of commercial value, number of meat animals, price premiums for farmers, and price to consumers. Consumer price represents the main limit to the expansion of organic meat purchasing; possible strategies to overcome this constraint are also discussed. An ample discussion is dedicated to the effects of organic farming (e.g. extensive rearing system, appropriate breed and feeding based on pasture) on meat quality (e.g. pH, colour, instrumental texture, sensory tenderness and flavour) and on differentiation from conventional products. Finally, we describe safety problems possibly arising in conventional meat (e.g. hormone, antibiotics and other growth promotants administration), and in organic products (limited use of conventional medicine to prevent and cure bacterial and parasitic infections). The reasons for possible increased healthiness of organic meat are also illustrated.

Key words: organic farming, organic meat quality, organic meat market, organic meat safety, organic meat healthiness.

17.1 Introduction

17.1.1 Organic standards

Intensive systems allow the production of meat at relatively low prices. However, for consumers from western countries price is not the only determinant behind animal-food purchases as they are acquiring an increasing interest in other aspects concerning farming practices and related to product safety (Verbeke and Viane, 1999), animal welfare (McInerney, 2004) and environmental pollution (McCarthy

et al., 2003). Currently, public concern is particularly centered on factory-farm methods of raising and slaughtering animals for meat production, which represent the main production system in Europe and North America, and also account for most of the market in the developing countries, including Brazil, Malaysia, the Philippines, Thailand, etc. These methods are considered responsible for public health threats, such as avian flu, mad cow disease and dioxin contamination, ecological problems concerning air and water pollution, loss of livestock genetic diversity, and massive instrumentalisation of animals into short-lived production machines (Verhoog *et al.*, 2004).

As a response to the increasing demand for products responding to these new societal needs, the International Federation of Organic Agriculture Movements (IFOAM) was founded with the aim to coordinate the international network of organic agriculture organizations. IFOAM in 1980 developed the first basic standard for organic agriculture, which has been repeatedly revised and updated. Such standards have the broad objective of bringing agricultural and animal production practices back to the principles of sustainability. In particular, farming should not rely on external feeds, and manure production should not exceed the absorption capability of the agricultural ecosystem. In addition, the ethological needs of the organically farmed animals should be respected in order to allow the expression of their natural behaviour.

Considerable variation among organic standards of different European countries induced EU to promulgate a common Council Regulation (EC 1804/1999) on organic farming (European Communities, 1999), which implemented the general Regulation EEC 2092/91 on organic agriculture (European Communities, 1991) recently repealed by Regulation EC 834/2007 (European Union, 2007). The EU Regulation had an important impact on the harmonization of international organic standards within and outside Europe, as all countries willing to export to EU need to meet the European standards. However, an analysis of different national and international standards shows that, although many aspects are similar (limits on non-organic feed, prohibition of growth promoters, prohibition of drugs in absence of disease), there are still a number of areas where harmonization is needed (housing, grazing areas, withholding periods for drugs, conversion time, age at weaning, nose ringing of pigs, etc.). In general, EU standards are more detailed than standards from the USA, developed within the National Organic Program (NOP) in 2002, or from Australia. In addition, the EU Regulation pays more attention to the animal ethological needs as compared to other international standards (Schmid, 2000). Variation among different standards can also be observed on general principles. IFOAM standards focus more on management, with the aim to promote health and welfare of the animals by respecting their physiological and ethological needs (IFOAM, 2002). Conversely, Codex Alimentarius, as issued by Food and Agriculture Organization (FAO), is more environmental oriented and gives the animals a role in closing the nutrient cycle, improving soil fertility through their manure, and controlling weeds through grazing (FAO, 2001). These differences may result in variations in specific aspects, which may limit the trade of organic meat.

17.1.2 Organic meat market

North America and Western Europe account for 95% of the world retail sales of organic food products, at US\$ 13 and 10.4 billion, respectively. Although consumer interest is growing in other regions, the demand is confined to the industrialized world largely because of the price premium of organic products. Many developing countries have large sections of their populations below the poverty line, and this makes it difficult for a market of organic products to develop (Willer and Yussefi, 2004). Rapid economic growth in countries such as China, Brazil, and South Africa is causing the upper social classes to expand, and this is creating a market for organic food. Most production in Asian and African countries will be for export markets, although regional markets are also developing.

The Western European market for organic meat was traditionally the largest in the world; however, it has now been equalled by North America. The European market has faced rapid expansion since the mid 1990s and is now reporting slowing growth rates as certain sectors approach maturity.

The average European expenditure rate on organic products is US\$ 27.2 per annum, ranging from US\$ 7.3 (Spain) to US\$ 105 (Switzerland) *per capita*.

In 2003, EU, including 15 countries, certified non-dairy cattle (mainly suckler cows) amounting to about 1 million heads and corresponding to 1.7% of the total non-dairy cattle herd (European Commission, 2005). Austria ranked first with 25% of EU, followed by Italy with 15%. Certified pigs amounted to 450 000 head, mainly located in Germany and France, while organic broilers were more than 9 million (France ranked first). Organic sheep and goats amounted to 2.4 million. They were located mainly in the United Kingdom and in Italy. According to Hamm *et al.* (2002), beef had the highest market share (1.7%) followed by sheep and goat meat (0.7%), pork (0.3%) and poultry (0.3%).

Only little and partial information is available on prices for organic meat and meat products as prices diverge depending on the country and on the product. For instance, farmer price for organic pork in 2002 was about 2.46 €/kg on EU-15 average. Price premiums for organic pork in EU were about 62%. Consumer price for organic pork cutlet was about 13.17 €/kg but differed considerably among the 15 member states. The high divergence in consumer prices and price premiums often reflects the different sale chains used, e.g. consumer prices usually are much higher in organic food shops than in supermarket chains. Similarly, there was considerable variability among the 15 Member States in farm gate prices for beef as well as in consumer prices for minced beef (European Commission, 2005).

The North American market for organic products is reporting the highest growth worldwide. The meat sector is the fastest growing organic food industry, with sales expanding by 51% in 2005 (Organic Monitor Ltd., 2006). High market growth rates have led to organic meat supply shortages, with producers unable to meet growing demand from retailers. The organic beef and pork markets are the most affected because of low production levels. Although production has increased since 2004, supply is expected to lag behind demand for a number of years. As a consequence, prices are rising for a range of organic meat products and an increasing number of North American processors are looking overseas for

supplies, particularly from Latin America, Australasia and Canada. Although organic beef has the fastest growing market, organic poultry, and broilers in particular, is the most widely available in North America (nearly two-thirds of this sector). The relatively short production cycle and low price premium are responsible for organic chicken being the most popular organic meat with consumers. USA retail sales of organic poultry were US\$ 161 million in 2005, well under 1% of conventional poultry sales. However, retail sales of organic poultry are rapidly growing (*Nutrition Business Journal*, 2006). Prices for organic broilers from July 2004 through early 2006 ranged from US\$ 1.89 to US\$ 2.45, while average prices for conventional broilers varied from US\$ 0.59 to US\$ 0.82 per pound (Oberholtzer *et al.*, 2006). Organic beef, in comparison, is more rarely found in retailers because of small-scale production, and inadequate distribution infrastructure, although the success of competing products such as 'natural' beef is decreasing. United States Department of Agriculture (USDA) rules for 'natural' beef are less strict than those for organic products: antibiotics, hormones and animal by-products are banned but conventional feedstuffs are allowed. In USA the number of certified animals in 2005 was over 36 000 beef cows, 10 000 hogs and pigs, 4400 sheep and lambs, and over 10 000 000 broilers.

The Argentine government has established national standards for organic products. These are at least as stringent as those of IFOAM and the European Union. The organic industry in Argentina is mainly export oriented (85% by value is exported, with an estimated value of US\$ 32 million). The European Union is the principal destination of Argentina's organic beef exports. Meat exporting began in the mid 1990s with beef and more recently Patagonian lamb became the predominant export for international markets. In 2002, there were 754 000 and 122 000 certified sheep and cattle, respectively, in Argentina. However, a domestic market is being developed in Buenos Aires (Willer and Yussefi, 2004).

Elsewhere, both Brazil and Uruguay are seeking to develop exports of organically produced meat (Willer and Yussefi, 2004). In the case of Brazil, organic beef production systems are being developed in the centre of the country. The authorities in Brazil see the organic production system as a way of boosting local cattle producers' income, while reducing the environmental damage that traditional methods of cattle raising have caused. While 90% of Brazil's overall organic production is exported, Brazil's internal market for organic foods is growing at around 25% a year.

Although the Australasian continent comprises almost 40% of global organic farmland, with 12.1 million hectares, the market represents a fraction of the global total (Willer and Yussefi, 2004). Sales of organic products were estimated at about US\$ 200 million in 2002, with Australia comprising the most. Beef cattle farmers use much of the organic farmland in Australia as pastureland. The Australian and New Zealand organic food industry is export-oriented, with significant quantities of primary products, including beef and lamb, going to other northern hemisphere countries and relatively low amounts sold in the domestic market. However, sales of organic products within Australia are growing at about 15 to 20% per annum.

Although trade liberalization may be removing tariff barriers, exporters are finding it increasingly difficult to meet supply gaps because of the differences in organic standards between regions.

17.1.3 Willingness to buy organic meat

Food quality is not an objective feature of the products as it is affected by consumer perception, and it is often referred to as perceived quality. Many aspects can be used by consumers to make their food choices. Intrinsic (e.g. cut, colour, fat rim) and extrinsic cues (price, origin, stamp of quality, production and nutritional information) are used to form expectations about product quality attributes. The latter can be classified in two categories: those experienced before or during consumption (experience quality attributes, e.g. price and sensory properties) and those not experienced directly, such as healthiness, naturalness and ethical aspects, which have to be communicated in order to be perceived as they are credence characteristics that cannot be confirmed either before or after purchase (Grunert *et al.*, 2004).

According to a recent on-line survey (ACNielsen, 2005), organic alternatives are purchased mainly for health reasons (respondents thought that organic foods were healthier for them and their children). European people seemed to be more conscious of the wider benefits of organic foods, such as protecting the environment and animals. As to the barriers to purchasing organic alternatives, availability and credibility were considered a problem. However, the general sense among consumers who would not buy organic products was that they were usually more costly.

Meeting organic certification requirements usually implies higher production costs. For example, it is reported that the cost of producing organic beef in the United Kingdom is 20% higher than that for conventional methods. In some cases, the high cost of converting to organic meat and dairy production has led to subsidies being paid to the farmer. However, this is not the case in many countries (e.g. USA and developing countries). Thus, the extra production costs have to be paid by consumers.

Provision of information about the manufacturing process (e.g. organic vs. OGM) can affect acceptability (Caporale and Monteleone, 2004) and consumer willingness to pay (Lange *et al.*, 2002), thus providing a means to cover the extra production costs sustained by organic farmers. The price that people are willing to pay is the major determinant of the market share of organic meat. Although intent to purchase depends upon the interactions of quality attributes such as appearance and colour (Brewer and McKeith, 1999), organic labeling has been found to have a more consistent effect as compared to some sensory characteristics on the price offered by consumers for organic pork (Dransfield *et al.*, 2005). Using questionnaires on organic foods in Spain, consumers appeared to be prepared to pay about 12% more for organic red meats and chicken (Gil *et al.*, 2000). In France and the Netherlands, questionnaire responses suggested that almost half of consumers would pay 20% more for pork from pigs raised outdoors (Carpentier *et al.*, 2004),

whereas Dranfield *et al.* (2005) suggested that people from different European countries would offer 5% extra for organic pork.

Meat is usually commercialized as undifferentiated product. Food differentiation can be based on both product and process characteristics. For animal-based products, such as meat, the process characteristics may be represented by the farming practices and the related organic standards. However, these characteristics are not easily evaluated or experienced by consumers, which indicates the need for a special, constant and reliable quality signaling system, given to consumers through appropriate information in order to motivate them and increase their willingness to buy and pay for organic meat (Bredahl, 2004; Grunert *et al.*, 2004).

Another element favoring the spread of organic meat into the general retail sector is that purchasers of organic food tend to be in the higher income segment (FAO, 2002). Consequently, supermarkets seek to attract such customers by providing a wide range of food, including organic meats. The increased involvement of supermarkets, with their centralized systems of purchasing and distribution, may result in pressure to reduce the current price differential between organic and conventional products.

17.2 The quality of organic meats as compared to conventional products

Organic rules markedly affect farming practices, which in turn can influence meat quality. This section will provide information about the effects of the main aspects covered by organic standards on the quality of meat obtained from the major farmed animal species (Table 17.1), in relation to the corresponding conventional products. However, in many studies, the overall production system was considered; thus the effects of single factors, such as rearing system, diet or genetic type, could not be precisely separated. For instance, Walshe *et al.* (2006) compared organic and conventional beef samples gathered from the retail market; thus the effect of the entire organic chain was evaluated. These authors found similar sensory characteristics in the two products. Combes *et al.* (2003a) compared rabbit meat obtained from organic and conventional production systems which were different for several aspects including housing, feeding and breed. In this study, trained panel members were able to distinguish the organic product from the conventional one, indicating that the former was more tender.

17.2.1 Rearing system

Organic rules promote the use of pasture and outdoor areas, while indoor space allowance is, in general, higher as compared to conventional/intensive systems.

Organic broilers housed in an indoor pen with access to a grass paddock show lower growth performance and lower amounts of abdominal fat compared with conventional birds housed in indoor pens (Castellini *et al.*, 2002a). This is

Table 17.1 Effects of the main aspects covered by the organic standards on meat quality characteristics

Property	Animal		Outdoor/Grazing		Forage-based diet		Rustic breed
IMF ¹	Pigs	–	Olsson <i>et al.</i> , 2003; Millet <i>et al.</i> , 2004	–	Hansen <i>et al.</i> , 2006		
	Broilers					+/–	Fanatico <i>et al.</i> , 2005
	Cattle	–	Vestergaard <i>et al.</i> , 2000a; Realini <i>et al.</i> , 2004	+/–	Marino <i>et al.</i> , 2006		
L* ²	Pigs	–	Bridi <i>et al.</i> , 1998	+	Millet <i>et al.</i> , 2004	+/–	Oliver <i>et al.</i> , 1994; Enfalt <i>et al.</i> , 1997
	Broilers	+	Castellini <i>et al.</i> , 2002			–	Fanatico <i>et al.</i> , 2005
	Cattle			+/–	French <i>et al.</i> , 2000		
WHC ³	Pigs	–	Olsson <i>et al.</i> , 2003			+/–	Oliver <i>et al.</i> , 1994; Enfalt <i>et al.</i> , 1997
	Broilers	–	Castellini <i>et al.</i> , 2002			–	Fanatico <i>et al.</i> , 2005
	Cattle	+/–	Realini <i>et al.</i> , 2004	+/–	Marino <i>et al.</i> , 2006		
pHu ⁴	Pigs	–	Enfalt <i>et al.</i> , 1997; Olsson <i>et al.</i> , 2003	–	Millet <i>et al.</i> , 2004		
	Broilers	–	Castellini <i>et al.</i> , 2002			+	Castellini <i>et al.</i> , 2002
	Cattle					+/–	Campo <i>et al.</i> , 1999
Flavour	Broilers					+	Fanatico <i>et al.</i> , 2006
	Cattle	+/–	Realini <i>et al.</i> , 2004	+/–	Marino <i>et al.</i> , 2006	+	Braghieri <i>et al.</i> , 2005
WBS ⁵	Pigs	+	Olsson <i>et al.</i> , 2003				
	Broilers	+	Castellini <i>et al.</i> , 2002			+	Fanatico <i>et al.</i> , 2005
Tenderness	Cattle			+/–	Marino <i>et al.</i> , 2006	–	Braghieri <i>et al.</i> , 2005
	Pigs			–	Hansen <i>et al.</i> , 2006		
	Broilers					–	Fanatico <i>et al.</i> , 2005
Crude protein content	Cattle			+/–	Marino <i>et al.</i> , 2006		
	Pigs	+	Olsson <i>et al.</i> , 2003				
	Broilers	+/–	Fanatico <i>et al.</i> , 2005				
	Cattle			+/–	Marino <i>et al.</i> , 2006	+/–	Fanatico <i>et al.</i> , 2005
Mineral content	Pigs	+	Olsson <i>et al.</i> , 2003				
	Broilers	+	Castellini <i>et al.</i> , 2002			–	Fanatico <i>et al.</i> , 2005
	Cattle	+	Nielsen and Thamsborg, 2005	+	Marino <i>et al.</i> , 2006; Nielsen and Thamsborg, 2005	+	Braghieri <i>et al.</i> , 2005
PUFA/CLA ⁶	Pigs	+	Nilzen <i>et al.</i> , 2001	+	Nilzen <i>et al.</i> , 2001; Oksbjerg <i>et al.</i> , 2005		
	Broilers		Castellini <i>et al.</i> , 2002	+	Castellini <i>et al.</i> , 2002		
	Cattle	+	Yang <i>et al.</i> , 2002; Descalzo <i>et al.</i> , 2005	+	Yang <i>et al.</i> , 2002; Descalzo <i>et al.</i> , 2005		
Antioxidants	Pigs	+	Nilzen <i>et al.</i> , 2001	+	Nilzen <i>et al.</i> , 2001; Hogberg <i>et al.</i> , 2002		

+: increase; –: decrease; +/-: no effect.

¹ Intramuscular fat; ² Lightness; ³ Water Holding Capacity; ⁴ Ultimate pH; ⁵ Warner–Bratzler Shear force; ⁶ Polyunsaturated-Conjugated Linoleic Acid.

probably due to increased activity (Lewis *et al.*, 1997), which also makes animals calmer and less sensitive to environmental stressors.

In general, free-range pigs display increased carcass lean meat contents (Enfält *et al.*, 1997; Sather *et al.*, 1997) and decreased back fat thickness (Warriss *et al.*, 1983) because of both higher energy requirements to maintain body temperature and higher levels of exercise performed in outdoor areas. However, different results were obtained by other authors: although organic pigs were kept in ample outdoor areas, their carcasses had lower lean meat percentages and thicker back fat than the corresponding conventionally grown animals (van der Wal *et al.*, 1993; Bridi *et al.*, 1998; Olsson *et al.*, 2003). Such different results are not necessarily in conflict as in the latter experiments the higher fatness may be explained on the basis of the higher energy content of the feed formulated according to organic standards, in combination with a rather mild climate during the production period. Enhanced physical activity may also cause shorter carcasses in organically grown pigs (Enfält *et al.*, 1993; Millet *et al.* 2004).

The organic production system may result in a decreased technological meat quality. Meat of organically raised broilers (Castellini *et al.*, 2002a) and pigs (Sather *et al.*, 1997; Enfält *et al.*, 1997; Olsson *et al.*, 2003; Millet *et al.*, 2004) had lower ultimate pH (pHu), higher drip and cooking losses and higher shear force values than conventionally grown animals. Exercise may also negatively affect beef, with higher shear values and reduced taste (Vestergaard *et al.*, 2000b).

The lower pHu of the organic chickens could be due to a lower consumption of glycogen as a consequence of reduced sensitivity to pre-slaughter stress of animals kept in good housing conditions (Castellini *et al.*, 2002a). The same effect was observed in outdoor reared pigs. Enfält *et al.* (1997) attributed this result to the capacity to utilize substrates other than glycogen during transport to the slaughterhouse. Increasing the pig physical fitness by spontaneous activity performed in ample outdoor rearing areas may determine adaptations of muscle metabolism, leading to increased aerobic capacity (Petersen *et al.*, 1998). These adaptations are glycogen sparing (Henckel *et al.*, 2000; Petersen *et al.*, 1997). In fact, it has been shown that moderate physical activity during growth can increase the pre-slaughter glycogen content of muscle (Essen-Gustavsson *et al.*, 1988). Accordingly, other studies found higher energy levels in the *Longissimus dorsi* of outdoor raised pigs at slaughter (Barton-Gade and Blaabjerg, 1989; Enfält *et al.*, 1997). In addition, it has been shown that very high levels of vitamin E in the fresh grass that is frequently associated with outdoor rearing may increase muscle glycogen stores (Lauridsen *et al.*, 1999). Lower pHu can shrink myofibrils, reducing their water-binding ability and increasing light scattering (Castellini *et al.*, 2002a). Furthermore, this condition can reduce the importance of myoglobin in selectively absorbing green light, resulting in less red and more yellow meat. However, other studies found no differences in the rate and extent of pH decrease post-mortem (Dufey, 1995), while the changes observed in water-holding capacity were explained by the slightly changed muscle characteristics and early post-mortem metabolic events of the exercised outdoor animals (Nilzen *et al.*, 2001). In fact, recent studies suggest that pHu accounts for a small part of the ability of meat to retain water

(Schafer *et al.*, 2002), while a high glycogen content in itself may negatively affect water-holding capacity by the ability to bind water released during post-mortem glycolysis (Fernandez, 1991). Conversely, in outdoor organically raised rabbits, higher pH_u and improved loin tenderness in comparison with conventionally reared indoor animals were observed (Combes *et al.*, 2003b).

Outdoor raising may have diverging effects on meat colour and heme pigment levels, as some authors found no influence of exercise (van der Wal *et al.*, 1993; Enfält *et al.*, 1993), whereas in more recent studies, increased space allowance and grazing induced a darker meat color in young bulls (Vestergaard *et al.*, 2000a) and pigs (Bridi *et al.*, 1998). Nuernberg *et al.* (2005) also observed that bulls fed on grass-based systems, including pasture, showed a color of muscle darker than concentrate-fed animals. These latter results are generally attributed to the increased activity of grazing animals (Millet *et al.*, 2004; Priolo *et al.*, 2001) leading either to high levels of myoglobin in the muscle (Shorthose and Harris, 1991) or to a greater frequency of Type II A than Type II B fibres or to an increased mean fibre cross-sectional area (Petersen *et al.*, 1998). Occasionally, the meat from free-range pigs has been found slightly paler or less red than that from conventionally raised animals (Sather *et al.*, 1997).

The higher shear and bite resistance value as well as the lower sensory tenderness observed in organic pig meat (Danielsen *et al.*, 2000) either with (Enfält *et al.*, 1997; Sather *et al.*, 1997) or without (van der Wal *et al.*, 1993) lower intramuscular fat can be due to the higher physical activity of the animals. The same effect has been observed in broilers as a consequence of a lower stocking density (Farmer *et al.*, 1997). The higher shear force values of organically produced meat may be due to the slower daily growth rate, which can cause a slower protein turnover in the muscle of more extensively raised animals, making their muscular tissue more difficult to tenderize during post-mortem storage (Nielsen and Thamsborg, 2005). In addition, collagen can adapt to functional demands such as physical activity, and a tendency toward increased amounts of heat stable collagen has been reported in the *Longissimus dorsi* of pigs performing physical exercise (Petersen *et al.*, 1997). Combes *et al.* (2003a) documented that collagen content is similar or slightly higher in organic outdoor rabbits than in confined animals, while the heat-solubility of collagen is not modified by the rearing system. However, differences in tenderness between organic and conventional meat may be very subtle as neither a descriptive sensory panel, nor a consumer preference test, could discriminate organic from conventional pig meat (Olsson *et al.*, 2003; Millet *et al.*, 2005). These results suggest that other factors, such as genetics, and slaughter age and procedures might interfere and prevail in determining the final product tenderness.

High space allowances and grazing are deemed to be related to high energy expenditure and low fatness (Enfält *et al.*, 1997; Sather *et al.*, 1997). Accordingly, Olsson *et al.* (2003) and Millett *et al.* (2004) observed that, despite a higher energy content in the feed and a lower estimated lean meat percentage for organically raised pigs, intramuscular fat was lower in this product than in that obtained from conventionally raised animals. Nevertheless, no general rule concerning the

influence of the outdoor rearing system on intramuscular fat content can be proposed because of confounding factors such as climatic conditions, level of space allowance, genotype, feeding level and diet composition offered to the animals. For instance, the meat of free-range or organic pigs may contain less intramuscular fat (Enfält *et al.*, 1997; Olsson *et al.*, 2003) than the meat of indoor animals, while increased (Lebret *et al.*, 2004) or similar (van der Wal *et al.*, 1993) intramuscular fat levels may be observed in pigs from semi-outdoor housing systems as compared to indoor rearing. An increase in intramuscular fat level can enhance consumer acceptability of pork (Fernandez *et al.*, 1999b), as several studies suggest a favourable relationship between intramuscular fat, juiciness and tenderness (Hodgson *et al.*, 1991; Fernandez *et al.*, 1999a).

A higher crude protein content and lower water/protein ratio seems to be one of the more consistent characteristics of meat from pigs raised in a more extensive way (Dworschak *et al.*, 1995; Enfält *et al.*, 1997; Olsson *et al.*, 2003). Another consequence of alternative production systems on meat chemical composition is the higher ash (Olsson *et al.*, 2003) and mineral (zinc, copper and iron) contents (Dworschak *et al.*, 1995), as the metal binding capacity of proteins may be higher in the muscles of pigs kept outdoors as compared to conventionally raised animals. In addition, higher iron content may be associated with the higher myoglobin levels often observed in the muscles of exercised and organically reared animals (Shorthose and Harris, 1991).

17.2.2 Feeding

Major differences with conventional/intensive systems include the assumption of spontaneous or cultivated fresh forages at the pasture, higher forage-to-concentrate ratio, the prevalent use of organically produced ingredients, and the ban of synthetic amino acids, antibiotics and growth promoters as feed ingredients. The effects of these latter two aspects will be discussed in the subsequent paragraph.

A higher nutrient content in organic crops has been observed in comparison with conventional feed. The latter may contain more water, thus causing nutrient dilution (Worthington, 1998). A higher crude protein content in organically produced feed may determine reduced intramuscular fat contents in organic pigs (Olsson *et al.*, 2003; Essen-Gustavsson *et al.*, 1994).

When animals can benefit from an outdoor area, energy requirements for activity and thermoregulation will increase. Hence, they may require a feed with a higher energy-to-amino acid ratio. However, the main problem in non-ruminant nutrition is the difficulty in finding protein sources with a well-balanced amino acid pattern. The exclusion of synthetic amino acids has to be compensated for by other protein sources; for instance, the use of grain legumes has been suggested (faba beans, peas and lupines) for pigs (Sundrum *et al.*, 2000). Although weight gain and feeding efficiency may be reduced, no amino acid supplementation (Sundrum *et al.*, 2000) or the consumption of diets with low protein-to-energy ratios (Blanchard *et al.*, 1999) favor the production of meat with high intramuscular fat, which is an important positive aspect of eating quality character-

istics (Fernandez *et al.*, 1999a). According to Millet *et al.* (2006), in the diet of finishing pigs, protein content has limited effects on meat characteristics. Thus, in this phase, reduced lysine levels, compared with those used in conventional pig diets, may be fed to organic pigs without relevant consequences on meat quality (Millet *et al.*, 2006).

In ruminant nutrition, protein sources alternative to soya bean (faba beans, peas and lupines) are being studied in order to minimize the risk of GMO contamination. Preliminary results indicate that faba beans did not change color, water-holding capacity (Sodo *et al.*, 2007), chemical composition, tenderness and cooking loss (Ragni *et al.*, 2006) of beef, whereas the use of lupin induced a lower intramuscular fat as compared to soya bean-based diets (Ragni *et al.*, 2007).

A restricted concentrate feeding plus *ad libitum* roughages resulted in lower intramuscular fat and slightly lower tenderness in pig meat (Danielsen *et al.*, 2000; Hansen *et al.*, 2006). The decreased tenderness of meat from the restricted-fed pigs may be due to a reduced daily gain, which results in slower muscle growth, slower protein turnover and lower meat proteolytic potential (Therkildsen *et al.*, 2002).

Higher levels of roughage and larger intakes of fresh grass can determine changes in the fatty acid profile of intramuscular and depot fat, resulting in higher ratios of unsaturated to saturated fat. A marked effect of grass-based diets was observed in organic beef cattle (Nielsen and Thamsborg, 2005), lambs (Enser *et al.*, 1998), free-range reared pigs (Dufey, 1995; Nilzen *et al.*, 2001; Oksbjerg *et al.*, 2005; Hansen *et al.*, 2006), broilers (Castellini *et al.*, 2002a) and rabbits (Pla *et al.*, 2007). These effects on fatty acid composition could depend either on high roughage intake or on restricted feeding leading to leaner carcasses (Oksbjerg *et al.*, 2005). A leaner meat, in fact, has a higher proportion of phospholipids that are richer in polyunsaturated fatty acids (PUFA) and particularly in C20 and C22 fatty acids (Elmore *et al.*, 1999). An improvement in fatty acid composition of bovine muscles, with a higher polyunsaturated to saturated ratio, was also found in Podolian young bulls fed with a higher forage-to-concentrate ratio in the diet, according to EC-Regulation 1804/1999 (Marino *et al.*, 2006). In addition, meat from cattle (French *et al.*, 2000; Yang *et al.*, 2002; Realini *et al.*, 2004; Nielsen and Thamsborg, 2005) and lambs (Santos *et al.*, 2002) fed large amounts of roughage, or fed on pasture, have a higher content of conjugated linoleic acid (CLA), an intermediate product of ruminal biohydrogenation, with beneficial effects on human health (Pariza *et al.*, 2001).

However, an increased amount of PUFA in muscles can make meat more susceptible to lipid oxidation. Meat from organic broilers shows higher 2-thiobarbituric acid reactive substances (TBARS) values in comparison with conventional products (Castellini *et al.*, 2002a; Lawlor *et al.*, 2003).

Thermal and oxidative degradation of unsaturated fatty acids yield a number of carbonyl compounds that influence flavor (Griebenow *et al.*, 1997). Carcasses from beef slaughtered directly from pasture can have a grassy flavor that may derive from the action of ruminal microorganisms breaking down chlorophyll (Griebenow *et al.*, 1997). However, finishing of steers with large amounts of roughage had only minor detrimental effects on eating quality as compared to

finishing with barley *ad libitum*, so it is questionable if the consumer can taste any differences (Andersen *et al.*, 2002).

The lower lipid stability of the meat from organic animals may also be due to the higher content of metallic ions (total and heme Fe) catalysing peroxidation (Fukozawa and Fuji, 1992). Physical exercise can increase the amount of heme-iron (Hoffmann, 1995), particularly in the more oxidative muscles (Petersen *et al.*, 1997).

In cattle, Walshe *et al.* (2006) found that organic and conventional beef had similar levels of natural antioxidants, but organic samples were higher in fat content and were therefore more susceptible to lipid oxidation and less color stable. However, in general, beef produced on pasture could have lower TBARS levels than meat from grain-fed animals, due to the protection conferred by natural products present in grass (Descalzo *et al.*, 2005; 2007). Feeding cattle on pasture, in fact, confers higher levels of vitamin E, β -carotene (Yang *et al.*, 2002; Descalzo *et al.*, 2005) and vitamin C (Descalzo *et al.*, 2005). In addition, feeding grass silage causes a higher vitamin E concentration in beef compared to maize silage (O'Sullivan *et al.*, 2003). Higher levels of α -tocopherol were also found in the meat from free-range pigs with access to pasture (Nilzen *et al.*, 2001). In pigs, the feed formulated according to the European regulation contained more vitamin E than the conventional feed (25.3 compared with 19.9 mg α -tocopherol/100 g feed), which led to higher vitamin E levels in the meat of organically raised animals (Hogberg *et al.*, 2002).

Grazing and high amounts of roughage can result in yellow fat, due to the high levels of carotene in the feedstuff that is not totally degraded to vitamin A in the intestinal mucosa (Therkildsen *et al.*, 1995): the longer cattle graze on pasture, the higher are β -carotene levels (up to 50% higher than those finished on grain) in plasma, muscle, and adipose tissues (Yang *et al.*, 2002).

Forage effects on tenderness are controversial as in some cases their consumption produces an improvement as compared to concentrate (Oltjen *et al.*, 1971), whereas in other studies no differences in tenderness between grain- and forage-finishing beef (French *et al.*, 2000, 2001) or higher tenderness in grain-fed cattle (Bennett *et al.*, 1995) was observed.

Flavor is highly dependent on diet, and, in general, high-energy grain diets induce a more acceptable intense flavour than low-energy forage or grass diets (Melton, 1990; Kerth *et al.*, 2007).

17.2.3 Genotype

Organic farming should be based on the use of indigenous genotypes, well adapted to extensive rearing conditions and the difficulty of the surrounding environment.

In Europe, organic poultry systems frequently employ slow-growing meat birds, which have a growing period of at least 81 d. Most USDA organic production, on the other hand, relies on the same fast-growing genotype used in conventional systems. According to Castellini *et al.* (2002b), slow-growing genotypes show a good adaptation to extensive rearing conditions, whereas fast-

growing genotypes show unbalanced muscle response to the greater activity and reduced oxidative stability of the meat.

The effect of genotype often interacts with age: for instance, slow-growing chickens require a longer rearing period (Farmer *et al.* 1997), but also local and rustic cattle and pig breeds may need a longer finishing period due to their lower weight gains which are a consequence of either a lower genetic selection or a more fibrous diet. Obviously, meat quality is affected by the degree of maturity of the animals at slaughter (Castellini *et al.*, 2002b).

Genotype effect on poultry meat color is not directly manifest. Slow-growing genotypes usually show redder and darker meat compared with fast-growing birds (Le Bihan-Duval *et al.*, 1999; Berri *et al.*, 2001). This is probably due the higher content of myoglobin in the muscles of slow-growing animals (Gordon and Charles, 2002), which achieve their slaughter weight at an older age (Fanatico *et al.*, 2006). Other authors ascribe differences in redness among genotypes to differences in the type of muscle fibre (Lonergan *et al.*, 2003). In addition, the longer period spent foraging by slow-growing birds may determine a more yellow meat (Fanatico *et al.*, 2005). In general, the meat from fast growing broilers has more fat, lower pHu and iron, and is paler (Castellini *et al.*, 2002b).

Genotype effects on broiler meat quality are also evident on water-holding capacity (Fanatico *et al.*, 2005; Lonergan *et al.*, 2003; Rizzi *et al.*, 2007) and tenderness (Fanatico *et al.*, 2005): both are lower in alternative genotypes. Texture differences may be due to the higher slaughter age of slow-growing genotypes and, consequently, to the higher content of mature collagen crosslinks (Fletcher, 2002). Differences in tenderness may be also ascribed to the fact that fast-growing genotypes have larger muscle fibers with different proteolytic potentials (Dransfield and Sosnicki, 1999).

The higher drip and cooking losses found for the muscles of slow-growing genotypes can affect the sensory profile of meat, which may be drier than in fast-growing birds (Fanatico *et al.*, 2006). Although a trained panel perceived some differences in texture and flavor between the meats from conventional and alternative genotypes, the consumer panel did not detect differences in liking (Fanatico *et al.*, 2006). The capacity of a panel to discriminate meat from slow- vs. fast-growing genotypes is largely debated (Richardson and Mead, 1999).

Organic pigs are required to eat large amounts of roughage. Thus it is necessary to find suitable genotypes in order to convert this roughage into useful nutrients (Nielsen and Thamsborg, 2001). In addition, animals should be pigmented and adapted to outdoor conditions. As a consequence, many organic farms rely either on commercial hybrids presenting large proportions of Duroc, or on the pure Duroc breed or on local pigmented breeds. The Duroc genotype has a positive influence on taste and juiciness in comparison with Landrace (Oliver *et al.*, 1994) or Yorkshire (Enfält *et al.*, 1997), whereas no effect on color or water holding capacity was observed (Oliver *et al.*, 1994; Enfält *et al.*, 1997). However, this breed may give rise to an increased amount of inter-muscular fat with detrimental effects on meat products such as ham (Wood *et al.*, 2004). Pork from local breeds is often characterized by higher fat percentage, which is detrimental for human

health (Zullo *et al.*, 2003). However, breeds of high fat production potential and high levels of subcutaneous fat tend to produce more saturated meat, which makes the product more suitable for transformation (Wood *et al.*, 1989).

In cattle, some Spanish rustic breeds (Aveleña-Negra Ibérica, Morucha and Retinta) do not differ from double muscled, dual-purpose or fast growth rate genotypes in terms of juiciness, fibrosity or overall flavor intensity (Campo *et al.*, 1999), whereas other native bovine genotypes, such as Podolian cattle, showed significant differences in comparison with Limousine \times Podolian crossbred with an improved fatty acid profile in terms of PUFA content and a lower tenderness (Braghieri *et al.*, 2005). Differences in shear force and sensory tenderness were found between Hereford, Hereford \times Friesian, and Friesian steer grazed at pasture when compared immediately after slaughter. Such differences were possibly associated with a higher calpastatin to μ -calpain ratio in the meat from Friesian steers, which was less tender. However, after an appropriate period of ageing (6–9 days) these differences disappeared (Muir *et al.*, 2000). Ageing time, in fact, seems to reduce toughness differences among breeds (Campo *et al.*, 1999).

17.2.4 Mutilations

A central concern in organic husbandry is the welfare of the farmed animals. This does not mean that it is important only to keep the animals healthy and to prevent anxiety, pain and suffering, but that it is also important to respect the integrity of the animals (Verhoog *et al.*, 2004). One aspect of the nature of cattle is that they use horns in the expression of social behavior. Therefore, in organic husbandry, dehorning should be avoided. The EU regulation states that these operations should be not carried out systematically, although for reasons of safety they may be authorized by the inspection authority. Many farmers see problems of working safely with horned cattle. In addition, the risk of injuries for the animals increases, with possible negative effects on carcass quality (higher incidence of bruised muscles), while demands regarding housing design, space allowances and management are higher. In organic farms, the use of tie stalls is limited in time; therefore, the percentage of herds with dehorned animals is increasing with the introduction of loose housing systems.

As for de-horning, the production of entire animals may be more in line with the organic principles of naturalness. However, many organic dairy farmers do not consider castration unethical as they prefer not to engage in outdoor young bull production due to handling and fencing problems, particularly when there are heifers nearby (Nielsen and Thamsborg, 2002). Security of the farmer and the public may be also at risk, when bulls are on pasture. Bulls are well suited to intensive feeding systems as they have a greater potential for muscle growth, with higher live-weight gains (about 20%), better feed efficiency (about 15%), and leaner carcasses (Jarrige and Auriol, 1992) than steers. Conversely, the latter tend to accumulate fat, thus they may be preferred in organic systems where the amount of roughage administered to the animals is higher and the grazing period longer (Nielsen and Thamsborg, 2005). Steer meat generally shows a better eating quality

in terms of tenderness, juiciness and taste (Andersen and Ingvarsen, 1984; Steen and Kilpatrick, 1995), as it is higher in marbling (intramuscular fat), lower in collagen content, and higher in collagen solubility (Temisan, 1989).

Castration is extensively performed also in heavy pig organic farming, when the objective is transformation into meat products such as cured ham, and boar taint may become a problem, as the animals are slaughtered after they are sexually mature.

17.2.5 Conclusive remarks concerning quality and ethics

Differences between organic and conventional meats may vary markedly according to the interpretation of organic rules by farmers. The diversity can be very small if the systems differ only in terms of origin of feed (organic vs. conventional) administered to the animals, or very large when a comprehensive organic approach, including breed, components of diet, percentage of forage in the ration, grazing and space allowance, is adopted. However, the higher quality of organic meats is related to process characteristics rather than to subtle differences in sensory attributes or nutritional characteristics: product safety, animal welfare, biodiversity and environment safeguard can provide an ethical value to the organic product, which may become even higher if associated with traditional farming systems and typical meat productions.

17.3 Safety and healthiness of organic meat

The IFOAM's principle of health states that 'Organic Agriculture should sustain and enhance the health of soil, plant, animal, human and planet as one and indivisible'. Health is the wholeness and integrity of living systems. In view of this the use of fertilizers, pesticides, animal drugs and food additives that may have adverse health effects should be avoided. Based on this principle, consumers usually perceive organic meat as safer and healthier compared to conventional product and this is the main reason for purchasing organic food. Organic processing and banning animal flour, GMO food and chemicals in animal feeding, gives consumers the assurance to avoid many diseases (bovine spongiform encephalopathy *Escherichia coli* O157 infections, dioxin toxicity, foot and mouth disease) affecting modern livestock (Magkos *et al.*, 2006).

17.3.1 Chemical residues

Organic regulations require that animals have to be fed on organically produced feedstuffs; thus the potential for contamination with pesticide residues and other agricultural chemicals is greatly reduced compared with conventional farming methods. However, organic agriculture, albeit reducing the global level of pollution, cannot avoid contaminations from persistent environmental pollutants, which can potentially be present in organic feedstuffs and hence in organic meat.

For instance, Smith *et al.* (1997) detected levels of pesticide residues above American threshold limits in liver from beef cattle produced under natural and organic conditions, although muscle and fat were not affected. However, the same authors recorded a much higher number of detectable, but non-violative, chlorinated-hydrocarbon and organophosphate residues in conventional samples as compared to organic meat, while natural beef showed intermediate levels. In another study, pesticides and polychlorinated biphenyl residues in both organic and conventional meat were lower than legal limits, while lead and cadmium residues were very low and did not differ between organic and conventional meat (Ghidini *et al.*, 2005).

17.3.2 Growth promotants

Organic rules do not allow the use of growth promotants, such as antibiotics and hormones, for growing and fattening animals. If antibiotics are used to restore an animal to health, that animal cannot be used for organic production or be sold as organic.

The practice of administering growth promoters to livestock has been an issue of scientific debate and public concern for many years. European Union banned anabolic steroids in 1986 and did not authorize the use of alternative substances such as β -agonists, the physiological analogue of adrenalin, for repartitioning purposes (increase lean meat to fat ratio) and feed conversion efficiency improvement (Allen *et al.*, 1987). In contrast, the USA beef cattle industry has adopted the use of anabolic implants as a routine management practice because of market incentives to increase growth rates and reduce costs of live weight gain. Approved anabolic implants are either estrogenic, or androgenic, or both (Morgan, 1997).

The risk associated with the use of hormones, in particular estradiol, is the carcinogenic effect exerted both in their initiating and promoting tumors (Devanesan *et al.*, 2001). Little information is available on hormone residues in conventional meat as compared to the organic product. Smith *et al.* (1997) detected no residues of anabolic steroids (testosterone, estradiol, progesterone) and xenobiotics (zearanol, trenbolone acetate) in conventional, natural and organic beef samples. However, a dose-dependent increase in residue levels of various hormones, particularly at the implantation sites, has been observed (Hageleit *et al.*, 2000). Thus, misplaced implants and repeated implanting, which seem to occur frequently, represent a considerable risk that contaminated meats could enter the food chain.

The term 'antibiotic growth promoter' is used to describe any medicine that destroys or inhibits bacteria and is administered at a low, sub-therapeutic dose. The use of antibiotics for growth promotion rose with the intensification of livestock farming. Infectious agents reduce the yield of farmed food animals and, to control these, the administration of sub-therapeutic antibiotics and antimicrobial agents has been shown to be effective. There is controversy surrounding the administration of growth promoters to animals used for meat production, as overuse of any antibiotic over a period of time leads to the local bacterial populations becoming

resistant to the antibiotic. In 2006, the European Union banned the feeding of all antibiotics and related drugs to livestock for growth promotion purposes, whereas in the USA a wide range of antibiotics is used as growth promoters for pigs, including some substances (penicillins, lincosamides and macrolides, including erythromycin and tetracyclines) considered to be 'medically important' (JETACAR, 1999). In addition, in USA pigs are exposed to a range of other antibiotic growth promoters such as bacitracin, flavophospholipol, pleuromutilins, quinoxalines, virginiamycin and arsenical compounds. Compounds used as antibiotic growth promoters for cattle and poultry include flavophospholipol and virginiamycin. Cattle are also exposed to ionophores such as monensin, while poultry are given arsenical compounds.

Human health can be affected directly through residues of antibiotic in meat, which can cause side-effects. However, the major concern connected to the administration of low levels of antimicrobial drugs to food-producing animals is the potential selection of antibiotic resistance determinants that may spread to a human pathogen (Hamer and Gill, 2002). The analyses of conventional, natural and organic beef detected no antibiotic residues (penicillin, tylosin, erythromycin, tetracycline) in the three product categories (Smith *et al.*, 1997).

Despite the improved carcass conformation and the larger *Longissimus* muscle areas induced by anabolic growth promotants and β -agonists, these compounds can compromise carcass (Roeber *et al.*, 2000) and meat eating quality (Lowman *et al.*, 1991). Although in a recent study Berthiaume *et al.* (2006) did not observe differences in marbling and lean colour between 'natural' beef cattle (i.e. produced without the use of ionophores and hormonal implants) and steers with hormone implants, generally hormonal growth promotants and β -agonists can reduce marbling and increase dark cutting incidence (Roeber *et al.*, 2000). Similar results were obtained by Woodward and Fernandez (1999) using ionophore-treated steer and organic steers. According to Duckett *et al.* (1999), implanting alters intramuscular lipid amount and composition through a dilution effect with the increase in muscle size. In other studies, these treatments induced lower sensory tenderness (Roeber *et al.*, 2000) and higher shear force, possibly associated to lower marbling levels (Roeber *et al.*, 2000; Platter *et al.*, 2003; Smith *et al.*, 2007). Through inhibition of the calpain/calpastatin proteolytic system (Simmons *et al.*, 1997), in fact, β -agonists can increase the shear-force of meat in a dose-related manner by up to threefold (Vestergaard *et al.*, 1994).

17.3.3 Parasite and bacterial contaminations

The growth of the organic food market has been supported by consumers' perception of organic products as safer (Sundrum, 2001). In fact, in organic farms, animals are generally kept in more appropriate environments where animal resistance to infections is promoted and risk of microbial contamination of meat is reduced. However, organic livestock production is not designed to reduce pathogen loads in food animals (Engvall, 2001; Thamsborg, 2001). The limited use of curative and preventive conventional medicines, the ban on antimicrobial

compounds, as well as the outdoor rearing, the incorporation of biological cycles within the farm (use of organic manure within a farm, may carry the risk of recirculating infectious pathogens) and the use of very small slaughtering facilities involve potentially higher microbiological safety risks.

Providing chickens with access to an outdoor area may increase the risk of poultry becoming infected with *Salmonella* and *Campylobacter* due to contact with wild birds and other animals and their faeces. *Campylobacter* is the most common cause of gastroenteritis in the United States (Altekruse *et al.*, 1999), the UK (Frost, 2000) and worldwide. The prevalent route of transmission to humans is the ingestion of raw or undercooked poultry meat (Javid and Ahmed, 2002). Engvall (2001) stated that almost 100% of the organically farmed flocks in Sweden might be infected with *Campylobacter*, compared with only 10% of the conventionally reared flocks. These findings were confirmed in recent Danish and Dutch studies (Heuer *et al.*, 2001; Rodenburg *et al.*, 2004). Organic poultry are at particular risk from *Campylobacter* probably because they are more likely to pick up the pathogen from the environment, than flocks in conventional housing systems (Engvall, 2001; Heuer *et al.*, 2001). A difference was found in *Campylobacter* strains between organic and conventional broiler farms. *C. coli* appeared to be the predominant species on organic farms, whereas in conventional broilers 70% of the strains were *C. jejuni*. However, only 7% of the cases of *Campylobacter*-related illness in humans are caused by the former species (Tam *et al.*, 2003; Rodenburg *et al.*, 2004). The Dutch data on *Campylobacter* are different from the Danish ones, where no difference in strains was found between organic and conventional systems (Heuer *et al.*, 2001).

Prevalence of *Salmonella* in organic poultry systems has been recently investigated in the Netherlands (Rodenburg *et al.*, 2004). The incidence of *Salmonella* infections in organic broilers was 13% in 2003, a percentage similar to that found in conventionally reared broilers. In addition, a high incidence of *Toxoplasma* infections has been reported in free-ranging chickens but no data are available on the presence of *Toxoplasma* infections in organic chickens (Dubey *et al.*, 2004). *Toxoplasma* does not pose a direct health problem to chickens, but is an important food safety issue (Mead *et al.*, 1999).

Outdoor rearing may imply an increased exposure to microbiological agents in pigs. A comparison of the *Salmonella*-seroprevalence in Danish organic, free-range, conventional and breeding pig herds (Wingstrand *et al.*, 1999) showed that the risk of meat juice samples being seropositive was higher for organic and free-range than for conventional herds (Jensen and Baggesen, 2005).

On the other hand, research carried out at Cornell University demonstrated that organic farming of cattle and sheep can reduce the risk of *Escherichia coli* infection. The main source for human infection with *E. coli* is meat contaminated during slaughter. Virulent strains of *E. coli*, such as *E. coli* 0157:H7, seem to develop in the digestive tract of ruminants when they are fed mainly with starchy grain, whereas ruminants fed grass, silage and hay generate less than 1% of the *E. coli* found in the faeces of grain-fed animals (Couzin, 1998).

Outdoor rearing has been related to high rates of endoparasite infections such as

several helminth and ascaris species in organic pigs (Carstensen *et al.*, 2002). Similar results have been documented for sheep, cattle, laying hens, and poultry. The prevalence and intensity of parasitic infections were higher in organically than in conventionally raised animals; helminth species diversity was also much higher (Thamsborg *et al.*, 1999; Permin *et al.*, 1999). In addition, there are parasites which are almost exclusively transmitted outdoors. *Hyoststrongylus rubidus* belong to this category together with parasites with indirect life cycles such as *Metastrongylus* spp., *Ascarops strongylina* and *Physocephalus sexalatus*. In organic pig farms, species with a wide host spectrum, such as *Fasciola hepatica*, *Dicrocoelium dendriticum* and *Trichostrongylus axei*, can be more frequently found (Nansen and Roepstorff, 1999). It has been shown that pigs' fibre-rich diets favor helminth infections, particularly *Oesophagostomum* spp., not only in experimental studies (Petkevicius *et al.*, 1999) but also at farm level (Pearce, 1999). This is an extremely important observation in relation to organic farming, as roughage/forage has to be offered to pigs on a daily basis in most countries.

Even if these parasites do not represent a real risk for human health, because they are destroyed either when the digestive tract is removed or by cooking, their single presence in animals is perceived negatively by consumers (Kouba, 2003).

Comparing organic and conventional carcass quality in terms of pathological effects, Hansson *et al.* (2000) observed that 28% of conventional and 17% of organic pig carcass showed abnormalities, while eosinophilic miositis was more prevalent in organically reared cattle.

17.3.4 Alternative strategies for animal medication

According to the European regulation, animals must be treated, when possible, with alternative remedies, such as homeopathic and phytotherapeutic treatments, rather than conventional veterinary medicines. Organic farming and homeopathy have similar views on health and disease, aiming to create a more balanced environment in and around the animal and to improve animal resistance to infections. The complementary medicine approach is growing in organic livestock farming practice as there are empirical evidences for the efficacy of alternative treatments (Baars *et al.*, 2003). Limited conventional scientific investigation has been conducted on this topic. However, it is important that the research is not simply carried out in conformity with currently valid scientific standards: it has to be also in line with the philosophy of homeopathy. Even less is known on the effect of homeopathic treatment on meat quality.

There is very little literature on the use of herbal veterinary remedies and their possible effects on meat quality. The administration of officinal herbs induced a higher cellular immune response and a lower incidence of puerperium pathologies in buffalo cows (Pacelli *et al.*, 2003), while Bodowski *et al.* (1992) reported the effect of a herbal mixture on muscle composition.

The consumption of forages containing compounds with a high content of condensed tannins (CT), such as *Hedysarum coronarium* and the trefoils (*Lotus* spp.), is able to reduce the impact of intestinal nematodes and nematode larvae in

lambs (Iqbal *et al.*, 2007). In the latter species, CT can also prevent bloated rumen (Waghorn and Jones, 1989). However, high concentrations of CT can have deleterious effects on animal performance (Pritchard *et al.*, 1992). These substances bind with and precipitate proteins in the rumen, reduce protein degradation (McNabb *et al.*, 1996), and reduce the fractional absorption of amino acids reaching the small intestine, resulting in low digestibility and low voluntary intakes (Waghorn *et al.*, 1999). The effects of CT on product quality has been poorly investigated. A lighter colour of meat has been obtained with lambs given CT-rich diets (Priolo *et al.*, 2000). The mechanism of action of is not clear. It is likely that tannins do not affect ruminant Fe absorption while inhibiting the successive utilization of the iron for synthesis, as suggested by Garg *et al.* (1992).

17.3.5 Nutritional properties

From a nutritional point of view, organic meat seems to have healthier properties compared with conventional product. As already mentioned in Section 17.2.2, owing to the frequent grazing, organic animals, and ruminants in particular, often produce a meat with high contents of PUFA, including omega 3 and CLA, that are known to have positive effect on human health (Nielsen and Thamsborg, 2005). In a recent study in the Netherlands (Rist *et al.*, 2007), the content of rumenic acid (the main CLA) and the level of *trans*-vaccenic acid (a precursor of vaccenic acid) in human breast milk increased, significantly moving in-line with a complete consumption of conventional meat to a moderately organic and to a strict organic meat intake. Since the fat from human breast milk is likely to be of dietary origin, the larger amounts of rumenic acid and *trans*-vaccenic acid (TVA) in breast milk from the organic groups may be due to the corresponding intake of organic meat products with higher levels of rumenic acid and *trans*-vaccenic acid. The authors assumed that the levels of CLA and TVA in human milk may be modulated if breastfeeding mothers replace conventional meat products by organic ones.

17.4 Future trends

The global organic meat market will soon face impediments to free trade as a consequence of differences in organic standards between countries. As conventional product tariff barriers are being removed, an effort is needed to make organic standards from different regions more alike in order to promote trade liberalization and allow further development of the organic meat market. However, the main limit to purchasing organic meat remains price because of high production costs, which are affected by organic rules (higher space allowance, origin of feedstuffs, etc.) and small-scale production systems. Two strategies to overcome this constraint have been identified: the spread of organic meat in supermarkets, which is likely to induce a reduction of current price (this approach may be suitable for meat which is organic but is otherwise undifferentiated from conventional products); and the induction of increased willingness to pay by

constant and reliable quality-signaling systems, capable of providing ethical value to the product, which may become even higher if associated with traditional farming systems and typical meat productions. The small-scale production, which often characterizes organic meat enterprises, highlights the need for structures collecting and distributing the organic meat aiming to organize and standardize the supply.

As to product quality, the main constraints to the fulfilment of high standards are dark colour and toughness, both due to the physical exercise of extensively reared animals and to the particular characteristics of local breeds. The most rapid strategies available for improving these aspects would be adequately extended post-mortem ageing and the crossbreeding of cows exceeding replacement needs with bulls of other breeds producing more tender and less dark meat. Research is also looking at protein sources alternative to soya bean in order to minimize the risk of GMO contamination. At least for ruminants raised in the Mediterranean regions, faba bean seems to be a valid substitute.

Increased microbiological and parasitic risks in organic meat are associated with the limited use of conventional medicine, outdoor raising of the animals and incorporation of biological cycles within the farm.

We conclude that, in general, the quality of organic meat may be considered higher than conventional products in terms of fatty acid profile, mineral and antioxidant contents, and risk of chemical and growth promotant residuals. Negative aspects such as toughness and color can be improved using appropriate techniques, while the risk of bacterial and parasitic infections should be carefully monitored, and if possible prevented, keeping the animals in a proper environment, and, when necessary, treated using herbal and/or homeopathic remedies. However, the higher quality of organic meats is related to process characteristics rather than to differences in the end product. Meat safety, animal welfare, biodiversity and environment safeguard are the specific aspects supporting the differentiation of organic meats.

17.5 Sources of further information and advice

Organic standards for meat producing animals

IFOAM provides a worldwide common system of standards, verification and market identity: <http://www.ifoam.org/>.

European regulations on organic agriculture and farming: <http://eur-lex.europa.eu/en/index.htm> and <http://ec.europa.eu/agriculture/qual/organic>. Further information on laws, research and market development at European level can be obtained at <http://www.organic-europe.net/>.

USA organic rules have been issued by USDA within the National Organic Program: <http://www.ams.usda.gov/nop/indexIE.htm>.

Organic meat market

The World of Organic Agriculture 2007 – Statistics and Emerging Trends 2007 – International Federation of Organic Agriculture Movements (IFOAM), Bonn, Germany: <http://www.organic-world.net/default.asp>.

Basic data on organic agriculture in Europe: www.europa.eu.int/comm/eurostat/ and <http://www.organic-market.info>.

Data on North American production and supply of organic products: <http://www.ers.usda.gov/Briefing/Organic> and <http://www.ota.com/index.html>.

FAO (2002), Market developments for organic meat and dairy products: implications for developing countries. Committee on commodity problems, Rome 27–29 August (2002). It is available at: <http://www.fao.org/DOCREP/MEETING/004/Y6976E.HTM>

Quality and safety of organic meat

The proceedings of the four thematic Workshops organized by the NAHWOA (Network for Animal Health and Welfare in Organic Agriculture) are published on: <http://www.veeru.reading.ac.uk/organic/proceedings.htm>.

The proceedings of the four thematic Workshops organised by the SAFO (Sustaining Animal Health and Food Safety in Organic Farming) are published on: <http://www.safonetwork.org/>
FAO (2000), *Food safety and quality as affected by organic farming*. 22nd FAO Regional Conference for Europe, Porto, Portugal, 24–28 July 2000: http://www.fao.org/docrep/meeting/X4983e.htm#P189_32631.

Organic meat and milk from ruminants (2002), EAAP Publication n.106, I. Kyriazakis and G. Zervas (eds), Wageningen Academic Publishers, the Netherlands.

Animal Health and Welfare in Organic Agriculture (2004), M. Vaarst, S. Roderick, V. Lund, W. Lockeretz (eds), Wallingford, UK, CABI Publishing.

Organizations promoting the research on organic production

CORE Organic: <http://www.coreorganic.org/>.

The International Research Association for Organic Food Quality and Health (FQH): www.organicfqhresearch.org.

The International Society of Organic Agriculture Research (ISO FAR): <http://www.isofar.org/>

Institutions involved in research on organic production

Research Institute of Organic Agriculture (FiBL): www.fibl.org.

University of Kassel: <http://www.uni-kassel.de/agrar/>

Danish Research Centre for Organic Farming (DARCOF): <http://www.darcof.dk/>.

Archive of papers related to research in organic agriculture (Organic Eprints): <http://www.orgprints.org/>

17.6 Acknowledgement

We are grateful to the Regione Marche for supporting the program E.Q.U.I.ZOO.BIO, 'Efficienza, Qualità e Innovazione nella Zootecnia Biologica', and thereby the effort of the authors.

17.7 References

- Nielsen A C (2005), *Organic and functional foods have plenty of room to grow according to new ACNielsen global study*. Available at <http://usacnielsen.com/news/>
- Allen P J F, Quirke and P V Tarrant (1987), Effects of cimaterol on the growth, food efficiency and carcass quality of Friesian cattle. In: *b-Agonists and their Effects on Animal Growth and Carcass Quality*, pp 83–89 Elsevier Applied Science, New York.
- Altekruse S F, Stern N J, Fields P I and Swerdlow D L (1999), *Campylobacter jejuni* – an emerging foodborne pathogen, *Emerg. Infect. Dis.*, 5, 28–35.

- Andersen H R and Ingvarsen K L (1984), The influence of energy level weight at slaughter and castration on growth and feed efficiency in cattle, *Livest. Prod. Sci.*, 11, 559–569.
- Andersen H R, Kristensen T, Bliggard H B and Thamsborg S M (2002), *Studeproduktion Ved Afgresning Af Ferske Enge*, Report from DIAS No 40, Danish Institute of Agricultural Science Foulum Denmark 87.
- Baars E, de Bruin A and Ellinger L (2003), Obstacles to homeopathy: Scientific and conceptual; In: E Baars and T Baars (eds) *Desk Study on Homeopathy in Organic Livestock Farming Principles, Obstacles and Recommendations for Practice and Research*, Louis Bolk Institute, 2003, Driebergen, pp 11–24.
- Barton-Gade P A and Blaabjerg L O (1989), Preliminary observations on the behaviour and meat quality of free range pigs, In *35th International Congress of Meat Science and Technology*, 20–25 August, Copenhagen, Denmark, 1002–1005.
- Bennett L L, Hammond A C, Williams M J, Kunkle W E, Johnson D D and Preston R L (1995), Performance carcass yield and carcass quality characteristics of steers finished on rhizome peanut (*Arachis glabrata*) – tropical grass pasture or concentrate, *J. Anim. Sci.*, 1881–1887.
- Berri C, Wacrenier N, Millet N and Le Bihan-Duval E (2001), Effect of selection for improved body composition on muscle and meat characteristics of broilers from experimental and commercial lines, *Poult. Sci.*, 80, 833–838.
- Berthiaume R, Mandell I, Faucitano L and Lafreniere C (2006), Comparison of alternative beef production systems based on forage finishing or grain–forage diets with or without growth promotants: 1 Feedlot performance, carcass quality, and production costs, *J. Anim. Sci.*, 84, 2168–2177.
- Blanchard P J, Ellis M, Warkup C C, Hardy B, Chadwick J P and Deans G A (1999), The influence of rate of lean and fat tissue development on pork eating quality, *Anim. Sci.*, 68, 477–485.
- Bodowski R, Patkowska S B and Szmanko T (1992), Effect of natural biostimulator supplements on meat, performance and profitability of lamb fattening, *Biul. Inf. Przem. Pasz.*, 31 (4), 35–46.
- Braghieri A, Cifuni G F, Girolami A, Riviezzzi A M, Marsico I and Napolitano F (2005), Chemical physical and sensory properties of meat from pure and crossbred Podolian bulls at different ageing times, *Meat Sci.*, 69, 681–689.
- Bredahl L (2004), Cue utilisation and quality perception with regard to branded beef, *Food Qual. Preference*, 15, 65–75.
- Brewer M S and McKeith F K (1999), Consumer related quality characteristics as related to purchase intent of fresh pork, *J. Food Sci.*, 64, 171–174.
- Bridi A M, Mueller L and Ribeiro J A R (1998), Indoor vs out-door-rearing of pigs performance carcass and meat quality In Diestre A and Monfort J M (Eds) *44th International Congress of Meat Sci Techn*, 30 August–4 September, Barcelona, Spain, 1056–1057 (1998).
- Campo M M, Sanudo C, Panea Alberti B P and Santolaria P (1999), Breed type and ageing time effects on sensory characteristics of beef strip loin steaks, *Meat Sci.*, 51, 383–390.
- Caporale G and Monteleone E (2004), Influence of information about manufacturing process on beer acceptability, *Food Qual. Pref.*, 15 (3), 271–278.
- Carpentier A, Latouche K, Meuwissen M P M and Van Des Lans I (2004), Consumer and citizen concerns and willingness to pay for green pork, *Communication au Séminaire Final de Green Piggery*, Février.
- Carstensen L M, Vaarst and Roepstorff A (2002), Helminth infections in Danish organic swine herds, *Vet. Par.*, 106, 253–264.
- Castellini C, Mugnai C and Dal Bosco A (2002a), Effect of organic production system on broiler carcass and meat quality, *Meat Sci.*, 60, 219–225.
- Castellini C, Mugnai C and Dal Bosco A (2002b), Meat quality of three chicken genotypes reared according to the organic system, *Italian J. Food Sci.*, 14 (4), 411–412.
- Combes S, Lebas F, Lebretton L, Martin T, Jehl N, Cauquil L, Darche B and Corboeuf M A

- (2003a), Comparison between organic and standard rabbit breeding system: Carcass traits and chemical composition of 6 muscles from the leg In: Itavi (Ed) *Proc. 10th Journ. Rech. Cunicole*, 133–136.
- Combes S, Lebas F, Juin H, Lebreton L, Martin T, Jehl N, Cauquil L, Darche B and Corboeuf M A (2003b), Comparison between organic and standard breeding system: Sensory analysis and mechanical toughness of meat In: Itavi (Ed) *Proc. 10th Journ. Rech. Cunicole*, 137–140.
- Couzin J (1998), Cattle Diet Linked to Bacterial Growth, *Science*, 281, 1578–1579.
- Danielsen V, Hansen LL, Moller F, Bejerholm C and Nielsen S (2000), Production results and sensory meat quality of pigs fed different amounts of concentrate ad lib Clover grass or clover grass silage. In: *Ecological Animal Husbandry in the Nordic Countries*, NJF seminar No 303, 79–86 16–17 September, Horsens Denmark.
- Descalzo A M, Insani E M, Biolatto A, Sancho A M, Garcia P T and Pensel N A (2005), Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef, *Meat Sci.*, 70 (1), 35–44.
- Descalzo A M, Rossetti L, Grigioni G, Irurueta M, Sancho A M, Carrete J and Pensel N A (2007), Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle, *Meat Sci.*, 75, 299–307.
- Devanesan P, Santen R J, Bocchinfuso W P, Korach R S, Rogan E G and Cavalieri E (2001), Catechol estrogen metabolites and conjugates in mammary tumors and hyperplastic tissue from estrogen receptor-alpha knock-out (ERKO)/Wnt-1 mice: Implications for initiation of mammary tumors, *Carcinogenesis*, 22, 1573–1576.
- Dransfield E and Sosnicki A A (1999), Relationship between muscle growth and poultry meat quality, *Poultry Sci.*, 78, 743.
- Dransfield E, Ngapo T M, Nielsen N A, Bredahl L, Sjoden P O, Magnusson M, Campo M M and Nute G R (2005), Consumer choice and suggested price for pork as influenced by its appearance, taste and information concerning country of origin and organic pig production, *Meat Sci.*, 69, 61–70.
- Dubey J P, Salant H, Sreekumar C, Dahl E, Vianna M C, Shen S K, Kwok O C, Spira D, Hamburger J and Lehmann T V (2004), High prevalence of *Toxoplasma gondii* in a commercial flock of chickens in Israel, and public health implications of free-range farming, *Vet. Par.*, 121, 317–322.
- Duckett S K, Wagner D G, Owens F N H, Dolezal G and Gill D R (1999), Effects of anabolic implants on beef intramuscular lipid content, *J. Anim. Sci.*, 77, 1100–1104.
- Dufey PA (1995), Fleisch und Fettqualität bei Schweinemast mit Weidegang, *Agrarforschung*, 2 (10), 453–456.
- Dworschak E, Barna E, Gergely A, Czuczy P, Hovari J, Kontraszti M, Gaal O, Radnoti L, Biro G and Kaltenecker J (1995), Comparison of some components of pigs kept in natural (free-range) and large-scale condition, *Meat Sci.*, 39, 79–86.
- Elmore J S, Mottram D S, Enser M and Wood J D (1999), Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles, *J. Agric. Food Chem.*, 47, 1619–1625.
- Enfält A C, Lundstrom K, Hansson I, Karlsson A, Essen-Gustavsson B and Hakansson J (1993), Moderate indoor exercise: Effect on production and carcass traits, muscle enzyme activities and meat quality in pigs, *Anim. Prod.*, 57, 127–135.
- Enfält A C, Lundstrom K, Hansson I, Lundeheim N and Nystrom P E (1997), Effects of outdoor rearing and sire breed (Duroc or Yorkshire) on carcass composition and sensory and technological meat quality, *Meat Sci.*, 45, 1–15.
- Engvall A (2001), May organically farmed animals pose a risk for *Campylobacter* infections in humans? *Acta Vet. Scand., Suppl.*, 95, 85–87.
- Enser M, Hallet K G, Hewett B, Fursey G A J, Wood J D and Harrington G (1998), Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition, *Meat Sci.*, 49, 329–341.
- Essen-Gustavsson B, Karlsson A, Lundstrom K and Enfält A C (1994), Intramuscular fat and

- muscle fibre lipid contents in halothane-gene-free pigs fed high or low protein diets and its relation to meat quality, *Meat Sci.*, 38, 269–277.
- Essen-Gustavsson B, Lundstrom K, Larsson G, Lindholm A, Nordin A C, Hansson I and Tornberg E (1988), The effect during growth of moderate exercise on muscle metabolic characteristics in vivo and relation to meat quality and sensory properties. In Chandler C S and Thornton R F (Eds), *34th International Congress of Meat Science and Technology*, 27–30 29 August–2 September, Brisbane Australia, (1988).
- European Commission (2005), *Report on Organic Farming in the European Union – Facts and Figures*. Bruxelles Belgium, Available at <http://ec.europa.eu/agriculture/qual/organic/index.htm>
- European Communities (1991), Council Regulation (EEC) No 2092/1991, Regulation on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs. *Off. J. Eur. Communities*, L 198, 1–15.
- European Communities (1999), Council Regulation (EC) No 1804/1999, Supplementing Regulation (EEC) No 2092/91 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs to include livestock production. *Off. J. Eur. Communities*, L 222, 1–28.
- European Communities (2007), Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. *Off. J. Eur. Communities*, L 189, 1–23.
- Fanatico A C, Cavitt L C, Pillai P B, Emmert J L and Owens C M (2005), Evaluation of slower-growing broiler genotypes grown with and without outdoor access: Meat quality, *Poultry Sci.*, 84, 1785–1790.
- Fanatico A, Pillai P, Emmert J, Meullenet J and Owens C M (2006), Impact of alternative boiler genotype and production system on sensory attributes, *Proc. of 95th Annual Meeting of Poultry Science*, Association July 16–19 Univ Alberta-Edmonton, Canada S-P172, 198 (2006).
- FAO (2001), *Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods*, GL 32-1999, rev 1–2001.
- FAO (2002), *Market developments for organic meat and dairy products: implications for developing countries*. Committee on commodity problems, Rome 27–29 August (2002) Available at <http://www.fao.org/>
- Farmer L J, Perry G C, Lewis P D, Nute G R, Piggott J R and Patterson R L S (1997), Responses of two genotypes of chicken to the diets and stocking densities of conventional UK and Label Rouge production systems. II. Sensory attributes, *Meat Sci.*, 47, 77.
- Fernandez X (1991), A review of the causes of variation in muscle glycogen content and ultimate pH in pigs, *J. Muscle Foods*, 2, 209–235.
- Fernandez X, Monin G, Talmant A, Mourot J and Lebret B (1999a), Influence of intramuscular fat content on the quality of pig meat. 1. Composition of the lipid fraction and sensory characteristics of *M longissimus lumborum*, *Meat Sci.*, 53, 59–65.
- Fernandez X, Monin G, Talmant A, Mourot J and Lebret B (1999b), Influence of intramuscular fat content on the quality of pig meat. 2. Consumer acceptability of *m longissimus lumborum*, *Meat Sci.*, 53, 67–72.
- Fletcher D L (2002), Poultry meat quality World's, *Poultry Sci. J.*, 58, 131–145.
- French P, Stanton C, Lawless F, O'Riordan E G, Monahan F J, Carey P J and Moloney A P (2000), Fatty acid composition including conjugated linoleic acid of intramuscular fat from steers on grazed grass, grass silage or concentrate-based diets, *J. Anim. Sci.*, 78, 2849–2855.
- French P, O'Riordan E G, Monahan F J, Caffrey P J, Mooney M T, Troy D J and Moloney A P (2001), The eating quality of meat of steers fed grass and/or concentrates, *Meat Sci.*, 57 (4), 379–386.
- Frost J A (2000), *Campylobacter* in humans and sources of infection. Abstract in conference proceeding: *Zoonotic infections in livestock and the risk to public health*, Anon (2000). Available at <http://www.defragovuk/animalh/diseases/zoonoses/conference/bookabsPDF>

- Fukozawa K and Fuji T (1992), Peroxide dependent and independent lipid peroxidation site-specific mechanism of initiation by chelated iron inhibition by α -tocopherol, *Lipids*, 27, 227–233.
- Garg S K, Makkar H P S, Nagal K B, Sharma S K, Wadhwa D R and Singh B (1992) Oak (*Quercus incana*) leaf poisoning in cattle, *Vet. Human Toxicol.*, 34, 161–164.
- Ghidini S, Zanardi E, Battaglia A, Varisco G, Ferretti E, Campanini G and Chizzolini R (2005), Comparison of contaminant and residue levels in organic and conventional milk and meat products from Northern Italy, *Food Addit. Contam.*, 22, 9–14.
- Gil J M, Gracia A and Sanchez M (2000), Market segmentation and willingness to pay for organic products in Spain, *International Food and Agribusiness Management Review*, 3, 207–226.
- Gordon S H and Charles D R (2002), *Niche and organic chicken products*, Nottingham University Press, Nottingham UK.
- Griebenow R L, Martz F A and Morrow R E (1997), Forage-based beef finishing systems: A review, *J. Prod. Agric.*, 10 (1), 84–91.
- Grunert K G, Bredahl L and Brunsø K (2004), Consumer perception of meat quality and implications for product development in the meat sector – A review *Meat Sci.*, 66(2), 259–272.
- Hageleit M, Daxenberger A, Kraetzl W-D, Kettler A and Meyer H H D (2000), Dose-dependent effects of melengestrol acetate (MGA) on plasma levels of estradiol, progesterone and luteinizing hormone in cycling heifers and influences on oestrogen residues in edible tissues, *Acta Pathologica, Microbiologica et Immunologica Scandinavica*, 108, 847–854.
- Hamer D H and Gill C J (2002), From the farm to the kitchen table: The negative impact of antimicrobial use in animals on humans, *Nutr. Rev.*, 60, 261–264.
- Hamm U, Gironefeld F and Halpin D (2002), *Analysis of the European Market for Organic Food: Organic Marketing Initiatives and Rural Development*, (Vol 1) School of Management and Business University of Wales, Aberystwyth, Wales, UK.
- Hansen L L, Claudi-Magnussen C, Jensen S K and Andersen H J (2006), Effect of organic pig production systems on performance and meat quality, *Meat Sci.*, 74, 605–615.
- Hansson I, Hamilton C, Ekman T and Forslund K (2000), Carcass quality in certified organic production compared with conventional livestock production, *J. Vet. Med.*, 47, 111–120.
- Henckel P, Karlsson A, Oksbjerg N and Soholm Petersen J (2000), Control of post mortem pH decrease in pig muscles: Experimental design and testing of animal models, *Meat Sci.*, 55 (1), 131–138.
- Heuer O E, Pedersen K, Andersen J S and Madsen M (2001), Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks, *Lett. Appl. Microbiol.*, 33 (4), 269–274.
- Hodgson R R, Davis G W, Smith G C, Savell J W and Cross H R (1991), Relationship between pork loin palatability traits and physical characteristics of cooked chops, *J. Anim. Sci.*, 69, 4858–4865.
- Hoffmann G (1995), Sport medical aspects of iron metabolism, *J. Inorganic Biochem.*, 59, 237.
- Hogberg A, Pickova J, Babol J, Andersson K and Dutta P C (2002), Muscle lipids vitamins E and A and lipid oxidation as affected by diet and RN genotype in female and castrated male Hampshire crossbreed pigs, *Meat Sci.*, 60 (4), 411–420.
- IFOAM (2002), *IFOAM Basic Standard*, International Federation of Organic Agriculture Movements, Tholet-Theley, Germany.
- Iqbal Z, Sarwar M, Jabbar A, Ahmed S, Nisa M, Sajid M S, Khan M N, Mufti K A and Yaseen M (2007), Direct and indirect anthelmintic effects of condensed tannins in sheep, *Vet. Par.*, 144, 125–131.
- Jarrige R and Auriol P (1992), An outline of world beef production. In: Jarrige R and Beranger C (Eds) *World Animal Science, Beef Cattle Production*, Elsevier, Amsterdam pp 3–30.

- Javid M and Ahmed S (2002), *Campylobacter* infections. Available at <http://www.wemedicinem.com/med/topic263.htm>
- Jensen A N and Baggesen D B (2005), *Salmonella* infection risk associated with outdoor organic pig production. *Proceedings of the 4th SAFO Workshop, Systems Development: Quality and Safety in Organic Livestock Products*, Frick, Switzerland, 87.
- Joint Expert Advisory Committee on Antibiotic Resistance (1999), *Report of the Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR) on the use of antibiotics in food producing animals: Antibiotic resistant bacteria in animals and humans*. Available at <http://www.health.gov.au/pubs/jetacarpdf> Last accessed 28 October 2001.
- Kerth C R, Braden K W, Cox R, Kerth L K and Rankins D L J (2007), Carcass sensory fat color and consumer acceptance characteristics of Angus-cross steers finished on ryegrass (*Lolium multiflorum*) forage or on a high-concentrate diet, *Meat Sci.*, 75, 324–331.
- Kouba M (2003), Quality of organic animal products, *Liv. Prod. Sci.*, 80 (2003) 33–40.
- Lange C, Martin C, Chabanet C, Combris P and Issanchou S (2002), Impact of the information provided to consumers on their willingness to pay for Champagne: Comparison with hedonic scores, *Food Qual. Pref.*, 13, 597–608.
- Lauridsen C, Nielsen J H, Henckel P and Sorensen M T (1999), Antioxidative and oxidative status in muscles of pigs fed rapeseed oil, vitamin E and copper, *J. Anim. Sci.*, 77 (1), 105–115.
- Lawlor J B, Sheehan E M, Delahunty P A, Morrissey C M and Kerry J P (2003), Oxidative stability of cooked chicken breast burgers obtained from organic free-range and conventionally reared animals, *International J. Poultry Sci.*, 2 (6), 398–403.
- Le Bihan-Duval E, Millet N and Remignon H (1999), Broiler meat quality: Effect of selection for increased carcass quality and estimates of genetic parameters, *Poultry Sci.*, 78, 822–826.
- Lebret B, Couvreur S, Dourmad J Y, Meunier-Salaun M C, Guingand N, Robin P, Hassouna M and Cariolet R (2004), Influence of husbandry method on animal welfare and meat quality traits in pigs, *J. Rech. Porcine*, 36, 53–62.
- Lewis P D, Perry G C, Farmer L J and Patterson R L S (1997), Responses of Two Genotypes of Chicken to the Diets and Stocking Densities Typical of UK and 'Label Rouge' Production Systems. I. Performance Behaviour and Carcass Composition, *Meat Sci.*, 45 (4), 501–516.
- Loneragan S M, Deeb N, Fedlet C A and Lamont S J (2003), Breast meat quality and composition in unique chicken population, *Poultry Sci.*, 82, 1990–1994.
- Lowman B G, Lewis M L, Neilson D R, Scott N A and Hunter E A (1991), Complementary influences of exogenous hormone implantation, antibiotic feed addition and supplementary undegradable dietary protein upon growth, feed intake and carcass characteristics of finishing beef cattle, *Livest. Prod. Sci.*, 28, 37–52.
- Magkos F, Arvaniti F and Zampelas A (2006), Organic Food: Buying More Safety or Just Peace of Mind? A Critical Review of the Literature, *Critical Reviews in Food Science and Nutrition*, 46, 23–56.
- Marino R, Albenzio M, Girolami A, Muscio A, Sevi A and Braghieri A (2006), Effect of forage to concentrate ratio on growth performance and on carcass and meat quality of Podolian young bulls meat quality, *Meat Sci.*, 38, 269–277.
- McCarthy M, de Boer M, O'Reilly S and Cotter L (2003), Factors influencing intention to purchase beef in the Irish market, *Meat Sci.*, 65(3), 1071–1083.
- McInerney J (2004), *Animal welfare, economics and policy. Report on a study undertaken for the Farm and Animal Health Economics Division of Defra* Available at <http://statistics.defra.gov.uk/esg/reports/animalwelfarepdf>
- McNabb W C, Waghorn G C, Peters J S and Barry T N (1996), The effect of condensed tannins in *Lotus pedunculatus* on the solubilization and degradation of ribulose-1,5-bisphosphate carboxylase (EC 41139; rubisco) protein in the rumen and the sites of rubisco digestion, *Br. J. Nutr.*, 76:535–549.
- Mead P S, Slutsker L and Dietz V (1999), Food-related illness and death in the United States, *Emerg. Infect. Dis.*, 5, 607–625.

- Melton S L (1990), Effects of feeds on flavour of red meat: A review, *Journal Anim. Sci.*, 68, 4421–4435.
- Millet S, Hesta M, Seynaeve M, Ongenae E, De Smet S, Debraekeleer J and Janssens G P J (2004), Performance meat and carcass traits of fattening pigs with organic versus conventional housing and nutrition, *Liv. Prod. Sci.*, 87, 109–119.
- Millet S, Raes K, Van den Broeck W, De Smet S and Janssens G P J (2005), Performance and meat quality of organically versus conventionally fed and housed pigs from weaning till slaughtering, *Meat Sci.*, 69, 335–341.
- Millet S, Ongenae E, Hesta M, Seynaeve M, De Smet S and Janssens G P J (2006), The feeding of ad libitum dietary protein to organic growing–finishing pigs, *The Vet. J.*, 171, 483–490.
- Morgan J B (1997), Implant program effects on USDA beef carcass quality grade traits and meat tenderness. In: *Proc Oklahoma State University Implant Symposium*, Stillwater, p 147.
- Muir P D, Wallace G J, Dobbie P M and Bown M D (2000), A comparison of animal performance and carcass and meat quality characteristics in Hereford Hereford × Friesian and Friesian steers grazed together at pasture, *New Zeal. J. Agr. Res.*, 43, 193–205.
- Nansen P and Roepstorff A (1999), Parasitic helminths of the pig: factors influencing transmission and infection levels, *Intern. J. Par.*, 29, 877–891.
- Nielsen B and Thamsborg S M (2001), Organic beef production with emphasis on feeding and health of dairy bred bull calves. Proc. of 4th NAHWOA Workshop, Wageningen, 24–27 March, Ed M Hovi and T Baars 155–168.
- Nielsen B and Thamsborg S M (2002), Dairybull calves as a resource for organic beef production: A farm survey in Denmark, *Liv. Prod. Sci.*, 75 (3), 245–255.
- Nielsen B K and Thamsborg S M (2005), Welfare health and product quality in organic beef production: A Danish perspective, *Liv. Prod. Sci.*, 94, 41–50.
- Nilzen E, Babol J, Dutta P C, Lundeheim N, Enfalt A C and Lundstrom K (2001), Free range rearing of pigs with access to pasture grazing. Effect on fatty acid composition and lipid oxidation products, *Meat Sci.*, 58, 267–275.
- Nuernberg K, Dannenberger D, Nuernberg G, Ender K, Voigt J, Scollan N D, Wood J D, Nute G R and Richardson R I (2005), Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of *longissimus* muscle in different cattle breeds, *Liv. Prod. Sci.*, 94, 137–147.
- Nutrition Business Journal (2006), US Organic Food Sales (\$mil) (1997–2010) e–Chart 22 Penton Media Inc USA.
- Oberholtzer L, Greene C and Lopez E (2006), *Organic Poultry and Eggs Capture High Price Premiums and Share of Specialty Markets*. USDA Outlook Report No LDPM-15001, Available at <http://www.usdagov/Publications/>
- Oksbjerg N, Strudsholm K, Gunilla L, Gunilla J and Hermansen E J (2005), Meat quality of fully or partly outdoor reared pigs in organic production, *Agric. Scand. Sect. A*, 55, 106–112.
- Oliver M A, Gou P, Gispert M, Diestre A, Arnau J L and Blasco A (1994), Comparison of five types of pig crosses. II. Fresh meat quality and sensory characteristics of dry cured ham, *Liv. Prod. Sci.*, 40, 179.
- Olsson V, Andersson K, Hansson I and Lundstrom K (2003), Differences in meat quality between organically and conventionally produced pigs, *Meat Sci.*, 64, 287–297.
- Oltjen R R, Rumsey T S and Putman P A (1971), All-forage diets for finishing beef steers, *J. Anim. Sci.*, 46, 716–724.
- Organic Monitor (2006), *The North American Market for Organic Meat Products*. Organic Monitor Ltd. Available at <http://www.mindbranch.com/>
- O’Sullivan A, Galvin K, Moloney A P, Troy D J, O’Sullivan K and Kerry J P (2003), Effect of pre-slaughter ration of forage and/or concentrates on the composition and quality of retail packaged beef, *Meat Sci.*, 63 (3), 279–286.

- Pacelli C, Braghieri A, Surianello F, Marsico I, Napolitano F and Girolami A (2003), Impiego di integratori alimentari a base di erbe officinali nella specie bufalina, *Atti 2° Congresso Nazionale sull'Allevamento del Bufalo*, 28–30 Agosto Monterotondo (Roma), 43–47.
- Pariza M W, Park Y and Cook M E (2001), The biological active isomers of conjugated linoleic acid, *Progress in Lipid Res.*, 40, 283–298.
- Pearce G P (1999), Interactions between dietary fibre, endo-parasites and *Lawsonia intracellularis* bacteria in grower-finisher pigs, *Vet. Par.*, 87, 51–61.
- Permin A M, Bisgaard F, Frandsen M, Pearman J, Kold and Nansen P (1999), Prevalence of gastrointestinal helminths in different poultry production systems, *Br. Poultry Sci.*, 40, 439–443.
- Petersen J S, Berge P, Henckel P and Sorensen M T (1997), Collagen characteristics and meat texture of pigs exposed to different levels of physical activity, *J. Muscle Foods*, 8, 47–61.
- Petersen J S, Henckel P, Oksbjerg N and Sorensen M T (1998), Adaptations in muscle fibre characteristics induced by physical activity in pigs, *Anim. Sci.*, 66, 733–740.
- Petkevicius S, Nansen P, Knudsen K E B and Skjoth F (1999), The effect of increasing levels of insoluble dietary fibre on the establishment and persistence of *Oesophagostomum dentatum* in pigs, *Parasite*, 6, 17–26.
- Pla M, Hernandez P, Arino B, Ramirez J A and Diaz I (2007), Prediction of fatty acid content in rabbit meat and discrimination between conventional and organic production systems by NIRS, *Method Food Chem.*, 100, 165–170.
- Platter W J, Tatum J D, Belk K E, Scanga J A and Smith G C (2003), Effects of repetitive use of hormonal implants on beef carcass quality, tenderness, and consumer ratings of beef palatability, *J. Anim. Sci.*, 81, 984–996.
- Priolo A, Waghorn G C, Lanza M, Biondi L and Pennisi P (2000), Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality, *J. Anim. Sci.*, 78, 810–816.
- Priolo A, Micol D and Agabriel J (2001), Effects of grass feeding systems on ruminant meat colour and flavour. A review, *Anim. Res.*, 50, 185–200.
- Pritchard D A, Martin P R and O'Rourke P K (1992), The role of condensed tannins in the nutritional value of mulga (*Acacia aneura*) for sheep, *Aust. J. Agric. Res.*, 42, 1739–1746.
- Ragni M, Cocca C, Di Turi L and Vicenti A (2006), Aspetti qualitativi della carne di vitelli Podolici allevati con differenti regimi alimentari, *Speciale Taurus*, Anno XVIII (7), Novembre/Dicembre.
- Ragni M, Vincenti F, Totoda F, Di Turi L, Facciolongo A and Lacitignola M (2007), Use of sweet lupin (*Lupinus albus* L) as alternative protein source in diets for podolian young bulls, *Ital. J. Anim. Sci.*, 6 (1).
- Realini C E, Duckett S K and Windham W R (2004), Effect of vitamin C addition to ground beef from grass-fed or grain-fed sources on colour and lipid stability and prediction of fatty acid composition by near-infrared reflectance analysis, *Meat Sci.*, 68, 35–43.
- Richardson R I and Mead G C (1999), *Poultry Meat Science*, Poultry Sci Symp Series, Vol 25, CABI Publishing, NY, USA.
- Rist L, Mueller A, Barthel C, Snijders B, Jansen M, Simoes-Wust M P A, Huber M, Kummeling I, von Mandachi U, Steinhart H and Thijs C (2007), Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands, *Br. J. Nutr.*, (2007), 97, 735–743.
- Rizzi C, Marangon A and Chiericato G M (2007), Effect of genotype on slaughtering performance and meat physical and sensory characteristics of organic laying hens, *Poultry Sci.*, 86, 128–135.
- Rodenburg T B, Van Der Hulst-Van Arkel M C and Kwakkel R P (2004), *Campylobacter* and *Salmonella* infections on organic broiler farms NJAS – Wageningen, *J. Life Sci.*, 52, 101–108.
- Roeber D L, Cannell R C, Belk K E, Miller R K, Tatum J D and Smith G C (2000), Implant

- strategies during feeding: Impact on carcass grades and consumer acceptability, *J. Anim. Sci.*, 78, 1867–1874.
- Santos S J, Bessa R J B and Santos S F (2002), Effect of genotype feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat, *Livest. Prod. Sci.*, 77 (2–3), 187–194.
- Sather A P, Jones S D M, Schaefer A L, Colyn J and Robertson W M (1997), Feedlot performance carcass composition and meat quality of free range reared pigs, *Canadian J. Anim. Sci.*, 77 (2), 225–232.
- Schafer A, Rosenvold K, Purslow P P, Andersen H J and Henckel P (2002), Physiological and structural events post mortem of importance for drip loss in pork, *Meat Sci.*, 61 (4), 355–366.
- Schmid O (2000), Comparison of European Organic Livestock Standards with national and international standards – problem of common standards development and future areas of interest. In: Hovi M and Trujillo R G (eds) *Diversity of Livestock Systems and Definition of Animal Welfare. Proceedings of the Second NAHWOA Workshop*, Cordoba, 8–11 January, University of Reading, Reading, UK.
- Shorthose R W and Harris P V (1991), Effects of growth and composition on meat quality. In A M Pearson and T R Dutson (Eds) *Advances in Meat Research*, London UK, 7th ed 515.
- Simmons N J, Young O A, Dobbie P M, Singh K, Thompson B C and Speck P A (1997), Post-mortem Calpain-system Kinetics in Lambs: Effects of Clenbuterol and Preslaughter Exercise, *Meat Sci.*, 47, 135–146.
- Smith G C, Heaton K L, Sofos J N, Tatum J D, Aaronson M J and Clayton R P (1997), Residues of Antibiotics, Hormones and Pesticides in Conventional, Natural and Organic Beef, *J. Muscle Foods*, 8, 157–172.
- Smith K R, Duckett S K, Azain M J, Sonon Jr R N and Pringle T D (2007), The effect of anabolic implants on intramuscular lipid deposition in finished beef cattle, *J. Anim. Sci.*, 85, 430–440.
- Sodo A, Napolitano F, Girolami A, Pacelli C, Piazzolla N and Braghieri A (2007), Impiego del favino per la produzione di carne bovina biologica: Colore e potere di ritenzione idrica, 3° Workshop GRAB-IT 22 maggio, Roma, *Sostenibilità e Qualità delle Produzioni Agricole Biologiche*.
- Steen R W J and Kilpatrick D J (1995), Effects of plane of nutrition and slaughter weight on the carcass composition of serially slaughtered bulls, steers and heifers of three breed crosses, *Livest. Prod. Sci.*, 43, 205–213.
- Sundrum A (2001), Organic livestock farming. A critical review, *Livest. Prod. Sci.*, 67, 207–215.
- Sundrum A, Butfering L, Henning M and Hoppenbrock K H (2000), Effects of on-farm diets for organic pig production on performance and carcass quality, *J. Anim. Sci.*, 78, 1199–1205.
- Tam C C, Rodrigues L C and O'Brien, S J (2003) Guillain–Barré syndrome associated with *Campylobacter jejuni* infection in England, 2000–2001 *Cl. Infectious Dis.*, 37(2), 307–310.
- Temisan V (1989), Bulls steers or heifers – Which should be fattened in future? *Tierzuchter*, 41 (7), 286–289.
- Thamsborg S M (2001), Organic farming in the Nordic countries: Animal health and production. In: *Veterinary challenges in Organic Farming. Proc 14th Nordic Committee for Veterinary Scientific Cooperation Symposium, Acta Vet. Scand.*, suppl, 95, 7–15.
- Thamsborg S M, Roepstorff A and Larsen M (1999), Integrated and biological control of parasites in organic and conventional production systems, *Vet. Par.*, 84, 169–186.
- Therkildsen M, Vestergaard M, Ramsgaard Jensen L and Andersen H R (1995), Betydningen af fodringsintensitet, afgræsning og slutfodning på produktion og slagtekvantitet af SDM ungtyre, *Forskningsrapport Nr. 35, Statens Husdyrbrugsforsøg*. Frederiksberg, 36.
- Therkildsen M R B, Karlsson A, Erthbjerg P and Purslow P P (2002), Compensatory growth

- response in pigs' muscle protein turnover and meat texture: Effects of restriction/realimentation period, *Anim Sci.*, 75, 367–377.
- Van der Wal P G, Mateman G, de Vries A W, Vonder G M A, Smulders F J M, Geesink G H and Engel B (1993), 'Scharrel' (free range) pigs: Carcass composition meat quality and taste-panel studies, *Meat Sci.*, 34, 27–37.
- Verbeke W and Viaene J (1999), Beliefs, attitude and behaviour towards fresh meat consumption in Belgium: Empirical evidence from a consumer survey, *Food Qual. Pref.*, 10(6), 437–445.
- Verhoog H, Lund V and Alroe H F (2004), Animal Welfare, Ethics and Organic Farming. In: M Vaarst, Roderick S, Lund V and Lockeretz W (Eds) *Animal Health and Welfare in Organic Agriculture*, Wallingford, UK, CABI Publishing, pp 73–94.
- Vestergaard M, Henckel P, Oksbjerg N and Sejrsen K (1994), The effect of cimaterol on muscle fiber characteristics, capillary supply, and metabolic potentials of *longissimus* and *semitendinosus* muscles from young Friesian bulls, *J. Anim. Sci.*, 72, 2298–2306.
- Vestergaard M, Oksbjerg N and Henckel P (2000a), Influence of feeding intensity grazing and finishing feeding on muscle fibre characteristics and meat colour of *semitendinosus* long *dorsi* and *supraspinatus* muscles of young bulls, *Meat Sci.*, 54, 177–185.
- Vestergaard M, Therkildsen M, Henckel P, Jensen L R, Andersen H R and Sejrsen K (2000b), Influence of feeding level grazing, and finishing feeding on meat and eating quality of young bulls and the relationship between muscle fibre characteristics, fibre fragmentation and meat tenderness, *Meat Sci.*, 54, 187–195.
- Waghorn G C and Jones W T (1989), Bloat in cattle, 46: Potential of dock (*Rumex obtusifolius*) as an antibiotic agent for cattle, *N. Z. J. Agric. Res.*, 32, 227–235.
- Waghorn G C, Reed J D and Ndlovu L R (1999), Condensed tannins and herbivore nutrition. In: *Proceedings of the 18th International Grassland Congress*, Vol III, Buchanan-Smith J G, Bailey L D and McCaughy P (Eds), Saskatchewan (Canada), 8–19 June 1997, Association Management Centre, Calgary, AB, Canada, pp 153–166.
- Walsh B E, Sheehan E M, Delahunty C M, Morrissey P A and Kerry J P (2006), Composition, sensory and shelf life stability analyses of *Longissimus dorsi* muscle from steers reared under organic and conventional production systems, *Meat Sci.*, 73, 319–325.
- Warriss P D, Kestin S C and Robinson J M (1983), A note on the influence of rearing environment on meat quality in pigs, *Meat Sci.*, 9, 271–279.
- Willer H and Yussefi M (2004), *The World of Organic Agriculture Statistics and Emerging Trends*, International Federation of Organic Agriculture Movements, Bonn, Germany.
- Wingstrand A, Dahl J and Lo Fo Wong D M A (1999), *Salmonella* – Prevalences in Danish Organic, Free-range, Conventional and Breeding Herds. In: *Proceedings of the 3rd International Symposium on the Epidemiology and Control of Salmonella in Pork*, p 186–189 August 5–7, 1999, Washington DC, USA.
- Wood J D, Enser M, Whittington F M, Moncrieff C B and Kempster A J (1989), Backfat composition in pigs: Differences between fat thickness and sexes, *Liv. Prod. Sci.*, 22, 351–362.
- Wood J D, Nute G R, Richardson R I, Whittington F M, Southwood O, Plastow G, Mansbridge R, da Costa N and Chang K C (2004), Effects of breed diet and muscle on fat deposition and eating quality in pigs, *Meat Sci.*, 67, 651–667.
- Woodward B W and Fernandez M I (1999), Comparison of conventional and organic beef production systems. II. Carcass characteristics, *Liv. Prod. Sci.*, 61, 225–231.
- Worthington V (1998), Effect of agricultural methods on nutritional quality: A comparison of organic with conventional crops, *Altern. Ther. Health Med.*, 4, 58–69.
- Yang A, Lanari M C, Brewster M and Tume R K (2002), Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement, *Meat Sci.*, 60, 41–50.
- Zullo A, Barone C M A, Colatruccio P, Girolami A and Matassino D (2003), Chemical composition of pig meat from the genetic type 'Casertana' and its crossbreeds, *Meat Sci.*, 63, 89–100.

Improving the quality of meat from ratites

K. W. McMillin, Louisiana State University Agricultural Center, USA and L. C. Hoffman, Stellenbosch University, South Africa

Abstract: An overview of the ratite industries and use of these birds for meat, with emphasis on ostrich, introduces this topic. Growth traits, carcass yields, and meat composition of the ostrich, rhea, and emu as the major ratite species are discussed. Diet, breed, transport, lairage, and slaughter practices influence pH decline and rigor mortis and may alter lipid components, fiber types, color, tenderness, juiciness, and flavor of ratite meat. Fiber diameter, sarcomere length, and collagen content are not highly related to tenderness of ostrich meat, which is variable with animal age, muscle, and aging period enzyme activity. The chemical and nutritional profiles of meat from ratites are altered by diet, breed, bird age, and muscle while microbiological profiles of ratite meat are more affected by sanitary practices and packaging. Value-added products such as mince, salami, jerky, ham, sausages, and cured meat products can be successfully manufactured from ostrich meat. While it is unlikely that the emu and rhea industries will develop into major meat-producing entities, the future of the well-established ostrich meat industry may depend upon control of the contagious avian diseases. Technological developments for slaughter and processing have potential for success in the ratite meat industries, with optimization of live bird production and meat harvest and processing desirable for highest meat quality and shelf-life.

Key words: ostrich, emu, rhea, meat quality, composition, value-added.

18.1 Introduction

Ratites are flightless, cursorial birds that lack a keel on the sternum and have no interlining structure of feathers. The ratites are ostrich, emu, rhea, cassowary and kiwi. Cassowaries are endangered natives of New Guinea and kiwis, which are not avian herbivores (Deeming and Angel, 1996), are endemic and endangered in New Zealand (Sales, 2006). Ratite meat is evaluated similarly for other red meat species through the determination of carcass traits, physical and chemical properties and

palatability traits. Meat production for ratites is primarily under controlled farming and management schemes so the same factors of diet, age, sex, handling, stress, slaughter practices, post-mortem ageing and processing that influence the properties of meat from domesticated species also can influence ratite meat. The size and economic impact of ostrich industries in South Africa and Australia and a few other countries compared with the size and impact of the rhea and emu industries has generated more information on ostrich meat compared with the other two ratite meat species. There is no indication that kiwi are commercially harvested for meat.

The chapter will briefly outline the current status of the ostrich industry and then give details on the influences of production, harvest, and processing on ratite meat chemical, physical, sensory and nutritional properties.

18.2 Ratite meat industries

A majority of the ostrich meat is produced in South Africa, which contributes up to 70% (950 000 tons per annum) of the total ostrich meat produced worldwide. Most ostrich producers use the variety, *Struthio camelus* var. *domesticus*, because it is the more common ostrich found in South Africa (Madeiros, 1995). Currently, the feathers, leather and the meat each contribute 5%, 50% and 45% to the income of ostrich production, but the proportional value of the meat relative to that of the whole bird has increased over the last few years, whilst the value of the skin has decreased (Burger, 2005). Emus and rheas are smaller avian species than ostrich and produce less meat per animal.

Most of the ostrich meat is exported and lesser amounts are consumed locally in the areas of ostrich production. The use of ostrich meat appears to be restricted to specific market channels, probably due both to consumer demand and to established meat trading systems. In a survey of ostrich meat use in south central US, 90% of retailers and 95% of restaurants had never handled ostrich meat and 49% of retailers and 66% of restaurants were not familiar with ostrich meat. Of the factors influencing purchase of ostrich meat, product form and purchase price were the most important attributes in preference ratings, with portion size and branding less important (Gillespie *et al.*, 1998).

Ostrich slaughter practices have been adapted specific to the ratite species to produce desired products and processing efficiencies (Sales and Oliver-Lyons, 1996). It has been suggested to slaughter ostriches at 85 kg (Jones *et al.*, 1994), about 14 months of age. When age is unknown, the ossification of the pectoral girdle could be used to separate carcasses from old and young ostriches (Sales and Mellett, 1995). Restraint and stunning require specialized facilities and procedures because of the long neck, head anatomy, and physiology of ratites. An electrical current in excess of 400 mA at 50 Hz AC applied only to the head would prevent recovery in more than 90% of the ostriches when bled within 60 s of stunning (Wotton and Sparrey, 2002). It was also noted that the first stages of recovery in the birds are accompanied by rhythmic breathing movements. While observations of

breathing could be a diagnostic tool on the effectiveness of the stunning, identification of rhythmic breathing movements in the ostrich after stunning is difficult because the spinal reflexes causing limb muscle contraction also result in almost rhythmic body movements that could be confused with breathing movements. The South African legislation requires a current of 400–600 mA, 90–110V for a duration of 4–6 seconds.

Stunning has traditionally been with hand-held tongs as birds are held in the restraining area by pressure normally applied by gently pushing from behind on the tail feathers. The area is often a V-shaped structure, high enough that the stunning operator is not kicked, since ostriches kick forwards. After (and sometimes during) stunning, the birds are rocked backwards and a rubberized leg clamp is placed over the legs at the tarso-metatarsal bone, thereby immobilizing them, and allowing the birds to be ring/chain shackled via the big toes. Birds are hoisted onto a 3.4 m overhead rail and manually conveyed to another area for exsanguination. This conventional stunning procedure has been replaced in many abattoirs with a new restraining and stunning mechanism that completely encompasses the ostrich in a padded clamp holder. Double padded sides restrain the bird's upper thighs and a rubberized foot clamp holds the feet so that there is no physical damage to the bird. As the bird is electrically stunned, the entire stunning box rotates 180° so that toe clamps can be applied without any danger to the stunning operators. The restraint is opened after stunning and the bird is hoisted and conveyed for exsanguination.

Within 20 seconds of stunning, the birds should be bled by means of a complete ventral cut to the neck and/or by thoracic sticking. The head is normally held between two horizontal metal bars to minimize blood spillage on the feathers and skin. Feathers are pulled and the carcasses skinned on the rail. Conventional slaughter practice is to remove the legs from the carcass before initiation of rigor and to chill the legs at 0 to 4 °C for 12 hours before removal of the individual muscle cuts (Sales and Horbanczuk, 1999). Thicker membranes are removed from the muscles as ostrich meat is sold as individual muscles. While the anatomy of the hip and thigh are typically avian, the ostrich has a *M. pectineus*, the ambiens muscle originates on the lateral surface of the ilium, there are four rather than three femorotibial muscles, and there is no *M. iliochantericus medius* (Mellett, 1994). There are different common trade names in different countries for the muscles from ostrich carcasses (Sales and Oliver-Lyons, 1996).

It is common practice for the South African trade to further cut and vacuum pack muscle portions (100 g) rather than the whole muscle for export. A survey of restaurants in the US indicated that a portion-controlled option was preferred to a non-portion option and ostrich filet was superior to either ground or processed ostrich meat (Gillespie *et al.*, 1998).

18.3 Body and carcass quality traits

The amount and characteristics of meat produced from animals will determine the

relative quality traits. Animals produced specifically for the meat trade are harvested at sufficiently heavy body weights and/or ages to provide the quantity of meat for production efficiencies while maintaining the desired physical, chemical, and palatability characteristics. Generally, ostriches are harvested at 8 to 14 months of age, with body weights varying from 50 to 125 kg (Sales and Oliver-Lyons, 1996). The meat from older or cull animals is also salvaged for use.

18.3.1 Live ostrich growth

Ostriches have a sigmoidal growth curve from hatching to 100 kg weight at 350 days of age, with decreased total body water with increased weight and age, which is typical of domestic meat animals (Degen *et al.*, 1991). The monitoring of ostrich growth (*Struthio camelus*) from different geographical areas during 700 days of age indicated there was no difference in weight between the sexes for ostriches from Zimbabwe or South Africa, while male ostriches from Namibia were heavier than the female ostriches at a given age (du Preez *et al.*, 1992). It was unknown if subspecies or production practices may have contributed to those findings.

It is desirable to be able to predict growth characteristics of the body and the different body parts to determine optimal times of bird sacrifice. Mellett and Randall (1994) reported that the growth of only the head could be described with the Gompertz function while growth of all body parts was overestimated in the early stages.

18.3.2 Ratite carcass yields and composition

The yield and composition of carcasses are influenced by breed or subspecies, diet, animal age, and other management factors. Increased energy in the diet increases growth rate, resulting in heavier, but fatter, carcasses. Pollok *et al.* (1997b) indicated that ostriches fed a forage diet had lighter carcass weights at slaughter than those fed concentrates. Increased energy levels in ostrich diets increased the backfat by 7.19 ± 1.06 mm per 1.5 mJ ME per kg DM of the diet (van Schalkwyk *et al.*, 2002). Ostriches fed hay had heavier, but not significantly so, carcasses than those fed concentrates. Grazing did not impair growth, carcass weight or dressing percentage (Nitzan *et al.*, 2002). There was increased slaughter weight with increased dietary energy concentration during the grower (8.5, 10.5, and 12.5 MJ ME per kg feed) and finisher (7.5, 9.5, and 11.5 MJ ME per kg feed) phases for ostriches sacrificed at 12 months of age (Brand *et al.*, 2004). A dietary protein concentration of 120 g protein per kg feed resulted in the highest final weights and slaughter weights compared with 80, 100, 140, and 160 g protein per kg feed (Brand *et al.*, 2004).

On a live weight basis, the separable lean, fat, and bone were 35.7, 5.2, and 15.3%, respectively, for ostrich (Morris *et al.*, 1995a) and 38.8, 6.6, and 13.3%, respectively, for rhea (Sales *et al.*, 1997). The body composition of ostriches (*Struthio camelus*) on a 17% crude protein 12.33 MJ per kg ME diet had slightly increased protein and ash content while moisture declined by 8.6% and fat

increased by 12.4% to 22.6%, with animal weight increase from 10 to 30 kg (Swart *et al.*, 1993). Berge *et al.* (1997) found only minor differences in moisture, protein, and lipids among different muscles from emus of different ages (6, 10, 14, 17, and > 20 months). Birds fed a forage diet that had lighter carcass weights at slaughter also had less knife separable fat than those fed a concentrate diet, but the fat deposition was attributed more to the effect of the lighter slaughter weight than to the type of diet (Pollok *et al.*, 1997b). The *M. fibularis longus* of ostriches that were fed concentrates on pasture had higher dry matter and less fat than those from ostriches given only concentrate, with grazing not impairing growth, carcass weight or dressing percentage (Nitzan *et al.*, 2002). The abdominal fat and total fat were increased with increased dietary energy concentration during the grower (8.5, 10.5, and 12.5 MJ ME per kg feed) and finisher (7.5, 9.5, and 11.5 MJ ME per kg feed) phases for ostriches sacrificed at 12 months of age, with highest total fat with 120 g protein/kg feed compared with dietary protein concentrations of 80, 100, 140, or 160 g protein/kg feed (Brand *et al.*, 2004). The overall carcass fat in the ostrich averages about 15% compared with 25%, 30%, and 10 to 15% in beef, pork, and poultry, respectively (Cooper and Horbańczuk, 2002).

18.4 Influences on composition and quality development

18.4.1 Ante-mortem influences on body and carcass composition

Even though transport conditions would be expected to influence the physiological status of the birds and thus the resulting meat quality, there are no reported studies on the influence of transport conditions (type of vehicle, duration, environmental conditions, road conditions, standing density, etc.) on the composition and yields of ratites. Lairage does influence carcass characteristics.

Direct shipment and slaughter of ostriches reduced weight shrinkage to 2% compared with 10% for 19-month-old ostriches held overnight before slaughter (Schaefer *et al.*, 1997). In that study, birds that received electrolyte treatment in alfalfa feed pellets for 24 hours before transport and immediate slaughter had slightly higher hot and cold carcass weights and higher moisture content in muscles compared with birds not receiving the electrolyte treatment and rested overnight before slaughter. Sabbioni *et al.* (2003) noted that the time of lairage from 2 to 26 hours significantly affected carcass weight. The effect of 2.5 days of lairage (a period simulating lairage of ostriches arriving at the abattoir over the weekend and being slaughtered on Monday) on the meat quality of ostriches weighing 50 to 100 kg was monitored, with the major effect being less weight loss (1.04 ± 0.51 kg) than for birds that had not received any feed (3.23 ± 0.56 weight loss) (van Schalkwyk *et al.*, 2005). The held birds also had a 1 °C higher temperature.

Dressing percentage is the proportion of live weight that is obtained as a carcass. Dressing percentages of mammals range from 48 to 75%, depending upon the species, age, weight, diet, gut fill, and sex. Dressing percentages were 55% for

female and 57% for male birds (Schaefer *et al.*, 1997), while higher dressing percentages of 58.6% for females and 60.9% for males had been previously reported (Jones *et al.*, 1994). Dressing percentages of 58.6% were also obtained for ostriches 10 to 14 months of age, but there were no differences in sex for slaughter yields (Morris *et al.*, 1995a). Slaughter weight and dressing percentages were increased with higher energy levels in feed or with 120 g protein per kg compared with 80, 100, 140, or 160 g levels (Brand *et al.*, 2004), but grazing of ostriches while feeding concentrates did not alter carcass weight or dressing percentage (Nitzan *et al.*, 2002). Emus had dressing percentages of 52.64% with lean meat, fat and bone of 14.1, 11.5, and 4.3%, respectively, of cold carcass weight (Sales *et al.*, 1999a). Dressing percentages were 63.1% for *Rhea americana* and 59.6% for *Pterocnemia pennata* with lean meat, fat and bone of 64.1, 10.8, and 21.9%, respectively, of carcass weight (Sales *et al.*, 1997). Wings and heads of rhea comprise more and the skin less of the live weight than in ostriches (Sales and Horbańczuk, 1998).

A 24-hour cooler shrinkage was 1 to 1.5% (Schaefer *et al.*, 1997), with a higher cooler shrink of 2.6% (minimum of 0.9 and maximum of 4.5%) (Pollok *et al.*, 1997a) also reported for ostrich carcasses. The weight loss during chilling is relatively high because ostriches do not have a full subcutaneous fat cover as do most of the traditionally farmed species. Changes in the chilling environment after slaughter, such as cooling rate or increased humidity through sprays, might minimize the carcass or leg weight shrinkage.

Lairage time affected the *M. fibularis longus* fat content and fat energy to total energy ratio, with the increased fat content attributed to dehydration caused by stress (Sabbioni *et al.*, 2003), but the weight of hot and cold drumsticks was independent of the length of lairage (van Schalkwyk *et al.*, 2005). The *M. fibularis longus* of ostriches that were fed concentrates on pasture had higher dry matter and less fat than those from ostriches given only concentrate (Nitzan *et al.*, 2002).

The value of different muscles in the carcass is based on their mass and characteristics. Ten of the major muscles from the ostrich carcasses account for about two-thirds of the recoverable lean meat (Sales and Oliver-Lyons, 1996). The different muscle proportions as a percentage of carcass weight or hind limb weight are shown in Table 18.1. Lean to bone ratios of 3.14 for female ostriches and 3.25 for male ostriches and fat to bone ratios of 1.06 and 0.84 for females and males, respectively, have been reported (Jones *et al.*, 1994). In another study, lean to bone ratios were 3.10 to 3.17 for female ostriches and 3.24 to 3.39 for male ostriches and fat to bone ratios were 0.75 to 0.96 for females and 0.71 to 0.76 for males (Schaefer *et al.*, 1997). Lean to fat, lean to bone, and fat to bone ratios on a live weight basis were 6.87, 2.33, and 0.34, respectively, for ostrich (Morris *et al.*, 1995a) and were 5.88, 2.91, and 0.50, respectively, for rhea (Sales *et al.*, 1997) while the lean to fat, lean to bone, and fat to bone ratios on a carcass weight basis were 6.79, 2.32, and 0.34, respectively, for ostrich (Morris *et al.*, 1995a) and were 5.94, 2.92, and 0.49, respectively, for rhea (Sales *et al.*, 1997).

Table 18.1 Different muscle proportions as percentage of hot carcass weight

Muscle	Ostrich ¹	Ostrich ²	Ostrich ³	Ostrich ⁴	Emu ⁵	Rhea ⁶
<i>Obturatorius medialis</i>	4.30	3.12		5.70	1.21	2.20
<i>Iliotibialis lateralis</i>	8.24	6.43	2.01	11.33	7.63	7.47
<i>Femorotibialis medius</i>	4.17		1.18	5.53	3.59	3.38
<i>Flexor cruris lateralis</i>	2.94	1.92	0.66	3.90	2.54	3.39
<i>Iliotibialis cranialis</i>	3.84	2.56	0.96	5.02	4.40	3.34
<i>Iliofibularis</i>	8.54	6.38	2.85	11.33	5.66	4.77
<i>Gastrocnemius pars interna</i>		7.99	1.71	9.10	5.43	3.76
<i>Gastrocnemius pars externa</i>				7.20	3.77	3.67
<i>Fibularis longus</i>		4.71	0.81	5.50	4.09	3.49
<i>Ambiens</i>	1.36		0.52		0.56	
<i>Iliofemoralis</i>	2.89	1.71	0.74	3.87	1.31	
<i>Femorotibialis externa et interna</i>	1.68	3.84			2.55	

¹ Mellett (1994) (percentage of hindquarter weight).

² Morris *et al.* (1995b).

³ Sales (1996).

⁴ Cooper and Horbańczuk (2002) (percentage boneless meat).

⁵ Sales *et al.* (1999a).

⁶ Sales *et al.* (1997).

18.4.2 Influences on quality development

The post-mortem pH and the decline in pH relative to temperature is known to have an influence on muscle and meat quality properties. In contrast to beef and lamb muscles, Morris *et al.* (1995a) found the lowest post-mortem pH value for ostrich *M. iliofibularis* and *M. gastrocnemius, pars interna* occurred within 30 min after slaughter. Sales and Mellett (1996) and Sales (1994) found ostrich *M. iliofibularis* to have a very rapid pH decline until 2 h post-mortem, after which the pH increased. The pH decline was not different among *M. femorotibialis*, *M. gastrocnemius lateralis*, or *M. fibularis longus* with animal age; however, pH decline was slower in stressed emus slaughtered immediately after transport without rest (Berge *et al.*, 1997). There was a 0.2 higher pH directly post-slaughter and at 26.5 h post-slaughter in birds with 2.5 days of lairage compared with ostriches slaughtered after transport (van Schalkwyk *et al.*, 2005).

Ostrich *M. flexor cruris* and *M. iliofibularis* had similar pH to beef *M. pectineus* and lower pH than turkey thigh meat (Paleari *et al.*, 1998). Sales *et al.* (1998) found that rhea *Gastrocnemius pars externa*, *Iliofibularis*, and *Iliotibialis lateralis* from two species (*Rhea americana* and *Pterocnemia pennata*) had ultimate pH between 5.8 and 6.2, similar to meat from ostriches (Sales and Mellett, 1996).

Stunning procedures will alter pH and the process of rigor mortis. The rigor mortis value in the tenderloin (*M. ambiens*) and the pH₁ (45 min post-mortem) and pH₂ (18 h post-mortem) in the big drum (*M. gastrocnemius*), tender loin and triangular fillet (*M. iliofemoralis*) muscles were lower when stunned with air pressure compared with electrical stunning (Lambooj *et al.*, 1999b). Those

authors also noted that a short stun-stick interval (5 versus 39 seconds) resulted in lower pH₂ values in the tender loin and triangular fillet muscles and higher water binding capacity in the big drum. They recommended that at least 500 mA be used with a short stun-stick time interval or to kill the birds by a long stunning duration. A captive needle pistol, using air pressure, can be an alternative for electrical head only stunning (Lambooij *et al.*, 1999a).

Some abattoirs in South Africa electrically stimulate (ES) carcasses during the bleeding phase using 45V, 0.4 mA; 10 seconds on, 10 seconds off for 3 minutes because it is presumed to help with bleeding and subsequent meat shelf-life. Morris *et al.* (1995a) noted that ES at 45 minutes post-mortem had no influence on muscle pH or temperature decline in five muscles. However, it is now known that ostrich muscle pH undergoes a very rapid decline post-mortem (Botha *et al.*, 2004a,b) and any benefit from ES would be gained only if ES were applied very early post-mortem.

Cold shortening is a phenomenon of muscles chilled very rapidly before rigor mortis that subsequently influences meat properties. At temperatures above 12 to 15 °C, muscle fibers contract at rigor while contraction occurs before rigor below this temperature range (Hwang *et al.*, 2003). Shortening during normal rigor or cold shortening is due to release of ionic calcium into the myofibrillar space at ATP concentrations sufficient for contraction (Honikel *et al.*, 1983), resulting in shortening to 80% of the original muscle sarcomere length (Lawrie, 1998). A high frequency of shortened (20 to 40%) sarcomeres in conventionally slaughtered and fabricated ostriches was found only in the *M. iliotibialis lateralis* and *M. iliofemoralis*, which was explained by hanging of the carcass initially by the feet so that there is no tension on those muscles (Sales and Horbańczuk, 1999).

There are contradictory results on the occurrence of cold shortening in hot deboned ostrich muscles. Sales and Horbańczuk (1999) analyzed six muscles removed after 12 hours chilling at 0 to 4 °C from legs removed from the carcass within 30 minutes post-mortem. Although the *M. iliotibialis* and *M. iliofemoralis* had a high frequency of sarcomere shortening (20 to 40%), the W-B shear values were similar to other muscles. Botha (2005) removed the *M. gastrocnemius, pars interna* from the ostrich carcass within one hour post-mortem and analyzed shortening compared with the muscle removed within one hour post-mortem and chilled for 24 hours at 0–4 °C and with the same muscle removed after cooling on the carcass for 24 hours at 0–4 °C. The shorter sarcomere lengths of the hot deboned muscles (1 hour post-mortem and 24 hours post-mortem) compared with the cold deboned muscles was attributed to the muscle fibers still contracting one hour post-mortem and shortening to a greater extent because there were no attachments to the leg bones. Shortening and rigor development in ostrich muscles are temperature dependent (Botha *et al.*, 2008), but both the *M. gastrocnemius, pars interna* and *M. iliofibularis* reached pH < 6.2 early post-mortem, with muscle temperatures above 10 °C, so there would be no risk of cold shortening if these muscles were hot-deboned 2 to 4 hours post-mortem (Hoffman *et al.*, 2007).

18.5 Raw chilled ratite meat characteristics

Meat can be evaluated by objective chemical or physical means and through measurement of sensory traits. Sensory properties of meat may be evaluated as flavor, odor, color, juiciness, and tenderness/texture, as perceived by trained sensory panels or consumers. These properties are influenced by the physical structure and chemical composition of meat.

18.5.1 Chemical composition

Ostrich *M. flexor cruris* and *M. iliofibularis* had similar moisture, ash, collagen, collagen as percent of protein, slightly higher protein, and a much lower fat to protein ratio compared with beef *M. pectineus* and turkey thigh meat (Paleari *et al.*, 1998). Ostriches fed on pasture only had significantly less intramuscular fat in the *M. fibularis longus* compared to those fed on pasture and concentrate (Nitzan *et al.*, 2002). Ash content was higher in the *M. fibularis longus* of birds that had consumed hay rather than just concentrates (Sabbioni *et al.*, 2003).

In general, the meat of younger ostriches contains less fat than that from older (breed stock) birds (Hoffman and Fisher, 2001). In young birds, however, there were no differences in intramuscular fat content of meat from ostriches 10 to 11 or 14 to 15 months of age at slaughter (Girolami *et al.*, 2003). In another study, age (10 to 54 months old) did not influence the fat content, although there was a linear increase in protein content (Sabbioni *et al.*, 2003).

Lairage time was also found to increase the *M. fibularis longus* fat content and fat energy to total energy ratio, with the increased fat content attributed to dehydration caused by stress (Sabbioni *et al.*, 2003).

The influences of subspecies and muscle type on the lipid content and composition of ostrich meat are not clear (Sales, 1994, 1996, 1998; Sales and Hayes, 1996; Horbańczuk *et al.*, 1998). There were no differences in total lipid content of *M. gastrocnemius*, and *M. iliofibularis* from Red Neck (*Struthio camelus massaicus*) and Blue Neck (*Struthio camelus australis*) ostriches (Horbańczuk *et al.*, 1998), although the lipid values for the *M. iliofibularis* were lower than the lipid values found for African Black (*Struthio camelus* var. *domesticus*) ostriches of similar ages (Sales and Hayes, 1996; Horbańczuk *et al.*, 1998). Girolami *et al.* (2003) found that the intramuscular fat content was higher in the *M. iliotibialis* than in the *M. iliofibularis* or the *M. gastrocnemius* of Blue Neck ostriches. This contradicted results of Sales and Hayes (1996), who found that the intramuscular fat content of the *M. femorotibialis medium* and the *M. gastrocnemius pars interna* were lower than in the *M. iliofibularis*.

The crude protein and fat content of freeze dried ostrich *M. ambiens*, *M. iliofibularis* and *M. gastrocnemius* could be predicted by near infrared reflectance spectroscopy (NIR) (Viljoen *et al.*, 2005), so it might be anticipated that NIR could also be used to successfully estimate the chemical composition of wet ostrich meat.

18.5.2 Physical composition

Muscles are composed of combinations of differing fiber (cell) types (Aberle *et al.*, 2001). Meat has properties depending upon the proportions of the fiber types (Aberle *et al.*, 2001), with the muscle fibers differing in contraction speed, metabolic type, and size (Patak and Baldwin, 1993). The *Pectoralis* muscles of ostrich and emu had varying proportions of fast-twitch glycolytic (FG), fast-twitch oxidative-glycolytic (FOG), and slow-tonic in cranial, central, and caudal portions, with FG fibers predominating in the *Pectoralis* muscle from male and female ostriches, while emus had somewhat homogenous proportions of slow-tonic, FOG, and FG fibers (Rosser and George, 1985).

Although the fiber types were not easily distinguished and appeared continuously variable in emu muscles, the fast fiber muscles had fast oxidative small (FO) and fast glycolytic large fibers (FG), while mixed fiber muscles had FG, fast intermediate glycolytic and oxidative capacities (FGO), and slow-twitch oxidative small (SO) fibers (Patak and Baldwin, 1993). The *Gastrocnemius lateralis*, *G. intermedius caudalis* and *G. intermedius medialis* muscles had 55 to 58% FG and 42 to 45% FO fibers while the *Gastrocnemius medialis* muscle had 72% FG and 28% FO. The *Flexor digitorum longus* muscle had 19 to 37% SO, 29 to 70% FGO, and 27 to 41% FG fibers, but myoglobin did not differ with muscle type (Patak and Baldwin, 1993).

Histochemical analyses showed the presence of FG, FOG, and slow-twitch oxidative fibres (SO) only in the *Gastrocnemius pars externa* muscle of ostriches; the FG fibers were absent in the other muscles (Velotto and Crasto, 2004). There were more SO fibers than FOG fibers in the *Tibialis cranialis caput femorale* and *Tibialis cranialis caput tibiale* muscles, while it was the opposite for *Fibularis longus tendo caudalis* muscle. The FG fibers outnumbered the other fibers, followed by the SO and FOG fibers in the *Gastrocnemius pars externa* muscle (Velotto and Crasto, 2004).

18.5.3 Color

Color influences meat purchasing decisions more than other factors of quality because consumers use discoloration to indicate freshness and wholesomeness (Mancini and Hunt, 2005). The raw ostrich *M. flexor cruris* and *M. iliofibularis* had similar L* values to beef and were darker than turkey thigh meat, and the ostrich muscle b* value was higher than for beef or turkey while the cooked ostrich meat was darker than turkey or beef (Paleari *et al.*, 1998).

Minolta L*, a*, and b* values of drum and thigh ostrich meat were not influenced by pre-slaughter electrolyte treatment of ostriches (Schaefer *et al.*, 1997). The color of muscles from birds fed just concentrates was significantly brighter after 6 days than that of the muscles of ostriches also fed hay (Sabbioni *et al.*, 2003). However, van Schalkwyk *et al.* (2002) noted that dietary energy and protein levels did not cause differences in the color of the muscles measured.

Color reflectance was decreased and a* and b* readings were increased with increased age at slaughter (Hoffman and Fisher, 2001). The lightness (L*) and

yellowness (b^*) increased in value until 4.5 to 9 hours post-mortem, when there was a decline in values, which then increased through 5 days post-mortem (Thomas *et al.*, 2004).

The heme pigment was lower in the *M. iliofibularis*, *M. gastrocnemius pars interna*, and *femorotibialis medius* than in the *M. iliotibialis lateralis*, *M. ambiens* and *M. iliofemoralis* (Sales, 1996). Iron pigments in the *M. iliofibularis* and *M. iliotibialis lateralis* were lower than in the *M. gastrocnemius lateralis*, *M. gastrocnemius medialis*, and *M. fibularis longus* of emu at slaughter ages of 6 to more than 20 months (Berge *et al.*, 1997). Variations in lightness (L^*) between different emu muscles were small, while the colour stability after air-permeable and vacuum packaging was more variable between muscles (Berge *et al.*, 1997).

18.5.4 Tenderness

Tenderness has been characterized as the most important factor of cooked meat that determines consumer acceptability as it plays a central role in determining consumer preference (Risvik, 1994). Tenderness was ranked most important by 51% of participants in a study asking consumers to rank the importance of quality traits, while 39% of participants chose flavor and 10% selected juiciness as the most important (Huffman *et al.*, 1996).

Tenderness is the force needed to shear, compress and grind meat during mastication and consumption (Lepetit and Culioli, 1994). Tenderness refers to the ease with which the consumer disorganizes the meat structure and so it is affected by many factors, including intramuscular fat; percentage of moisture and fat; collagen content and percentage soluble collagen; level of enzymes; glycogen content; sarcomere length; and juiciness (Davis *et al.*, 1979). Other factors are crosslinking of collagen with increased animal age, and the crossbridge formation and contractile state of myofibrillar proteins (Aberle *et al.*, 2001). Tenderness is influenced by ante-mortem factors of species; age; sex; and nutritional status while post-mortem factors that influence tenderness include slaughtering methods; pre-slaughtering stress; handling; processing; and cooking temperatures (Lawrie, 1998). Objective tenderness of cooked meat is often measured by Warner–Bratzler (W–B) shear for red meat and Kramer shear for poultry; sensory determinations of initial force to bite, number of chews, and other measures of tenderness may be measured by trained or consumer panels.

The *M. flexor cruris* and *M. iliofibularis* from ostrich had similar shear force to turkey thigh meat while the shear force of beef *M. pectineus* was much higher (Paleari *et al.*, 1998).

Dietary and lairage effects on tenderness of ostrich muscles are minimal. W–B shear forces of the same muscle were not different in ostriches fed on pasture only or fed on pasture and concentrate (Nitzan *et al.*, 2002). The lack of differences in W–B shear due to bird diet was confirmed in ostriches fed concentrates or with hay (Sabbioni *et al.*, 2003). There were no differences in shear values of *M. iliofibularis* between control and treatment birds due to lairage time (van Schalkwyk *et al.*, 2005).

It had been indicated that W–B shear force was not affected by ostrich age of 8, 10, 12, or 14 months (Sales, 1994; Mellett and Sales, 1997; Girolami *et al.*, 2003). However, in other studies (Mellett and Sales, 1997; Girolami *et al.*, 2003), sensory panel results indicated that tenderness of ostrich meat was affected by slaughter age. Meat from 8-month-old birds was significantly more tender than meat from 10-, 12- and 14-month-old birds, and meat from 10-month-old birds was more tender than that from 12- and 14-month-old birds as measured by sensory analysis. Only Girolami *et al.* (2003) gave the subspecies (*Struthio camelus australis*) of ostrich. The W–B shear values of muscles from 8-year-old ostriches were higher than the published W–B values for muscles from 14-month-old birds, indicating that ostrich age did have an effect on tenderness (Hoffman and Fisher, 2001). Although the subspecies were not mentioned, it can be assumed that the variety in the studies of Sales (1994), Mellett and Sales (1997) and Hoffman and Fisher (2001) was *Struthio camelus* var. *domesticus*.

Less post-mortem aging time is needed for tenderization of ostrich meat compared with other red meat species. The shear force values for ostrich meat aged for 1 h, 24 h and 7 d were all lower (more tender) than for beef aged for 7 d (Marks *et al.*, 1998). It was suggested by Pollok *et al.* (1997d) that aging of ostrich meat is not necessary since ostrich meat appears to be sufficiently tender and tenderness improvement with aging would be offset by a potential increase in microbial growth and the subsequent decrease in shelf-life. Aging decreased W–B shear in muscles from ostriches ranging from 8 to 14 months (Sales *et al.*, 1996b). The *M. iliofibularis* and *M. iliofemoralis* aged for 10.5 days had decreased W–B shear compared with aging for 3.5 days, while the *M. femorotibialis medius* and *M. iliotibialis lateralis* had lower, but not significantly so, W–B shear with increased aging time and the *M. gastrocnemius pars externa* and *M. ambiens* had increased, but not significant, W–B shear at 10.5 days compared with 3.5 days. Ultrastructural examination of the *M. gastrocnemius pars externa* and *M. iliofibularis* in that study attributed the tenderization with aging time to proteolysis rather than Z-disk degradation. Hedonic sensory scores for tenderness increased with time post-mortem from 1 hour to 1 day to 1 week (Marks *et al.*, 1998). Shear force increased through 9 days of storage at 4 °C and then was lower at 12 days post-mortem (Thomas *et al.*, 2004).

After storage for 48 h post-mortem, hot deboned muscles were less tender according to both sensory tenderness scores and W–B shear force values than cold deboned muscles (Hoffman *et al.*, 2006). In contrast, cold deboning resulted in less variation, and therefore would produce meat with more consistent eating quality in terms of texture than hot deboned muscles. The exponential decline in W–B shear over 21 days post-mortem ageing was similar in hot- and cold-deboned muscles (Fig. 18.1), but the faster rate for hot-deboned muscles resulted in an exponential decline in Warner–Bratzler shear force values over a 21-day post-mortem aging period. The hot-deboned muscles were initially tougher than cold-deboned muscles, but reached the shear level of the initial cold-deboned muscles after 5 days of post-mortem aging (Botha, 2005).

Fiber diameters and sarcomere lengths of different ostrich muscles did not seem

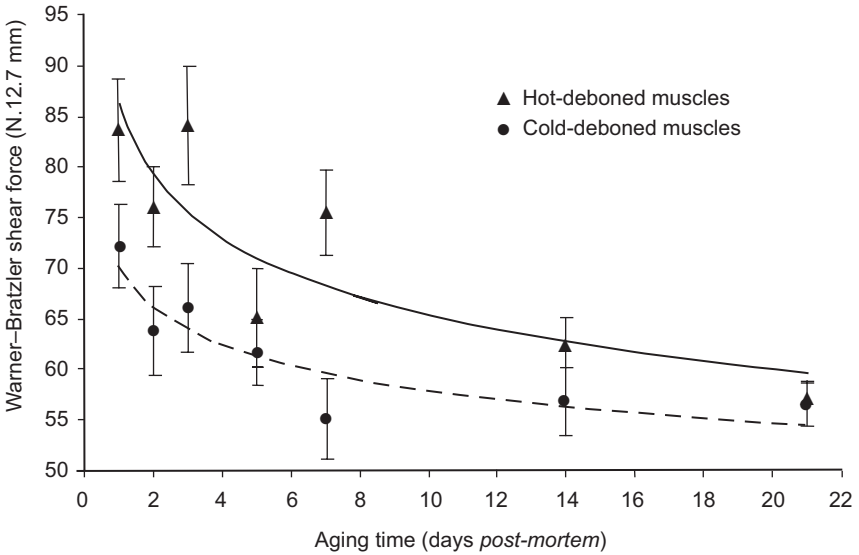


Fig. 18.1 Decrease in shear force (\pm s.e.) with *post-mortem* aging time hot-deboned and cold-deboned ostrich muscles (adapted from Botha, 2005; Botha *et al.*, 2006).

related to W-B shear values (Sales, 1996) because the *M. iliofibularis* had the smallest fiber diameter and second longest sarcomere length, but highest W-B shear, while the *M. iliofemoralis* had the second smallest fiber diameter, shortest sarcomere length, and lowest W-B shear value. The correlation between collagen content and W-B shear was very low (Sales, 1996).

Muscle type will influence the tenderness of ostrich meat (Cooper and Horbańczuk, 2002). There were no significant differences in sensory tenderness of the *M. iliofibularis*, *M. iliotibialis lateralis* and *M. femorotibialis medius*., but the *M. iliotibialis lateralis* and *M. femorotibialis medialis* had lower W-B shear than *M. iliofibularis* (Mellett and Sales, 1997). One week of post-mortem aging decreased Kramer shear force in all muscles except the *M. femorotibialis medius*, *M. iliofemoralis externus*, *M. oburatorius lateralis*, *M. fibularis longus*, and *M. ambiens*, which had higher shear forces after ageing (Marks *et al.*, 1998). A consumer panel ranked the tenderness of ostrich muscles in order of increased liking as *M. fibularis longus*, *M. oburatorius medialis*, *M. gastrocnemius*, *M. iliofibularis*, and *M. iliofemoralis externu* (Marks *et al.*, 1998). Instrumental measurements and sensory analysis ranked *M. iliofibularis* as the most tender and *M. gastrocnemius* as the least tender, with *M. iliotibialis* having intermediate tenderness (Pollok *et al.*, 1997a; Girolami *et al.*, 2003). Shear force values for different ostrich muscles are in Table 18.2.

The *M. iliotibialis lateralis* muscles from 14-month old emus were rated higher for tenderness while the *M. iliotibialis cranialis* had highest tenderness from 10 month old emus compared with other ages (Berge *et al.*, 1997). The *M. iliofibularis* and *M. iliotibialis cranialis* were the most tender while *M. gastrocnemius medialis*

Table 18.2 Shear force of different ostrich muscles

Muscle	W-B shear, kg ¹	Kramer shear (kg force/g weight) ²	W-B shear (N/1.27 cm) ³
<i>Iliofemoralis</i>	2.64 ^c	10.1	
<i>Femorotibialis medius</i>	2.94 ^c	8.9	
<i>Gastrocnemius pars interna</i>	2.97 ^c		
<i>Iliotibialis lateralis</i>	3.46 ^b		
<i>Ambiens</i>	3.67 ^b	11.4	
<i>Iliofibularis</i>	4.44 ^a	10.8	59
<i>Obturatorius medialis</i>		8.9	52
<i>Femorotibialis accessorius</i>		6.7	
<i>Iliofemoralis externus</i>		8.6	
<i>Iliotibialis lateralis</i>		9.8	69
<i>Gastrocnemius</i>		9.9	63
<i>Flexor cruris lateralis</i>		10.4	
<i>Fibularis longus</i>		12.4	
<i>Oburatorius lateralis</i>		12.2	
<i>Iliotibialis cranialis</i>			48

¹ Sales (1996), 24 hours post-mortem; means with different letters (a,b,c) are different ($P < 0.05$).

² Marks *et al.* (1998), after 1 week of aging.

³ Brand (2006), 24 hours post-mortem.

and *M. fibularis longus* received the lowest tenderness ranking by trained sensory panellists. The *M. iliofibularis* had less total intramuscular collagen and 20% less heat-soluble collagen than other muscles measured in 6-month to more than 20-month-old emu (Berge *et al.*, 1997). Tenderness of different muscles increased with decreased total collagen or insoluble collagen when cooked to 60 °C, showing that the content and heat stability of intramuscular connective tissue in emu meat are major factors influencing tenderness. Sales (1996) reported a ranking for total collagen content that was similar in the ostrich muscles to those reported by Berge *et al.* (1997) for emu muscles, but Sales (1996) found no consistent relationship between shear values of meat cooked at 75 °C and total collagen content.

It was concluded that Ca²⁺-dependent proteases (CDP) were more likely to have caused myofibrillar changes in *M. iliofibularis* than cathepsin enzymes because incubation of myofibrils with CDP mimicked the post-mortem changes in W-B shear force during 12 days at 2 to 4 °C (van Jaarsveld *et al.*, 1997a). The enzyme activities in *M. iliofibularis* during 12 days post-mortem were high, especially of the proteasome, but the mean shear force values showed no improvement in tenderness during that time (Thomas *et al.*, 2004). The activity of ostrich matrix metalloproteinases increased from 14 to 20 °C, with a large activity increase at 38 °C until reaching the optimal temperature of 45 °C, which was followed by a sharp decrease in the activity. The activity of the matrix metalloproteinase gradually increased during 2 to 21 days post-mortem aging, implying the enzymes could have a role in post-mortem maturation of ostrich meat (Pambuka *et al.*, 2007).

Cooking temperatures have a great influence on W–B shear. Heating the *M. iliotibialis lateralis* to 60, 70, and 80 °C gave W–B shear values of 4.1, 3.6 and 4.4, respectively, while heating the *M. iliofemoralis* to the same temperatures resulted in shear forces of 1.3, 3.8, and 4.8 (Sales, 1997).

18.5.5 Juiciness

Consumers consider tenderness and juiciness to be the most important quality attributes of fresh meat and meat products (Xiong, 2005). Sensory analyses should measure the two components of juiciness, the initial impression of fluid, primarily moisture, exuded on the meat surface and sustained juiciness upon chewing, which is dependent upon both water and fat (AMSA, 1978). Ostrich meat is less juicy (3.38) than meat derived from lamb (4.68) and pork (4.60) and juicier than meat derived from beef (3.25) and chicken (2.89) (Rødbotten *et al.*, 2004), which can be directly linked to the fat content of ostrich meat.

Increased water and/or fat content at the time of consumption are generally associated with increased juiciness. Decreased water binding capacity and loss of moisture or fat through drip or cooking would decrease juiciness. Lairage time caused no differences in drip loss or cooking loss of *M. iliofibularis* (van Schalkwyk *et al.*, 2005). Cooking losses were 31.9 to 37.7% for ostrich muscles cooked to 75 °C (Sales, 1996), while cooking *M. iliofibularis* to 70 °C resulted in a cooking loss of only 17% (Sales *et al.*, 1996a). Cooking losses from heating the *M. iliotibialis lateralis* to 60, 70, and 80 °C were 12.0, 25.6, and 27.3%, respectively, while cooking losses in the *M. iliofemoralis* after heating to the same temperatures were 1.3, 3.8, and 4.8% (Sales, 1997). The *M. iliotibialis lateralis* muscle had opposing trends for drip and cooking losses; increased shear force values were related to decreased drip losses and increased cooking losses (Thomas *et al.*, 2004).

Lower lipid values would be expected to decrease perceptions of juiciness. Total lipid content was not different in the *M. gastrocnemius*, and *M. iliofibularis* from Red Neck (*Struthio camelus massaicus*) and Blue Neck (*Struthio camelus australis*) (Horbańczuk *et al.*, 1998), but the lipid values for the *M. iliofibularis* were lower than the lipid values found for African Blacks (*Struthio camelus* var. *domesticus*) at similar ages (Sales and Hayes, 1996; Horbańczuk *et al.*, 1998). Intramuscular fat contents of individual muscles may vary among subspecies of ostriches. Intramuscular fat content of the *M. iliofibularis* was reported to be higher than in the *M. femorotibialis medius* and the *M. gastrocnemius pars interna*, presumably from *Struthio camelus* var. *domesticus* (Sales and Hayes, 1996), while intramuscular fat content was higher in the *M. iliotibialis* than in either the *M. iliofibularis* or the *M. gastrocnemius* of Blue Neck ostriches (Girolami *et al.*, 2003).

Ostriches fed on pasture and concentrate had higher intramuscular fat in *M. fibularis longus* than the same muscles from birds fed on pasture only (Nitzan *et al.*, 2002).

Hot deboning appears to decrease juiciness; after storage for 48 h post-mortem,

the hot deboned muscles were less juicy than cold deboned muscles (Botha, 2005; Hoffman *et al.*, 2006).

The *M. iliotibialis cranialis* had the highest juiciness scores from 10-month-old emus compared with other ages, but the *M. iliotibialis lateralis* muscles from 14-month-old emus were rated higher for juiciness (Berge *et al.*, 1997).

18.5.6 Flavor

Meat flavor is a function of the sensory sensations of odor and taste. Odor has many different chemical components due to release of volatiles, while taste is sweet, salty, sour, bitter, and the savory characteristic of meat umami. Peptides, lipids, carbohydrates, nucleic acids and many other compounds contribute to taste (Miller, 2004).

Ostrich meat has a characteristic aftertaste, which is seldom observed in beef (Harris *et al.*, 1993; Paleari *et al.*, 1995). However, the panelists in those studies also considered ostrich meat to be bland, with the *M. gastrocnemius* identified as bland more frequently than the *M. iliofibularis*, *M. obturatorius medialis* and *M. iliotibialis lateralis* and the *M. obturatorius medialis* was described as the most intense in flavor (Harris *et al.*, 1993). The perceived blandness may be attributed to the high ultimate pH and low intramuscular fat content of ostrich meat (Lawrie, 1998; Cooper and Horbańczuk, 2002). Ostrich muscle type and slaughter age were found to have no effect on meat flavor intensity (Pollock *et al.*, 1997c; Girolami *et al.*, 2003). A consumer panel ranked the hedonic flavor and overall likeness scores of ostrich muscles in order of increased liking as *M. fibularis longus*, *M. oburatorius medialis*, *M. gastrocnemius*, *M. iliofibularis*, and *M. iliofemoralis externus*, which was also the same order for sensory tenderness scores (Marks *et al.*, 1998). Beef and ostrich were characterized by relatively strong intensities for most flavor and odor attributes (Kubberød *et al.*, 2002). Interestingly, the evaluation of gender specific preferences and attitudes towards meat indicated that dislike of red meat varieties, including ostriches, was more prevalent in females than in males (Kubberød *et al.*, 2002).

Flavor was highest in the *M. iliotibialis cranialis* from 10-month-old emus while the *M. iliotibialis lateralis* muscles from 14-month-old emus were rated higher for flavor compared with other ages of emus (Berge *et al.*, 1997). Feeding fish oil at 43.5 g per day to ostriches had no effect on sensory characteristics of ostrich meat, but did influence flavor of abdominal fat pads (Hoffman *et al.*, 2005). However, feeding fish oil at higher levels might result in undesirable meat sensory attributes.

18.5.7 Nutritional properties

The nutritional properties of meat are due to the chemical composition and relative proportions of proteins, lipids, vitamins, and minerals. Generally, meat is considered an excellent source of proteins, particularly the essential amino acids. Meat is also a major contributor of B vitamins and minerals in the diet (Lawrie,

1998). Fat in the diet has been linked to obesity in many studies (Melanson, 2003). Meat is perceived to be a major source of fat in the diet, especially with saturated fats, which have been implicated for many diseases in modern society. The polyunsaturated to saturated fatty acid ratio (P:S) in meat is about 0.1 while the recommended ratio is 0.4 (Wood *et al.*, 2004). The quantities of essential fatty acids and proportions of cholesterol, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in meat are major concerns for the healthfulness of meat in the diet. The main reference for amino acid and mineral composition of ostrich meat is Sales and Hayes (1996). The low sodium content of ostrich meat (43 mg per 100 g) (Sales and Hayes, 1996) would be advantageous for individuals who wish to consume low sodium foods.

Ostrich *M. flexor cruris* and *M. iliofibularis* had slightly higher protein, less cholesterol and much lower fat to protein ratio than turkey and beef (Paleari *et al.*, 1998). The fatty acid profile of ostrich meat was generally closer to that of beef than to turkey thigh meat (Paleari *et al.*, 1998). Oleic acid (C18:1) is the fatty acid with the highest concentration in ostrich meat, followed by palmitic acid (C16:0) and then linoleic acid (C18:2n-6) (Sales, 1994; Horbańczuk *et al.*, 1998; Paleari *et al.*, 1998; Sales, 1998; Hoffman and Fisher, 2001). Ostrich fat might be a source of PUFA or essential fatty acids in human and livestock diets because there is a high PUFA to SFA in breast fat from culled breeding ostrich females ((Horbańczuk *et al.*, 2003) and high contents of C18:2, C18:3, and C20:4 from breast and back fat depots in 14-month-old ostriches (Horbańczuk *et al.*, 2004).

Inclusion of citrus pulp in ostrich diets decreased intramuscular SFA and MUFA and increased PUFA, compared with meat from ostriches fed control diets (Lanza *et al.*, 2004). The fatty acid profile of both extramuscular fat and ostrich muscle was altered as a result of the consumption of increased levels of fish oil by the birds (Hoffman *et al.*, 2005). The C16:0 was increased with increased fish oil, as were SFA, while PUFA were decreased in the *M. iliofibularis*. Changes in fatty acids with increased fish oil were less clear for the abdominal fat pad (Hoffman *et al.*, 2005).

Differences in fatty acid content were found in meat from ostriches of 14 months compared with 8 years of age (Hoffman and Fisher, 2001). Although ostrich age from 10 to 54 months did not influence the fat content, it caused a linear increase in protein content and decreased the unsaturated to saturated ratio in the *M. fibularis longus* (Sabbioni *et al.*, 2003). Pre-slaughter rest reduced SFA and PUFA and increased oxidative processes in the meat, which was linked to the change in fatty acid profiles (Sabbioni *et al.*, 2003).

Within the normal ages at slaughtering, the fatty acid profile of ostrich meat varied with bird age (10 to 11 compared with 14 to 15 months) and muscles (*M. iliofibularis*, *M. gastrocnemius*, *M. iliotibialis*). Muscles from older birds had increased total SFA and MUFA and decreased total PUFA (Girolami *et al.*, 2003), which would decrease the PUFA:SFA ratio. In general, the meat of younger animals generally contains a higher percentage of PUFA and less SFA than that of older animals (Lawrie, 1998). Higher PUFA and lower MUFA were in meat of ostriches slaughtered at 10–11 months (Girolami *et al.*, 2003). The meat from

young ostriches would therefore contribute towards improved PUFA:MFA ratios in meat for consumers.

In a comparison of ostrich subspecies, the percentage of the C14:0 fatty acids in both *M. gastrocnemius*, and *M. iliofibularis* and the C16:0 fatty acids in the *M. iliofibularis* were higher in the Blue necks than in the Red Necks (Horbańczuk *et al.*, 1998). The MUFA C16:1 in the *M. gastrocnemius* of the Red Necks was higher than in the Blue Necks, while the MUFA C20:1 was higher in the Blue Necks than in the Red Necks in both muscles (Horbańczuk *et al.*, 1998).

Differences in individual fatty acids result in different ratios of SFA:MUFA:PUFA and FSA:UFA as well as *n-3:n-6* ratios. Ostriches fed a concentrate diet had higher levels of PUFA in the meat (Pollok *et al.*, 1997c). In African Black ostriches (*Struthio camelus* var. *domesticus*), higher percentages of total PUFA were found in the *M. gastrocnemius* and the *M. iliofibularis* (Sales, 1994). The percentage of total SFA, MUFA and PUFA varied between muscles in African Black ostriches, with SFA (C16:0, C18:0) being the lowest in the *M. iliofibularis* (Sales, 1998). The highest percentage of total MUFA was in the *M. ambiens*, followed by the *M. iliofemorotibialis*. Because the *M. iliofibularis* contained the highest total PUFA, the PUFA:SFA was higher and MUFA:PUFA was lower than in other muscles. The *M. gastrocnemius*, *M. femortibialis* and the *M. iliofibularis* also had the highest content of *n-3*-fatty acids (Sales, 1998).

In another study with ostrich subspecies, the total SFA and MUFA were similar between subspecies in *M. gastrocnemius* and *M. iliofibularis* and there were no differences in total PUFA between subspecies in the *M. iliofibularis*, but PUFA were higher in *M. gastrocnemius* from Blue Necks than from Red Necks (Horbańczuk *et al.*, 1998). The *M. flexor cruris* and *M. iliofibularis* from ostrich had slightly lower total SFA, slightly higher total UFA, and a lower SFA:UFA (0.9) than turkey (1.1) and beef (1.0) (Palairet *et al.*, 1998). The highest PUFA was in the *M. gastrocnemius*, whereas the highest SFA and MUFA were in the *M. iliofibularis* (Girolami *et al.*, 2003). The intramuscular lipids of emu meat had high levels of linoleic, arachidonic, α -linolenic, and docosahexanoic fatty acids, with a PUFA to SFA of 0.72 (Sales and Horbańczuk, 1998).

The ratios of *n-6:n-3* for *M. iliofibularis*, *M. gastrocnemius*, and *M. iliotibialis* were 7.57, 8.31 and 7.77, respectively, but did not differ significantly (Girolami *et al.*, 2003). These findings supported the work of Sales (1998) who noted that, for different muscles of African Black ostriches (*Struthio camelus* var. *domesticus*), the *n-6:n-3* ratio was the highest in the *M. iliofemoralis*, but constant in all other muscles even though the *M. gastrocnemius*, *M. femortibialis* and the *M. iliofibularis* had the highest content of *n-3*-fatty acids. However, with age groups of 10 to 11 months and 14 to 15 months, the *n-6:n-3* ratios were 6.82 and 8.95, respectively (Girolami *et al.*, 2003).

Total lipid and cholesterol content was not different between *M. gastrocnemius* and *M. iliofibularis* from 14-month-old ostriches, but total PUFA was higher in *M. iliofibularis* than in *M. gastrocnemius* (Horbańczuk and Sales, 1998). There were no differences in fatty acids in intramuscular fat among muscles or between

Greater or Lesser rhea species (Sales *et al.*, 1999b). Total SFA did not differ between species, but MUFA was lower and PUFA was higher for Greater rhea than for Lesser rhea species (Sales *et al.*, 1999b). The overall fatty acid profile of muscle from grey nandu (*Rhea americana*) was similar to ostrich meat and the *M. gastrocnemius* had mean lipid and cholesterol of 3.87 g and 75 mg per 100 g tissue, respectively (Horbańczuk *et al.*, 2004).

Cholesterol is a structural component of cell membranes and the sub-cellular distribution of cholesterol differs in muscle tissue (Sales *et al.*, 1999b; Cooper and Horbańczuk, 2002) so cholesterol does not necessarily increase with increased intramuscular fat. Cholesterol content was shown to differ among muscles in studies by Sales (1996, 1998), but Horbańczuk *et al.* (1998) noted no differences between the cholesterol content of the *M. gastrocnemius* and the *M. iliofibularis* (65.63 mg/100 g for both muscles). In that study, cholesterol content also did not differ between the subspecies *Struthio camelus massaicus* and the subspecies *Struthio camelus australis*. Girolami *et al.* (2003) also observed that cholesterol content did not differ among muscles or with ostrich age. The cholesterol content of back fat was higher than in breast fat of ostriches aged 14 months (Horbańczuk *et al.*, 2004). Cholesterol did not vary with muscle or species in rhea meat (Sales *et al.*, 1999b).

Compared with raw meat, cooking *Iliofibularis* to 70 °C decreased the moisture and increased the fat content, with a corresponding increase in cholesterol from 57 to 72 mg per 100 g and a slight increase in *n*-6:*n*-3 from 2.5 to 3.33 (Sales *et al.*, 1996a).

18.5.8 Microbiological quality and shelf-life

Total aerobic counts were 2.15 cfu per cm² for ostrich carcasses and 2.82 cfu per cm² for emu carcasses slaughtered in a small abattoir, but no coliforms or *E. coli* were reported (Gill *et al.*, 2000). Immediately after skinning of ostrich carcasses, aerobic plate counts ranged from 0.84 to 1.34 log₁₀ cfu per cm² and from 1.60 to 2.22 log₁₀ cfu per cm² at the end of carcass dressing in three abattoirs (Severini *et al.*, 2003).

The use of air inflation to assist in skin removal did not negatively affect the microbial load of ostrich carcasses (Severini *et al.*, 2003). A pre- de-feathering shower did not affect the carcass microbial load, but a final carcass washing did appear to reduce bacterial counts (Severini *et al.*, 2003). There was more than 90% reduction in total viable counts and *Enterobacteriaceae* when ozone gas or UV lights were used in chilling rooms for overnight cooling of ostrich legs immediately after slaughter (McKinnon *et al.*, 2005).

The shelf-life of vacuum packed retail ostrich meat decreased with storage time and there was a positive correlation between microbiological load and pH, with the lowest bacterial counts at pH ≤ 5.8 (Alonso-Calleja *et al.*, 2004). As previously described, the pH of most ostrich meat is higher than 5.8 so it would provide excellent conditions for microbial growth. The total viable counts, psychrotrophic counts, and *Enterobacteriaceae* on ostrich meat steaks through nine days were

higher at 10 °C than at 4 °C during both air and vacuum storage, with total counts, *Enterobacteriaceae*, and *Pseudomonas* approximately one log less and psychrotrophic counts two logs less in vacuum than in air (Capita *et al.*, 2006). There are differing reports on the contamination of ostrich meat with pathogenic bacteria, which may reflect different sanitary practices at different abattoirs or differences in live bird bacterial infections (Gill, 2007).

When there are outbreaks of avian flu virus in ratites, the export of fresh vacuum packed ostrich meat is halted and large volumes of ostrich meat are stored frozen. Examination of the refrigerated shelf-life of vacuum packaged, previously frozen (five-day) ostrich meat indicated that aerobic plate counts and psychrotrophic bacteria increased after 7 days and color decreased and unacceptable aroma developed by 14 days, prompting the recommendation that previously frozen vacuum packaged ostrich meat be used within 10 days (Otremba *et al.*, 1999). No studies on the quality characteristics of ostrich meat frozen for longer times have been reported, although more rapid thawing rates improve quality of frozen and thawed ostrich meat by increasing water holding capacity, and decreasing cooking losses compared with slower thawing rates (Hong *et al.*, 2005).

Deteriorative oxidative reactions in meat lead to losses of both nutritional value and food quality. Endogenous antioxidants added in feed can reduce oxidative processes in muscle tissues. Feed with 3.75% more vitamin E combined with vacuum packaging of the ostrich meat did not affect sensory evaluations of off-meat aroma, sourness, juiciness or mealiness, or influence rancidity or total viable counts during refrigerated storage, although coliform counts were slightly suppressed by vitamin E in the diet (Joubert *et al.*, 2003a, b; Hoffman *et al.*, 2007). Glutathione peroxidase is an endogenous enzyme that can function as an antioxidant. Ostrich muscle had lower levels of glutathione peroxidase and total selenium compared with chicken, turkey, duck, and lamb (Daun and Åkesson, 2004).

18.6 Value-added products from ostrich meat

Ostrich meat has been usually sold as vacuum-packaged muscles, but purge is highly increased with storage time of chilled meat (Joubert *et al.*, 2003b). Many processed products for avian species have been derived from transferring of red meat technologies.

Ground ostrich was rated lower than ground beef in a stew and a stir-fry, with both stews rated higher than stir-fries, while there was no influence on the relative ratings when the panellists had knowledge of the meat origin (Walter *et al.*, 2000). Low-fat ostrich meat patties made with pork lard or fat replacer (corn starch, soya isolate, water) were not different in trained sensory panel evaluations, even though the types of ostrich muscles for the products could be distinguished and patties from meat with a higher collagen content were more preferred (Hoffman and Mellett, 2003). Patties with the fat replacer had a higher PUFA profile and so this value-added product would maintain a 'healthier' chemical composition of the

ostrich muscles than incorporating pork fat. Addition of 0.2% rosemary extract to vacuum packaged ground ostrich meat patties inhibited lipid oxidation and protected color, while 2% sodium lactate also delayed oxidation and inhibited microorganism growth, but decreased redness of treated patties compared with controls (Seydim *et al.*, 2006b). Packaging of ground ostrich meat in high oxygen increased lipid oxidation, surface lightness, redness, and total color difference compared with nitrogen, vacuum and air packaging, which limited shelf-life to less than three days while meat in the other packaging was below saleable quality in less than six days (Seydim *et al.*, 2006a).

Salami from ostrich meat had higher texture and sensory evaluation with starter cultures rather than using glucono-delta-lactone (Böhme *et al.*, 1996). *Lactobacillus* starter cultures with bacteriocins inhibited *Listeria monocytogenes* in ostrich salami (Dicks *et al.*, 2004). Spanish ostrich salchichon had higher sensory acceptance when pork fat or ham fat was added, although this slowed the ripening process (Soriano *et al.*, 2007).

Dried plum puree incorporated into comminuted jerky from emu created a slightly sweeter, but tastier, product (Pegg *et al.*, 2006). The proximate compositions of finished emu and beef jerky (*Semimembranosus* muscles) were similar, with higher creatine levels in emu than beef jerky. The emu snack stick might be considered as a functional food for athletes who wish to enhance performance by consuming greater quantities of creatine from food rather than supplements (Pegg *et al.*, 2006).

Chopped hams and vienna from ostrich meat were highly acceptable, although color of ostrich viennas was darker, more red, and more blue than commercially purchased meat viennas (Fisher *et al.*, 2000). Bologna from ostrich meat was darker, more red, more blue, and was harder than bologna from beef, but the bologna from both *M. iliofibularis* and *M. femorotibialis* were acceptable in chemical composition and sensory evaluation, with bologna from *M. iliofibularis* receiving the highest sensory scores for general quality by experienced panellists (Fernández-López *et al.*, 2003). Traditional Thai 'Yor' sausage made with hydrocolloids (xanthan gum, carboxymethylcellulose and locust bean gum) had smaller fat droplets than control sausage, but use of 600 MPa and 50 °C for 40 min, which had completely denatured meat protein in previous experiments, gave fat surfactant behavior by the hydrocolloids rather than formation of networks as in conventional steaming to set the texture (Chatotong *et al.*, 2007). Increased pressure and temperature reduced water release and increased gel strength, lightness and blueness of 'Yor' sausage, but holding the given pressure and temperature for 40 minutes reduced released water compared with 60 minutes (Supavitpatana and Apichartsrangkoon, 2007).

Pâté campagne and pâté spread made with ostrich liver and pork fat had similar ratings for color intensity, odor, saltiness, rancidity, juiciness, and product quality by an experienced panel (Fernández-López *et al.*, 2004). The pâté spread was softer, more cohesive, gummier and less chewy in texture, had less fat, and was darker, more red and more yellow than the pâté campagne. Redness and oxidative stability (TBARS) of each pâté decreased with refrigerated storage time in vacuum

packaging, with color and fat oxidation changes more rapid in light than in dark storage (Fernández-López *et al.*, 2004).

Ponce-Alquicira *et al.* (2002) studied the effect of (brine) pH on ostrich meat color during curing and found that the meat became darker as the pH of the brine increased. The darker color of ostrich meat is attributed to higher myoglobin content than other red meats. The total iron was not higher, but there were higher proportions of non-heme iron in ostrich than for other red meats (Lombardi-Boccia *et al.*, 2002). Ostrich meat was also the only meat along with pork chump chop to have more of the total iron as heme iron in cooked form than in raw form (Lombardi-Boccia *et al.*, 2002), which indicated that the iron in ostrich does not readily dissociate to non-heme during heating. There would likely be more bioavailable iron and less initiation of oxidative processes by the more highly bound heme iron.

CDP and cathepsin D enzymes remained active under normal curing and processing conditions of pH and temperature (van Jaarsveld *et al.*, 1997b). Cathepsins B and L might have an important proteolytic role throughout the three stages of dry-curing of ostrich ham, but cathepsin H was active only during the middle and end of the dry-curing process (van Jaarsveld *et al.*, 1998).

18.7 Future trends

Based upon previous history, it is unlikely that the emu and rhea industries will develop into major meat-producing entities. The ostrich industry seems well established, particularly due to continuing demand for feathers and skins. The export of raw chilled ostrich meat is highly dependent upon control of the contagious avian diseases that is essential in the animal and meat trade. The surplus of ostrich meat in frozen storage due to past trade restrictions will dictate that methods of utilization for this meat as domestic or further processed products be developed. Heated or ready-to-eat products will be developed for export, but must be of superior quality to satisfy the upscale market that presently consumes ostrich meat.

Processors in Europe and America prefer to receive unprocessed muscles and manufacture cured and sausage products in their facilities. Expansion of domestic markets will depend upon suitable thawing and processing techniques to minimize economic losses and the examination of domestic meat competition and marketing strategies to allow surplus meat to gradually enter into domestic meat channels. Presently, ostrich steaks are vacuum packaged and sold as portions. Use of modified atmosphere packaging may enhance the color and shelf-life stability of ostrich meat as processors desire a minimum shelf-life of 40 days for chilled (0 °C) meat. With the slightly increased toughness of hot deboned meat negated within 7 days of aging at 4 °C, use of hot boning would result in one day less of storage time and shrinkage and provide refrigeration energy savings.

In order to produce meat of high quality and safety, the entire production line has to be considered and optimized, from pre-harvest (production, fattening house,

transportation of live animals), harvesting (slaughter) through post-harvest (processing, distribution, sale, consumer handling) (Blaha, 1997). The durability of stored meat depends both on its microbiological quality and its sensory quality (pH value, tenderness, etc.). Consequently, all people involved in the meat production and consumption chain must ultimately strive for the same goals (Nowak *et al.*, 2006). Improvements in each segment of the ostrich industry will require additional scientific studies and technological developments to improve ostrich meat quality.

18.8 Conclusions

Information about ostrich meat from research and other sources is more readily available than for emu or rhea meat. Concentrate feeding increases weight gains and generally results in greater fat deposition in the carcass, even though ratite meat has generally less intramuscular fat than other red meats. It is advised that ostriches not be held in lairage overnight because of loss of yields and pH changes. Dressing percentages of ratites are generally 52% for emus, 58% for ostriches, and 60% for rheas. The higher pH in meat from ratites results in higher water binding and darker color than for other red meats. Diet and lairage do not seem to influence ratite meat tenderness and juiciness, but there is conflicting information on the effect of animal age. It does not appear that post-mortem ageing is necessary for increased tenderization of ostrich muscles, but hot-boned meat requires aging to reach equivalent tenderness of cold-deboned muscle. There are differences in sensory properties among muscles, but all muscles have increased toughness with excessive heating. The limited information on value-added products indicates that the same unit operations used to process and preserve other red meat and avian species result in generally satisfactory products if the inherent properties of colour, low fat, and high pH are considered in designing them. The characteristics of ratite meats that make them different from other red meat also provide nutrition and health benefits through the decreased fat and sodium, and increased heme iron in cooked meat.

18.9 Sources of further information and advice

Sources of information about the ostrich industry are primarily books, trade associations, and private internet sites. Books include the *Ratite Encyclopedia* (1995, C Drenowatz, C Elrod and H Wilborn, Ratite Records Inc, 480 pages), *Ratite Production: Ostrich, Emu and Rhea* (2001, L E Gegner, ATTRA, 8 pages), *The Ostrich: Biology, Production and Health* (1999, D C Deeming, CABI Publishing, 368 pages), and *Ostrich Farming* (1994, J Batty, Silvio Mattacchione, 110 pages). Several ostrich associations have internet resources, including the World Ostrich Association (<http://www.world-ostrich.org>), American Ostrich Association (<http://www.ostriches.org/index.html>), and Canadian Ostrich Association (<http://www.ostrich.ca>). An international ostrich workshop is held periodically in different countries.

18.10 References

- Aberle, E D, Forrest, J C, Gerrard, D E and Mills, E W (2001), *Principles of Meat Science* (4th ed.), Dubuque, IA: Kendall/Hunt Publishing Company.
- Alonso-Calleja, C, Martínez-Fernández, B, Prieto, M and Capita, R (2004), 'Microbiological quality of vacuum-packed retail ostrich meat in Spain', *Fd. Microbiol.*, 21, 241–246.
- AMSA (1978), *Guidelines for Cookery and Sensory Evaluation of Meat*, Chicago, IL, American Meat Science Association, National Livestock and Meat Board.
- Berge, P, Lepetit, J, Renerre, M and Touraille, C (1997), 'Meat quality traits in the emu (*Dromaius novohollanae*) as affected by muscle type and animal age,' *Meat Science*, 45, 209–221.
- Blaha, T (1997), 'Public health and pork: Pre-harvest food safety and slaughter perspectives,' *Revue. Scien. Techn.*, 16, 489–495.
- Böhme, H M, Mellett, F D, Dicks, L M T and Basson, D S (1996), 'The use of ostrich meat in Italian type salami production,' *Meat Sci.*, 44, 173–180.
- Botha, S St C, Hoffman, L C and Britz, T J (2006), 'Effect of hot-deboning on the physical quality characteristics of ostrich meat,' *S. Afr. J. Anim. Sci.*, 36, 197–208.
- Botha, S St C, Hoffman, L C and Britz, T J (2004a), 'Muscle pH and temperature changes in ostrich *M. iliofibularis* and *M. gastrocnemius, pars interna* during the first 24 hours post-mortem,' In: *Proc. 2nd Joint Congr. Grassland Soc. Southern Africa and S. African Soc. Anim. Sci.*, p.152.
- Botha, S St C, Hoffman, L C, Britz, T J, Nilsen, B N and Slinde, E (2004b), 'The effect of rigor-temperature on isometric tension, shortening and pH for ostrich *M. gastrocnemius, pars interna*,' *Proc. 50th Intl. Congr. Meat Sci. Technol.*, p. 74.
- Botha, S St C (2005), *The effects of hot-deboning on the physical quality characteristics of ostrich (Struthio camelus) Muscularis gastrocnemius, pars interna and Muscularis iliofibularis*, MSc thesis, University of Stellenbosch, Stellenbosch, South Africa.
- Botha, S St C, Hoffman, L C and Britz, T J (2008), 'Effects of post-mortem temperature on isometric tension, shortening and pH in ostrich muscle,' *S. Afr. J. Anim. Sci.*, 38, 184–192.
- Brand, M M (2006), *Reproduction criteria and meat quality of South African Black (Struthio camelus var. Domesticus), Zimbabwean Blue (Struthio camelus Australis) and South African Black × Zimbabwean Blue ostriches*, MSc in Food Science, University of Stellenbosch, Stellenbosch, South Africa.
- Brand, T S, Gous, R M, HorbaDzuk, J O, Kruger, A C M, Aucamp, B B and Brand, Z. (2004), 'The effect of dietary energy and protein (amino acid) concentrations on the end products of slaughter ostriches,' *S. Afr. J. Anim. Sci.*, 34 Supplement 2, 107–109.
- Burger, W G (2005), 'Personal communication,' *Klein Karoo Cooperative*, Oudtshoorn, South Africa.
- Capita R, Díaz-Rodríguez, N, Prieto, M and Alonso-Calleja, C (2006), 'Effects of temperature, oxygen exclusion, and storage on the microbial loads and pH of packed ostrich steaks,' *Meat Sci.*, 73, 498–502.
- Chatotong, U, Apichartsrangkoon, A and Bell, A E (2007), 'Effects of hydrocolloid addition and high pressure processing on the rheological properties and microstructure of a commercial ostrich meat product 'Yor' (Thai sausage),' *Meat Sci.*, 76, 548–554.
- Cooper, R G and Horbańczuk, J O (2002), 'Anatomical and physiological characteristics of ostrich (*Struthio camelus* var. *domesticus*) meat determine its nutritional importance for man,' *Anim. Sci. J.*, 73, 167–173.
- Daun, C and Åkesson, B (2004), 'Comparison of glutathione peroxidase activity, and of total and soluble selenium content in two muscles from chicken, turkey, duck, ostrich and lamb,' *Fd. Chem.*, 85, 295–303.
- Davis, G W, Smith, G C, Carpenter, Z L, Dutson, T C and Cross, H R (1979), 'Tenderness variations among beef steaks from carcasses with the same USDA quality grade,' *J. Anim. Sci.*, 49, 103–107.
- Deeming, D C and Angel, C R (1996), 'Introduction to the ratites and farming

- operations around the world,' *First Intl. Sci. Ratite Congr. Proc.*, 2–3.
- Degen, A A, Kam, M, Rosenstrach, A and Plavnik, I (1991), 'Growth rate, total body water volume, dry-matter intake and water consumption of domesticated ostriches (*Struthio camelus*),' *Anim. Prod.*, 52, 225–232.
- Dicks, L M T, Mellett, F D and Hoffman, L C (2004), 'Use of bacteriocin-producing starter cultures of *Lactobacillus plantarum* and *Lactobacillus curvatus* in production of ostrich salami,' *Meat Sci.*, 66, 703–708.
- du Preez, J J, Jarvis, M J F, Capatos, D and de Kock, J (1992), 'A note on growth curves for the ostrich (*Struthio camelus*),' *Anim. Prod.*, 54, 150–152.
- Fernández-López, J, Sayas-Barberá, E and Pérez-Alvarez, J A (2004), 'Quality characteristics of ostrich liver pâté,' *J. Fd. Sci.*, 69, 85–91.
- Fernández-López, J, Sayas-Barberá, E, Navarro, C, Sendra, E and Pérez-Alvarez, J A (2003), 'Physical, chemical, and sensory properties of Bologna sausage made from ostrich meat,' *J. Fd. Sci.*, 68, 1511–1515.
- Fisher, P, Hoffman, L C and Mellett, F D (2000), 'Processing and nutritional characteristics of value added ostrich products,' *Meat Sci.*, 55, 251–254.
- Gill, C O (2007), 'Review microbiological conditions of meats from large game animals and birds,' *Meat Sci.*, 77, 149–160.
- Gill, C O, Jones, T, Bryant, J and Brereton, D A (2000), 'The microbiological conditions of the carcasses of six species after dressing at a small abattoir,' *Fd. Microbiol.*, 17, 233–239.
- Gillespie, J, Taylor, G, Schupp, A and Wirth, F (1998), 'Opinions of professional buyers towards a new, alternative red meat: Ostrich,' *Agribus.*, 14(3), 247–256.
- Girolami, A, Marsico, I, D'Andrea, G, Braghieri, A Napolitano, F and Cifuni, G F (2003), 'Fatty acid profile, cholesterol content and tenderness of ostrich meat as influenced by age at slaughter and muscle type,' *Meat Sci.*, 64, 309–315.
- Harris, S D, Morris, C A, May, S G, Lucia, L M, Jackson, T C, Hale, D S, Miller, R K, Keeton, J T, Savell, J W and Acuff, G R (1993), *Ostrich Meat Industry Final Report*, Am Ostrich Assoc, Fort Worth, Texas.
- Hoffman, L C and Fisher, P (2001), 'Comparison of meat quality characteristics between young and old ostriches,' *Meat Sci.*, 59, 335–337.
- Hoffman, L C and Mellett, F D (2003), 'Quality characteristics of low fat ostrich meat patties formulated with either pork lard or modified corn starch, soya isolate and water,' *Meat Sci.*, 65, 869–875.
- Hoffman, L C, Botha, S St C and Britz, T J (2006), 'Sensory properties of hot-deboned ostrich (*Struthio camelus* var. *domesticus*) *Muscularis gastrocnemius, pars interna*,' *Meat Sci.*, 72, 734–740.
- Hoffman, L C, Botha, S St C and Britz, T J (2007), 'Muscle pH and temperature changes in hot- and cold-deboned ostrich (*Struthio camelus* var. *domesticus*) *Muscularis gastrocnemius, pars interna* and *Muscularis iliofibularis* during the first 23 h post-mortem,' *Meat Sci.*, 75, 343–349.
- Hoffman, L C, Joubert, M, Brand, T S and Manley, M (2005), 'The effect of dietary fish oil rich in *n*-3 fatty acids on the organoleptic, fatty acid and physicochemical characteristics of ostrich meat,' *Meat Sci.*, 70, 45–53.
- Hong, G-P, Park, S-H, Kim, J-Y, Lee, C-H, Lee, S and Min, S-G (2005), 'The effect of thawing rate on the physicochemical properties of frozen ostrich meat,' *Fd. Sci. Biotech.*, 14, 676–680.
- Honikel, K O, Roncalés, P and Hamm, R (1983), 'The influence of temperature on shortening and rigor onset in beef muscle,' *Meat Sci.*, 8, 221–241.
- Horbańczuk, J O and Sales, J (1998), 'Total lipid and cholesterol content and fatty acid composition of meat obtained from ostriches reared on a commercial farm,' *Anim. Sci. Pap. Rep.*, 1, 51–55.
- Horbańczuk, J, Sales, J, Celeda, T, Konecka, A, Zieba, G and Kawka, P (1998), 'Cholesterol content and fatty acid composition of ostrich meat as influenced by subspecies,' *Meat Sci.*, 50, 385–388.

- Horbańczuk, J O, Cooper, R G, Józwik, A, Klewec, J, Krzyewski, J, Malecki, I, Chyliński, W, Wójcik, A and Kawka, M (2003), 'Cholesterol content and fatty acid composition of fat from culled breeding ostriches (*Struthio camelus*),' *Anim. Sci. Pap. Rep.*, 21, 271–275.
- Horbańczuk, J O, Cooper, R G, Józwik, A, Klewec, J, Krzyewski, J, Chyliński, W, Kubasik, W, Wójcik, A and Kawka, M (2004), 'Total fat, cholesterol and fatty acids of meat of grey nandu (*Rhea americana*),' *Anim. Sci. Pap. Rep.*, 22(2), 253–257.
- Horbańczuk, J O, Malecki, I, Cooper, R G, Józwik, A, Klewec, J, Krzyewski, J, Khalifa, H, Chyliński, W, Wójcik, A and Kawka, M (2004), 'Cholesterol content and fatty acid composition of two fat depots from slaughter ostriches (*Struthio camelus*) aged 14 months,' *Anim. Sci. Pap. Rep.*, 22, 247–251.
- Huffman, K L, Miller, M F, Hoover, L C, Wu, C K, Brittin, H C, and Ramsey, C B (1996), 'Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant,' *J. Anim. Sci.*, 74(1), 91–97.
- Hwang, I H, Devine, C E and Hopkins, D L (2003), 'The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness,' *Meat Sci.*, 65, 677–691.
- Jones, S D M, Robertson, W M and Brereton, D A (1994), 'The ostrich as a meat animal,' *Report for the Canadian Ostrich Association*, Agriculture and Agri-Food, Canada, Lacombe, Alberta, 20 pages.
- Joubert, M, Hoffman, L C, Brand, T and Manley, M (2003a), 'The effect of dietary vitamin E and organic selenium on the shelf life of ostrich meat,' *Consistency of Quality: Abstr. Proc. 11th Intl. Meat. Symp.*, Centurion, South Africa.
- Joubert, M, Hoffman, L C, Brand, T and Manley, M (2003b), 'The effect of heat shrink treatment on the shelf life of ostrich meat,' *Consistency of Quality: Abstr. Proc. 11th Intl. Meat Symp.*, Centurion, South Africa.
- Kubberød, E, Ueland, Ø, Rødbotten, M, Westad, F and Risvik, E (2002), 'Gender specific preferences and attitudes towards meat,' *Fd. Qual. Pref.*, 13, 285–294.
- Lambooij, E, Potgieter, C M, Britz, C M, Nortjé, G L and Pieterse, C (1999a), 'Effects of electrical stunning methods on meat quality in ostriches,' *Meat Sci.*, 52, 331–337.
- Lambooij, E, Pieterse, C, Potgieter, C M, Snyman, J D and Nortjé, G L (1999b), 'Some neural and behavioural aspects of electrical and mechanical stunning in ostriches,' *Meat Sci.*, 52, 339–345.
- Lanza, M, Fasone, V, Galofaro, V, Barbagallo, D, Bella, M and Pennisi, P (2004), 'Citrus pulp as an ingredient in ostrich diet: Effects on meat quality,' *Meat Sci.*, 68, 269–275.
- Lawrie, R A (1998), *Lawrie's Meat Science* (6th ed), Cambridge, England: Woodhead Publishing Limited.
- Lepetit, J and Culioli, J (1994), 'Mechanical properties of meat,' *Meat Sci.*, 36, 203–237.
- Lombardi-Boccia, G, Martinez-Dominguez, B and Aguzzi, A (2002), 'Total heme and non-heme iron from raw and cooked meats,' *J. Fd. Sci.*, 67, 1738–1741.
- Madeiros, C A (1995), 'An introduction to the ostrich,' In: 2 *TOE, The Practical Guide to Ostrich Farming*, West Bar Veterinary Hospital, Banbury, Oxfordshire.
- Mancini, R A and Hunt, M C (2005), 'Current research in meat color,' *Meat Sci.*, 71, 100–212.
- Marks, J, Stadelman, W, Linton, R, Schmieder, H and Adams, R (1998), 'Tenderness analysis and consumer sensory evaluation of ostrich meat from different muscles and different aging times,' *J. Fd. Qual.*, 21, 369–381.
- McKinnon, D C, Heyneke, P G, Olivier, A J, Mulder, C, Britz, T J and Hoffman, L C (2005), 'The effect of treatment of ostrich carcasses with ozone and UV on muscle surface microbial load,' In: *Proc. 3rd Intl. Ratite Sci. Symp. World's Poul. Sci. Assoc.*, Madrid, p 276.
- Melanson, K, Gootman, J, Myrdal, A, Kline, G. and Rippe, J M (2003), 'Weight loss and total lipid profile changes in overweight women consuming beef or chicken as the primary protein source,' *Nutr.*, 19, 409–414.
- Mellet, F D (1994), 'A note on the musculature of the proximal part of the pelvic limb of the ostrich (*Struthio camelus*),' *J. So. Afr. Vet. Assoc.*, 65, 5–9.

- Mellet, F D and Randall, J H (1994), 'A note on the growth of body parts of the ostrich (*Struthio camelus*),' *Anim. Prod.*, 58, 291–293.
- Mellet, F D and Sales, J (1997), 'Tenderness of ostrich meat,' *So. Afr. J. Fd. Nutr.*, 9(1), 27–29.
- Miller, R K (2004), 'Palatability,' In W K Jensen, C Devine and M Dikeman (eds), *Encyclopedia of Meat Sciences*, Elsevier, Amsterdam, 256–266.
- Morris, C A, Harris, S D, May, S G, Jackson, T C, Hale, D S, Miller, R K, Keeton, J T, Acuff, G R, Lucia, L M and Savell, J W (1995a), 'Ostrich slaughter and fabrication. 1. Slaughter yields of carcasses and effects of electrical stimulation and post-mortem pH,' *Poul. Sci.*, 74, 1683–1687.
- Morris, C A, Harris, S D, May, S G, Jackson, T C, Hale, D S, Miller, R K, Keeton, J T, Acuff, G R, Lucia, L M and Savell, J W (1995b), 'Carcass weights, fabrication yields and muscle color evaluation,' *Poul. Sci.*, 74, 1688–1692.
- Nitzan, R, Barkai, D, Nitzan, Z and Landau, S (2002), 'Intake, growth and carcass characteristics of young ostriches given concentrates with and without pasture,' *Anim. Sci.*, 74, 71–79.
- Nowak, B, Sammet, K, Klein, G and van Mueffling, T (2006), 'Trends in the production and storage of fresh meat – the holistic approach to bacteriological meat quality,' *Int. J. Fd. Sci. Technol.*, 41, 303–310.
- Otremba, M M, Dikeman, M E and Boyle, E A E (1999), 'Refrigerated shelf life of vacuum-packaged, previously frozen ostrich meat,' *Meat Sci.*, 52, 279–283.
- Paleari, M A, Camisasca, S, Beretta, G, Renon, P, Corisco, P, Bertolo, G and Crivelli, G (1998) 'Ostrich meat: Physico-chemical characteristics and comparison with turkey and bovine meat,' *Meat Sci.*, 48 (3/4), 205–210.
- Paleari, M A, Corsico, P and Beretta, G (1995), 'The ostrich: breeding, reproduction, slaughtering and nutritional value of the meat,' *Fleischwirts.*, 75 (9), 1120–1123.
- Pambuka, S E, Adebisi, A P, Muramoto, K and Naudé, R J (2007), 'Purification and partial characterisation of a matrix metalloproteinase from ostrich skeletal muscle, and its activity during meat maturation,' *Meat Sci.*, 76, 481–488.
- Patak, A and Baldwin, J (1993), 'Structural and metabolic characterization of the muscles used for power running in the emu (*Dromaius novaehollandiae*), a giant flightless bird,' *J. Exp. Biol.*, 175, 233–249.
- Pegg, R B, Amarowicz, R and Code, W E (2006), 'Nutritional characteristics of emu (*Dromaius novaehollandiae*) meat and its value-added products,' *Food Chem.*, 97, 193–202.
- Pollok, K D, Hale, D S, Miller, R K, Angel, R, Blue-McLendon, A, Baltmanis, B and Keeton, J T (1997a), 'Ostrich slaughter and by-product yields,' *Am. Ostrich*, 4, 31–35.
- Pollok, K D, Miller, R K, Hale, D S, Angel, R, Blue-McLendon, A, Baltmanis, B, Keeton, J T and Maca, J V (1997b), 'Ostrich carcass and meat yields,' *Am. Ostrich*, 4, 36–38.
- Pollok, K D, Hale, D S, Herber-McNeill, S, Miller, R K, Angel, R, Blue-McLendon, A, Baltmanis, B and Keeton, J T (1997c), 'The nutritional profile of cooked and raw ostrich meat,' *Am. Ostrich*, 4, 39–45.
- Pollok, K D, Miller, R K, Hale, D S, Angel, R, Blue-McLendon, A, Baltmanis, B, Keeton, J T and Maca, J V (1997d), 'Quality of ostrich steaks as affected by vacuum-package storage, retail display and differences in animal feeding regime,' *Am. Ostrich*, 4, 46–52.
- Ponce-Alquicira, E, Kuri-Rojas, R, Signorini, M, Pérez-Chabela, L and Guerrero-Legarreta, I (2002), 'Changing ostrich meat color during curing as affected by pH,' *Proc. 48th Intl. Congr. Meat Sci. Technol.*, 520–521.
- Risvik, E (1994), 'Sensory properties and preferences,' *Meat Sci.*, 36, 67–77.
- Rødbotten, M, Kubberød, E, Lea, P and Ueland, Ø (2004), 'A sensory map of the meat universe – sensory profile of meat from 15 species,' *Meat Sci.*, 68, 137–144.
- Rosser, B W C and George, J C (1985), 'Histochemical characterization and distribution of fiber types in the pectoralis muscle of the ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*),' *Acta Zool.*, 66 (4), 191–198.

- Sabbioni, A, Superchi, P, Sussi, C, Quarantelli, A, Bracchi, P G, Pizza, A, Barbieri, G, Beretti, V, Zanon, A, Zambini, E M and Renzi, M (2003), 'Factors affecting ostrich meat composition and quality,' *Ann. Fac. Med. Vet. Di Parma*, 23, 243–252.
- Sales, J (1994), *Die Identifisering en Verbetering van Kwaliteitsienskappe van Volstruisvleis*, PhD in Animal Science Thesis, University of Stellenbosch, Stellenbosch, South Africa.
- Sales, J (1996), 'Histological, biophysical, physical and chemical characteristics of different ostrich muscles,' *J. Sci. Fd. Agric.*, 70, 109–114.
- Sales, J (1997), 'Effect of cooking on the quality of ostrich muscles,' *J. Fd. Sci. Technol.*, 34(6), 501–502.
- Sales, J (1998), 'Fatty acid composition and cholesterol content of different ostrich muscles,' *Meat Sci.*, 49, 489–492.
- Sales, J (1999), 'Slaughter and products,' In: *The Ostrich, Biology, Production and Health* (ed. D.C. Deeming), Wallingford, Oxon, UK: CABI Publishing, pp. 231–274.
- Sales, J (2006), 'Digestive physiology and nutrition of ratites,' *Avian Poul. Biol. Rev.*, 17(2), 41–55.
- Sales, J and Hayes, J P (1996), 'Proximate, amino acid and mineral composition of ostrich meat,' *Fd. Chem.*, 56, 167–170.
- Sales, J and Horbańczuk, J (1998), 'Ratite meat,' *World's Poul. Sci. J.*, 54, 59–67.
- Sales, J and Horbańczuk, J (1999), 'Cold-shortening in different ostrich muscles,' *Czech. J. Anim. Sci.*, 44, 273–275.
- Sales, J and Mellett, F D (1995), 'The determination of the age of ostrich carcasses from ossification of the pectoral girdle (*ossa cinguli membrii thoracici*),' *Anim. Sci.*, 60, 499–501.
- Sales, J and Mellett, F D (1996), 'Post-mortem pH decline in different ostrich muscles,' *Meat Sci.*, 42, 235–238.
- Sales, J and Oliver-Lyons, B (1996), 'Ostrich meat: A review,' *Fd. Australia*, 48, 504–511.
- Sales, J, Horbańczuk, J, Dingle, J, Coleman, R and Sensik, S (1999a), 'Carcass characteristics of emus (*Dromaius novaehollandiae*),' *Br. Poul. Sci.*, 40, 145–147.
- Sales, J, Marais, D and Kruger, M (1996a), 'Fat content, caloric value, cholesterol content, and fatty acid composition of raw and cooked ostrich meat,' *J. Fd. Comp. Anal.*, 9, 85–89.
- Sales, J, Mellett, F D and Heydenrych, H J (1996b), 'Ultrastructural changes in ostrich muscles during post-mortem aging,' *So. Afr. J. Fd. Nutr.*, 8, 23–25.
- Sales, J, Navarro, J L, Bellis, L, Manero, A, Lizurume, M, and Martella, M B (1997), 'Carcass and component yields of rheas,' *Br. Poul. Sci.*, 38, 378–380.
- Sales, J, Navarro, J L, Bellis, L, Manero, A, Lizurume, M, and Martella, M B (1998), 'Post mortem pH decline as influenced by species in different rhea muscles,' *Vet. J.*, 155, 201–211.
- Sales, J, Navarro, J L, Martella, M B, Lizurume, M E, Manero, A, Bellis, L and Garcia, P T (1999b), 'Cholesterol content and fatty acid composition in rhea meat,' *Meat Sci.*, 53, 73–75.
- Schaefer, A L, Robertson W M, and Brereton, D A (1997), 'Carcass yield and meat quality of ostriches under two different antemortem management regimes. (II),' *Final Report for the Canadian Ostrich Assoc.*, 35 pages.
- Severini, M, Ranucci, D, Miraglia, D and Branciari, R (2003), 'Preliminary study on the microbiological quality of ostrich (*Struthio camelus*) carcasses dressed in small Italian abattoirs,' *Ital. J. Fd. Sci.*, 15, 295–300.
- Seydim, A C, Acton, J C, Hall, M A and Dawson P L (2006a), 'Effects of packaging atmospheres on shelf-life quality of ground ostrich meat,' *Meat Sci.*, 73, 503–510.
- Seydim, A C, Guzel-Seydim, Z B, Acton, J C and Dawson, P L (2006b), 'Effects of rosemary extract and sodium lactate on quality of vacuum-packaged ground ostrich meat,' *J. Fd. Sci.*, 71, S71–S76.
- Soriano A, Garcia Ruiz, A, Gómez, E, Pardon, R, Gálan and González Viñas, M A (2007),

- 'Lipolysis, proteolysis, physicochemical and sensory characteristics of different types of Spanish ostrich salchicon,' *Meat Sci.*, 75, 661–668.
- Supavititpatana, T and Apichartsrangkoon, A (2007), 'Combination effects of ultra-high pressure and temperature on the physical and thermal properties of ostrich meat sausage,' *Meat Sci.*, 76, 555–560.
- Swart, D, Siebrits, F K, Hayes, J P (1993), 'Growth, feed intake and body composition of ostriches (*Struthio camelus*) between 10 and 30 kg live mass,' *S. Afr. J. Anim. Sci.*, 23, 142–150.
- Thomas, A R, Gondoza, H, Hoffman, L C, Oosthuizen, V. and Naudé, R J (2004), 'The roles of the proteasome and cathepsins B, L, H and D, in ostrich meat tenderisation', *Meat Sci.*, 67, 113–120.
- van Jaarsveld, F P, Naudé, R J and Oelofsen, W (1997a), 'The effects of Ca ions, EGTA and storage time on myofibrillar protein degradation, levels of Ca²⁺-dependent proteases and cathepsins B, H, L and D of ostrich skeletal muscle', *Meat Sci.*, 45 (4), 517–529.
- van Jaarsveld, F P, Naudé, R J and Oelofsen, W (1997b), 'Optimisation of calcium-dependent protease and cathepsin D assays in ostrich muscle and the effect of chemical and physical dry-curing parameters', *Meat Sci.*, 47 (3/4), 287–299.
- van Jaarsveld, F P, Naudé, R J and Oelofsen, W (1998), 'Effect of chemical and physical dry-curing parameters on cathepsins B, H, and L from ostrich muscle', *Meat Sci.*, 50 (2), 223–233.
- van Schalkwyk, S J, Cloete, S W P, Hoffman, L C, Brand, T S, Brand, Z and Lambrechts, H (2002), 'The effect of dietary energy and protein levels on meat quality,' In: *Proc. 2nd Joint Cong. Grassland Soc. So. Afr. and S. Afr. Soc. Anim. Sci.*, Christiana, p 84.
- van Schalkwyk, S J, Hoffman, L C, Cloete, S W P and Mellett, F D (2005), 'The effect of feed withdrawal during lairage on meat quality characteristics in ostriches,' *Meat Sci.*, 69, 647–651.
- Velotto, S and Crasto, A (2004), 'Histochemical and morphometrical characterization and distribution of fibre types in four muscles of ostrich (*Struthio camelus*),' *Anat. Histol. Embryol. J. Vet. Med. Series C*, 33 (4), 251–256.
- Viljoen, M, Hoffman, L C and Brand, T S (2005), 'Prediction of the chemical composition of freeze dried ostrich meat with near infrared reflectance spectroscopy,' *Meat Sci.*, 69, 255–261.
- Walter, J M, Soliah, L and Dorsett, D (2000), 'Ground ostrich: A comparison with ground beef,' *J. Amer. Diet Assoc.*, 100, 244–245.
- Wood, J D, Richardson, R I, Nute, G R, Fisher, A V, Camp, M M, Kasapidou E, Sheard, P R, and Enser, M (2004), 'Effects of fatty acids on meat quality: A review,' *Meat Sci.*, 66, 21–32.
- Wotton, S and Sparrey, J (2002), 'Stunning and slaughter of ostriches,' *Meat Sci.*, 60, 389–394.
- Xiong, L Y (2005), 'Role of myofibrillar proteins in water-binding in brine-enhanced meats,' *Fd. Res. Intl.*, 38, 281–287.

Improving the meat quality of venison and other exotic game

L. C. Hoffman, Stellenbosch University, South Africa and K. W. McMillin, Louisiana State University Agricultural Center, USA

Abstract: The effect of various external factors on the sensory quality of venison and game meat is reviewed. As cervid species and wild boar have been domesticated and farmed for a number of years, there are more data available on these effects than there are for the wild free-roaming African game species. Most of these effects are similar in magnitude to that experienced in the traditionally farmed monogastric animals and ruminants, e.g. change in fatty acid profiles to reflect that of the diet in monogastrics. With the farmed species, greater control over the transport, lairage and stunning/ slaughter processes is possible, resulting in more uniform practices being developed that ultimately lead to a more consistent meat quality. However, with the wild game species this is not the case and research has focused on finding methodologies that best control the harvesting of the various species. Various value-added techniques used in the traditional red meat processing have been adapted and are used successfully in these wild species.

Key words: deer, wild boar, African game, fatty acids, value adding, wildlife utilization.

19.1 Introduction

A large number of the deer species found in the world have adapted well to domestication because most of the deer harvested for human consumption originate from herds where man has some form of control over the production process. The production may be in the form of a free-ranging herd under the control of herdsman, as found in Alaska (Wiklund and Malmfors, 2004) or a herd finished in a feedlot (Volpelli *et al.*, 2002). Fletcher (1994) reported on a historical perspective of why farmers choose to domesticate deer and farm with these cervids; some advantages and disadvantages that are still relevant today to the semi-intensive domestication of deer are also listed. The history of reindeer

production (particularly by the Saami people) in the Northern hemisphere has changed from an intensive herding system to a more extensive system using modern aids such as trucks, helicopters, motorbikes and snowmobiles to help with the herding (Malmfors and Wiklund, 1996; Wiklund and Malmfors, 2004). A production system that could be considered as an intermediate between the wild animals in Africa and intensively farmed deer is the herding of semi-domestic reindeer (*Rangifer tarandus tarandus*) in the Nordic countries and Alaska. Reindeer are free-ranging out in the forest or mountain tundra, but handled twice a year, in July, for marking of new calves and in December to be slaughtered (Hoffman and Wiklund, 2006).

The Africa game species that are harvested, on the other hand, are either feral or loosely confined in large commercial farms by various forms of fencing (Eloff, 2002). Confinement of many African game species is difficult, however, as many of them, such as the kudu (*Tragelaphus strepsiceros*) are able to jump over 2 m high fences. The concept of ranching African game arose in the late 1950s with research being conducted in Zimbabwe (Dasmann and Mossman, 1960). Prior to this, game was found throughout Africa, but had very little commercial value. It was only in the late 1960s that the potential of game for meat production was recognized (Ledger, 1963; Ledger, Sachs and Smith, 1967; Von la Chevallerie, 1970). Most of the studies conducted towards the end of the 1900s consisted of recording basic information such as growth and yields (Von la Chevallerie and van Zyl, 1971; Von la Chevallerie, 1972), and any data collected on the meat composition (or quality) were focused more towards the effects of extreme environmental conditions, such as the droughts experienced towards the end of winter in Africa (see, for example, Van Rooyen, 1993).

Some of the attributes required by wild ungulate species for domestication include non-territorial behaviour, a tolerance for other males, a high fecundity, a pre-disposal towards herding, and social behavioural patterns compatible with that of humans (Clutton-Brock, 1992). Such species should be gregarious, breed readily in captivity, and have a wide home range and a short flight distance. An example of such a species is the eland (*Taurotragus oryx*). However, a trial in Zimbabwe that began in the early 1950s and ran for 30 years in an attempt to domesticate this species and farm it, together with cattle, failed due to a number of reasons. The dominant causes for the lack of successful domestication were parasite burdens, a density-dependent decrease in their fertility, and a decrease in their overall body condition because most wild ungulates are concentrate selectors whilst the farmed animals are generalized feeders (Kyle, 1994). Goats and sheep and most cervids fulfil these conditions, but gazelles (such as the Springbok *Antidorcas marsupialis*) do not and thus are not suitable candidates for domestication (Skinner and Louw, 1996).

The wild boar (*Sus scrofa scrofa* L.) has been present in parts of Europe and Asia since historical times and has disappeared from regions due to man's influence (normally due to deforestation) and then reappeared again, either from wild invasions or from man's introduction. Various strategies have been employed to manage these wild populations by means of hunting – normally by hunting

teams using hound packs (The third issue of the *Ibex Journal of Mountain Ecology*, 1995, provides a good overview of the management strategies employed to control population numbers). McIlroy (1995) reviews the various strategies such as use of helicopters for shooting and poisoning campaigns that have been used to control feral wild boar populations in Australia. By contrast with most European countries, the European wild boar has not existed in the wild in the UK for 700 years (Booth, 1995). In the late 1980s, people started to farm with wild boar in the UK. The communication by Booth (1995) provides an overview of the history and progress of wild boar farming in the UK. Most of the farmed systems now consist of an extensive system with large paddocks (over 1 ha each) for a family herd, e.g. a boar with up to 10 sows, dry sows and weaners/growers, rotated to new paddocks after 1 year. These animals are fed a mixture of commercial feed and forage vegetables. As wild boars do not occur in the wild in the UK, they are classified as pig meat and therefore they have been slaughtered in abattoirs. However, this is a hazardous procedure for both the animals and the handlers, and shooting on the farm (as for deer) is now considered the most desirable method. These carcasses then go through the normal veterinarian health inspections to ensure that they are fit for human consumption.

In the review of the prevalent microbes found on wild boar and feral pig meat, Gill (2007) also noted that these animals are normally skinned (and not scalded, dehaired and singed as is the practice with commercially farmed pigs). This is mainly to remove the thick black hairs found in these 'wild' pigs. This is also the practice for the wild warthog (*Phacochoerus africanus*) found in Africa.

As soon as a species has become domesticated, man can then start manipulating the ante-mortem factors (diet, age at slaughter, selection of breeding stock for a specific trait, etc.) that can improve the meat and sensory quality of the end product. Similarly, the factors prior to the slaughter of the animal are under control resulting in a better quality product. This is the reason why a large number of the deer species are farmed and managed under a production system very similar to that used for the traditionally farmed domestic species. On the other hand, the African ungulate species are, with a few exceptions, not suitable for domestication, and the control over the end product is not always ideal. Most of the exotic game species are inherently wild and have developed survival instincts that make them successful in the wild. Thus, these species are perhaps even more pre-disposed to the effects of stress than many of the domesticated species because the first reaction to any external stimuli (such as the stress induced during harvesting) typically is the flight or fight reaction, which increases the associated hormone levels. For this reason, behaviour must be considered when designing the handling, transporting (if applicable), and holding facilities at the farm and/or abattoir if optimal meat quality and the welfare of the animal are to be achieved (Renecker *et al.*, 2001). Of the species harvested in southern Africa, the Springbok is the most prevalent and most of the research to date has been conducted on the growth and production, and lately, on the meat quality of this species.

19.2 Improving meat quality by means of the production system

19.2.1 Genetic selection

Although deer farmers do select the stags that they wish to use as future breeding stock according to size, growth rate, antler characteristics and other desired traits, there are no reports of the effects of selection on the meat quality of the offspring. Hybrid (75% red deer *Cervus elaphus*: 25% Elk) stags and hinds had significantly high growth rates, carcass weights and dressout percentages when slaughtered at the same age and fed the same diet. The stags also grew faster and were heavier than the hinds (Hoskin *et al.*, 1999). Similar results were also found with Scottish red deer (*C. elaphus scoticus*) compared with hybrids containing Père David's deer (*Elaphurus davidianus*, PD) (Goosen *et al.*, 1991). The stags were also noted to contain less total carcass fat than the hinds. An interesting phenomenon in that study was the significantly different muscle tissue distribution in Père David's hybrids than in the red deer. The authors postulated that the 5% larger hind leg total in the hybrids could be indicative of a major gene effect similar to double-muscling observed in cattle and callipyge in sheep. The hybrids had larger *vastus* (6%), *rectus femoris* (6%), *semitendinosus* (9%), *gastrocnemius* (10%) muscles compared with the red deer. There were no significant differences in shear force values of muscles between the genotypes, but the *M. longissimus dorsi* of the stags were tougher than the hinds.

Bison (*Bison bison*) and their hybrids with cattle (*Bos taurus*) had more lean meat and less fat trim than purebred cattle. However, the bison had a thicker fat depth at the 12th rib than cattle since most of the carcass fat of bison is located over the thoracic area. The bison and their hybrid also had a lower proportion of their carcass in the hindquarter than the *Bos taurus*. Bison meat was more tender as shear force and tenderness scores and had a flavour different from that of *Bos taurus* (Koch *et al.*, 1995).

Most of the African wildlife species have musculature similar to that of the general bovid pattern. There are, however, some species-specific variations. For example, the springbok has exceptionally large *M. longissimus thoracis et lumborum* (Skinner *et al.*, 1971), but the fibre diameter is very small compared to other African ungulates, resulting in it having a low level of toughness (Table 19.1). Most of the increase in fibre diameter in the springbok occurs within the first 28 weeks of age (Von la Chevallerie and van Zyl, 1971).

The only anecdotal report of improvement of African game species is that where a number of farmers have imported the larger Kalahari springbok (body weights of males 41.6 and females 35.4 kg) into the Karoo region in South Africa to breed with the smaller local animals (body weights of males 31.2 and females 26.5 kg, Skinner and Louw, 1996), the resulting offspring are larger, although this is attributed to heterosis as their offspring are once more smaller in weight. It is postulated that the South African springbok are smaller due to insufficient nutrition.

Wild boar readily cross-breed with domestic pigs and this phenomenon has been

Table 19.1 Dressing percentage and meat quality in mature males of four antelope species (adapted from Skinner and Louw, 1996)

	Springbok	Eland	Impala	Blesbok
Number of animals	72	6	18	23
Dressing (%)	56	51	59	53
Moisture content (%)	74.5	74.8	75.7	75.5
Buttock fat content	1.7	2.4	1.4	1.7
Colour	7.3	5.9	7.4	7.9
Fibre diameter (μm)	45.5	66.3	56.7	53.8
Toughness (g/cm)	1181	3366	2751	2323
Taste panel scores for flavour (10 points = highest)				
Number of animals	36	3	9	11
Intensity	4.2	4.4	4.0	4.9
Acceptability	6.1	5.3	5.2	5.8

exploited by producers/farmers/hunters (even in Japan – Kanzaki and Kodera, 1995) to improve the litter size, growth rate and survival rate of the offspring. Using the wild boar in a cross-breeding program, Muller and co-workers (2000) showed that lean cuts and meat-to-fat ratio indicated a higher meat percentage in wild boar than for Meishan (the latter were more fat), although the wild boar (and its crosses) had slower growth rates. The cooling loss was also significantly higher for the wild boar than for the Meishan or Pietrain breeds. Wild boar also had the lowest dressing yield – caused by their heavier heart and liver weights. As pertaining to the meat quality, the wild boars and their crosses had darker meat (this was ascribed to a higher red fibre proportion – 28.4% for wild boar compared to 14.7% for Large White breeds; Essén-Gustavsson and Lindholm, 1984). The authors also noted that all the Wild boars tested were stress resistant (CK_{20}^- , CRC- and Halothane tests). Marchiori and de Felicio (2003) characterized the meat quality of wild boar (raised in a semiconfinement area) found in Brazil with local domestic breeds (crosses between Large White, Landrace and Pietrain, raised in a confinement system) and found that the muscle pH decrease was more gradual in the wild boar. The commercial pigs also had a lower 48 h pH than the wild boar. A comparison of the L^* , a^* and b^* values indicated that the wild boar also had the darker muscles (in both the *Longissimus dorsi* and *Semimembranosus* muscles).

There have been indications that game meat sold as Japanese wild boar is adulterated by cross-breeding between pigs and wild boars or by contamination with meat from domestic pigs or European wild boars (Naya *et al.*, 2003). In Japan there are two subspecies of wild boar: the Japanese wild boar (*S. s. leucomystax*) and the Ryukyu (*S. s. riukiuanus*).

Nii and co-workers (2005) analysed the quantitative trait loci (QTL) of a number of meat traits in a cross-population of wild boar \times Large White pigs and found that for muscle fibre composition, wild boar alleles had favourable effects on meat quality. These authors further speculated that wild boar containing these

Table 19.2 Mean values for fatty acid composition (g/kg total fatty acids) in *M. longissimus* from pasture and pellet-fed reindeer (*Rangifer tarandus tarandus* L) and red deer (*Cervus elaphus*), respectively

Fatty acid	Reindeer ^a			Red deer ^b		
	Pasture (n = 9)	Pellets (n = 6)	Degree of significance	Pasture (n = 7)	Pellets (n = 7)	Degree of significance
Polar lipids						
14:0	2.1	2.9	n.s.			
16:0	12.6	13.8	n.s.	10.1	10.3	n.s.
16:1	0.6	0.9	**	1.1	0.4	**
17:0	0.4	0.2	***			
17:1	0.4	0.2	***			
18:0	12.4	13.4	*	15.8	14.1	*
18:1	3.4	2.0	***	12.3	12.4	n.s.
18:1 (<i>trans</i>)	0.4	0.3	**			
18:1 (<i>n-9</i>)	11.5	12.0	n.s.			
18:1 (<i>n-7</i>)	1.0	1.7	***			
18:2 (<i>n-6</i>)	21.1	27.6	***	20.3	29.8	***
18:3 (<i>n-3</i>)	6.1	1.2	***	5.2	0.2	***
20:3 (<i>n-3</i>)	6.0	8.0	***	1.0	1.3	***
20:4 (<i>n-6</i>)	10.2	9.5	n.s.	9.0	12.1	***
20:5 (<i>n-3</i>)	2.7	1.6	***	3.0	0.8	***
22:4 (<i>n-6</i>)	6.0	6.0	n.s.			
22:5 (<i>n-3</i>)	4.6	3.3	***	4.0	1.9	***
22:6 (<i>n-3</i>)	2.0	2.0	*	0.9	0.2	***
SFA	25.4	26.3	n.s.	25.9	24.4	n.s.
MUFA	17.3	16.0	*	13.8	12.4	n.s.
PUFA (<i>n-6</i>)	31.9	39.4	***	29.3	41.9	***
PUFA (<i>n-3</i>)	14.2	7.5	***	14.2	4.5	***
(<i>n-6</i>)/(<i>n-3</i>)	2.2	0.53	***	2.1	9.3	***
Neutral lipids						
12:0	4.5	3.5	**			
14:0	1.7	1.8	n.s.	5.0	6.1	n.s.
14:1				1.6	2.2	*
15:1				0	0.1	n.s.
16:0	23.8	27.2	***	33.3	34.6	n.s.
16:1 (<i>trans</i>)	0.3	0.3	n.s.			
16:1	0.9	1.6	***	9.3	11.9	*
17:0	1.0	0.8	***	0.6	0.4	n.s.
18:0	21.4	21.0	n.s.	15.7	9.3	***
18:1				24.7	25.7	n.s.
18:1 (<i>trans</i>)	1.3	0.6	***			
18:1 (<i>n-9</i>)	34.1	35.6	*			
18:1 (<i>n-7</i>)	1.0	1.1	*			
18:2 (<i>n-6</i>)	2.2	2.1	n.s.	3.8	5.3	*
18:3 (<i>n-3</i>)	1.0	0.2	***	1.5	0.3	***
20:0	0.5	0.2	***	0.1	0.1	n.s.
20:3 (<i>n-3</i>)				0	0.1	*
20:4 (<i>n-6</i>)	0.4	0.2	***	0.7	0.8	n.s.
20:5 (<i>n-3</i>)				0.3	0	***
22:5 (<i>n-3</i>)	4.0	0.1	***	0.6	0.2	***

Table 19.2 continued

Fatty acid	Reindeer ^a			Red deer ^b		
	Pasture (n = 9)	Pellets (n = 6)	Degree of significance	Pasture (n = 7)	Pellets (n = 7)	Degree of significance
SFA	53.0	54.6	n.s.	54.7	50.6	**
MUFA	37.6	39.2	*	36.4	39.8	*
PUFA (n-6)	2.6	2.3	n.s.	4.3	6.6	**
PUFA (n-3)	1.4	0.3	***	2.5	0.6	***
(n-6)/(n-3)	1.9	7.7	***	1.7	11.0	***

^a adapted from Wiklund *et al.* (2001c).

^b Wiklund *et al.* (2003a).

n.s. Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

alleles are expected to be used in breeding programs, such as marker-assisted introgression, to improve meat quality traits of pork.

19.2.2 Artificial feeding

The farming systems for deer range from wild, extensive to intensive systems that utilize strategic feeding such as over-wintering (Webster *et al.*, 2001) or strategic feeding for finishing (Volpelli *et al.*, 2002).

In the Cervids, a large number of experiments have compared the meat quality of wild/free range deer to that from feedlot finished animals. Although venison is renowned for its low muscle lipid content (Aidoo and Haworth, 1995; Wiklund *et al.*, 2001c), higher levels (4.5% in red deer; Kay *et al.*, 1981; 4.2% in female reindeer, Sampels *et al.*, 2005) than in meat from African ungulates have also been noted. The later phenomenon is particularly noted when the animals have been finished on pelleted diets (Wiklund *et al.*, 2001c, 2003a; Phillip *et al.*, 2007; Table 19.2). The effects of age, gender (including castration), region, and production system on the meat composition (Volpelli *et al.*, 2002; Stevenson *et al.*, 1992), including the fatty acid profile of the meat (Garton and Duncan, 1971; Manley and Forss, 1979; Wiklund *et al.*, 2003a; Sampels *et al.*, 2005), have been reported for fallow deer, red deer and reindeer. By and large, deer respond in a manner similar to any ruminant (Raes *et al.*, 2004; Wood *et al.*, 2003). The effects of these factors on the palatability and sensory characteristics of the venison have also been explored (Britten *et al.*, 1982; Stevenson *et al.*, 1992; Forss *et al.*, 1979; Wiklund *et al.*, 2003a). Those findings were similar to findings on the traditionally farmed ruminant species, *viz* large variation between animals within a treatment. A sensory panel could distinguish between the meat from animals that had been fed grain-based pellets and those grazed on natural pasture as pertaining to the attribute 'grassy flavour', with significantly higher scores for this attribute in meat from deer grazing pasture (Wiklund *et al.*, 2003a). Similar results have been reported for reindeer venison, where both a trained panel and consumers found that meat from

free-ranging reindeer had a stronger gamey flavour compared with meat from animals fed commercial grain-based pellets (Wiklund *et al.*, 2003b). The consumer preference test showed that 50% of the consumers preferred meat from grazing reindeer and 50% preferred meat from pellet-fed animals. Recently, seasonal variation in sensory attributes has been studied in meat from Alaskan free-ranging reindeer slaughtered at three different times of the year: July, November and March. The strong and gamey flavour of the meat increased over the season, so that reindeer slaughtered in July produced meat with a milder flavour, while meat from animals killed in March had a clear gamey flavour (Wiklund *et al.*, 2006a). It is also suggested that these results were related to the variation in diet composition over the year for the free-ranging reindeer.

Free-range red deer had higher muscle pigment than pellet fed deer, although the pigment content of the meat samples did not seem to have an influence on the colour stability or oxidation product formation (Wiklund *et al.*, 2006b). Type of diet also did not have an influence. As expected, the oxidation products (TBARS) increased during refrigerated storage. However pasture-fed fallow deer (*Dama dama*) had a longer colour display life after 2 and 3 weeks under refrigerated conditions than deer fed a barley and hay diet (Wiklund *et al.*, 2005).

Although it is known that diet influences the fatty acid profile of ruminants to a lesser degree than that of monogastric animals (MacRae *et al.*, 2005), dietary effects can still be noticed (Table 19.3). An illustration is the comparison of values of the kudu, predominantly a browser, to those of the springbok, predominantly a grazer. The influence of diet has been further reported in impala, an ungulate that grazes and browsers, depending on the available food. Animals from two different habitats (predominantly grass and predominantly bush) had different fatty acid profiles (Hoffman *et al.*, 2005). Comparison of the muscle tissue lipids of domestic cattle with those of African buffalo (*Synceros caffer*), giraffe (*Giraffa camelopardalis*), eland (*Taurotragus oryx*), kongoni (*Acephalus buselaphus*) and topi (*Damaliscus korrigum*) in East Africa showed that meat of the game species reflected the lipid composition of the diet (Crawford *et al.*, 1970). The game species also contained high amounts of the long chain (C>20) unsaturated fatty acids. The fatty acid profiles of the species in Table 19.3 all had P:S ratios above 0.4 and *n*-6:*n*-3 ratios below 4.0. With a desirable fatty acid profile, game meat can compete well with domesticated meat as it contains high levels of PUFA, yet has high P:S and low *n*-6:*n*-3 ratios (Table 19.3). The game species listed have high levels of linoleic acid and knowledge of the concentrations of the different isomers would allow quantification of the potential health aspects of the isomers in game meat (Schmid *et al.*, 2006). As early as 1968, it had been noted that the proportion of polyunsaturated to non-essential fatty acids in tissues was in the order of 1/50 for domestic bovids whilst that in the free-living animal was 1/2.3 (Crawford, 1968), but no data has been found on the muscle chemical composition of African ungulate game species fed formulated diets.

The chemical composition, especially the fatty acid profiles of monogastric animals, is strongly influenced by diet. Townsend and co-workers (1978) evaluated the chemical, physical and sensory properties of loins from Yorkshire,

cross-bred and wild pigs that had all been reared under the same conditions, and found differences between the breeds in the percentage moisture, protein and total lipids. The loins of the wild pigs had significantly higher levels of pentadecanoic acid (C15:0) and palmitoleic acid (C16:1), although the total percentage of the former was less than 1.3 and the difference between the breeds of the latter fatty acid was less than 1.5%. A trained taste panel could not distinguish among the three genotypes as pertaining to the juiciness, flavour intensity and flavour desirability. However, the wild pigs had a lower overall desirability which was due mainly to the meat being perceived to be significantly less tender, a finding that also correlated to the higher (less tender) measurements of the Warner–Bratzler apparatus. The meat of the wild pigs was also darker than that from the cross-bred or pure Yorkshire breed.

19.2.3 Effect of animal age and gender on meat quality

Similar to other animals, the meat quality of the exotic species, especially tenderness, tends to decrease with age, although the effect does not seem as pronounced in the wild ungulates as in the traditionally farmed species.

The meat from 13–16 month Blackbuck antelope (*Antilope cervicapva*) stags was leaner, had higher ultimate pH, and was slightly less tender than that of castrates or younger (7–10 months) stags (Woodford *et al.*, 1996). In fallow deer (*Dama dama*), older (30-month-old) animals had more insoluble collagen and higher shear force values than 18-month-old animals (Volpelli *et al.*, 2003). The *M. longissimus thoracis et lumborum* in older animals were fatter and the *M. semitendinosus* provided lower PUFA, both in the *n*-6 and *n*-3 fraction. In free-range reindeer (*Rangifer tarandus*), the older animals had less tender meat (sensory as well as shear force values) than younger animals (Renecker *et al.*, 2005). The taste panel noted no significant differences between age and gender for either juiciness or gamey flavour of the loin steaks. Although the older males were found to be more lean in all their fat reserves, this was attributed to the fact that they had just completed their rut.

For the African game species, an extensive study on meat quality with springbok age indicated that the loins from young animals (24–60 weeks old) were deemed to be too tender! (Zondagh *et al.*, 1995). Remember that young springbok reach their growth inflection point at 28 weeks (Skinner and Louw, 1996). In another study, however, neither age nor gender had an effect on the shear values of Springbok meat, which varied from 2.04 to 2.31 kg/1.27 cm diameter for the different age categories (Hoffman *et al.*, 2007a). The shear force values obtained for springbok were low compared with values of 3.21–4.08 reported for impala (*Aepyceros melampus*) (Hoffman, 2000), 3.23–4.28 for black wildebeest (*Connochaetus gnou*), 3.77–4.60 for blue wildebeest (*C. taurinus*), 2.95–3.00 for mountain reedbuck (*Redunca fulvorufula*) (Van Schalkwyk, 2004), and 2.03–7.74 for beef (Belew *et al.*, 2003) as kg/1.27 cm diameter. Females had higher intramuscular fat than males and, although fat levels increased as animals aged, the fat levels were still below 3.5% (Hoffman *et al.*, 2007b). The specific fatty acids

Table 19.3 Mean total fat (%), fatty acid composition (%) and total cholesterol content (mg.100 g⁻¹) of the *M longissimus dorsi* of the common duiker (*Sylvicapra grimmia*), kudu (*Tragelaphus strepsiceros*), blesbok (*Damaliscus dorcas phillipsi*), springbok (*Antidorcas marsupialis*), impala (*Aepyceros melampus*), red hartebeest (*Alcelaphus buselaphus caama*), black wildebeest (*Connochaetes gnou*), blue wildebeest (*Connochaetes taurinus*), warthog (*Phacochoerus aethiopicus*), buffalo (*Syncerus caffer*) and zebra (*Equus zebra*) (from Hoffman and Wiklund, 2006)

Fatty acid	Common duiker (male) ^a	Kudu (male) ^b	Blesbok (male) ^c	Springbok (male) ^d	Impala (male) ^c	Red hartebeest (male) ^c	Black wildebeest (male) ^f	Blue wildebeest (male) ^f	Warthog ^g	Buffalo ^g	Zebra ^g
Total fat	2.12	1.58	0.76	1.07	–	4.69	0.97	2.94	–	–	–
14:0	0.75	–	–	–	0.32	–	–	–	0.80	0.64	1.13
16:00	0.86	16.10	16.44	13.93	15.04	18.27	13.2	16.12	20.00	18.03	22.50
16:1 (<i>n</i> -7)	18.58	0.52	0.00	0.07	0.57	0.00	0.19	0.18	0.70	1.50	2.02
18:00	19.68	19.72	24.7	25.32	22.25	36.08	26.21	21.47	14.7	18.83	10.22
18:1 (<i>n</i> -9)	18.70	19.91	17.98	16.33	19.34	16.01	14.37	16.75	15.8	30.02	20.55
18:2 (<i>n</i> -6)	19.91	20.53	18.89	21.62	19.67	14.55	20.97	20.45	26.10	12.93	24.01
18:3 (<i>n</i> -6)	0.12	0.05	0.08	0.13	0.14	0.26	0	0.13	0.20	0.08	0.11
18:3 (<i>n</i> -3)	4.10	4.85	3.72	3.37	5.09	4.06	4.47	4.57	7.30	3.79	11.46
20:00	0.81	0.11	0.31	0.31	0.14	0.49	0.39	0.33	0.10	0.62	0.14
20:1 (<i>n</i> -9)	0.23	0.06	0.04	0.10	0.10	0.38	0.19	0.12	0.10	0.31	0.30
20:2 (<i>n</i> -6)	0.29	0.15	0.03	0.28	0.18	0.08	0.19	0.20	0.30	1.00	0.39
20:3 (<i>n</i> -9)	–	–	–	–	–	1.11	0.78	–	–	–	–
20:3 (<i>n</i> -6)	2.94	1.14	1.85	–	0.86	–	–	–	1.10	0.95	0.75
20:3 (<i>n</i> -3)	0.19	–	–	–	0.09	–	–	–	0.90	0.20	0.59
20:4 (<i>n</i> -6)	7.83	8.44	10.96	9.30	7.87	7.01	9.9	7.72b	7.50	5.71	3.29

20:5 (<i>n</i> -3)	2.10	3.17	2.39	2.38	3.44	2.38	3.11	3.28	0.90	1.55	0.41
22:00	0.08	0.31	0.31	0.26	0.16	0.46	0.39	0.22	0.10	0.56	0.07
22:2 (<i>n</i> -6)	0.01	–	–	–	0.14	–	–	–	0.10	0.06	0.06
22:3 (<i>n</i> -3)	0.14	–	–	–	–	–	–	–	–	0.30	0.00
22:4 (<i>n</i> -6)	0.31	–	0.22	0.27	0.43	0.28	0.58	0.28	0.40	0.27	0.26
22:5 (<i>n</i> -3)	1.14	2.75	2.43	2.60	2.82	2.31	3.69	5.38	2.40	1.65	1.24
22:6 (<i>n</i> -3)	1.09	2.50	0.39	0.94	1.00	0.37	0.58	0.98	0.40	0.83	0.39
24:00	0.06	–	0.57	0.53	0.19	0.88	0.78	0.41	0.10	0.10	0.06
24:1 (<i>n</i> -9)	–	–	0.49	0.17	0.14	11.71	0.58	0.18	0.10	0.78	0.04
SFA	22.24	35.93	42.33	40.35	38.11	56.18	40.97	38.55	35.8	38.78	34.12
MUFA	37.51	20.48	18.51	16.67	20.15	28.1	15.33	17.23	16.7	31.61	22.91
PUFA	40.26	43.59	40.96	31.59	41.74	32.41	44.27	42.99	47.6	29.32	42.96
PUFA:SFA	1.81	1.23	0.97	0.79	1.10	0.58	1.01	1.15	1.33	0.76	1.26
(<i>n</i> -6)/(<i>n</i> -3)	–	2.29	3.62	3.28	–	2.75	2.82	2.07	–	–	–
Cholesterol (mg.100 g ⁻¹ meat sample)	–	–	51.38	56.9	–	50.9	46.05	51.08	–	–	–

Adapted from:

^a Hoffman and Ferreira (2004).

^b Mostert and Hoffman (2007).

^c Smit (2004).

^d Hoffman *et al.* (2007c).

^e Hoffman *et al.* (2005).

^f van Schalkwyk (2004).

^g Unpublished data chemically analysed as described in Hoffman *et al.* (2005).

were also quantified (Hoffman *et al.*, 2007c) and the major fatty acid of the *M. longissimus dorsi* was stearic acid (C18:0), which contributed 23.92–27.02%. Oleic acid (C18:1) represented the largest component (16.33–20.45%) of the mono-unsaturated fatty acids (MUFA). The major *n*-6 polyunsaturated fatty acid (PUFA) was C18:2*n*-6, which formed 18.77–21.62%, whereas C18:3*n*-3 (3.33–4.00%) was the most abundant *n*-3 PUFA. The *n*-6:*n*-3 ratio of the meat varied from 3.02 to 3.35, with an average ratio of 3.2. Polyunsaturated to saturated (P:S) ratios varied between 0.96 and 1.18 and averaged at 1.06. Total MUFA was found to be higher in males (20.99%) than females (16.67%). In the same study, the regional effect was greater on the sensory characteristics of springbok than either gender or age (Hoffman *et al.*, 2007d). Production region influenced the game meat aroma, initial juiciness, sustained juiciness and residual tissue ratings of the meat, whilst gender and age had only a significant effect on the residual tissue rating of the meat. Gender had no effect on the chemical (proximate, amino and fatty acids, minerals) composition of kudu (*Tragelaphus strepsiceros*) (Mostert and Hoffman, 2007).

Żochowska and co-workers (2005) quantified the effect of carcass weight (age) on the muscle fibre characteristics of free ranging wild boar aged either 0.5 or 3 yrs (carcass weights of 20 or 60 kg, respectively). The young animals showed significantly lower muscle texture (hardness, cohesiveness, springiness, chewiness) than the older animals, which was linked to the latter having thicker perimysia and endomysia, fibres of higher cross-sectional area and also a higher content of red fibres (Type I). In a later study, Żochowska-Kujawska *et al.* (2007) found similar results as pertaining to the age effects on wild boar muscle. They also found that the older animals had lower percentages of Type IIB fibres. No effect of age on the rheological properties was found.

19.2.4 Other farming strategies

There have also been reports of deer farmers' velvetting as well as castrating stags to ensure less fighting and more space at the feed trough (MacDougall *et al.*, 1979; Mulley and English, 1985; Woodford *et al.*, 1996; Sookhareea *et al.*, 2001). A shift in muscle distribution between castrate and entire red deer showed the castrate forequarter muscle to be proportionately 7% lighter and hindquarter muscle proportionately 7% heavier than entire males (Tan and Fennessy, 1981). With castration and intensive feeding, the next logical steps are concerns for herd health and vaccination programmes, the use of AI (artificial insemination) and embryo transfer, as well as the inclusion of growth promotants to improve the productivity of the animals (Knox *et al.*, 1991; Mulley *et al.*, 1996). However, the deer industries worldwide have rejected use of growth hormones to increase meat production.

19.3 Transport, lairage and slaughtering techniques

As deer have become more domesticated, greater control has been practised on the transport, lairage and slaughter techniques to minimise the effects of stress on meat quality. However, it must still be remembered that these are wild animals and therefore they have more highly developed flight or fight reflexes than most domesticated species. The instinctive behaviour of these species must be known when the handling, transporting (if applicable), and holding facilities at the farm and/or abattoir are designed if optimal meat quality and the welfare of the animal are to be achieved (Renecker *et al.*, 2001). Evaluation of the bruising of deer carcasses at a slaughter plant over a three-year period revealed that transport and lairage were the main causes of the down grading of carcasses (Jago *et al.*, 1996).

The effects of different pre-slaughter handling routines (reindeer: Wiklund *et al.*, 1996a; Wiklund *et al.*, 1997), transport (reindeer: Wiklund *et al.*, 1995; Wiklund *et al.*, 2001a; red deer and fallow deer: Pollard *et al.*, 1999) and lairage (red deer: MacDougall *et al.*, 1979; Pollard *et al.*, 1999; reindeer: Wiklund *et al.*, 1996b) on several of the physical meat quality attributes have been reviewed (Hoffman and Wiklund, 2006). Most of the responses of the animals to these ante mortem stressors are similar to those noted for domesticated animals. For example, diet had a stronger influence on muscle metabolism post-mortem than transport or lairage (Wiklund *et al.*, 1996b). The reindeer (*Rangifer tarandus tarandus* L.) that had received a supplementary diet two months prior to transport and lairage had lower blood metabolites indicative of stress than non-supplemented deer.

In red deer killed either in a pasture or in a slaughter plant, pre-slaughter handling created moderate stress (as determined by measuring various biochemical parameters in the blood) and the high levels of muscular exertion or damage noted on the deer killed in the slaughter plant were possibly related to antagonism during lairage (Pollard *et al.*, 2002). However, muscle glycogen, pH and meat quality measurements showed only minor muscle specific differences between the two treatments.

These various ante-mortem factors and their effects on the meat quality of deer has also been reported and reviewed by Malmfors and Wiklund (1996), Renecker *et al.* (2001) and Wiklund and Malmfors (2004). Conclusions of all these studies were identification of possibilities to improve pre-slaughter handling routines for all of the included deer species that would further reduce the frequency of DFD (Dark, Firm, Dry) meat with high ultimate pH values.

The harvesting techniques used for African game species have been reviewed in detail (Hoffman and Wiklund, 2006). The normal harvest at night is the least stressful while harvesting in the day time from a hide creates little stress, but the harvest rates are very restricted. Shooting in a boma gives high take-off rates but is stressful to the animals and shooting from a helicopter is highly stressful and results in a large amount of bullet damage in the neck and back region of the animals. Meat from most game animals tends towards DFD due to the stress of the cropping process; however, PSE meat has been observed in animals that have experienced acute stress during the killing process (Hoffman, 2001). For example,

Table 19.4 The effect of ante-mortem stress on the rate of pH change and drip loss of warthogs (Hoffman and Sales, 2007e)

No.	Description of ante-mortem stress	Meat class	Constants for the exponential function ($y = a + be^c$)			% Drip loss
			<i>a</i>	<i>b</i>	<i>c</i>	
1	Shot behind the shoulder, run 100 m before dying	Pale, soft, exudative	5.41	1.27	−0.31	3.35
2	Head shot, died immediately	Slightly dark, firm, dry	5.24	1.65	−0.06	2.45
3	Shoulder shot, dropped immediately	Normal	5.53	1.17	−0.14	2.16
4	Head shot, paralysed, frantic kicking movements	Pale, soft, exudative	5.34	1.18	−0.65	6.76
5	Neck shot, dropped immediately, frantic kicking movements	Pale, soft, exudative	5.47	0.84	−0.58	3.14

Note: y = pH at time t ; a = ultimate pH; e = base of natural logarithm and b and c are the function parameters describing the shape of the curve.

buffalo (*Syncerus caffer*) that were killed using scoline had meat that was PSE (Hoffman, 2001). This phenomenon was also similar to a condition called white muscle myopathy that is sometimes found during the live capture of game (Harthoorn and van der Walt, 1974). Warthogs, similar to the domesticated pig, can be prone to PSE (Hoffman and Sales, 2007e) depending on the ante-mortem stress that is experienced (Table 19.4, Fig. 19.1). The time and manner of harvesting does have an impact on the meat quality of the wild animals (Kritzinger *et al.*, 2003). A light calibre silenced rifle used at night seems to have the least effect on the meat quality and also has the least effect on the herd behaviour as a whole (Lewis *et al.*, 1997).

19.4 Post-mortem intervention to improve the meat quality

Some of the post-mortem interventions that have been utilized to improve quality attributes (mainly meat tenderness) include electrical stimulation (Chrystal and Devine, 1983; Wiklund *et al.*, 2001b) combined with ageing (Drew *et al.*, 1988). Studies have also focused on some of the interactions between muscle glycogen and technological meat quality attributes (Wiklund *et al.*, 2004).

19.4.1 Pelvic suspension

The technique of pelvic suspension (‘tenderstretching’) of the carcass resulted in positive effects on tenderness in several valuable cuts from fallow deer carcasses (Woodford *et al.*, 1996; Sims *et al.*, 2004).

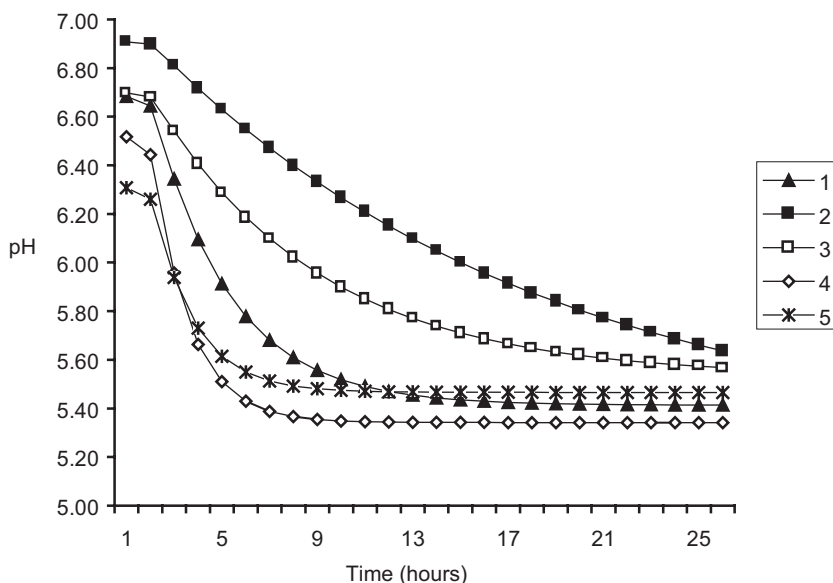


Fig. 19.1 Post-mortem pH change in the *M. longissimus dorsi* of warthogs (Warthogs 1, 4 and 5 showing a post-mortem pH decrease characteristic of the pale, soft, exudative condition, Warthog 2 a pattern illustrating a slightly dark, firm, dry condition, and Warthog 3 a pattern of normal meat). From Hoffman and Sales (2007e).

19.4.2 Electrical stimulation (ES)

As mentioned in other chapters, a chilling rate that is too rapid may induce cold shortening and thus result in tougher meat. This is particularly pronounced when the carcasses have very little, or only localized, subcutaneous fat. Conventional carcass chilling is also a lengthy and energy-expensive process. Blast chilling can reduce cooling time and associated shrink loss, although its application may compromise meat quality, particularly in lean carcasses or those with localized finish such as most exotic game species (with the exception of zebra and very fat eland). Low voltage electrical stimulation (LVES) can reduce the risk of decreased meat quality by inducing rapid rigor onset prior to exposure of the musculature to extreme cold temperature. Blast chilling (-20°C , 3 m/s air velocity, 2 h) accelerated the temperature decline of bison (*Bison bison bison*) *Longissimus lumborum* muscle and significantly reduced the cooler shrink loss when compared to conventional chilling ($0 \pm 2^{\circ}\text{C}$, 24 h) (Janz *et al.*, 2001). Although blast chilling tended to produce darker bison meat, this effect was tempered by the application of LVES, and samples from the combined treatments were significantly lighter than conventional chilling. Blast chilling also resulted in reduced tenderness in the *M. Longissimus lumborum*, as assessed by shear force measurement, in part due to significantly shorter sarcomere length in blast-chilled samples. Taste panellists,

however, were unable to detect a significant or detrimental blast chilling effect. Where LVES was incorporated, there was an improved tenderness response with ageing. The combined LVES/blast chilling treatment of bison carcasses was thus recommended for rapid processing of bison without compromising meat quality.

Low ES (80 V peak, unidirectional half sine-wave pulses of 10 ms duration) for a 90 s period resulted in beef loin muscles having a 'conditioned' status with tenderness similar to muscles that had been maintained at 10 °C for 24 h followed by 24 h at 2 °C (Chrystall and Devine, 1983). The control muscles, chilled rapidly at 2 h post mortem, were very tough and showed characteristics typical of cold shortening. LVES also increased the rate of post-mortem glycolysis in red deer (Wiklund *et al.*, 2001b). This improvement in tenderness was maintained for at least 3 weeks post-mortem, but the differences disappeared by 6 and 12 weeks post-mortem. After 1 week of refrigerated storage, ES significantly reduced the display life (hours of Minolta a* value 512), but this difference disappeared at 3, 6 and 12 weeks of ageing. ES did not affect drip at any ageing time point during that study.

High voltage electrical stimulation (HVES) may be used instead of LVES. The efficiency of high voltage electrical stimulation (700 V, 1400 V peak, pulses 1 s on/1 s off, 60 Hz, 2 A) on buffalo (*Bubalus bubalis*) carcass muscle conditioning during nine days storage resulted in a significantly more rapid pH fall as compared with controls (Soares *et al.*, 1995). The IMP/ATP ratio clearly indicated that ES reduced the time period necessary for the onset of rigor mortis. Myofibrillar fragmentation index differences between the control and ES muscles increased throughout storage at 2 °C. On the 6th day of post-mortem conditioning, the SDS electrophoretic patterns of the myofibrillar proteins indicated light weakening of Troponin T (37 000D).

Another alternative to ES for alleviation of cold shortening effects is elevated temperature conditioning (ETC: 10 °C until 10 h post-mortem) until the critical decrease in energy reserves required to minimize cold shortening is achieved. ETC allowed avoidance of the cold-induced meat quality defects that are a risk with conventional bison carcass chilling (0 ± 2 °C for 24 h) (Janz *et al.*, 2000). The ETC treatment maintained internal *M. Longissimus lumborum* and *M. Semimembranosus* temperatures above 10 °C within the first 10 h post mortem. The time/temperature combination did not result in significant evaporative loss, although loss of weight during carcass cooling can represent a practical economic loss. ETC accelerated post-mortem glycolysis and pH decline, and resulted in samples of lighter, more intense red colour than those conventionally chilled. Significant improvement in both initial tenderness and tenderization during ageing was also realized with the use of ETC.

In Africa, the various game species are frequently harvested during winter when the ambient temperatures are below 4 °C and cold shortening of muscles in smaller game species may occur. A pilot study on tenderization of tenderloin muscle in Springbok with LVES indicated that, although there was a tendency for the meat from ES carcasses to have less Warner–Bratzler shear force, a trained taste panel could not distinguish any differences with treatments. The results may have been

confounded by the freezing of the muscles prior to preparation for sensory analysis, with the small differences becoming undetectable due to physical damage caused by ice crystal formation.

Most of the research results demonstrate that the benefits of ES on tenderness are not permanent, and the procedure is not necessary for a product that is to be maintained in chilled storage for a long period.

19.5 Improving or maintaining the meat quality post-mortem

19.5.1 Colour

It has been well recorded in the literature that most of the free-ranging exotic species have higher muscle pigmentation (myoglobin; Mb) and thus are darker than the animals farmed and/or finished in a feedlot. This darker pigmented muscle is usually attributed to a higher level of exercise. The retail storage time of a meat product is generally limited by colour deterioration, which occurs at some time before microbial spoilage. Such colour deterioration severely restricts the possibilities for widespread distribution of retail-ready cuts because the deterioration process commences immediately when a meat surface is exposed to O_2 .

Venison and game meat have high concentrations of myoglobin (7.3 mg/g muscle for impala – Hoffman *et al.*, 2005) and mitochondria that increase the rate of metabolic oxygen consumption, so the bloomed appearance does not last long and thus results in poor colour stability when compared to meat from other species. Venison has a short retail display life, which was decreased even further by the duration of storage under vacuum prior to being displayed under oxygen (Seman *et al.*, 1988). This was linked to the extended period of auto-oxidation and lipid oxidation that occurs whilst the meat is stored under vacuum (Pietrasik *et al.*, 2006). The rapid formation of metmyoglobin (MetMb) is enhanced when the meat has been stored frozen for long periods prior to being thawed, cut and displayed under chilled retail conditions. The colour stability of previously frozen bison meat cuts was very low in the retail case compared with stability of beef (Dhanda *et al.*, 2002). The formation of ice crystals in the meat causes structural damage in the muscle cells, thereby allowing cellular components that are normally kept apart to mix. Also, as ice crystals exclude salts, the ionic strength of the remaining unfrozen water is increased. In the event of exposure to oxygen, these events lead to the formation of free radicals that accelerate tissue degradation and oxidation, which is linked to colour deterioration. It was concluded that vacuum packaging appeared to be the most economic method used and produced meat of better colour stability (Seman *et al.*, 1988). However, this method entails repackaging into oxygen permeable packages for retail display.

Investigations on the post-mortem MetMb reduction in fresh venison to gain more insight into methods suitable for regenerating the bright red oxymyoglobin state resulted in the conclusion that the MetMb reducing activity occurs

anaerobically in completely discoloured venison following storage display (Bekhit *et al.*, 2007). Any visible effects of MetMb reducing activity would only be possible when oxidative processes were slowed down or eliminated by techniques such as vacuum packaging. However, it was also noted that, in a vacuum system for regenerating the red colour in venison, any practical benefits from the packaging reduction system to regenerate the desirable cherry-red colour, which only lasts for a short time (<30 h), may be negated or prohibited by the cost of re-packaging for retail sale.

19.5.2 Sensory attributes

Similar to other red meat types, venison and game meat becomes more tender as refrigerated time increases. However, some of the sensory characteristics also become less desirable with storage, with the negative characteristics enhanced by the auto-oxidation reactions caused by the higher myoglobin and mitochondria levels. Similarly, as venison and game meat have a low lipid level, there is a higher proportion of phospholipids (Sampels *et al.*, 2005) that cause higher susceptibility to lipid oxidation. For example, red deer loin samples became significantly more tender, less desirable, more intense in flavour and exhibited a higher degree of off-flavour as storage time increased from 1 to 18 weeks (Seman *et al.*, 1988).

The animals of non-domesticated species are wild and thus susceptible to ante-mortem stress that may result in DFD meat. In evaluations of the proteolysis and tenderization in reindeer (*Rangifer tarandus tarandus* L.) *M. longissimus thoracis*, the muscles in the high pH (> 5.80) group had significantly higher activity of μ -calpain compared with the low pH group (< 5.79) at 1 day post-mortem (Wiklund *et al.*, 2003b). No differences in shear force, myofibrillar protein degradation by SDS-PAGE, m-calpain and calpastatin activities, cathepsin B + L activities or the levels of cystatin-like inhibitors were found between the two pH groups. In the three carcasses with the highest ultimate pH values (6.11, 6.34 and 6.38), the sarcomere lengths were around or below 40% of the resting length (1.37 μ m, 1.25 μ m and 1.25 μ m, respectively), which was presumed to be associated with the occurrence of heat shortening.

19.5.3 Packaging

In the meat industry, vacuum packaging (VP) is used to maximize the shelf-life of meat, whereas modified atmosphere packaging (MAP) containing high levels of O₂ is used to attain the bright red colour of meat through oxygenation of deoxymyoglobin. As with other red meat species, the evaluation of MAP has also enjoyed some attention in venison and bison. Vacuum packaging resulted in a lower incidence of off-odours and higher colour acceptability scores than did 100% CO₂ flushed packaging systems (Seman *et al.*, 1988). MAP with CO₂ conferred little additional shelf-life to chilled venison loins if the fabrication methodology was such that minimal microbiological contamination took place (Seman *et al.*, 1989).

Beef *Longissimus lumborum* steaks in MAP (70% O₂/30% CO₂) retained their bright red colour longer than bison steaks (Pietrasik *et al.*, 2006). Bison steaks had developed higher 2-thiobarbituric acid reactive substances (TBARS) during storage, which may have influenced the resulting rapid loss of redness from the bloomed meat. It was also noted that storage under MAP resulted in higher TBARS than storage under vacuum. Storage at -1 °C in MAP provided greater colour stability and a longer storage-life for both beef and bison. Steaks stored overnight under MAP before retail display maintained the highest *a** values for up to 5 d, compared with those stored under vacuum. MAP steaks stored overnight generally maintained the highest oxymyoglobin content for up to 5 d during retail display compared with those stored under vacuum. Nevertheless, oxymyoglobin levels were significantly lower in bison steaks compared with those of beef, irrespective of packaging treatments.

The greatest changes in the instrumental values of the parameters occurred during the first 9 days post-packing in evaluations of the effects of different gas mixtures in MAP on the quality (pH, colour as L* *a** *b** values, drip loss (DL), cooking loss (CL) and shear force (SF) of deer (*Cervus elaphus*) (Vergara *et al.*, 2003). The pH and water loss increased in all treatments (40% CO₂ + 60% N₂; 80% CO₂ + 20% O₂; 80% CO₂ + 20% N₂), thus making it impossible to maintain the initial meat quality in any of the groups tested. Also, the samples in all the groups turned yellow with time (increased *b** value), with the change more rapid in groups packed in MAP containing oxygen. The colour values (redness and yellowness) of the meat in MAP containing 40% CO₂ + 60% N₂ suggest that this mixture is the most appropriate for the preservation of deer meat.

19.5.4 Microbiological shelf-life

It would seem that, with modern slaughter and processing technologies, microbiological spoilage would be less of a problem than auto-oxidation and lipid oxidation. A recent review on the microbiological contamination of exotic meats indicates that the slaughter techniques play an obvious role in microbiological contamination (Gill, 2007). When springbok were harvested by standard commercial techniques (Hoffman and Wiklund, 2006), the microbiological status (mean total count and initial bacterial composition) indicated that introduction of certain critical control points into the harvesting system will be necessary to produce a fresh product with an acceptable shelf-life (Buys *et al.*, 1996). Ageing of springbok meat had no influence on bacterial counts (Buys and Kruger, 1995). As the ageing period increased beyond 12 days of refrigeration storage, vacuum packaging inhibited the growth of spoilage bacteria and the *Enterobacteriaceae* group.

Muscles of bison attain final pH values <5.7 (Janz *et al.*, 2001) and bison steaks vacuum packed or displayed in oxygen permeable packaging had storage lives comparable to that of beef steaks prepared under good hygienic conditions (Janz and Aalhus, 2006). Similarly, the storage life of ground bison meat maintained at chiller temperatures was comparable to that of ground beef (Li and Logue, 2005).

Venison can remain acceptable for up to 18 weeks when stored and distributed at temperatures around -1°C (Seman *et al.*, 1989). This has allowed the development in the meat trade of chilled venison transport from New Zealand and chilled game meat transport from South Africa and Namibia.

Wild boar meat proved to have a much better storage life than pork and resembles that of beef (Boers *et al.*, 1994). This was attributed to a high level of glucose in the wild boar muscle which may contribute to its long shelf-life through a delay of glucose limitation and subsequent amino acid breakdown by micro-organisms.

19.5.5 Improving meat quality by means of injecting/enhancement

The beef and pork industries have recently adopted moisture enhancement of fresh meat for widespread commercial use to help ensure a more consistent, juicy, and tender product. This technology has also been used on exotic meat types, with similar results as noted for beef. A salt/phosphate blend injected into bison steaks had a beneficial effect on the colour stability of steaks during retail display (Pietrasik *et al.*, 2006). However, this positive effect was more pronounced for bison steaks compared with those of beef injected steaks, with steaks stored at -1°C having significantly higher oxymyoglobin levels compared with non-injected counterparts and those stored at $+4^{\circ}\text{C}$, respectively. The TBARs values were also found to be lower in the injected bison samples than in the non-injected steaks.

The palatability of bison *semimembranosus* muscle and the effects of injection with sodium chloride (0.5%) and sodium tripolyphosphate (0.3%) on cooking yield, colour, shear force and consumer acceptability has been investigated (Dhanda *et al.*, 2002). Although the Hunter Lab a^* (redness) and b^* (yellowness) values did not differ between injection treatments, the injected steaks had lower L^* values (were darker) compared with the controls. Control samples were very lean and high in protein, but not very tender. Marination by injection significantly reduced shear force values compared to control samples. Cooking yields for the steaks/roasts from the injected sections were also significantly higher compared with control non-injected sections after being cooked to either 71°C or 77°C . Bison samples cooked by moist heat had significantly lower cooking losses and shear force values compared with those cooked by dry-heat. As expected, steaks/roasts were more tender and had higher cooking yields when cooked to a medium level of doneness (71°C) compared to an internal temperature of 77°C (well done). A panel of 80 consumers preferred injected steaks cooked to 77°C over other treatment combinations, followed by non-injected steaks cooked to 71°C , whereas injected steaks cooked to 71°C and non-injected steaks cooked to 77°C were equally, but least, preferred. Hence, injection seems to be protecting against moisture loss at high end-point cooking temperatures.

Enhanced (injected) springbok and Blesbok samples were more tender (W–B shear values and taste panel) and more juicy, with consumers preferring the enhanced muscles (Du Buisson, 2006). The enhancement of game meat with an

inorganic salt solution might be a useful processing tool to further improve the acceptability of game meat tenderness and juiciness, since game meat is often perceived as being dry and less tender because of its lower fat content and the use of slaughter techniques that stress the animals and subsequently lower meat quality.

Żochowska-Kujawska *et al.* (2007) evaluated the effect of injecting a standard brine solution and then massaging the wild boar muscles intermittently, on the hardness, rheological properties and structure of four muscles. They concluded that the lower the initial values of textural and structural parameters and percentage of Type I fibres of a muscle were (typically as found with a young animal compared to an old animal), the higher was the muscle's susceptibility to massage.

19.6 Value-added products as a means to improve the quality attributes of exotic meats

Venison and game meat are not only consumed fresh but also in various processed forms (Paleari *et al.*, 2000). A large number of these processed forms are from traditional recipes and few of the processed meats have had a scientific analysis of their nutritional and sensory quality characteristics. However, increased globalization of the food market will allow a large number of these products to move from being a local or domestic product to becoming a niche market item.

One of the most popular meat products is dried meat, which is known in South Africa as biltong and in the US as jerky. The meat can be in various forms, as whole muscles, muscle strips, or ground/mince for structuring into desired forms prior to processing for dried, salted and dried, or salted, smoked and dried products. Most jerky in the US is cured with sodium nitrate, whereas salt and pepper form the basis of the spices added to biltong. A hot smoking process slightly changes the fatty acid composition, lipid class composition and vitamin content, whereas drying results in major changes in these chemical components in reindeer *M. semimembranosus* (Sampels *et al.*, 2004). With curing and/or fermenting and drying, there is normally an increase in most of the chemical constituents due to the drying process. Comparison of fermented and dried cured products (similar to the traditionally prepared beef bresaola) with fresh meat showed that, surprisingly, the amount of lipid between the fresh and cured deer product was similar whilst lipid in the boar meat (and other meat species used) was higher in the cured product (Paleari *et al.*, 2003; Soriano *et al.*, 2006). The protein and ash contents in the cured products were also higher, with a high content of free amino acids and high levels of polyunsaturated fatty acids. Evaluation of the microflora of the cured products in the same study revealed only flora typical of processed products (Paleari *et al.*, 2002).

Sausages are another group of popular products where the meat is minced and then restructured into the final product. These are then either fermented and dried (typical salami-like products) or dried. During the ripening of fermented sausages, the proteins and lipids undergo major changes. For example, ten commercial

chorizos and saucissons (dry sausages found in Spain) were made from either wild boar or deer meat (Soriano *et al.*, 2006). These sausages are made following similar procedures and are mainly differentiated by the higher concentration of spices, particularly paprika in chorizo, which gives the typical red colour. The proteins in the myofibrillar fraction were higher than in the sarcoplasmic fraction. The chorizos made with deer or wild boar meat had higher percentages of poly-unsaturated free fatty acids, linoleic and linolenic acids and lower percentages of the mono unsaturated 11-eicosenoic acid than the saucissons.

Ripening of venison (*Cervus elaphus*) chorizo sausages was influenced by stage of the hunting season and natural or controlled drying rooms (Ruiz *et al.*, 2007). The myofibrillar protein decreased and proteolysis indices were between 4.6 and 14.4% after ripening, but variations were minimal after 45 days in vacuum packaging. Processing in controlled conditions showed similar myofibrillar changes, but there was more variation with natural drying rooms, with pH of sausages lower with controlled than natural drying. Hunting season stage influenced the initial meat pH before sausage production and the relative density of the 49 kDa band after 21 days of ripening. Changes in proteins profiles were found after storage of the four treatment batches.

19.7 Future trends

Storage of raw chilled venison, bison and game meat under MAP is an excellent option for short-term storage due to its positive effects on meat colour, but VP may be necessary for longer storage with minimal quality changes. An option to increase shelf-life and have retail display with desirable colour might be storage of meat under vacuum and then placing it under MAP just before retail display (Pietrasik *et al.*, 2006).

The marketing of portions derived from individual muscles is a strategy followed by the South African game meat export industry and has been adapted from the marketing of ostrich meat, which is another form of exotic meat discussed in Chapter 18.

The enhancement of meat quality traits through infusion, marination or injection or massaging (frequently under vacuum) of various lactate, salt, and phosphate blends would improve the tenderness and juiciness of most game meats, particularly if harvest and chilling conditions are not tightly controlled. Information on meat quality during extended shelf-life and subsequent consumer preferences is needed.

Consumers increasingly desire more convenient and value-added meat products. There may be a niche market for precooked ready to heat or ready-to-eat (RTE) products made from game, venison or other exotic meats. The large sales portion of meat in prepared form through in-store or restaurant purchases rather than raw form indicates that there is increased purchasing power for value-added items, particularly by the patrons who regularly consume game or exotic meats.

Continued attention to sanitary harvest and processing conditions is necessary

for all of the game, venison, and exotic species to maintain the highest possible meat quality through marketing channels. The increased production of the deer species and bison and wild boar (and its hybrids with domesticated swine breeds) under more controlled farm practices will allow management over the harvest and processing factors that affect food safety and meat quality. However, producers will have to remain sensitive to the fact that, as their husbandry management practices increase, their product may lose its 'exotic' image and become perceived to be yet another farmed animal species.

Different training and practices will also be necessary to minimize the potential for a negative impact of the less specialized field harvesting and processing conditions under which wild game meat is obtained and distributed. Here, two scenarios will be important, the first is the ethical procedures used during the harvesting and the second is the potential for microbiological contamination.

19.8 References

- Aidoo K E and Haworth R J P (1995), 'Nutritional and chemical composition of farmed venison', *J. Hum. Nutr. Diet.*, 8, 441–446.
- Bekhit A E D, Cassidy L, Hurst R D and Farouk M M (2007), 'Post-mortem metmyoglobin reduction in fresh venison', *Meat Sci.*, 75, 53–60.
- Belew J B, Brooks J R, McKenna D R and Savell J W (2003), 'Warner–Bratzler shear evaluations of 40 bovine muscles', *Meat Sci.*, 64, 507–512.
- Boers R H, Dijkman K E and Wijngaards G (1994), 'Shelf-life of vacuum-packaged wild boar meat in relation to that of vacuum-packaged pork: Relevance of intrinsic factors', *Meat Sci.*, 37, 91–102.
- Booth W D (1995), 'Wild boar farming in the United Kingdom', *J. Mount. Ecol.*, 3, 245–248.
- Britten H C, Armes C L, Ramsey C B and Simpson C D (1982), 'Consumer acceptability of ground venison', *J. Amer. Diet. Assoc.*, 80, 557–560.
- Buyes E M, Nortjé G L and van Rensburg D (1996), 'Bacteriological quality of springbok (*Antidorcas marsupialis marsupialis*) carcasses harvested during the 1994 hunting season in South Africa', *S. Afr. J. Food Sci. Nutr.*, 8, 56–59.
- Buyes E M and Kruger J (1995), 'Influence of ageing treatment on the bacterial quality of South African springbok (*Antidorcas marsupialis*) wholesale cuts. In *Proc. of the 13th Biennial Cong. of S. Afr. Assoc. Food Sci. Techn.*, Durban, South Africa, August pp1–27 mimeographed.
- Chrystall B B and Devine C E (1983), 'Electrical stimulation of deer carcasses', *N. Zeal. J. Agric. Res.*, 26, 89–92.
- Clutton-Brock J (1992), 'The process of domestication', *Mamm. Rev.*, 2, 79–85.
- Crawford M A, Gale M M, Woodford M H and Casped N M (1970), 'Comparative studies on fatty acid composition of wild and domestic meats', *Int. J. Biochem.*, 1, 295–305.
- Crawford M A (1968), 'Fatty-acid ratios in free-living and domestic animals', *Lancet*, June 22, 1329–1333.
- Dasman R F and Mossman A S (1960), 'The economic value of Rhodesian game', *Rhodes Farm.*, 30, 17–20.
- Dhanda J S, Pegg R B, Janz J A M, Aalhus J L, Shand P J (2002), 'Palatability of bison *semimembranosus* and effects of marination', *Meat Sci.*, 62, 19–26.
- Drew K R, Crosbie S F, Forss D A, Manley T R and Pearce A J (1988), 'Electrical stimulation and ageing of carcasses from red, fallow and New Zealand Wapiti-type male deer', *J. Sci. Food Agric.*, 43, 245–259.

- Du Buisson P-M (2006), *Improving the meat quality of Blesbok (Damalsicus dorcas Phillipsi) and springbok (Antidorcas masupialis) through enhancement with inorganic salts*, MSc thesis, Stellenbosch University, South Africa.
- Eloff T (2002), 'The economic realities of the game meat industry in South Africa', in Ebedes H, Reilly B, van Hoven W and Penzhorn B, *Sustainable Utilization – Conservation in Practice, Proc. 5th Int. Wildl. Ranch. Symp.*, 2001, 78–86.
- Essén-Gustavsson B and Lindholm A, (1984) 'Fiber types and metabolic characteristics in muscles of wild boars, normal and halothane sensitive Swedish Landrace pigs', *CompBiochem. Physiol.*, 78A, 67–71.
- Fletcher J T (1994), 'Why farm deer? – A historical perspective'. In Van Hoven W and Ebedes H, *Wildlife Ranching: A celebration of diversity*, Promedia, Pretoria, South Africa, 253–257.
- Forss D A, Manley T R, Platt M P and Moore V J (1979), 'Palatability of venison from farmed and feral red deer', *J. Sci. Food Agric.*, 30, 932–935.
- Garton G A and Duncan W R (1971), 'Fatty acid composition and intramuscular structure of triglycerides from adipose tissue of the red deer and reindeer', *J. Sci. Food Agric.*, 22, 29–33.
- Gill C O (2007), 'Microbiological conditions of meats from large game animals and birds', *Meat Sci.*, 77, 149–160.
- Goosen G J, Fennessy P F and Pearse A J (1991), 'Carcass composition comparison of male and female red deer and hybrids with Père David's deer', *N. Zeal. J. Agric. Res.*, 42, 483–491.
- Harthoorn A M and van der Walt K (1974), 'Physiological aspects of forced exercise in wild ungulates with special references to (so called) overstraining disease', *J. Sth. Afr. Wildl. Mgmt. Assoc.*, 4, 25–28.
- Hoffman L C, Kroucamp M and Manley M (2007a), 'Meat quality characteristics of springbok (*Antidorcas marsupialis*). 1. Physical meat attributes as influenced by age, gender and production region', *Meat Sci.*, 76, 755–761.
- Hoffman LC, Kroucamp M and Manley M (2007b), 'Meat quality characteristics of springbok (*Antidorcas marsupialis*). 2. Chemical composition as influenced by age, gender and production region', *Meat Sci.*, 76, 762–767.
- Hoffman LC, Kroucamp M and Manley M (2007c), 'Meat quality characteristics of springbok (*Antidorcas marsupialis*). 3. Fatty acid composition as influenced by age, gender and production region', *Meat Sci.*, 76, 768–773.
- Hoffman LC, Kroucamp M and Manley M (2007d), 'Meat quality characteristics of springbok (*Antidorcas marsupialis*). 4. Sensory meat evaluation as influenced by age, gender and production region', *Meat Sci.*, 76, 774–778.
- Hoffman L C and Sales J (2007e), 'Physical and chemical quality characteristics of warthog (*Phacochoerus africanus*) meat', *Livest. Res. Rural. Dev.*, 19, Article # 153. from <http://www.cipav.org.co/lrrd/lrrd19/10/hoff19153.htm>
- Hoffman LC and Wiklund E (2006), 'Game and venison – meat for the modern consumer', *Meat Sci.*, 74, 197–208.
- Hoffman L C, Kritzing B and Ferreira A V (2005), 'The effects of region and gender on the fatty acid, amino acid, mineral, myoglobin and collagen contents of impala', *Meat Sci.*, 69, 551–558.
- Hoffman L C and Webb E C (2005), 'Effect of electrical stimulation on the sensory characteristics of springbok (*Antidorcas marsupialis*)', *Proc. 50th Int. Cong. Meat Sci. Techn.*, Baltimore, Maryland, USA, 189–192.
- Hoffman L C and Ferreira A V (2004), 'Chemical composition of two muscles of the common duiker (*Sylvicapra grimmia*)', *J. Sc. Food Agric.*, 84, 1541–1544.
- Hoffman L C (2001), 'The effect of different culling methodologies on the physical meat quality attributes of various game species', In: H. Ebedes, B. Reilly, W. van Hoven, and B. Penzhorn (Eds.), *Proceedings of the 5th International Wildlife Ranching Symposium Sustainable Utilization – Conservation in Practice 2001* (pp. 212–221).

- Hoffman L C (2000) 'Meat quality attributes of night-cropped impala (*Aepyceros melampus*)', *S. Afr. J. Anim. Sci.*, 30, 133–137.
- Hoskin S O, Barry T N, Wilson P R, Charleston W A G and Kemp P D (1999), 'Growth and carcass production of young farmed deer grazing sulla (*Hedysarum coronarium*), chicory (*Cichorium intybus*), or perennial ryegrass (*Lolium perenne*)/White clover (*Trifolium repens*) pasture in New Zealand', *N. Zeal. J. Agric. Res.*, 42, 83–92.
- Jago J G, Hargreaves A L, Harcourt R G and Matthew L R (1996), 'Risk Factors Associated with Bruising in Red Deer at a Commercial Slaughter Plant', *Meat Sci.*, 44, 181–191.
- Janz J A M and Aalhus J L (2006), 'Meat quality, bacteriology and retail case life of bison *longissimus lumborum* following spray chilling', *J. Musc. Foods*, 17, 330–342.
- Janz J A M, Aalhus J L and Price M A (2001), 'Blast chilling and low voltage electrical stimulation influences on bison (*Bison bison bison*) meat quality', *Meat Sci.*, 57, 402–411.
- Janz J A M, Aalhus J L, Price M A and Schaefer A L (2000), 'The influence of elevated temperature conditioning on bison (*Bison bison bison*) meat quality', *Meat Sci.*, 56, 279–284.
- Kanzaki N and Kodera Y (1995), 'Present status of feral crossbred of pig × Wild boar in Japan', *J. Mount. Ecol.*, 3, 250.
- Kay R N B, Sharman G A M, Hamilton W J, Goodall E D, Pennie K and Coutts A G P (1981), 'Carcass characteristics of young red deer farmed on hill pasture', *J. Agric. Sci., Camb.*, 96, 79–87.
- Knox C M, Hattings J and Raath J P (1991), 'The effect of zeranol on body mass and physiological responses to repeated capture in boma-confined impala', *S. Afr. J. Wildl. Res.*, 21, 38–42.
- Koch R M, Jung H G, Crouse J D, Varel V H and Cundiff L V (1995), 'Growth, digestive capability, carcass, and meat characteristics of Bison bison, Bos Taurus, and Bos × Bison', *J. Anim. Sci.*, 73, 1271–1281.
- Kritzinger B, Hoffman L C and Ferreira A V (2003), 'A comparison between the effects of two cropping methods on the meat quality of impala (*Aepyceros melampus*)', *SA J. Anim. Sci.*, 33, 233–241.
- Kyle R (1994), 'Review: New species for meat production', *J. Agric. Sci., Camb.*, 123, 1–8.
- Ledger H P (1963), 'Animal husbandry research and wildlife in East Africa', *E. Afr. Wildl. J.*, 1, 18–29.
- Ledger H P, Sachs R and Smith N S (1967), 'Wildlife and food production', *Wrlld. Rev. Anim. Prod.*, 3, 13–36.
- Lewis A R, Pinchin A M and Kestin S C (1997), 'Welfare implications of the night shooting of wild impala (*Aepyceros melampus*)', *Anim. Welf.*, 6, 123–131.
- Li Q and Logue C M (2005), 'The growth and survival of *Escherichia coli* O157:H7 on minced bison and pieces of bison meat stored at 5 and 10 °C', *Food Micro.*, 22, 415–421.
- MacDougall D B, Shaw B G, Nute G R and Rhodes D N (1979), 'Effect of pre-slaughter handling on the quality and microbiology of venison from farmed young red deer', *J. Sci. Food Agric.*, 30, 1160–1167.
- MacRae J, O'Reilly L and Morgan P (2005), 'Desirable characteristics of animal products from a human health perspective', *Livest. Prod. Sci.*, 94, 95–103.
- Malmfors G and Wiklund E (1996), 'Pre-slaughter handling of reindeer – Effects on meat quality', *Meat Sci.*, 43, S257–S264.
- Manley T R and Forss D A (1979), 'Fatty acids of meat lipids from young red deer (*Cervus elaphus*)', *J. Sci. Food Agric.*, 30, 927–931.
- Marchiori A M and de Felicio P E (2003), 'Quality of wild boar meat and commercial pork', *Scient. Agrícola*, 60, 1–5.
- McIlroy J C (1995), 'New techniques for an old problem – recent advances in feral pig control in Australia', *J. Mount. Ecol.*, 3, 241–244.
- Mostert R and Hoffman L C (2007), 'Effect of gender on the meat quality characteristics and

- chemical composition of kudu (*Tragelaphus strepsiceros*), an African antelope species', *Food Chem.*, 104, 565–570.
- Muller E, Moser G, Bartenschlager H and Geldermann H (2000), 'Trait values of growth, carcass and meat quality in Wild Boar, Meishan and Pietrain pigs as well as their crossbred generations', *J. Anim. Bred. Genet.*, 117, 189–202.
- Mulley R C, English A W, Thompson J M, Butterfield R M and Martin P (1996), 'Growth and body composition of entire and castrated fallow bucks (*Dama dama*) treated with zeranol', *Meat Sci.*, 63, 159–165.
- Mulley R C and English A W (1985), 'The effect of castration of fallow deer on body growth and venison production', *Anim. Prod.*, 41, 359–361.
- Naya Y, Horiuchi M, Ishiguro N and Shinagawa M (2003), 'Bacteriological and genetic assessment of game meat from Japanese Wild boars', *J. Agric. Food Chem.*, 51, 345–349.
- Nii M, Hayashi T, Mikawa S, Tani F, Niki A, Mori N, Uchida Y, Fujishima-Kanaya N, Komatsu M and Awata T (2005), 'Quantitative trait loci mapping for meat quality and muscle fiber traits in a Japanese wild boar × Large White intercross', *J. Anim. Sci.*, 83, 308–315.
- Paleari M A, Moretti V M, Beretta G, Mentasti T and Bersani C (2003), 'Cured products from different animal species', *Meat Sci.*, 63, 485–489.
- Paleari M A, Bersani C, Vittorio M M and Beretta G (2002), 'Effect of curing and fermentation on the microflora of meat of various animal species', *Food Cont.*, 13, 195–197.
- Paleari M A, Beretta G, Colombo F, Foschini S, Bertolo G and Camisasca S, (2000), 'Buffalo meat as a salted and cured product', *Meat Sci.*, 54, 565–567.
- Phillip L E, Oresanya T F, St. Jacques J (2007), 'Fatty acid profile, carcass traits and growth rate of red deer fed diets varying in the ratio of concentrate:dried and pelleted roughage, and raised for venison production', *Small Rum. Res.*, 71, 215–221.
- Pietrasik Z, Dhanda J S, Shand P J and Pegg R B (2006), 'Influence of injection, packaging (modified atmosphere packaging [MAP] with 70% O₂/30% CO₂ and vacuum packaging [VP]), storage temperature (–1 °C and +4 °C), storage time on the colour, microbial and oxidative stability of beef and bison', *J. Food Sci.*, 71, S110–S118.
- Pollard J C, Littlejohn R P, Asher G W, Pearse A J T, Stevenson-Barry J M, McGregor S K, Manley T R, Duncan S J, Sutton C M, Pollock K L and Prescott J (2002), 'A comparison of biochemical and meat quality variables in red deer (*Cervus elaphus*) following either slaughter at pasture or killing at a deer slaughter plant', *Meat Sci.*, 60, 85–94.
- Pollard J C, Stevenson-Barry J M and Littlejohn R P (1999), 'Factors affecting behaviour, bruising and pH_u in a deer slaughter premises', *Proceed. N. Z. Soc. Anim. Prod.*, 59, 148–151.
- Raes K, De Smet S and Demeyer D (2004), 'Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review', *Anim. Food Sci. Techn.*, 113, 199–221.
- Renecker T A, Wiklund E and Stevenson-Barry J (2001), 'Research overview: Pre-slaughter handling effects on ultimate pH and tenderness in reindeer, red deer, and North American Wapiti meat. In: Game conservation and sustainability', in Renecker L A and Renecker T A, *Biodiversity, Management, Ecotourism, Traditional Medicine & Health*, Renecker & Assoc. Inc., Stratford, Ontario, Canada, 396–409.
- Renecker T A, Renecker L A and Mallory F F (2005), 'Relationships between carcass characteristics, meat quality, age and sex of free-ranging Alaskan reindeer: A pilot study', *Rangifer*, 25, 107–121.
- Ruiz, A G, Mariscal C and Soriano A (2007), 'Influence of hunting-season stage and ripening conditions on nitrogen fractions and degradation of myofibrillar proteins in venison (*Cervus elaphus*) chorizo sausages', *Meat Sci.*, 76, 74–85.
- Sampels S, Pickova J and Wiklund E, (2005), 'Influence of production system, age and sex on carcass parameters and some biochemical meat quality characteristics of reindeer (*Rangifer tarandus tarandus* L.)', *Rangifer*, 25, 85–96.

- Sampels S, Pickova J and Wiklund E, (2004), 'Fatty acids, antioxidants and oxidation stability of processed reindeer meat', *Meat Sci.*, 67, 523–532.
- Schmid A, Collomb M, Sieber R and Bee G (2006), 'Conjugated linoleic acid in meat and meat products: A review', *Meat Sci.*, 73, 29–41.
- Seman D L, Drew K R, and Littlejohn R P (1989), 'Packaging venison for extended chilled storage: Comparison of vacuum and modified atmosphere packaging containing 100% carbon dioxide', *J. Food Prot.*, 52, 886–893.
- Seman D L, Drew K R, Clarken P A and Littlejohn R P (1988), 'Influence of packaging method and length of chilled storage on microflora, tenderness and colour stability of venison loins', *Meat Sci.*, 22, 267–282.
- Sims K L, Wiklund E, Hutchison C L, Mulley R C and Littlejohn R P (2004), 'Effects of pelvic suspension on the tenderness of meat from fallow deer (*Dama dama*)', *Proc. 50th Int. Cong. Meat Sci. Techn.*, Helsinki, Finland.
- Skinner J D, von la Chevallerie M and van Zyl J H M (1971), 'An appraisal of the springbok as a farm animal in Africa', *Anim Breed Abstr.*, 39, 215–224.
- Skinner J D and Louw G N (1996), *The Springbok Antidorcas marsupialis* (Zimmermann, 1780). Trans Mus Monograph 10, Transvaal Museum, Pretoria.
- Smit K (2004), 'Meat quality characteristics of blesbok (*Damaliscus dorcas phillipsi*) and red hartebeest (*Alcelaphus buselaphus caama*) meat', M. Cons Sc. (Food) thesis. Depart Cons Sci. University of Stellenbosch, Stellenbosch.
- Soares G J D, Arêas J A G and Batistuti J P (1995), 'Effect of high voltage electrical stimulation on Buffalo meat conditioning', *Rev. Bras. de Agrociência*, 1, 61–68.
- Sookhareea R, Taylor D G, Dryden G McL and Woodford K B (2001), 'Primal joints and hind-leg cuts of entire and castrated Javan rusa (*Cervus timorensis russa*) stags', *Meat Sci.*, 58, 9–15.
- Soriano A, Cruz B, Gómez L, Mariscal C and García Ruiz A (2006), 'Proteolysis, physicochemical characteristics and free fatty acid composition of dry sausages made with deer (*Cervus elaphus*) or wild boar (*Sus scrofa*) meat: A preliminary study', *Food Chem.*, 96, 173–184.
- Stevenson J M, Seman D L and Littlejohn R P (1992), 'Seasonal variation in venison quality of mature, farmed red deer stags in New Zealand', *J. Anim. Sci.*, 70, 1389–1396.
- Tan G Y and Fennessy P F (1981), The effect of castration on some muscles of red deer (*Cervus elaphus* L.), *N. Zeal. J. Agric. Res.*, 24, 1–3.
- Townsend W E, Brown W L, McCampbell H C and Davis C E (1978), Comparison of chemical, physical and sensory properties of loins from Yorkshire, crossbred and wild pigs', *J. Anim. Sci.*, 46, 646–650, 1838.
- Van Rooyen A F (1993), 'Variation in body composition of impala and nyala in relation to social status and reproduction', *S. Afr. J. Wildl. Res.*, 23, 36–38.
- Van Schalkwyk S (2004), Meat quality characteristics of three South African game species: Black wildebeest (*Connochaetus gnou*), blue wildebeest (*Connochaetus taurinus*), and mountain reedbuck (*Redunca fulvorufula*). M.Sc. thesis. South Africa: University of Stellenbosch.
- Vergara H, Gallegoa L, Garcia A and Landete-Castillejos T (2003), 'Conservation of *Cervus elaphus* meat in modified atmospheres', *Meat Sci.*, 65, 779–783.
- Volpelli L A, Valusso R and Piasentier E (2002), 'Carcass quality in male fallow deer (*Dama dama*): Effects of age and supplementary feeding', *Meat Sci.*, 60, 427–423.
- Volpelli L A, Valusso R, Morgante M, Pittia P and Piasentier E (2003), 'Meat quality in male fallow deer (*Dama dama*): effects of age and supplementary feeding', *Meat Sci.*, 65, 555–562.
- Von la Chevallerie M (1970), 'Meat production from wild ungulates'. *Proc. S. A. Soc. Anim. Prod.*, 9, 73–87.
- Von la Chevallerie M and van Zyl J H M (1971), 'Growth and carcass development of the springbok *Antidorcas marsupialis marsupialis*', *Agroanim.*, 3, 115–121.

- Von la Chevallerie M (1972), 'Meat quality of seven wild ungulate species', *S. A. J. Anim Sci.*, 2, 101–103.
- Webster J R, Corson I D and Littlejohn R P (2001), 'Effect of feeding supplements on the intake and live-weight gain of male red deer given silage during winter', *Anim. Sci.*, 73, 555–561.
- Wiklund E, Johansson L, Aguiar G, Bechtel P J and Finstad G (2006a), 'Seasonal variation in sensory quality of meat from Alaskan reindeer bulls and steers', *Proc. 14th Nordic Conf. Reindeer Res.*, 21–22 March, Helsinki, Finland.
- Wiklund E, Sampels S, Manley T R, Pickova J and Littlejohn R P (2006b), 'Effects of feeding regimen and chilled storage on water-holding capacity, colour stability, pigment content and oxidation in red deer (*Cervus elaphus*) meat', *J. Sci. Food Agric.*, 86, 98–106.
- Wiklund E, Hutchinson C, Flesch J, Mulley R and Littlejohn R P (2005), 'Colour stability and water-holding capacity of *M. longissimus* and carcass characteristics in fallow deer (*Dama dama*) grazed on natural pasture or fed Barley. *Rangifer*, 25, 97–105.
- Wiklund E and Malmfors G (2004), 'The effects of pre-slaughter handling on reindeer meat quality – A review', *Anim. Breed. Abst.*, 72, 1N–6N.
- Wiklund E, Manley T R and Littlejohn R P (2004), 'Glycolytic potential and ultimate muscle pH values in red deer (*Cervus elaphus*) and fallow deer (*Dama dama*)' *Rangifer*, 24, 87–94.
- Wiklund E, Manley T R, Littlejohn R P and Stevenson-Barry J M (2003a), 'Fatty acid composition and sensory quality of *Musculus longissimus* and carcass parameters in red deer (*Cervus elaphus*) grazed on natural pasture or fed a commercial feed mixture', *J. Sci. Food Agric.*, 83, 419–424.
- Wiklund E, Barnier V M H, Smulders F J M, Lundström K and Malmfors G (2003b), 'Proteolysis and tenderisation in Reindeer (*Rangifer tarandus tarandus* L.) Bull *longissimus thoracis* muscle of varying ultimate pH', *Meat Sci.*, 46, 33–43.
- Wiklund E, Johansson L and Malmfors G (2003c), 'Sensory meat quality, ultimate pH values, blood parameters and carcass characteristics in reindeer (*Rangifer tarandus tarandus* L.) grazed on natural pastures or fed a commercial feed mixture' *Food Qual. Pref.*, 14, 573–581.
- Wiklund E, Reh binder C, Malmfors G, Hansson I and Danielsson-Tham M-L (2001a), 'Ultimate pH values and bacteriological condition of meat and stress metabolites in blood of transported reindeer bulls', *Rangifer*, 21, 3–12.
- Wiklund E, Stevenson-Barry J M, Duncan S J and Littlejohn R P (2001b), 'Electrical stimulation of red deer (*Cervus elaphus*) carcasses – effects on rate of pH-decline, meat tenderness, colour stability and water-holding capacity', *Meat Sci.*, 59, 211–220.
- Wiklund E, Pickova J, Sampels S and Lundström K (2001c), 'Fatty acid composition of *M. longissimus lumborum*, ultimate muscle pH values and carcass parameters in reindeer (*Rangifer tarandus tarandus* L.) grazed on natural pasture or fed a commercial feed mixture', *Meat Sci.*, 58, 293–298.
- Wiklund E, Malmfors G and Lundström K (1997), 'The effects of pre-slaughter selection of reindeer bulls (*Rangifer tarandus tarandus* L.) on technological and sensory meat quality, blood metabolites and abomasal lesions', *Rangifer*, 17, 65–72.
- Wiklund E, Malmfors G, Lundström K and Reh binder C (1996a), 'Pre-slaughter handling of reindeer bulls (*Rangifer tarandus tarandus* L.) – effects on technological and sensory meat quality, blood metabolites and muscular and abomasal lesions', *Rangifer*, 16, 109–117.
- Wiklund E, Andersson A, Malmfors G and Lundström (1996b), 'Muscle glycogen levels and blood metabolites in reindeer (*Rangifer tarandus tarandus* L.) after transport and lairage' *Meat Sci.*, 42, 133–144.
- Wiklund E, Andersson A, Malmfors G, Lundström K and Danell Ö (1995), 'Ultimate pH values in reindeer meat with particular regard to animal sex and age, muscle and transport distance', *Rangifer*, 15, 47–54.
- Wood J D, Richardson R I, Nute G R, Fisher A V, Campo M M, Kasapidou E, Sheard P R

- and Enser M (2003), 'Effects of fatty acids on meat quality: A review', *Meat Sci.*, 66, 21–32.
- Woodford K B, Shorthose W R, Stark J L and Johnson G W (1996), 'Carcass composition and meat quality parameters of entire and castrate farmed Blackbuck antelope (*Antelope cervicapva*)', *Meat Sci.*, 43, 25–36.
- Żochowska J, Lachowicz K, Gajowiecki L, Sobczak M, Kotowics M and Żych A (2005), 'Effects of carcass weight and muscle on texture, structure and myofibre characteristics of wild boar meat', *Meat Sci.*, 71, 244–248.
- Żochowska-Kujawska J, Lachowicz K, Sobczak M, Gajowiecki L, Kotowics M, Żych A and Medrala D (2007), 'Effects of massaging on hardness, rheological properties, and structure of four wild boar muscles of different fibre type content and age', *Meat Sci.*, 75, 595–602.
- Zondagh, I B, Illsley, J L L, van Rensburg, D M J and Müller, H (1995), 'Sensory quality characteristics of springbok (*Antidorcas marsupialis marsupialis*) with the emphasis on roasted loins'. *Proc. of the 13th Biennial Congr. of the S.A. Assoc. for Food Sci. and Techn.*, Durban, August 1995, pp 1–27.

Part IV

Improving the quality of fresh meat: processing strategies

Automated grading of beef carcasses

P. Allen, Ashtown Food Research Centre, Teagasc, Ireland

Abstract: Carcass grading forms the basis for quality-based payments to producers and is a common language to facilitate trade in carcasses. A good grading scheme can lead to improvements in efficiency, as producers have a financial incentive to modify their production methods to produce carcasses that are the most desired by consumers. Different beef carcass grading schemes were introduced in the main beef-producing regions, but they all used trained classifiers to assess certain carcass characteristics. They were therefore seen as being subjective and prone to influence, thereby limiting their effectiveness. The development of instrumental objective methods to grade beef carcasses has mainly concentrated on using video image analysis technology (VIA) to mimic grader assessments. In Europe, the rules governing beef carcass grading were changed in 2003 to allow mechanical grading. This has led to many installations in several countries of three different systems. In the USA, handheld VIA systems have been authorised to augment the grader assessments. Future developments in Europe may include using saleable yield assessments from the VIA systems as the basis for quality-based payments; and in the USA there is a move towards fully automated assessment. Ideally, grading should be based on palatability, the consumer perception of quality, but reliable methods to measure this on-line are lacking.

Key words: beef, carcass, grading, video image analysis, saleable yield.

20.1 Introduction

For around 50 years, beef carcass grading has been used in many countries to sort carcasses into quality classes as a basis for producer payment and to assist in trading. Early grading schemes relied on the visual assessment by trained personnel of attributes that were considered to reflect the relative value of a carcass. This was because accurate instrumental methods were not available at that time. Instrumental methods were subsequently developed for grading pig carcasses but when these were tested on beef carcasses they failed to reach an acceptable level of accuracy. One of the reasons for this was that, at least in the

European Union, grading was based on visual criteria rather than on subjective measures such as fat thickness or lean content, as is the case for pig carcasses. In the 1980s work began in Europe on developing Video Image Analysis (VIA) technology to mimic the visual assessments made by trained classifiers. This resulted in a number of commercial systems from Europe, North America and Australia, which found application on a commercial scale in Europe after several trials and changes to the EU grading regulations in 2003. Adoption on a smaller scale has happened elsewhere, particularly in North America.

The object of this chapter is to describe the development and application of automated beef carcass grading, outlining the barriers to progress that were overcome and possible future developments.

20.2 The purpose of carcass grading

Carcass grading is the sorting of carcasses into classes (or grades) based on criteria that are related to their quality or value. The objective is to manage the considerable natural variation in attributes that is commonly seen between carcasses, such that carcasses within a class are more similar to each other than they are to carcasses within other classes. More discrimination is possible as the number of classes increases, provided the method of assessment is sufficiently accurate to classify with a reasonably high degree of certainty.

Carcass grading is used both for quality-based payments to producers and to facilitate trade in carcasses by incorporating the grades into buying specifications. If a grading scheme and its associated pricing structure are working correctly, producers will be provided with a financial incentive to modify their production systems to produce carcasses of the quality that is most valued by the industry. In turn, this should reflect the desires of consumers. An effective grading scheme is therefore an essential part of the information flow from consumer to producer and can bring about changes in production practices that will maximise the proportion of carcasses that meet the top quality criteria and minimise the proportion that fall into the less desirable grades.

In order to be effective in this way, a grading scheme must be based on criteria that are closely related to the consumer perception of quality; it must be reasonably accurate at placing carcasses into the correct classes; the pricing structure must reflect the differences in the actual value of the various quality classes; and it must be applied fairly and consistently. Grading schemes that rely on human judgements are often perceived by producers to be subjective. Producers are then reluctant to accept quality based payments schemes if they are not convinced of the fairness and consistency of the assessments. Flat rate pricing with no discrimination on quality may then become common, particularly if there is overcapacity in slaughtering and therefore competition for cattle. This then undermines the whole purpose of quality-based grading schemes and introduces inefficiency into the industry as it becomes more difficult over time to meet consumer requirements.

20.3 Carcass grading based on visual assessment

Grading schemes based on visual assessment of attributes related to carcass quality are often referred to as 'subjective' schemes, but this is not correct as they are based on photographic and descriptive standards and graders are highly trained and monitored. However, it is generally true that it is difficult to demonstrate the objectivity of such schemes to interested parties such as producers.

20.3.1 European Union (EU) beef carcass classification scheme

The EU beef carcass classification scheme requires that carcasses are classified for conformation and external fat cover (Commission Regulation (EEC) No. 219/93, O.J. No. L196, 5.8.93, p18). For conformation, there are five classes denoted by the letters E, U, R, O and P, with E having the best conformation; hence it is often referred to as the EUROP scheme. Countries have the option of using an additional S class for carcasses with very superior conformation, mainly double muscled animals. Fat classes are denoted 1, 2, 3, 4 and 5, with 1 having the least external fat cover. Both conformation and fat classes may be subdivided into three subclasses. Several countries use this so-called 15-point scale while others subdivide only the most common classes. The classes each have descriptions and photographic standards. Classifiers may be employed by the state, by an independent grading organisation or by the processor. They are highly trained and must be regularly monitored by the responsible national organisation and retrained if necessary. Standards throughout the EU are maintained by an expert panel who visit each country on a regular basis to check that the grading is in line with the EU standards. The classification scheme is used by the EU to define a standard carcass for price reporting and to specify the quality of carcasses eligible for market intervention purposes. It is used by the industry for quality-based payments to producers and in buying specifications for carcass trading.

20.3.2 Beef carcass grading in the USA

In the USA, beef carcasses are graded according to quality and yield grades. The yield grades estimate the amount of boneless, closely trimmed retail cuts from the high-value parts of the carcass – the round, loin, rib and chuck. The grades are numbered 1 to 5, YG1 having the highest expected yield and YG5 the lowest. The grades are calculated from a formula that includes the fat depth over the ribeye, the percentage kidney, pelvic and heart fat (KPH), carcass weight and ribeye area. The ribeye fat depth is measured at the 12th rib, three-quarters of the length of the ribeye from the chine bone, but skilled graders make an adjustment of this measurement to reflect unusual amounts of fat in other parts of the carcass. In other words, they assess how representative this fat depth is of total carcass fat. The amount of kidney, pelvic and heart (KPH) fat is evaluated subjectively and expressed as a percentage of carcass weight, which is the hot carcass weight recorded by the scales. The area of the ribeye muscle is measured using a dot-grid.

The yield grades have descriptions in terms of the external and internal fat deposits and a stepwise procedure is used to assess the yield grade. Firstly, the preliminary yield grade (PYG) is determined from the ribeye fat measurement. This is then adjusted for the carcass weight, using 600 lb (270 kg approx.) as the baseline, then the percentage KPH, and finally the ribeye area.

Beef quality grades are designed to sort carcasses according to their expected palatability, i.e. tenderness, juiciness and flavour. The quality grading is based primarily on marbling fat and the amount of visible intramuscular fat, but also on maturity. Graders evaluate the amount of marbling fat in the ribeye muscle after the carcass has been ribbed between the 12th and 13th ribs. Quality grades are called Prime (most marbling), Choice, Select and Standard (least marbling). Each quality grade is divided into three marbling score subclasses, e.g. Prime is divided into Abundant, Moderately Abundant and Slightly Abundant. Each degree of marbling is divided into 100 subunits, but in practice marbling scores are generally referred to in tenths within each marbling grade, e.g. Slightly Abundant⁹⁰, Moderately Abundant⁵⁰.

Maturity is the second criterion of beef quality grading. Maturity refers to the physiological age of an animal rather than the chronological age. The indicators of maturity are the bone characteristics; that is, the degree of ossification of the cartilage of the sacral and lumbar vertebrae and the spinous processes of the thoracic vertebrae (increases with age) and the colour (darkens with age) and texture (becomes more coarse with age) of the ribeye muscle. Carcass maturity grades are labelled A to E, A being 9–30 months and E being over 96 months. Lean maturity grades are also labelled A to E and when the two do not agree a balancing is carried out with slightly more weighting on the bone score.

The final quality grade is determined by combining the marbling and maturity grades according to a plan. A stepwise procedure is used to determine the final quality grades of Prime, Choice, Select, Standard, Commercial, Utility and Cutter.

20.4 Development and application of automated methods: Video Image Analysis (VIA)

In Europe, the need for mechanical systems to mimic human assessments of conformation and fat cover according to the EUROP scheme undoubtedly limits the potential technologies that could be applied to beef carcass classification and probably has delayed their development for several years. Automated systems based on VIA technology were developed from the early 1980s. VIA involves taking images of a carcass, then using software to extract data such as linear measurements, volumes, angles, curvatures and colours, then using these to predict the conformation and fat class. They can also predict more objective indicators of carcass value, such as percentage hindquarter, yield of high-priced cuts and saleable yield. By the end of the 1990s, when there was a real possibility of the EU regulation being changed to allow mechanical systems to replace trained classifiers, five systems were available commercially.

20.4.1 Description of VIA systems

BCC2 was developed by SFK Technology in Denmark. This uses a colour camera, a holding frame with a coloured background to keep the half carcass steady and a lighting system including structured (striped) light to give 3D information. Three images of the outer side of the half carcass are taken while it is stationary, one with the lights on, another with them off, and a third with the structured light. The first two images are subtracted from each other to take account of any changes in ambient light. The third is used to gain 3D information about the carcass from the degree of curvature of the stripes. Neural network analysis is used to predict the conformation and fat classes, as well as saleable yield from all the available data.

VBS2000 was developed by E + V in Germany. This also uses a colour camera, a holding frame with a coloured background to keep the half carcass steady and a lighting system with structured light. Only two images are taken, one with the main lights on and one with structured light, as the developers did not consider it necessary to adjust for ambient light. Regression analysis is used to predict the conformation and fat class and saleable yield from image data. A cold cut face camera (VCS2000) is also available for grading at the quartering point.

MAC was developed by Normaclass in France. This uses six monochrome cameras set at different heights and viewing angles and a rotating dual holding frame with a background. The first half carcass rests against the frame rib side out and is imaged by two of the cameras (one for the hind the other for the fore). These images are used to determine the outline of the carcass and to assess the fat on the inside. The table then rotates 180 degrees, the first side is released and the second half carcass then comes to rest against the other frame. This side is moved into three different positions and all six cameras take images at each orientation. 3D information is gained from these different viewing angles. The MAC includes an automatic washing system. Regression analysis is used to predict the conformation and fat class and saleable yield from image data.

VIAScan was developed by Meat and Livestock Australia. A major difference between this system and the three European systems described previously is that it does not have a holding frame and does not stop the carcass. It takes pictures with a colour camera while the half carcass is moving so it can operate at much higher speeds. The camera, lighting system and computer are all contained in a stainless steel box. The system is the most compact and is placed only about half as far from the line as the others. The hot carcass system is one of three systems developed by VIAScan, the other two are for grading cold carcasses after quartering and for grading cuts. Data from all three systems can be linked to improve predictions, which are made using regression analysis.

CVS was developed by Lacombe University in Canada (Tong *et al.*, 1997). It is similar to VIAScan, using a single camera. It also operates at high line speeds as the line is not stopped. A cold cut face camera is also available for grading at the quartering point.

20.4.2 Performance of VIA systems

Adoption of VIA grading systems by the beef industry depends among other things

upon their effectiveness in accurately discriminating carcasses according to criteria that are related to their commercial value. Accuracy and repeatability are important criteria in making the decision to install systems and this will be the main focus of this section. Cost, both initial and running costs, practicality and reliability are also important considerations but these will not be discussed. Regulatory issues will also be a factor in the timing of the adoption of the technology by the industry.

Prediction of EUROP conformation and fatness

The EUROP system has been described in Section 20.3.1. Before commencing a review of the performance of VIA systems at predicting EUROP scores for conformation and fatness, it is important to note that the VIA systems have to be trained and calibrated using 'reference' scores determined by one or more human classifiers. Thus, any inaccuracy and inconsistency in the 'reference' scores is included in the measured error of the VIA systems. Their performance is then subsequently judged against a similar 'reference' so that any inherent inaccuracy in the 'reference' will be compounded. It is therefore important that the best possible reference is used when systems are being calibrated or assessed. This may involve using a panel of experienced classifiers rather than a single classifier.

The systems have been demonstrated to be accurate and repeatable in several trials. Madsen *et al.* (1996) reported a large trial where the BCC2 was compared with a classifier and an inspector. The results showed that the BCC2 was more accurate than the plant classifier for both conformation (SEP = 0.57 for BCC2 v 0.75 for classifier) and for fat class (SEP = 0.97 for BCC2 v 1.15 for classifier). Moreover, the BCC2 was more repeatable than the inspector when carcasses were reclassified within 1 hour (RMSE = 0.12 v 0.51 for conformation and RMSE = 0.17 v 0.80 for fat, for BCC2 and inspector, respectively).

Two trials of the VBS2000 system were reported by Sonnichsen *et al.* (1998). In the first trial, a single classifier was compared with the VIA system on 301 young bulls of three breeds. The performance differed little between breeds and was better for conformation class ($R^2 = 0.90$, SEP = 0.93) than for fat class ($R^2 = 0.75$, SEP = 1.20). In the second trial, two experienced classifiers were used as the reference and the results were improved ($R^2 = 0.91$, SEP = 0.81 for conformation class and $R^2 = 0.80$, SEP = 0.91 for fat class).

The MAC system was tested by the French national research organisation INRA (unpublished data). For conformation class, 100% of young bulls and 99% of cows were classified within 2 subclasses of the reference (15-point scale). Corresponding figures for fat class were 98% and 89%.

A common feature of all the trials of VIA systems is that conformation class is predicted with greater accuracy and repeatability than fat class. This may reflect the fact that human classifiers also find it more difficult to accurately determine fat class than to determine conformation class.

A comparative trial of three VIA systems

In 1999, the first comparative trial anywhere of VIA systems was undertaken in Ireland (Allen and Finnerty, 2000, 2001). Three systems, BCC2, VBS2000 and

VIAScan were installed side by side in the Dawn Meats factory in Midleton, County Cork. The first two systems, being European, had both been developed for predicting EUROP grades, but the VIAScan system from Australia had little previous experience of EUROP grading.

A panel of three experienced classifiers scored the carcasses using the 15 × 15 grid (i.e. three subclasses for each conformation class and fat class) and agreed a consensus score when they gave different scores. This consensus score was used as the reference. It was believed that this would give a more accurate reference than using the scores of individual classifiers. A total data set of over 7000 carcasses was divided into a calibration set ($n = 4278$) and a validation set ($n = 2969$). As none of the systems had previously been trained on Irish carcasses, the calibration set was used by the operators of the VIA systems to derive suitable algorithms for conformation class and fat class predictions. These were then tested on the validation set. For conformation, the percentage classified to within one subclass (1/3 of a class) of the reference was 96.5, 92.8 and 91.0% for VBS200, BCC2 and VIAScan respectively. The corresponding errors (RSD) were 0.75, 0.70 and 0.80. In common with previous tests of the systems individually, the performance for fat class predictions was poorer than for conformation, with the percentage predicted to within one subclass of the reference being 74.6, 80.4 and 72.0% for VBS200, BCC2 and VIAScan, respectively, and errors of 1.38, 1.14 and 1.38, respectively.

A second validation trial was undertaken at the same factory in Ireland in 2000. All the data from the first trial (calibration and validation sets) were made available to the companies to optimise their algorithms prior to this second trial. These were then tested on over 2000 carcasses. The performance of all three systems was again very acceptable for conformation with the percentage classified to within one subclass of the reference being 95.4, 97.0 and 94.2 for VBS200, BCC2 and VIAScan respectively. For fat class the percentage classified to within one subclass of the reference was again lower than for conformation and was unchanged for two systems with VIAScan showing an improvement to 76.1%.

The main conclusions from the two trials were that there were relatively small differences between the three systems in their performance and that all three systems performed markedly better at predicting conformation class than they did for fat class. As stated earlier, this may at least partly reflect problems with the reference fat scores. Another important finding was that all three systems showed some biases. In other words there was systematic under- or over- scoring by the systems either for the whole sample or for subsets. In particular, well-conformed carcasses tended to be underscored by all systems, something which producers would see as putting them at a disadvantage.

Authorisation trial

In 2003 the EC regulation was amended to allow automated grading and rules were agreed for conducting authorisation trials. The first authorisation trial was carried out in Ireland on the same three systems used in the earlier trials and at the same factory. A panel of five classifiers, three from other EU countries, was used and a representative sample of 1290 carcasses was classified independently by each

Table 20.1 Scoring system for authorisation of automated grading equipment

	Conformation	Fat cover
No error	10	10
Error of 1 subclass	6	6
Error of 2 subclasses	-9	0
Error of 3 subclasses	-27	-13
Error of more than 3 subclasses	-48	-30
Total score	>600	>600
Bias	± 0.3	± 0.60
Slope of the regression line	1 ± 0.15	1 ± 0.30

classifier and by the three VIA systems. The median result of the panel was taken as the reference for each carcass and the predictions of the systems were compared with this. Scores were allocated as shown in Table 20.1 and all three systems passed the 600 point threshold for authorisation. They also passed the bias and slope limits shown in the table.

The lower penalties for errors in fat class predictions in the scoring system reflect the eventual recognition by the authorities that fat class is more difficult to assess than conformation class. Acceptance of this principle delayed the process of agreeing the rules for authorisation of automated grading equipment for some time. The results from the comparative trials in Ireland and all other published research on VIA systems indicate that both classifiers and automated systems are less accurate at assessing fat class than they are at assessing conformation class. This may be due to the fact that on fatter carcasses the depth of fat becomes important in addition to the total area of the carcass covered by fat and the depth is difficult to visualise. It should be noted that, if classifiers have more difficulty in assessing fat cover, then so will the automated systems as these are calibrated against classifiers. Although the scoring system imposes lower penalties for misclassifying the fat class than for misclassifying the conformation class, the same threshold of 600 points applies to both.

Other countries have followed with authorisation trials for VBS2000, BCC2 and MAC and the number of installations in European abattoirs has slowly increased.

Prediction of saleable yield

Conformation and fatness as assessed in the EUROP scheme are both related to the commercial value of a carcass, but this relationship is mostly, though not totally, due to their effect on the saleable yield. When the EU scheme was devised, there were no satisfactory instrumental methods of measuring yield on-line, so the best tool available was a visual assessment of conformation and fat cover. No attempt was made to convert the different classes into yield percentages, as there was no standard definition of yield. If VIA systems are able to predict saleable yield and

other quality-related traits more accurately than the EUROP grading, then they would have an additional value to the industry.

Borggaard *et al.* (1996) showed that the BCC2 was more accurate than a classifier in predicting the percentage saleable yield (SEP = 1.34 v 1.63), the percentage hindquarter (SEP = 1.01 v 1.26) and the ribeye area (SEP = 5.8 v 6.7). VIAScan was shown to be more accurate at predicting saleable yield than the existing grading system that used weight and a fat depth for three out of four types of carcasses (Ferguson *et al.*, 1995). Standard errors for the VIAScan were between 0.98 and 1.52%. Sonnichsen *et al.* (1998) reported a slightly higher SEP of 1.8% for predicting the saleable yield of 301 young bulls of three breeds. However, it is not meaningful to compare the results of different trials due to differences in the variability of the samples and in the specification of saleable yield.

A test of the ability of the VIA systems to predict saleable yield was carried out as part of the first Irish trial described previously. This is the only comparative trial of VIA systems to predict saleable yield to have been conducted (Allen and Finnerty, 2000). A sample of 393 steer half carcasses was boned out and trimmed to a commercial specification. Roughly two-thirds of these were used to calibrate the three VIA systems and the rest ($n = 133$) were used for validation. There were only small differences between the three systems in their ability to predict saleable yield, with RSD's between 1.12 and 1.20%. Surprisingly though, the classification scores plus carcass weight were equally accurate (RSD = 1.21). This may have been due to the fact that a panel of three classifiers was used and the consensus scores were probably more reliable than those of individual classifiers as used in other trials. Such a result would not be expected for a single on-line classifier. Also, the fact that the specification did not involve heavy trimming of fat may have been a factor in this surprising result. In fact the percentage saleable yield actually increased as fat class increased, which is the opposite of what would be expected. Primal yield was therefore calculated by excluding the trim, the plate and the flank cuts. In this case the primal yield followed the expected trend, decreasing with increasing fatness. However, the VIA systems were less accurate than the classification scores and weight at predicting primal yield (RSD = 1.44 v 1.50–1.56).

20.4.3 Installations in Ireland

Following authorisation, the Irish beef industry selected the VBS2000 system and 25 systems were installed at all the main export plants in 2004. At the same time, the Department of Agriculture and Food withdrew their classification service. Smaller plants have to train their own operatives to continue with the visual system. After a few initial teething problems, all systems worked well. Early claims by producers that the systems underscored good conformation carcasses were shown to be unfounded as the manufacturer had corrected the algorithms for this bias that had become apparent in the original trials. The number of complaints from producers about grades quickly declined after the introduction of the

automated systems and the issue has ceased to be a major source of disagreement between producers and processors. Some companies have indicated their interest in having saleable yield predictions but as yet there is no move towards a national scheme based on yield. In the absence of this, each company will probably have its own calibrations based on their main cutting specifications.

20.4.4 VIA cut-face systems

In the USA and in Australia, grading is done after chilling and quartering. This allows the eye muscle and overlying fat to be seen. In the USDA scheme described earlier, carcasses are graded based on estimated yield, using factors such as weight, eye muscle area and fat depth, and on quality using factors such as ossification, marbling fat, lean texture, lean colour and fat colour. Jones *et al.* (1992) identified the need for rapid and automated methods to grade carcasses and the potential of VIA hand-held systems to replace manual grading. More recently, grading standards have been shown to vary significantly across packing plants, and graders are more likely to be accurate when grading relatively low-quality carcasses (Hueth *et al.*, 2007). Hand-held VIA systems have been developed to do at least part of this automatically. Cannell *et al.* (1999) showed that the dual component VIAScan was superior to the online grader at all three trimming specifications tested, but was less accurate than an expert grader working without time constraints. However, when the VIAScan ribeye area was combined with the expert grader's estimates of the other yield grade factors, the percentage of the variation in yield at all trim levels was greater than for the expert grader alone. This illustrates the potential of VIA systems for augmenting the grader and allowing more time for the other factors to be assessed by the grader. Interestingly, the percentage variation explained increased as the trim level increased from commodity to closely trimmed for all models, but there was a further increase as the trim increased to very closely trimmed only for the VIAScan system alone. At this level of trim, the advantage of augmenting the grader compared to using the VIAScan alone was marginal (75 v 71%). Similar results were found using the dual component CVS system (Cannell *et al.*, 2002). The CVS system was far superior at predicting closely trimmed wholesale yield than the online grader (% variation explained = 64 v 39) but almost as accurate as the expert grader working offline when used alone or to augment the expert grader. Shackelford *et al.* (2003) conducted trials in two commercial beef processing facilities of the Meat Animal Research Centre (MARC) system. Prediction equations that included image analysis variables and hot carcass weight accounted for 90% of the variation in calculated yield grade compared to 73% for the online graders. MARC data plus carcass weight also accounted for 88, 90, 88 and 76% of the variation in eye muscle area, preliminary yield grade, adjusted preliminary yield grade and marbling score, respectively. The authors concluded that the system could be used to determine yield grade on-line but is not accurate enough to be used alone for predicting marbling score.

20.5 Future trends

20.5.1 Eating quality

Adoption of hand-held VIA cameras within the EU to take images at the quartering point to augment the on-line VIA data could potentially improve the accuracy of yield predictions. It would also give information related to the potential eating quality of carcasses, such as marbling fat and fat and lean colour that could be used by the industry to apply quality-based grading schemes, but also could feed into the breeding schemes to improve yield and quality in the future.

It is important to remember that quality to the consumer really means eating quality: mainly tenderness, juiciness and flavour. The current EU grading system has little to do with this. With increasing competition internationally, an industry that doesn't seek to satisfy the consumer is unlikely to prosper. A change towards grading and quality payments based on consumers' expectations which will feed into breeding schemes and production methods is the way forward.

Grading based on eating quality depends on being able to measure or predict eating quality. As yet there are no very reliable methods to measure eating quality on-line or shortly after slaughter. Belt grill cooking and shear force determination of *longissimus* steaks cut at 1–2 days post-mortem has been shown to give repeatable and reliable predictions of sensory panel ratings of tenderness. Shackelford *et al.* (1999) and Vote *et al.* (2003) concluded that on-line measurements by a CVS VIA system equipped with a BeefCam were useful for predicting the tenderness of beef *longissimus* muscle steaks. Wheeler *et al.* (2003) showed that beef colour, either measured by the BeefCam VIA system or by colorimetry, was sufficiently accurate at predicting tenderness. More recently, VIS-NIR spectroscopy has been demonstrated to be useful for sorting carcasses into acceptable and unacceptable categories (Shackelford *et al.*, 2005; Price *et al.*, 2008; Rust *et al.*, 2008). VIA may also have potential for assessing ossification objectively, though further development is probably necessary (Hattem *et al.*, 2003).

In Australia, the approach taken by Meat and Livestock Australia is to predict eating quality from a number of live animal and post slaughter factors using a model, the MSA palatability model, developed over 10 years and based on over 70 000 samples tasted by large numbers of consumers. The development of this model is described elsewhere in this book. Briefly, the MSA model predicts palatability scores for all the main cuts in the carcass cooked by a number of different methods. This approach recognises the reality of the variability in eating quality between different muscles within a carcass and the interaction between muscle and cooking method. The MSA model has been tested for application in other countries, including Ireland and the USA.

20.5.2 Other technologies for carcass grading

Other technologies that have either been applied to carcass grading or have potential for this application include whole body electrical conductivity (TOBEC),

bio-electrical impedance analysis (BIA), ultrasound, and magnetic inductance technology (MIT). Berg *et al.* (1994) showed that TOBEC could predict the fat and lean content of lamb carcasses with sufficient accuracy for grading applications but the technology is not applicable to beef carcass grading due to size limitations. However, scanning individual cuts with TOBEC can give accurate predictions of carcass wholesale value (Weide *et al.*, 1995). BIA has also been shown to be a useful predictor of beef carcass composition (Bohuslavek *et al.*, 2004). It is feasible that BIA could be used to augment the VIA data as BIA measures internal rather than the external fat which is assessed by VIA. There are practical difficulties, however, related to the placement of electrodes on the carcass. MIT is similar in principle to TOBEC, but whereas the latter generates a field inside a large coil through which the carcass passes, with MIT the carcass passes through the field generated between pairs of coils. By using a series of coil pairs, it should be possible to gain information about the distribution of the lean tissues as well as the total amount of lean in the carcass. Ultrasound has been successfully applied to the measurement of fat and muscle depths in pig carcasses but in beef and sheep carcasses these measurements are less repeatable and have poorer correlation with overall composition. Reasons for this include the uneven nature of the subcutaneous fat cover on beef and sheep carcasses, which is exacerbated by hide removal, and by the fact that the subcutaneous fat depot is a smaller proportion of the total carcass fat in beef and sheep than in pork carcasses (Kempster *et al.*, 1982). Finally, X-ray CT and MRI have been shown to be highly accurate at estimating carcass composition (Dobrowolski *et al.*, 2004; Collewet *et al.*, 2005; Romvari *et al.*, 2006) but have little prospect of being used on-line due to cost and technical issues. Another X-ray system, the DEXA (Dual Energy X-Ray Absorption) system has been investigated for lamb and pork carcass grading, but size limitations and other practical issues probably rule it out for beef carcass grading.

20.6 Sources of further information and advice

- Allen, P. (2003), Beef Carcass Grading in Europe and the U.S.A. – The Prospects for Using VIA Systems. *Brazilian Journal of Food Technology*, Special Issue, 96–101.
- Overbeke, D.L. van (ed) (2007), *Handbook of Beef Safety and Quality*. Haworth Food and Agricultural Products Press, Binghampton, USA.
- Quality and Grading of Carcasses of Meat Animals* (1995), CRC Press Inc., Boca Raton, USA, 234 pp. ISBN: 0-8493-5023-9.

20.7 References

- Allen, P. and Finnerty, N. (2000), *Objective Beef Carcass Classification – A Report of a Trial of Three VIA Classification Systems*. Teagasc, Dublin, Ireland.
- Allen, P. and Finnerty, N. (2001), *Mechanical Grading of Beef Carcasses*. Teagasc End of Project Report, No. 45, Teagasc, Dublin, Ireland.
- Berg, E.P. and Marchello, M.J. (1994), Bioelectrical impedance analysis for the prediction of fat-free mass in lambs and lamb carcasses. *Journal of Animal Science*, 72 (2), 322–329.

- Bohuslavek, Z., Branscheid, W. and Augustini, C. (2004), Prediction of beef grades by means of the bioelectrical impedance analysis (BIA). *Fleischwirtschaft*, 84 (1), 108–111.
- Borggaard, C., Madsen, N. T. and Thodberg, H. H. (1996), In-line image analysis in the slaughter industry, illustrated by beef carcass classification, *Meat Science*, 43, S151–S163.
- Cannell, R. C., Tatum, J. D., Belk, K. E., Wise, J. W., Clayton, R. P. and Smith, G. C. (1999), Dual-component video image analysis system (VIAScan™) as a predictor of beef red meat yield percentage and for augmenting application of USDA yield grades. *Journal of Animal Science*, 77, 2942–2950.
- Cannell, R. C., Belk, K. E., Tatum, T. D., Wise, J. W., Chapman, P. L., Scanga, J. A. and Smith, G. C. (2002), Online evaluation of a commercial video image analysis system (Computer Vision System) to predict beef carcass red meat yield and for augmenting the assignment of USDA yield grades. *Journal of Animal Science*, 80, 1195–1201.
- Collewet, G., Bogner, P., Allen, P., Busk, H., Dobrowolski, A., Olsen, E. and Davenel, A. (2005), Determination of the lean meat percentage of pig carcasses using magnetic resonance imaging. *Meat Science*, 70, 563–572.
- Dobrowolski, A., Romvari, R., Allen, P., Branscheid, W. and Horn, P. (2004), X-ray computed tomography as possible reference for the pig carcass evaluation. (In German with summary in English). *Fleischwirtschaft*, 84 (3), 109–112.
- Ferguson, D. M., Thompson, J. M., Barrett-Lennard, D. and Sorensen, B. (1995), Prediction of beef carcass yield using whole carcass VIAScan, *Proceedings 41st ICoMST*, San Antonio, USA, Paper B16, 183–184.
- Hatem, I., Tan, J. and Gerrard, D.E. (2003), Determination of animal skeletal maturity by image processing. *Meat Science*, 65 (3), 999–1004.
- Hueth, B., Marcoul, P. and Lawrence, J. (2007), Grader bias in cattle markets? Evidence from Iowa. *American Journal of Agricultural Economics*, 89 (4), 890–903.
- Jones, S.D.M., Lang, D., Tong, A.K.W. and Robertson, W. (1992), A commercial evaluation of image analysis in the grading of beef carcasses. In: *Proceedings of the 38th ICoMST*, Clermont Ferrand, Vol 5, 915–918.
- Kempster, A.J., Cuthbertson, A. and Harrington, G. (1982), *Carcass Evaluation in Livestock Breeding, Production and Marketing*. Granada Publishing Ltd., London, UK.,
- Madsen, N. T., Thodberg, H. H., Flig, T. and Ovesen, E. (1996), BCC-2 for objective beef carcass classification and prediction of carcass composition. *Proceedings 42nd Annual Reciprocal Meat Conference*, Lillehammer, Norway, Paper G-6, 244–245.
- Price, D.M., Hilton, G.G., VanOverbeke, D.L. and Morgan, J.B. (2008), Using near-infrared system to sort various beef middle and end muscle cuts into tenderness categories. *Journal of Animal Science*, 86 (2), 413–418.
- Romvari, R., Dobrowolski, A., Repa, I., Allen, P., Olsen, E., Szabo, A. and Horn, P. (2006), Development of a computed tomographic calibration method for the determination of lean meat content in pig carcasses. *Acta Veterinaria Hungarica*, 54 (1), 1–10.
- Rust, S.R., Price, D.M., Subbah, J., Kranzier, G., Hilton, G.G., Vanoverbeke, D.L. and Morgan, J.B. (2008), Predicting beef tenderness using near infrared spectroscopy. *Journal of Animal Science*, 86 (1), 211–219.
- Shackelford, S.D., Wheeler, T. L. and Koohmaraie, M. (1999), Evaluation of slice shear force as an objective method of assessing beef *longissimus* tenderness. *Journal of Animal Science*, 77 (10), 2693–2699.
- Shackelford, S.D., Wheeler, T. L. and Koohmaraie, M. (2003), On-line prediction of yield grade, longissimus muscle area, preliminary yield grade, adjusted preliminary yield grade and marbling score using the MARC beef carcass image analysis system. *Journal of Animal Science*, 81, 150–155.
- Shackelford, S.D., Wheeler, T. L. and Koohmaraie, M. (2005), On-line classification of US Select beef carcasses for longissimus tenderness using visible and near-infrared reflectance spectroscopy. *Meat Science*, 69 (3), 409–415.
- Sonnichsen, M., Augustini, C., Dobrowolski, A. and Branscheid, W. (1998), Objective

- classification of beef carcasses and prediction of carcass composition by video image analysis, *Proceedings 44th ICoMST*, Barcelona, Spain, Paper C59, 938–939.
- Tong, A.K.W., Richmond, R.J., Jones, S.D.M., Robinson, D.J., Chabot, B.P., Zawadski, S.M., Robertson, W.M., Li, X. and Liu, T., 1997. *Development of the Lacombe Computer Vision System (Lacombe CVS) for Beef Carcass Grading*, Agriculture and Agri-food Canada, Lacombe Research Centre, AB., Canada.
- Vote, D. J., Belk, K. E., Tatum, J. D., Scanga, J. A. and Smith, G. C. (2003), Online prediction of beef tenderness using a computer vision system equipped with a BeefCam module. *Journal of Animal Science*, 81, 457–465.
- Weide, L.R., Calkins, C.R. and Wheeler, T.L. (1995), Predicting beef carcass subprimal weight with electromagnetic scanning and USDA yield grade, In: *Proceedings of the 41st ICoMST*, San Antonio, Volume 2, 177–178.
- Wheeler, T. L., Vote, D., Leheska, J. M., Shackelford, S. D., Belk, K. E., Wulf, D. M., Gwartney, B. L. and Koohmaraie M. (2003), The efficacy of three objective systems for identifying beef cuts that can be guaranteed tender. *Journal of Animal Science*, 80, 3315–3327.

Determining the lean content of pork carcasses

C. Pomar and M. Marcoux, Agriculture and Agri-Food Canada, Canada, M. Gisbert and M. Font i Furnols, IRTA, Spain and G. Daumas, IFIP Institut du Porc, France

Abstract: Carcass lean yield is defined as the proportion of tissues of interest of a carcass obtained according to a reference method. Carcass lean yield is associated with the carcass commercial value. This chapter reviews the across-countries definitions of pork carcass lean yield, the technologies that are available today for both the on-line determination of these lean yields and the precise measure of the weight of the tissues of interest. The limits of actual definitions of lean yields and of the methods used for predicting carcass lean composition are discussed. Future directions for the on-line determination of carcass value are given.

Key words: pork, carcass composition, lean yield, carcass grading, carcass value.

21.1 Introduction

Meat, in its broadest definition, is animal tissues used as food. Generally, it refers to skeletal muscle and associated fat. Lean is that part of flesh which consists principally of muscles, with no fat other than that naturally present within skeletal muscles, and which can be separated from the carcass by dissection. Skeletal muscle and fat contents are critical attributes of the meat and they determine the quality and the commercial value of pork carcasses and cuts.

The need to place an objective value on pork carcasses has been recognized by the pig industry for more than fifty years. Although the grading of live pigs was developed in the early 1920s, it was not until the 1960s that an effective value-based system was introduced in many countries, following the capacity to measure fat depths with automatic probes in slaughter plants. In most pig-producing countries today, the pork carcass grading system uses weight and lean yield to

determine the commercial value of the carcasses. Lean yield definition differs greatly between countries but generally carcass lean yield is predicted using the strong relationship that exists between this parameter and the subcutaneous fat and muscle depths measured at specific locations of the carcass (Engel *et al.*, 2003). These two thicknesses are measured using instruments specifically developed for grading pig carcasses on the slaughter line. Today, novel methods for grading pork carcasses on the slaughter line are available. These new methods are based on video analysis techniques combined, or not, with other measurements of fat and muscle thicknesses (Fortin *et al.*, 2003; McClure *et al.*, 2003) and dual energy X-ray absorptiometry (Mitchell *et al.*, 2003). These methods are described in the following sections.

Carcass lean yield is defined as the proportion of tissues of interest in a carcass, obtained according to a reference method in which carcass preparation and the number and extent of dissected tissues is precisely described. The tissues of interest are mainly dissected lean but they can also include some fat and bones. However, carcass dissection is time-consuming (4–9 h of work per half carcass), expensive, and subject to biases resulting from the dexterity or fatigue of the butchers (Daumas, 1999; Nissen *et al.*, 2006). Therefore, magnetic resonance and various X-ray technologies are proposed as non-destructive methods for measuring carcass tissue masses to replace actual dissection methods (Suster *et al.*, 2003; Marcoux *et al.*, 2003). Although these methods are too slow, expensive and complex to be used on slaughter lines, they are proposed for use in dissection projects aimed at developing new grading systems or their periodical calibration. A description of these methods is presented in the following sections of this chapter.

The objective of this chapter is to review the across-countries definitions of lean yield, to review the technologies that are available today for on-line determination of carcass lean yield, to review the technologies available to precisely measure the weight of the tissues of interest in the carcasses, and to indicate the limits of actual methods for evaluating and predicting carcass composition and carcass value. Future directions of on-line determination of carcass value are also given.

21.2 Determination of carcass lean yield

Pork carcass lean yield is generally expressed as a percentage and is obtained by dividing the weight of the tissues of interest (numerator) over the overall or partial carcass weight (denominator), depending on the reference method. The tissues of interest can include lean and/or fat and/or bones, depending on the consumer and industry demands or on other practical reasons. From the consumers' perspective, there is increasing demand for leaner carcasses and, in this context, a series of cut-out and dissection methods have been proposed in many countries for establishing or updating actual carcass grading systems. Thus, time and cost savings prompted the European Union to create a simplified dissection method (Walstra and Merkus, 1996) from which the definition of lean meat percentage was established following

an amendment to the European Community regulations on pig carcass grading that came into effect on January 1, 1995 (Daumas and Dhorne, 1996; Gispert and Font, 2005). This yield is calculated as the weight of the muscles of the shoulder, belly, loin, ham and tenderloin over the weight of 12 cuts separated from the half carcass (Walstra and Merkus, 1996). More recently, the denominator of this lean yield definition has been modified in the European Union to include only the weight of the dissected cuts (EU Regulation 1197/2006). Nonetheless, the tissues of interest (numerator) of all lean yield definitions used in the European Union include only red striated dissected muscles.

In the United States, carcass grading harmonization efforts appear to be leading stakeholders in the pork sector to adopt a definition of lean yield that is expressed in terms of standardized fat-free lean yield (NPPC, 2000; Johnson *et al.*, 2004). Fat-free lean (FFL) is a measure of the dissected carcass lean muscle after removing the estimated amount of fat tissue remaining in the dissected lean (Schinkel *et al.*, 2001). Ether lipid extraction provides an accurate measure of the extractable lipid component of the carcass soft tissue, the latter being obtained after the removal of the skin and bones from the carcass. However, total fat tissue mass includes the connective tissue, the water, and the ash masses associated with adipose tissue which must be taken into account in the estimation of the carcass FFL. The use of a correction factor to account for these components was first suggested by Fahey *et al.* (1977) and subsequently used by Orcutt *et al.* (1990), Schinkel *et al.* (2001) and Johnson *et al.* (2004). The definition of FFL as originally proposed by Fahey *et al.* (1977) was used for development of the National Pork Producers Council (NPPC; now the National Pork Board) standard for assessment of fat-free pork carcass lean (NPPC, 1994). Nonetheless, different pork packing plants in the United States use different carcass preparation and cutting methods, different assessment equipment and measurement sites and therefore proper comparisons between all these methods is difficult. Some efforts have, however, been made to provide statistically based methods to standardize data and facilitate the consistency of price reporting data (Johnson *et al.*, 2004). However, the success of these efforts has been limited.

In Canada, the pork carcass grading system tries to segregate the carcasses in relation to their content of saleable meat, which is believed to represent their true commercial value. Saleable meat yield is defined in the Canadian context as being the proportion of the weight of the lean in picnic, butt, loin, tenderloin and ham, plus the belly (skinless and trimmed) and the side ribs (Fortin *et al.*, 2004). This yield takes into consideration the weight of the commercial cuts, which include not only dissected lean but also some fat tissues such as those from the belly and some bones such as those from the side ribs. Although this carcass evaluation and grading system was initially proposed for use across Canada, its utilisation is not mandatory today and some slaughter plants are using modified versions of the original grading system or giving premiums or penalties to carcasses with specific muscle and backfat depths.

The differences among the various carcass lean yield definitions in terms of the selected tissues of interest and their denominator contribute to the carcass lean

yield values in different ways. First, a yield definition that includes a higher number and weight of tissues (i.e., lean, fat and bone) in the numerator of its formula necessarily gives higher absolute values than other definitions in which lower weight of tissues are included. Similarly, the dispersion of the values across the mean will also be affected by the yield definition. It is, however, possible to standardize lean yield values across definitions in order to compare them to similar average values and proportional variances. Using this approach, Marcoux *et al.* (2007) demonstrated that, after adjustment of several carcass lean yield definitions measured on the same carcasses, the spread of the observations around the same average yield value was strongly affected by the yield definition. Indeed, the standard deviation and the range values of the leaner yield definitions were nearly four times greater than those of the fatter definitions (Marcoux *et al.*, 2007). It was also observed that the greater the proportion of fat and bone included in the numerator of the yield definition formula, the less this yield definition was able to discriminate between lean and fat carcasses. In fact, the inclusion of the belly and its fat content in the numerator of the formula reduces the differences in yield values among carcasses since the weight increases with carcass fat content (Fredeen, 1980; Gibson and VanderVoort, 1999). These results indicate that, in a context where the evaluation of overall carcass leanness is the most important criterion for carcass value, it would be preferable to predict the yield of a carcass in terms of the dissected lean, avoiding the inclusion of fat and bone. Furthermore, based on the results of Marcoux *et al.* (2007) and, assuming that there is a positive correlation between intramuscular fat and the overall carcass fatness (Latorre *et al.*, 2007), it can be expected that lean yields using fat-free lean masses as tissues of interest will be theoretically more discriminating in terms of carcass leanness than those yields in which dissected tissues are the measured tissues.

Carcass evaluation and grading systems are frequently linked to a system of premiums to producers, the assumption being that the premiums represent the true commercial value of the carcass. The payment systems are the bridge between producers and processors, both stakeholders having the common objective of profit maximization. Producers choose the animals' genetics, the feeds and management practices that maximize their net revenue. The premium systems are established by the stakeholders to promote the production of carcasses that maximize the processor's economic return. The production sectors' response to the processor's priorities always depends on the clarity of the signal that comes from a given grading and payment system (Cross and Savell, 1994). Therefore, if processors are interested in increasing overall carcass leanness, then the producers' response will be faster and more efficient when using the leaner yield definitions rather than yield definitions in which fat and bones are part of the numerator. However, overall carcass leanness may not be the only criterion to be taken into account when the processor's objective is to obtain carcasses with increased commercial value from the producers. This aspect will be developed in the following sections.

Table 21.1 Equipment used or approved for pig carcass grading by country (August, 2007)

Country	Manual		Semiautomatic				Automatic		
	ZP	OP	Reflectance		Ultrasound		AUTOFOM	Others	
			FOM	HGP	Others	UFOM	UMEATER		
Argentina			X	X					
Australia				X		X			CVT
Belgium					CGM				
Brazil				X		X		X	
Canada				X	DES				
Czech Republic	X		X	X	ISD	X			
Germany	X		X					X	
Denmark			X					X	KC
Estonia		X				X			
Greece			X	X	DES				
Spain			X					X	
Finland		X		X					
France	X				CGM		X	X	
Hungary			X			X		X	
Ireland			X	X					
Italy			X	X					
Mexico						X			
Netherlands				X					VCS 2000
New Zealand				X					
Northern Ireland		X		X				X	UP Mark II
Poland					CGM/ IM03	X		X	
Portugal		X	X	X					
Sweden		X		X				X	
United Kingdom		X	X	X			X	X	
United States			X	X	DES			X	TOBEC

Note: AUTOFOM: Automatic Carcass Grading; CGM: Capter Gras-Maigre; CVT: Carcass Value Technology; DES: Destron; FOM: Fat-o-meat'er; HGP: Hennessy Grading Probe; KC: Classification Center, SFK Ltd, Denmark; OP: Optical Probe; TOBEC: Total Body Electrical Conductivity; UFOM: Ultrafom; UMEATER: Ultrameater; UP: Ulster Probe; VCS2000: Vision Carcass System; ZP: Zwei-Punkt Messverfahren.

21.3 On-line determination of carcass composition and lean yield

Over many years, numerous types of equipment for pig carcass evaluation and grading have been developed and tested (Evans and Kempster, 1979; Fortin *et al.*, 1984; Diestre and Kempster, 1985). Most of these technologies are based on the relationship that exists between these parameters and fat and lean depths. The various devices are classified, based on their degree of automation, as manual, semiautomatic or fully automatic equipment (Table 21.1). The prediction errors associated with the different devices vary according to the study sample, the country, the definition of lean content, etc. (Table 21.2).

Table 21.2 Accuracy of different equipment

Equipment	Country	RSD/RMSEP*	Reference
Manual			
OP	Italy	2.38–2.45	Daumas and Dhorne, 1998
	Poland	2.38	Daumas and Dhorne, 1998
	Sweden	2.47	Daumas and Dhorne, 1998
	United Kingdom	2.31	Daumas and Dhorne, 1998
ZP	Germany	2.44	Brøndum <i>et al.</i> , 1998
		2.52	Daumas and Dhorne, 1998
	France	2.45–2.49	Daumas and Dhorne, 1998
	Sweden	2.38	Daumas and Dhorne, 1998
Semiautomatic			
CGM	Canada	2.28	Pomar <i>et al.</i> , 2001b
	Belgium	2.08	Daumas and Dhorne, 1998
	France	1.92–2.06	Daumas and Dhorne, 1998
CVT	Canada	2.14–2.27	Pomar <i>et al.</i> , 2001b
	Canada	1.57	Fortin <i>et al.</i> , 2004, 2005
DES	Belgium	2.45	Daumas and Dhorne, 1998
	Canada	1.712.432.15	Usborne <i>et al.</i> , 1987; Pomar <i>et al.</i> , 2001b ; Fortin <i>et al.</i> , 2003
FOM	Poland	2.49	Piechocki <i>et al.</i> , 1994
	Germany	2.01	Brøndum <i>et al.</i> , 1998
	Germany	1.80	Adapted from Bransheid <i>et al.</i> , 2004
	Denmark	2.02	Daumas and Dhorne, 1998
	Spain	1.58	Gispert and Diestre, 1994
	Spain	2.18–2.33	Font i Furnols <i>et al.</i> , 2004
	Ireland	2.2	Allen and McGeehin, 2001
	Italy	2.30–2.37	Daumas and Dhorne, 1998
	United States	2.351.87	Brøndum <i>et al.</i> , 1998; McClure <i>et al.</i> , 2003
HGP	Canada	1.712.181.56	Usborne <i>et al.</i> , 1987; Pomar <i>et al.</i> , 2001b; Fortin <i>et al.</i> , 2004; 2005
	Germany	2.05	Daumas and Dhorne, 1998
	Finland	2.17	Daumas and Dhorne, 1998
	Ireland	2.3	Allen and McGeehin, 2001
	Netherlands	2.242.37	Engel <i>et al.</i> , 2006; Hulsegge <i>et al.</i> , 1994; Hulsegge and Merkus, 1997
			Fortin <i>et al.</i> , 2004 ; 2005
UFOM	Canada	1.70	Piechocki <i>et al.</i> , 1994
	Poland	2.36	Daumas and Dhorne, 1998
	United Kingdom	2.38	Daumas and Dhorne, 1998
UMEATER	Germany	2.12	Daumas and Dhorne, 1998
	France	2.13–2.17	Daumas and Dhorne, 1998
	United Kingdom	1.96	Daumas and Dhorne, 1998

Table 21.2 continued

Equipment	Country	RSD/RMSEP*	Reference
Automatic			
AUTOFOM	Canada	1.681.80	Fortin <i>et al.</i> , 2004, 2005; Jones, 1997
	Germany	1.581.83	Brøndum <i>et al.</i> , 1998; Brøndum and Jensen, 1996
	Germany	1.80	Adapted from Bransheid <i>et al.</i> , 2004
	Denmark	1.941.84	Brøndum and Jensen, 1996; Busk <i>et al.</i> , 1999; Brøndum <i>et al.</i> , 1998;
	Spain	2.02	Gispert <i>et al.</i> , 2002
	Poland	2.0	Strzelecki <i>et al.</i> , 1998
	United States	1.70	Brøndum <i>et al.</i> , 1998
	Denmark	1.70	Busk <i>et al.</i> , 1999
	Ireland	2.3	Allen and McGeehin, 2001
	United State	0.81	Higbie <i>et al.</i> , 2002
TOBEC	United States	1.59	Berg <i>et al.</i> , 1994
VCS2000	Canada	1.81–2.04	McClure <i>et al.</i> , 2003
	Netherlands	2.19	Engel <i>et al.</i> , 2006

* The definition of lean meat content (Marcoux *et al.*, 2007) varies depending on the country.

RSD: Residual Standard Deviation.

RMSEP: Root Mean Squared Error of Prediction.

AUTOFOM: Fully Automatic Carcass Grading; CGM: Capteur Gras-Maigre; CVT: Carcass Value Technology; DES: Destron; FOM: Fat-o-meat'er; HGP: Hennessy Grading Probe; OP: Optical Probe; TOBEC: Total Body Electrical Conductivity; UFOM: Ultrafom; UMEATER: Ultrameater; ZP: Zwei-Punkt Messverfahren.

21.3.1 Manual devices

Manual devices depend completely on the operator. The operator carries out the measurement and the reading of the result. Manual devices can measure tissue depths either at the split line (Zwei-Punkt-Messverfahren, ZP method) or laterally. At the split line, the ZP method can be performed with rulers or callipers (manual or electronic). Laterally to the split line, the measurement is carried out with the intrascope (or Optical Probe, OP) with which the operator visually detects the depths between fat and muscle. These devices are of low cost and are used mainly in low volume slaughterhouses.

Rulers or callipers are used to measure fat and muscle thicknesses at the midline (ZP parameters), so they are not invasive. However, in low volume slaughterhouses in which these devices are used, carcasses are often split in two halves manually and the error of prediction for lean yield tends to be high (Diestre and Kempster, 1985). The intrascope avoids this problem because it measures at a fixed distance from the midline. However, it is an invasive device and measures only fat thicknesses.

In these devices the operator is an important source of uncontrolled variation

(i.e. measurements taken in the wrong location, the pressure applied by the operator when measuring with callipers, etc.) which increase the lean yield prediction errors. Reported prediction errors vary from 2.38 to 2.52 for rulers and callipers and from 2.31 to 2.47 for intrascopes (Table 21.2).

21.3.2 Semiautomatic devices

Semiautomatic devices were developed to decrease the operator effect but they only partially succeeded. Semiautomatic devices use light reflectance (optical) or ultrasound probes to automatically determine fat and loin depth. Optical probes are invasive and all have a rigid shaft fitted with a sharp blade and a light-emitting device. Light reflection is measured as the tip passes through the various tissues of the carcass (Fortin *et al.*, 1984). In ultrasound probes, the image representing the interfaces between the ultrasound transducer and the animal skin, skin and fat, fat and muscle, and muscle and bone appears bright, whereas the image of the homogenous part of a tissue appears dark. According to these characteristics of the ultrasonic image, simple algorithms for automatic edge detection of animal fat and muscle have been developed (Whittaker *et al.*, 1992; Liu and Stouffer, 1995). For both probe types, however, an operator has to carry the device and introduce the shaft or place the transducer in the appropriate location of the carcass in which fat and muscle depths have to be measured.

Light reflectance devices use an invasive tip that perforates the tissues and those based on ultrasound use a non-invasive transducer that takes the measurement from the surface of the skin. Both avoid most of the splitting effect due to the fact that measurements are taken on the carcass after slaughter at specific locations which are a fixed distance from the midline. In the semiautomatic devices, measuring errors can originate from the device itself or from the operator. Measuring errors appear when the device has not been properly calibrated or has technical problems. In the invasive probes, a badly sharpened, blunt or too narrow knife can also reduce measurement accuracy. Moreover, due to the fact that they are semiautomatic devices, operators need to be trained to reduce errors due to the improper identification of the measurement location, angle of penetration, transducer position and pressure, etc. As the measurement is tiring for the operators, it is necessary to frequently relieve the operator to avoid probe insertion errors.

The most common semiautomatic invasive devices are the following:

- Fat-O-Meat'er (FOM) (SFK Technology A/S, Herlev, Denmark),
- Hennessy Grading Probe (HGP) (Hennessy Grading Systems Ltd., Auckland, New Zealand),
- Capteur Gras-Maigre (CGM) (Sydel, Lorient, France),
- Destron (DES) (Viewtrak Technologies Inc., Markham, Ontario, Canada).

Although all the probes are based on reflectance measurement, each of them works differently due to differences in the type of light emitted, sensitivity of the reflection-measuring device and the software used for light-reflectance reading interpretation. For that reason it is necessary to have a prediction equation specific

for each probe. The prediction error for these devices as described in different studies varies from 1.58 to 2.37 for FOM, from 1.56 to 2.37 for HGP, from 1.92 to 2.28 for CGM and from 1.71 to 2.49 for DES (Table 21.2).

All the non-invasive semi-automatic devices take the measurements by means of ultrasound. They are less vulnerable and hardly subject to interference (Hulsegge and Merkus, 1997). As they are not invasive, they can be used to measure fat thicknesses in other cuts such as the ham. This is an important issue in countries like Spain and Italy where the ham and has to have narrow fat depths to be properly cured. The most commonly used are:

- Ultrafom (UFOM) (SFK Ltd., Denmark),
- Ultrameater (UMEATER) (CSB-System, Geilenkirchen, Germany),
- Carcass Value Technology (CVT) (Animal Ultrasound Services (AUS), Inc, Ithaca, NY, USA),

As they are based on ultrasound, carcasses must be warm and moistened to ensure good contact between the probe and the surface of the meat. Ultrafom measures a specific point of the loin (or ham) by means of 64 sensor elements that send pulses into the carcass. The CVT2 instrument measures a 7 cm longitudinal area of the loin; 1 cm at the top and 1 cm at the bottom are excluded and the middle 5 cm of the scanned image are used to calculate the average fat and loin depths. The reported prediction error of these devices varies from 1.70 to 2.38 for UFOM, from 1.96 to 2.17 for UMEATER and from 1.57 to 2.27 for CVT (Table 21.2).

21.3.3 Automatic devices

Fully automatic devices have the advantage of avoiding the operator effect. However they have to be tested periodically to ensure they are calibrated and work correctly. Usually they take numerous measurements from which they estimate the parameters of interest.

They are used in large slaughterhouses and are based on different technologies. The main technologies and devices that can be found in the market are:

- Reflectance: Classification Center (KC) (SFK Ltd., Denmark),
- Ultrasound: Automatic Carcass Grading (AUTOFOM) (SFK Ltd., Denmark),
- Electromagnetism: Total Body Electrical Conductivity (TOBEC) (Meat Quality Inc., Springfield, USA),
- Vision: Vision Carcass System (VCS) (e+V Technology GmbH, Oranienburg, Germany).

The KC system evaluates overall carcasses at line speeds of 360 to 400 carcasses per hour. When carcasses are moved into the system, their dimensions are measured mechanically (carcass length from gambrel to snout, forelimb position, and height of pubis) to enable correct positioning of a head-holder and the probes. There are seven probes mounted on three carriages. Two probes are inserted in the ham, three in the loin and the last two in the forepart of the carcass. Probes measure light reflectance as well as distances to give a profile of reflectance when the probe

is pressed through the carcass. Measurement errors can be due to badly sharpened knives, non-adequate pressure of the probes or error in the placement of the probes in the carcass. The prediction error has been reported as being 1.70 (Table 21.2).

The AUTOFOM equipment is a fully automatic 3D ultrasound scanner. It has 16 transducers embedded in a U-shape array that measure every 5 mm of the carcass for 1 m length. It takes a maximum of 3200 measurements. From these measurements 127 variables are extracted and used to predict the lean yield of the whole carcass and the different cuts. The measure has to be taken in moistened entire carcasses as found between dehairing and singeing. Carcasses hung on gambrels move over the equipment at on-line speeds ranging from 300 to 1150 carcasses/h. Measurement errors can happen if carcasses twist and have poor contact with transducers. The reported prediction error varies from 1.58 to 2.02 (Table 21.2).

The electromagnetic device TOBEC measures the absorption by a carcass or cut of electrical energy from an electromagnetic field. Due to the fact that the water content of the carcass lean and fat are different, the conductivity differs depending on the tissue composition. It is probably most useful to scan warm pre-rigor carcasses because the temperature is relatively constant and is not needed in the prediction equations (Jones, 1997). Measurement errors are related to high ambient humidity and to carcass or cut geometry. The reported prediction error varies from 0.81 to 2.3 (Table 21.2).

The Vision Carcass System (VCS) for pork grading consists of two components. The ultrasound imaging component scans a cross-section of the loin muscle and the video imaging component captures a two-dimensional (2D) and a three-dimensional (3D) image of the carcass. The VCS has three cameras, two installed to visualize the carcass from the internal side and the other one to visualize the carcass from the external side. From the images, different measurements of fat and muscle thickness, areas, angles and ratios are taken, which are used to predict lean yield. The reported prediction error varies from 1.81 to 2.19 (Table 21.2).

21.4 Current technologies available to accurately determine carcass composition and lean yield

Carcass composition, carcass lean yield and any eventual grading system developed for pork have traditionally been estimated by dissection. To avoid the inherent limitations of dissection, many alternative methods have been developed and evaluated (Kempster *et al.*, 1982), with the objective of finding an accurate, fast and inexpensive method to determine carcass composition for a wide range of animals or species of differing carcass fat content. Since the pioneering work in Norway in the early 1980s (Skjervold *et al.*, 1981), computed tomography (CT) and magnetic resonance imaging (MRI) techniques, as well as image analysis, have made huge progress. In 1999, Szabo *et al.* reviewed the application of digital imaging techniques in the *in vivo* estimation of the body composition of pigs.

A new interest in CT for pig grading emerged as a result of the European

research project EUPIGCLASS (<http://www.eupigclass.net/>) aimed at increased harmonization of pig classification. The reference lean meat percentage needed to calibrate the classification methods is obtained by manual dissection. As dissection is laborious, costly and difficult to standardize, the EUPIGCLASS consortium studied the potential of CT and MRI to replace dissection. The consortium concluded (Daumas, 2004) that both methods are candidates to replace dissection, with decisive benefits, which are rate of scanning, repeatability, reproducibility, standardization of the measuring protocol and better tracking of pig population evolution. Because CT is generally less expensive than MRI, eight European countries are today equipped with CT for animal research purposes and three with MRI. Most of the applications of these technologies concern sheep and pig production. Countries with significant sheep production have developed CT breeding programs.

21.4.1 Magnetic resonance imaging

Magnetic resonance imaging is today one of the most powerful diagnostic tools of modern medicine. MRI is based on the magnetic resonant properties of protons associated with water and lipid molecules of tissues, which results in a range of signal intensities capable of distinguishing numerous tissues and organs, including fat and muscle. The magnetic resonance signal is produced by placing the carcass in a static magnetic field and exciting it with radiofrequency waves. The resonant frequency is determined by the magnetic field strength and the nucleus to be studied. After excitation, the studied section of the carcass emits radiofrequency signals that can be detected by a coil placed around the carcass. The intensity of the emitted signal is related to the number of protons present in a given volume and on the spin-lattice (T1) and the spin-spin relaxation times (T2) of the excited section of the carcass, which are specific to each tissue (Mitchell *et al.*, 2001).

MRI is seldom used in animal research and therefore limited information is available. Most body composition studies using MRI on pigs were made *in vivo*, as reviewed by Szabo *et al.* (1999), citing Baulain (1997), Fowler *et al.* (1992), Henning (1992) and Scholz *et al.* (1993). The estimates of the proportion of tissues were less accurate than those obtained by dissection. The coefficients of determination (R^2) obtained for tissues' weight ranged from 0.8–0.9, while Baulain *et al.* (1998) obtained coefficients of correlation ranging from 0.72 to 0.94 between dissected and MRI lean content in belly cuts. Furthermore, Kastelic *et al.* (1996) used MRI volume measurements to determine the allometric growth of muscle and fat areas of 20, 35, 50 and 70 kg body-weight pigs.

Due to the practical limitations of total body MRI scanning, the prediction of body muscle and adipose tissue composition based on volume measurements at specific regions was investigated. Five body regions studied by Baulain *et al.* (1996) achieved R^2 greater than 0.9 for fat and lean weight. Mitchell *et al.* (2001) found the best prediction of total body fat percentage using the fat volume from a 10-cm section of the *longissimus* muscle and the fat/muscle ratio from a 15-cm section of the ham ($R^2 = 0.9$; Standard error of estimate; SEE = 1.5%). The best

prediction of the percentage of total body protein was obtained by this same author using a combination of the volumes (as a percentage of BW) of jowl fat, backfat, shoulder muscle, and ham muscle ($r^2 = 0.62$; $SEE = 0.46\%$). Total body lean was predicted with an r^2 of 0.88 ($SEE = 1.54\%$), combining the fat volume from the 10-cm section of longissimus muscle, the fat:muscle ratio from the 15-cm section of the ham, and the lean volume percentage from a 15-cm section of ham.

On pork carcasses, Daumas *et al.* (2003) investigated the capabilities MRI scanning of 20-cm sections to estimate overall muscle percentage. The most predictive region was in the ham. More recently, Daumas *et al.* (2005) reported that in a European dataset, the combination of a 20-cm section scan in the ham and another in the trunk decreased the RMSE to 0.6–0.7 when estimating carcass muscle percentage. On the same dataset, Collewet *et al.* (2005) predicted the dissected carcass lean weight using the whole carcass MRI information and found a SEP of 465 g when using PLS regression analysis on signal intensity histograms.

Monziols *et al.* (2006a,b) estimated the muscle and fat contents in carcasses and cuts using fully automated image analysis software specially developed for the estimation of muscle and fat contents in carcasses and cuts. The purpose of using fully automated image processing was to avoid manual image segmentation which is both tedious and less reproducible (Wang and Doddrell, 2001). Images were analysed to identify and quantify pixels representing muscle, subcutaneous fat and intermuscular fat fractions. MRI analysis provided a good prediction of muscle content in cuts and carcasses, with R^2 ranging from 0.970 to 0.997. The prediction of total fat was slightly less accurate ($0.951 < r^2 < 0.986$) or subcutaneous fat ($0.918 < r^2 < 0.994$). Monziols *et al.* (2006b) found that the prediction of intermuscular fat content considering intermuscular fat classified pixels was acceptable only for the belly ($r^2 = 0.837$). Monziols *et al.* (2005) developed a partial volume detection method and applied it to pig belly, giving a prediction error of the muscle content of 82 g and of the fat content of 107 g. To improve the estimation of fat contents, Monziols *et al.* (2006b) suggested several paths of investigation, such as alternative acquisition protocols which can provide better differentiation of fat and bone signals, improvement of morphological procedures and thickness reduction of the transverse sections.

21.4.2 Computed tomography

Computed tomography scanners are equipped with X-ray tubes positioned opposite to an array of a large number of detectors. Both rotate around the 'patient'. X-ray projections are emitted at regular intervals and the detectors measure the X-ray absorption. The detectors' information is computed for producing reconstructed cross-sectional images of the 'patient'. Each picture element is a pixel and is characterized by a CT value or density value, expressed in Hounsfield units (Hounsfield, 1979). Most CT scanners are able to differentiate between 2000 CT values. Water has a typical density of zero and air has a value of -1000. Fat is around -100, muscle around +100 and hard bone +1000. The CT values are reconstructed into a grey-scale image.

CT scanners were developed for human medical purposes, mainly to detect anatomical and physiological disorders. The potential of this technique in animal science studies was first recognized in Norway (Skjervold *et al.*, 1981) and some promising results were published by Allen and Vangen (1984), Vangen (1984) and Vangen and Standal (1984). In order to reduce costs connected to calibration and recalibration of carcass grading instruments, the Norwegian Meat Marketing Board financed a CT experiment with dissection. Sehested and Vangen (1989) reported a RSD of 1.02 meat percentage using six cross-sectional scans made on a half carcass. No significant effect of carcass weight, sex (gilt vs castrate) and breed was found in this study. Eleven scans were used in the Norwegian programs for predicting the amount of protein, fat and water in the carcasses but 7 were considered sufficient (Vangen, 1992).

Hungary was equipped with a CT scanner in 1990 with the objective of studying whether the CT scanning of live pigs could replace slaughterhouse evaluations in progeny tests conducted in central stations (Horn, 1995). During the 1990s, CT was used in the selection of pig breeds and lines.

Studies based on CT from Luiting *et al.* (1995), Kolstad and Vangen (1996) and Kolstad *et al.* (1996) showed differences in maintenance requirements, fat distribution and fat mobilization between pig breeds. According to Kolstad (2001), CT could be included in the breeding evaluation of boars to improve efficiency, as well as meat quality traits related to the distribution of fat.

Jopson *et al.* (1995) as well as Glasbey and Robinson (1999, 2002) have suggested the utilisation of CT as an alternative method to replace the dissection in cut-out studies. From the EUPIGCLASS data, Dobrowolski *et al.* (2004) have recommended the utilization of CT as a reference method for the estimation of lean meat percentage but taking into account that both half carcasses should be scanned because of the morphologic differences between them. Judas *et al.* (2005) found an estimation error of 1% of average carcass weight and concluded that carcass CT scans, with a defined protocol and combined with PLS-regression, provided an alternative to manual dissection. Judas *et al.* (2006) proposed a spectral analysis of X-ray attenuation data by PLS regression, refined by a smoothing of the PLS calibration model.

In Denmark, Christensen and Borggaard (2005) first focused on the reproducibility of CT images and measurements. They suggested using a pre-processing step prior to applying multivariate modelling methods for predicting the lean meat content. Then, in order to take into account the spatial context during CT scans, Lyckegaard *et al.* (2006) applied contextual methods of image analysis combining Bayesian classifier algorithms (Larsen, 2000) with a post-processing analysis. Finally, Christensen *et al.* (2006) compared contextual volume grading with spectral calibration and found that contextual volume analysis was a potential alternative to the conventional spectral analysis of the Hounsfield spectrum. Nevertheless, further improvements are needed for avoiding misclassification of skin as meat instead of fat during CT scanning. In Europe, where this technology is being proposed as a reference method, the challenges are:

- to develop robust procedures for measuring the lean meat percentage of a carcass with CT,
- to adapt the present dissection procedure to a new standard, close to the CT procedure,
- to ensure an average equivalency between both methods,
- to promote the adaptation of the EC regulations, and
- to apply the new regulations and to implement the new methods in European slaughterhouses for improved harmonization.

No doubt the research planned for the coming years on pig classification in Europe will benefit the world community, as well as the animal production sector in general.

21.4.3 Dual energy X-ray absorptiometry

Dual energy X-ray absorptiometry (DXA) is an alternative technique that has been successfully used to predict the chemical composition of live pigs (Mitchell *et al.*, 1996; Pomar and Rivest, 1996; Suster *et al.*, 2003) and pig carcasses (Mitchell *et al.*, 1998), as well as the composition of carcass dissected tissues in pigs (Marcoux *et al.*, 2003) and sheep (Mercier *et al.*, 2006). Because of its speed, accuracy, reliability and ease of use, DXA holds promise as an indirect method of estimation of the composition of carcasses, thus avoiding their dissection. DXA technology is effective for predicting the weight of the different half-carcass tissues and primal cuts (Suster *et al.*, 2003; Marcoux *et al.*, 2005). The coefficients of determination for the prediction of total weight are high (i.e. $r^2 > 0.95$) and the residuals are low (i.e. $CVe < 1.6\%$; Marcoux *et al.*, 2005). Total weight of the half-carcasses and the primal cuts are, however, the variables that are predicted most accurately. Lean, fat and bone weight follow in terms of prediction accuracy (Suster *et al.*, 2003, Marcoux *et al.*, 2003, 2005). Marcoux *et al.* (2003) concluded, however, that this technology is not to be recommended for predicting dissected bone weights.

Few studies have addressed the potential of this technology to predict lean yield of pig carcasses. Marcoux *et al.* (2003) found that the weight of the tissues included in the numerator of the Canadian lean yield or in the European lean yield were accurately predicted by the DXA lean variable (r^2 : 0.88 and 0.82; RSD: 0.55 and 0.60, respectively). However, the respective yields were predicted less accurately (r^2 : 0.76 and 0.70; RSD: 1.32 and 2.05, respectively), making this technology less suitable for use as a reference method in cut-out projects.

21.5 Limits of current technologies for estimating carcass composition and carcass value

21.5.1 Prediction accuracy

Most of the current technologies reviewed in the previous section measure backfat and muscle thicknesses, from which lean carcass lean yield can be estimated.

These measurements, however, vary with type of probe (manual, optical, ultrasound, etc.) and therefore the precision of the probes varies and the equations developed for predicting the various yields are specific to each instrument (Sather *et al.*, 1989; Swatland, 2001; Pomar and Marcoux, 2003) and measurement site. The accuracy of the different devices when predicting carcass lean yields was presented in the previous section but care should be taken when comparing the determination coefficients across experiments. In fact, evaluating the accuracy of an instrument implies the evaluation of the closeness between its measurements and the accepted reference values in terms of trueness and precision. The trueness of a measurement indicates the degree of agreement between the expected and reference value, while the precision indicates the degree of internal agreement between independent measurements made under specific conditions. A device is said to be accurate when it is true, i.e. when its measurements adjust to the true values, and precise when there is no spread around the true value (International Standardization Organization, 1993).

The lack of accuracy of grading systems for predicting carcass lean yield can originate both from errors in measuring the predicting parameters (i.e. fat and muscle depths) and from the ability of these parameters to predict carcass lean yield. Differences between measuring devices for fat and muscle depths can result from the fact that backfat and muscle thickness measurements are specific to each probe, since the probes operate in their own distinctive ways. For example, Pomar and Marcoux (2003, 2005) found average differences of 1.4 and 3.7 mm between some devices when measuring fat and muscle depths in the same carcass.

The accuracy of the measurements of backfat thickness, and loin or intercostal muscle thickness is difficult to evaluate because the accurate dimensions of the measurements are not available. However, the measuring accuracy of the various instruments can be evaluated by comparing the readings obtained by the grading probes in the hot carcass with the equivalent measurement taken on the digitized images of the cold loin chops (Pomar *et al.*, 2001a). However, thickness differences between the readings of the grading devices on hanging hot carcasses and the equivalent measurements made on the digitized image of the refrigerated loin might be explained by differences in fat layers and muscle shape. In fact, hot carcasses undergo complex shifts in muscle and fat areas as they hang in the vertical position (Mersmann, 1982). Furthermore, the effect of cooling on backfat and muscle thickness may have also contributed to these differences. Differences between the readings of the measuring devices, or between the measurements taken in the hot carcass and in the cold carcass chops, can statistically be corrected by decomposing the prediction errors as suggested by Benchaar *et al.* (1998). Using this procedure, Pomar and Marcoux (2005) observed that, besides the absolute differences observed between image and instrumental measurements, the evaluated optical grading probes (DES PG-100, HGP2 and CGM) were generally more precise than the evaluated ultrasound probes (CVT) in measuring backfat thickness, while the ultrasound grading probes were more precise than the optical probes in measuring loin muscle thickness. Furthermore, random errors, as estimated by the RSD, were relatively large in all the evaluated probes, as they

range from 1.3 to 1.9 mm for backfat thickness and from 3.6 to 5.3 mm for loin thickness. Measures of the intercostal muscles were not reliable in either of the probes able to take that measure. These biases would be expected to be larger in commercial conditions, where carcasses are graded on the moving rail at high speed.

Besides the accuracy of the instruments for measuring fat and muscle depths, we should be questioning the capability of these parameters for predicting the overall carcass lean yield. Backfat thickness can predict more accurately carcass lean yield than loin thickness. However, even when taken together, these two parameters measured on a digitalized image can explain about 67% of the observed variation on lean meat percentage or have residual prediction errors lower than 2.1% (Pomar *et al.*, 2001a). When the digitalized image measurements, which can be assumed to be the most precise measurements of backfat and muscle thickness, are used to predict the Canadian lean yields, both parameters together can explain 69% of the observed variation while the prediction residuals decrease to near 1.3%. These lower residuals result from the fact that the observed variation is lower for the Canadian than for the European lean yield. It can also be estimated that, when using instrumental measurements of thicknesses instead of the more accurate image measurements, the explained variation for lean meat percentage decreases to 59% for the optical probes and to 62% for the ultrasound probes; that is, a loss of 11% and 6%, respectively, in accuracy (Pomar *et al.*, 2001a). Similarly, the residual prediction error increases to 2.3 and to 2.1 mm for the optical and ultrasound probes, respectively, which correspond to losses in accuracy of 11% and 7%. Similar losses were observed when predicting the Canadian lean yield.

Most of the actual technologies used in the on-line determination of carcass composition and lean yield, whether they are manual, automatic or semiautomatic or if they use rulers, callipers, light reflectance or ultrasound, use the relationship between the carcass lean yield and the thickness of backfat and muscle measured at one, two or many specific locations. These latter values can vary significantly between measuring devices, lean yields and authors (see previous section) but in all these cases, one important part of the observed variation on carcass lean yields cannot be explained by these measurements. There are several factors that can explain the inability of these measurements to account for the remaining observed variation. Increasing the number of measurements (Hulsegge *et al.*, 1994; Hulsegge and Merkus, 1997; Pomar *et al.*, 2001b) or including the sex effect (Pomar *et al.*, 2001b) does not significantly improve the prediction accuracy. On the other hand, in the reference method, carcasses are manually dissected in the different tissue components, normally by experienced operators. Differences between operators can be important (Nissen *et al.*, 2006), but these effects can only partially explain the observed prediction error. Therefore, the value of backfat and muscle tissue depth measurements taken at specific carcass locations is limited by both the accuracy of the measurements and the capability of these measurements to explain the observed variation in carcass lean yields; therefore, grading systems using backfat and muscle measurements seem to have reached maximum accuracy.

Several new systems for grading pork carcasses combining ultrasound measurements and video imaging, capturing two-dimensional and/or three-dimensional images of the carcass have been recently developed, such the VCS2000 (McClure *et al.*, 2003; Engel *et al.*, 2006) and Computer Video System (Fortin *et al.*, 2003), and they show some improvement in the ability to improve carcass lean yield prediction accuracy. The use of numerous ultrasound readings, generating a three-dimensional ultrasound image, followed by a complex noise reduction and data analysis procedure, as used in the AUTOFOM system, seems to improve lean yield prediction accuracy (Brøndum *et al.*, 1998; Busk *et al.*, 1999), although in some circumstances this technology did not show a clear advantage over other commercially available ultrasound devices (Fortin *et al.*, 2004).

21.5.2 The estimation of carcass value

The compositional evaluation of the carcass and its subsequent grading is used to establish its commercial value. The carcass grading and payment system is the inherent bridge between producers and processors. Pork stakeholders should use the payment system to encourage the production of carcasses whose composition meets consumer demand (Cross and Savell, 1994).

Carcass leanness has been directly associated with carcass value since the early 60s (Fredeen *et al.*, 1964) and this still applies in many countries, whether the carcasses are evaluated by their lean content (e.g. European Union), saleable meat (e.g. Canada), standardized fat-free lean (e.g. United States) or backfat and loin muscle depth (e.g. United Kingdom, New Zealand, Australia). These carcass evaluation systems assume that increasing the value of these yield criteria increases the commercial value of carcasses, which means that lean has a better market price than fat. In a recent study, Marcoux *et al.* (2007) found that, in the Canadian pork market, carcass value has only a weak link with the various definitions of lean yield studied. More precisely, the correlation between the actual Canadian sealable lean definition and carcass market value was only 0.14. This correlation was slightly higher (0.23) when carcass lean yield was evaluated with the European lean yield definition. In the current context of pig carcass grading in Canada, the adjusted prices paid to producers do not reflect the variation in the market carcass value and it is probable that the same figures apply to Europe and other economic contexts.

The low correlations between lean yield and market value are explained by the influence of the weights and market prices of the primal carcass cuts (loin, ham, belly and shoulder) on the overall market carcass value. The proportion of the weight of the various cuts relative to total carcass weight is a measure of the conformation of the carcass. Due to variations in conformation, carcasses of identical weight can have different cut sizes and weights. Therefore, two carcasses with similar weight and fat content can have different market value based solely on their conformation. In theory, since the loin and the belly are the two most profitable cuts in North America, carcasses with good conformation for these two cuts are more profitable for processors than the carcasses with proportionally

heavier hams and shoulders. In other areas where the ham is one of the most valuable cuts, the most profitable carcasses are those with heavier hams.

In a study by Marcoux *et al.* (2007), the relative economic contributions of the loin, ham, belly and shoulder to the total value of the carcass were 35.4%, 19.8%, 26.6% and 18.2%, respectively. The relative economic contributions of the loin, ham and shoulder was positively correlated with the various lean yields, but these correlations were always low, that is between 0.07 and 0.36 for the loin, ham and shoulder cuts and between -0.35 and -0.57 for the belly. It appears that the correlation between the contribution of primal cuts to the carcass market value and the different lean yields was always negative for the belly. This means that, on average, the leaner the carcass, the less the belly contributes to the market value of the carcass and conversely, the fatter the carcass, the more the belly contributes to its market value. It should be noted that the cuts contributing the most to carcass value have an opposite influence, which indicates that the more profitable the loin on a carcass becomes, the greater is the value loss on the belly. These figures may change with economic context.

In addition to the effect of conformation and of the market prices of cuts, the rate of defatting of the cuts is also a factor explaining the weak relationship between yields and market value. Indeed, during the commercial cut production process, the primal loin is the cut that undergoes the most defatting, as the backfat thickness must not exceed 9 mm in Canada. This also contributes to the lack of a relationship between yield and market value. To our knowledge, the relationship between lean yields and carcass market value has not been studied outside Canada. In North America, the most valuable cuts are the central cuts of the carcass, while in other countries or regions the ham is the cut contributing the most to the value of the carcass. In all the cases, however, it should be recognized that the value of the lean and fat of a carcass is strongly related to its anatomical position and therefore, overall carcass lean yield is not necessarily the best indicator of carcass value. Furthermore, the quality of pork meat varies significantly between carcasses. Pale, soft and exsudative (PSE) and dark, firm and dry (DFD) meat are the extremes in meat quality attributes related to meat pH values. The water-holding capacity of pork reflects its value in further processing. Meat colour, fat softness and fat composition are other pork quality attributes that the consumer and the pork processing industry increasingly demand to be included in carcass evaluation and grading systems.

21.6 Future trends

The objective of producing lean pigs seems to have been achieved, while the slaughter sector suggests that certain carcasses are now too lean, as they are associated with meat quality problems, such as belly softness, pale, soft and exudative pork, etc. It therefore appears that the signal given via current grading and payment systems may not be the only criterion to consider if one wants to reward the producer according to the market value of the carcass. However,

consumer demands regarding meat quality change over time and new expectations concerning ethics, environment and animal welfare, for example, are contributing to a complex reformulation of the concept of meat quality (Andersen *et al.*, 2005). The challenge for future carcass grading systems will be to send a clear signal to the production sector reflecting both the continuously changing meat market values and evolving consumer requirements, while maintaining reasonable stability and profitability for all stakeholders.

In North America, the belly and loin, including the tenderloin and the side ribs, are the cuts that fetch the best prices. It should be noted that the value of the fat content in a carcass differs depending on the anatomical location. While consumers do not appreciate visible fat on the meat chop surface (Brewer *et al.*, 2001), bacon fat has a high commercial value. To meet consumers' demands while maintaining the profitability of the pork sector, future carcass evaluation systems will need to take into consideration consumer demands for pork meat (fat content, lean colour, safety, etc.) while also encouraging the production of carcasses whose conformation maximizes the weight of the most profitable cuts on the market. Reconciling the interests of consumers and the industry is important, since they sometimes differ (Person *et al.*, 2005). Furthermore, meat market value and, at times, consumers' demands may change at a faster pace than the pork industry can handle. Grading systems must monitor market price cycles and changing consumer demands, ensuring some stability for the production sector.

An effective carcass evaluation system must also facilitate the development of niche markets that will have different specifications related to the quality of the meat and cuts. The production of carcasses with a conformation that favours the most profitable cuts for one or more markets will have a very positive impact for the production and marketing sector by increasing potential income. Currently, the signal given to the production sector concerning carcass conformation specifications, such as cut weights, is ambiguous since most of the current evaluation and grading systems emphasize the overall lean content of the carcasses without taking into account the anatomical localisation of that lean.

From the standpoint of maximizing the potential income from carcasses, commercial carcass value is an essential quality criterion that the industry may want to introduce more clearly in future grading and payment systems. Total carcass weight does not provide any information on the weight proportion of each cut and measurements of fat and muscle thickness at the grading site do not result in accurate estimates of the fat content of the ham, shoulder and belly. Actually, there is no reference to market prices of the various cuts in the estimation of the carcass value. The main issue raised by a carcass evaluation system based on market value therefore involves the ability to evaluate the specific characteristics of the cuts. It is the considered opinion that future grading systems will require that the weight of each cut (ham, loin, belly and shoulder), its economic value on the market and the proportion of fat and lean that it contains, be estimated (Marcoux *et al.*, 2007). The need to determine the weight of the cuts and their leanness on the slaughter line will necessarily involve a major change in current technology and grading methods. Despite a few new grading approaches using complex ultra-

sound or imaging approaches (Brøndum *et al.*, 1998; Fortin *et al.*, 2003; McClure *et al.*, 2003), to our knowledge there is currently no fully developed technology capable of estimating, with reasonable precision, the weight of the cuts and their leanness on the slaughter line before cut-out. Investigation into a method or technology capable of obtaining this information would therefore be desirable. Finally, besides the estimation of the amount of fat and muscle provided by the different carcass cuts, consumers' demands increasingly include pork quality attributes such as meat colour, intramuscular fat content, fat composition, and others. Non-invasive, efficient and effective technologies for meat quality assessment need to be included in future carcass evaluation and grading systems.

21.7 Conclusions

Most of the actual on-line carcass evaluation systems use backfat and muscle depths to estimate overall carcass lean yield. The capability of these measurements to explain the observed variation on carcass yields seems to have reached maximum accuracy. New carcass evaluation approaches are therefore necessary to improve their accuracy. On the other hand, carcass lean yield is defined as the proportion of tissues of interest in the carcass and they are obtained according to a reference method in which carcass preparation and tissue dissection is precisely described. Carcass dissection is time-consuming, expensive and subject to biases and therefore, new methodologies based on magnetic resonance and various X-ray technologies are proposed. Further research is, however, needed to develop robust procedures for measuring lean meat percentage in the carcasses.

The definition of lean yield influences its ability to accurately determine carcass leanness and to discriminate carcasses in relation to lean yield. Carcass evaluation and grading systems are used in many countries to establish payment systems and therefore the carcass lean definition should be established in relation to the processors' economical interest. If processors are interested in increasing overall carcass leanness, then the leaner yield definitions, rather than yield definitions in which fat and bones are part of the numerator, should be used. However, overall carcass leanness should not be the only criterion to be taken into account when the processor's objective is to obtain from the producers carcasses an increased commercial market value. In fact, it appears that carcass market value is not always adequately correlated with carcass lean yield and therefore the signal given to the production sector concerning the production of carcasses of optimal market value is confusing. In fact, most of the current evaluation and grading systems emphasize the overall lean content of the carcasses without taking into account the market value of the cuts. Future carcass evaluation and grading systems will require estimating the weight of each commercial cut, its economic market value and the proportion of fat and lean that it contains. Additionally, future carcass evaluation and grading systems will need to include pork quality attributes such as meat colour, intramuscular fat, fat composition, and other parameters.

21.8 References

- Allen P and Vangen O (1984), 'X-ray tomography of pigs – some preliminary results', in Lister D, *In Vivo Measurement of Body Composition in Meat Animals*, Elsevier, London, 52–66.
- Allen P and McGeehin B (2001), *Measuring the Lean Content of Carcasses using TOBEC*. Final report. Project Armis n 4054. Research Report 40. Teagasc. (www.teagasc.ie/research/reports/foodprocessing/4896/eopr-4896.htm)
- Andersen H J, Oksbjerg N, Young J F and Therkildsen M (2005), Feeding and meat quality – A future approach. 50th International Congress of Meat Science and Technology, (ICoMST), 8–13 August 2004, Helsinki, Finland. *Meat Sci.*, 70, 543–554.
- Baulain U (1997), 'Magnetic resonance imaging for the in vivo determination of body composition in animal science', *Comput. Electron. Agric.*, 17, 189–203.
- Baulain U, Henning M, and Kallweit E (1996), 'Bestimmung der Körperzusammensetzung von Landrasse-Schweinen unterschiedlichen Alters mittels MRI. [Determination of body composition in German Landrace pigs of various ages by means of MRI]', *Arch. Tierz.*, 39, 431–440.
- Baulain U, Henning M, Tholen E, Wittmann W, and Peschke W (1998), 'Objektive Erfassung des Fleischanteils im Schweinebauch. 2. Mitteilung: Verwendung von Bildinformationen aus dem MR-Imaging', *Zuechtungskunde*, 70, 202–212.
- Benchaar C, Rivest J, Pomar C and Chiquette J (1998), 'Prediction of methane production from dairy cows using existing mechanistic models and regression equations', *J. Anim. Sci.*, 76, 617–627.
- Berg E P, Forrest J C and Fisher J E (1994), 'Electromagnetic scanning of pork carcasses in an on-line industrial configuration', *J. Anim. Sci.*, 72, 2642–2652.
- Bransheid W, Höreth R, Baulain U, Tholen E and Dobrowolski A (2004), 'Schätzung der schlachtkörperzusammensetzung auf der basis der kombinationen von klassifizierungsgeräten mit der videobildauswertung', *Fleischwirtschaft*, 2, 98–104.
- Brewer M S, Zhu L G and McKeith F K (2001), 'Marbling effects on quality characteristics of pork loin chops: Consumer purchase intent, visual and sensory characteristics', *Meat Sci.*, 59, 153–163.
- Brøndum J and Jensen S A (1996), 'Carcass grading using the Autofom ultrasound system', *42nd International Congress of Meat Science and Technology*, 238–239.
- Brøndum J, Egebo M, Agerskov C, and Busk H (1998), 'On-line pork carcass grading with the Autofom ultrasound system', *J. Anim. Sci.*, 76, 1859–1868.
- Busk, H., Olsen, E. V. and Brøndum, J. (1999), Determination of lean meat in pig carcasses with the Autofom classification system. *Meat Sci.*, 52, 307–314.
- Christensen L B and Borggaard C (2005), 'Challenges in the approval of CT as future reference for grading of farmed animals', *51st ICoMST*, Baltimore, Maryland USA, 260–269.
- Christensen L B, Lyckegaard A, Borggaard C, Romvari R, Olsen E V, Branscheid W, and Judas M (2006), 'Contextual volume grading vs. spectral calibration', *52nd ICoMST*, Dublin, Ireland, 205–206.
- Collewet G, Bogner P, Allen P, Busk H, Dobrowolski A, Olsen E, and Davenel A, (2005), 'Determination of the lean meat percentage of pig carcasses using magnetic resonance imaging', *Meat Sci.*, 70, 563–572.
- Cross H R and Savell J W (1994), 'What do we need for a value-based beef marketing system?' *Meat Sci.*, 36, 19–27.
- Daumas G (2004), 'Pig classification in Europe: Why and how to change the reference?' *Proceedings of the 39th Simposio Internazionale di Zootecnica 'Meat Science & Research'*, CNR Roma 10 giugno 2004, 23–42.
- Daumas G, Davenel A, Collewet G, Quellec S, and Bogner P, (2005), 'Contributions of magnetic resonance imaging in the search of predictors of the lean meat proportion of pig carcasses', *Journ. Rech. Porcine*, 37, 159–164.

- Daumas G, Davenel A, Quellec S, Collewet G, and Mignot J, (2003), 'Searching for new predictors of the pig lean meat proportion by Magnetic Resonance Imaging (MRI)', *Proceedings of the 49th ICoMST*, Campinas, Brazil, Paper No 88.
- Daumas G (1999), 'Classification des carcasses de porcs: Principes, Résultats, Perspectives', *Techni-Porc.*, 22, 35–42.
- Daumas G and Dhorne T (1996), 'Historique et futur du classement objectif des carcasses de porc en France', *Journ. Rech. Porcine*, 28, 171–180.
- Daumas G and Dhorne T (1998), 'Pig carcass grading in European Union', *Proceedings of the 44th International Congress of Meat Science and Technology*, 946–947.
- Diestre A and Kempster A J (1985), 'The estimation of pig carcass composition from different measurements with especial reference to the classification', *Anim. Prod.*, 41, 383–391.
- Dobrowolski A, Branscheid W, Romvári R, Horn P and Allen P, (2004), 'X-ray computed tomography as possible reference for the pig carcass evaluation', *Fleischwirtschaft*, 84, 109–112.
- Engel B, Buist W G, Walstra P, Olsen E and Daumas G (2003), 'Accuracy of prediction of percentage lean meat and authorization of carcass measurement instruments : Adverse effects of incorrect sampling of carcasses in pig classification', *Anim. Sci.*, 76, 199–209.
- Engel B, Lambooij E, Buist W G, Reimert H and Mateman G (2006), 'Prediction of the percentage lean of pig carcasses with a small or a large number of instrumental carcass measurements – an illustration with HGP and Vision', *Anim. Sci.*, 82, 919–928.
- Evans D G and Kempster A J (1979), 'A comparison of different predictors of the lean content of pigs' carcasses', *Anim. Prod.*, 28, 97–108.
- Fahey T J, Schaefer D M, Kauffman R G, Epley R J, Gould P F, Romans J R, Smith G C and Topel D G (1977), 'A comparison of practical methods to estimate pork carcass composition', *J. Anim. Sci.*, 44, 8–17 (Abstract).
- Font i Furnols M, Engel B and Gispert M (2004), 'Short communication: Validation of the Spanish equation to predict the lean meat percentage of pig carcasses with the Fat-O-Meat'er', *Spanish J. Agri. Res.*, 2, 545–549.
- Fortin A, Jones S D M and Haworth C R (1984), 'A note on the accuracy of the New Zealand Hennessy Grading Probe and the Danish Fat-O-Meater in measuring fat and muscle thickness in pig carcasses', *Anim. Prod.*, 38, 507–510.
- Fortin A, Tong A K W and Roberston W M (2004), 'Evaluation of three instruments, CVT-2, UltraFom300 and AutoFom for predicting salable meat yield and weight of lean in the primals of pork carcasses', *Meat Sci.*, 86, 537–549.
- Fortin A, Tong A K W and Roberston W M (2005), 'Comparaison de différentes sondes à ultrasons pour la prédiction du rendement boucher' *Journ. Rech. Porcine*, 37, 165–170..
- Fortin A, Tong A K W, Robertson W M, Zawadski, S M, Landry S J, Robinson D J, Liu T and Mockford R J (2003), 'A novel approach to grading pork carcasses: Computer vision and ultrasound', *Meat Sci.*, 63, 451–462.
- Fowler P A, Fuller M F, Glasbey C A, Cameron G C, and Foster M A (1992), 'Validation of the in vivo measurement of adipose tissue by MRI of lean and obese pigs', *Am. J. Clin. Nutr.*, 56, 7–13.
- Fredeen H T, Berg R T, Bowland J P and Doornenbal H (1964), 'Prediction of yield and value of hog carcasses', *Can. J. Anim. Sci.*, 44, 334–346.
- Fredeen H T (1980), 'Yields and dimensions of pork bellies in relation to carcass measurements', *J. Anim. Sci.*, 51, 59–68.
- Gibson J P and VanderVoort G (1999), 'Grading equations to predict carcass lean yield and carcass wholesale value', *Report to Ontario Pork*, 7.
- Gispert M and Font M (2005), 'Classificació de canals porcines: Equips i mètodes. In: *Sistema de Pesatge i Classificació de Canals Porcines*, J. Tarragó (Dir.) and A. Pallí (coord), Generalitat de Catalunya, Departament d'Agricultura, Ramaderia i Pesca. Barcelona, Spain.

- Gispert M and Diestre A (1994), 'Classement des carcasses de porc en Espagne: Un pas vers l'harmonization communautaire', *Techniporc*, 17, 29–32.
- Gispert M, Font i Furnols M, Batallé J and Diestre A (2002), 'El Autofom: Nuevo equipo de clasificación de canales aprobado en España', *Eurocarne*, 110, 69–74.
- Glasbey C A and Robinson C D (2002), 'Estimators of tissue proportions from X-ray CT images', *Biometrics*, 58, 928–936.
- Glasbey C A and Robinson C D, (1999), 'Inference from X-ray CT images of sheep', Invited talk at 52nd ISI Session, Helsinki, Finland.
- Henning MD (1992), 'Magnetic resonance imaging for the assessment of body composition in pigs', *Pig News Info.*, 13, 163N–166N.
- Higbie A D, Bidner T D, Matthews J O, Southern L L, Page T G, Persica M A, Sanders M B and Monlezun C J (2002), 'Prediction of swine carcass composition by total body electric conductivity (TOBEC)', *J. Anim. Sci.*, 80, 113–122.
- Horn P (1995), 'Using X-ray Computed Tomography to Predict Carcass Leanness in Pigs', *National Swine Improvement Federation Conference and Annual Meeting*, Clive, Iowa.
- Hounsfield G N (1979), 'Computed medical imaging', Nobel lecture, *J. Computer Assisted Tomography*, 4, 665–674.
- Hulsegge B and Merkus G S M (1997), 'A comparison of the optical probe HGP and the ultrasonic devices Renco and Pie Medical for the estimation of the lean meat proportion in pig carcasses', *Anim. Sci.*, 64, 379–383.
- Hulsegge B, Sterrenburg P and Merkus G S M (1994), 'Prediction of lean meat proportion in pig carcasses and in the major cuts from multiple measurements made with the Hennessy Grading Probe', *Anim. Prod.*, 59, 119–123.
- International Organization for Standardization (ISO) (1993), *Statistics – Vocabulary and Symbols Part 1: Probability and General Statistical Terms*, International Organization for Standardization, Geneva, Switzerland.
- Johnson R K, Berg E P, Goodwin R, Mabry J W, Miller R K, Robison O W, Sellers H and Tokach MD (2004), 'Evaluation of procedures to predict fat-free lean in swine carcasses', *J. Anim. Sci.*, 82, 2428–2441.
- Jones S D M (1997), *The Canadian Pork Carcass Grading System and the 1992 National Carcass Cut Out*, Consulted at: <http://mark.asci.ncsu.edu/nsif/96proc/jones.htm>.
- Jopson N B, Kolstad K, Sehested E, and Vangen O (1995), 'Computed tomography as an accurate and cost effective alternative to carcass dissection', *Proc. Aust. Assoc. Anim. Breed. Gen.*, 11, 635–639.
- Judas M, Höreth R, and Dobrowolski A (2005), 'Computertomographie als Methode zur Analyse der Schlachtkörper von Schweinen [Computed tomography as an analytical method for pig carcasses]', *Mitteilungsblatt der Fleischforschung Kulmbach*, 44, 145–151.
- Judas M, Höreth R, Dobrowolski A, and Branscheid W (2006), 'The measurement of pig carcass lean meat percentage with X-ray computed tomography', *52nd ICoMST*, Dublin, Ireland, 641–642.
- Kastelic M, Baulain U and Kallweit E, (1996), 'Allometric growth of muscle and fat areas in German Landrace pigs', *Proc. 47th Annu. Mtg. Europ. Assoc. Anim. Prod.*, Lillehammer, Norway, p 279 (Abstr.).
- Kempster A J, Cuthbertson A and Harrington C (1982), *Carcass Evaluation in Livestock Breeding, Production and Marketing*, Toronto, Granada Publishing Ltd.
- Kolstad K and Vangen O (1996), 'Genetic differences in maintenance efficiency when accounting for changes in body composition', *Livest. Prod. Sci.*, 47, 23–32.
- Kolstad K (2001), 'Evaluation of lean and fat deposition in swine selection. The use of computer tomography to measure lean and fat deposition in live pigs', *2nd Int. Virtual Conf. Pork Quality*, via Internet, 2001.
- Kolstad K, Jopson N B and Vangen O (1996), 'Breed and sex differences in fat distribution and mobilisation in growing pigs fed at maintenance', *Livest. Prod. Sci.*, 47, 33–41.

- Larsen R (2000), '3-D contextual Bayesian classifiers', *IEEE Transactions on Image Processing*, 9, 518–524.
- Latorre M A, Pomar C, Faucitano L, Gariepy C and Methot S (2007), 'The relationship within and between production performance and meat quality characteristics in pigs from three different genetic lines', *Livest. Sci.*, 115, 258–267.
- Liu Y and Stouffer J R (1995), 'Pork Carcass Evaluation with an Automated and Computerized Ultrasonic System', *J. Anim. Sci.*, 73, 29–38.
- Luiting P, Kolstad K, Enting H and Vangen O (1995), 'Pig breed comparison for body-composition at maintenance – analysis of computerized-tomography data by mixture distributions', *Livest. Prod. Sci.*, 43, 225–234.
- Lyckegaard A, Larsen R, Christensen L B, Vester-Christensen M, and Olsen E V (2006), 'Contextual analysis of CT scanned pig carcasses', *52nd ICoMST*, Dublin, Ireland.
- Marcoux M, Bernier J F and Pomar C (2003), 'Estimation of Canadian and European lean yields and composition of pig carcasses by dual-energy X-ray absorptiometry', *Meat Sci.*, 63, 359–365.
- Marcoux M, Faucitano L and Pomar C (2005), 'The accuracy of predicting carcass composition of three different pig genetic lines by dual-energy X-ray absorptiometry', *Meat Sci.*, 70, 655–663.
- Marcoux M, Pomar C, Faucitano L and Brodeur C (2007), 'The relationship between different pork carcass lean yield definitions and market carcass value', *Meat Sci.*, 75: 94–102.
- McClure E K, Scanga J A, Belk K E and Smith G C (2003), 'Evaluation of the E + V video image analysis system as a predictor of pork carcass meat yield' *J. Anim. Sci.*, 81, 1193–1201.
- Mercier J, Pomar C, Thériault M, Goulet F, Marcoux M and Castonguay F (2006), 'The use of dual-energy X-ray absorptiometry to estimate the dissected composition of lamb carcasses', *Meat Sci.*, 73, 249–257.
- Mersmann H J (1982), 'Ultrasonic determination of backfat depth and loin area in swine', *J. Anim. Sci.*, 54, 268–284.
- Mitchell A D, Scholz A M, Wang P C and Song H (2001), 'Body composition analysis of the pig by magnetic resonance imaging', *J. Anim. Sci.*, 79, 1800–1813.
- Mitchell A D, Conway J M and Pott W J E (1996), 'Body Composition Analysis of Pigs by Dual-energy X-ray Absorptiometry', *J. Anim. Sci.*, 74, 2663–2671.
- Mitchell A D, Scholz A M and Pursel V G (2003), 'Prediction of pork carcass composition based on cross-sectional region analysis of dual energy X-ray absorptiometry (DXA) scans', *Meat Sci.*, 63, 265–271.
- Mitchell A D, Scholz A M, Pursel V G and Evock-Clover C M (1998), 'Composition Analysis of Pork Carcasses by Dual-energy X-ray Absorptiometry', *J. Anim. Sci.*, 76, 2104–2114.
- Monziols M, Collewet G, Bonneau M, Mariette F, Davenel A and Kouba M (2005), 'Quantification of muscle, subcutaneous fat and intermuscular fat in pig carcasses and cuts by magnetic resonance imaging', *Meat Sci.*, 72, 146–154.
- Monziols M, Collewet G, Bonneau M, Mariette F, Davenel A, and Kouba M (2006a), 'Quantification des tissus musculaire et adipeux dans les carcasses et les pièces de découpe de porc à l'aide de l'imagerie par résonance magnétique', *Journ. Rech. Porcine*, 37, 151–158.
- Monziols M, Collewet G, Mariette F, Kouba M, and Davenel A (2006b), 'Muscle and fat quantification in MRI gradient echo images using a partial volume detection method. Application to the characterization of pig belly tissue', *Magnetic Resonance Imaging*, 23, 745–755.
- Nissen P M, Busk H, Oksama M, Seynaeve M, Gispert M, Walstra P, Hansson I and Olsen E (2006), 'The estimated accuracy of the EU reference dissection method for pig carcass classification', *Meat Sci.*, 73, 22–28.

- NPPC (1994), *Fat-free lean index users guide*. National Pork Producers Council, Des Moines, IA, USA.
- NPPC (2000), *Pork composition and quality assessment procedures*, NPPC, Des Moines, IA, USA.
- Orcutt M W, Forrest J C, Judge M D, Schinckel A P and Kuei C H (1990), 'Practical means for estimating pork carcass composition', *J. Anim. Sci.*, 68, 3987–3997.
- Persson R C, McKenna D R, Griffin D B, McKeith F K, Scanga J A, Belk K E, Smith G C and Savell J W (2005), 'Benchmarking value in the pork supply chain: Processing characteristics and consumer evaluations of pork bellies of different thicknesses when manufactured into bacon', *Meat Sci.*, 70, 121–131.
- Piechocki T, Borzuta K and Grzeskowiak E (1994), 'The usefulness of classifying instruments Ultra-Fom and PG-200 for estimation of pork carcasses meatness in Poland', *Proc. 40th Int. Meat Sci. and Tech.*, S-III, 14.
- Pomar C and Marcoux M (2003), 'Comparing the Canadian pork lean yields and grading indexes predicted from grading methods based on Destron and Hennessy probe measurements', *Can. J. Anim. Sci.*, 83, 451–458.
- Pomar C and Marcoux M (2005), 'The accuracy of measuring backfat and loin muscle thicknesses on pork carcasses by the Hennessy HGP2, Destron PG-100, CGM and ultrasound CVT grading probes', *Can. J. Anim. Sci.*, 85, 481–492.
- Pomar C, Fortin A and Marcoux M (2001b), 'Estimation du rendement boucher et de la teneur en viande maigre (TVM) des carcasses de porc r1 l'aide de différentes méthodologies de mesure de l'épaisseur de gras et du muscle dorsal', *Journ. Rech. Porcine*, 33, 71–77.
- Pomar C, Rivest J, Jean dit Bailleul P and Marcoux M (2001a), 'Predicting loin-eye area from ultrasound and grading probe measurements of fat and muscle depths in pork carcasses', *Can. J. Anim. Sci.*, 81, 429–434.
- Pomar C and Rivest J (1996), 'The effect of body position and data analysis on the estimation of body composition of pigs by dual energy x-ray absorptiometry (DEXA)', *Proceedings of the 46th Annual Conference of the Can. Soc. Anim. Sci.*, Lethbridge, Alberta, 26 (Abstr.).
- Sather A P, Jones S D M and Robertson W M (1989), 'The effect of genotype on predicted lean yield in heavy pig carcasses using the Hennessy Grading Probe, the Destron PG-100 and the Fat-O-Meater Electronic grading probes', *Can. J. Anim. Sci.*, 69, 93–101.
- Schinckel A P, Wagner J R, Forrest J C and Einstein M E (2001), Evaluation of alternative measures of pork carcass composition. *J. Anim. Sci.*, 79, 1093–1119.
- Scholz A, Baulain U and Kallweit E (1993), 'Quantitative Analyse von Schnittbildern lebender Schweine aus der Magnet-Resonanz-Tomographie', *Züchtungskunde*, 65, 206–215.
- Sehested E and Vangen O (1989), 'Computer tomography, a non-destructive method of carcass evaluation', *Proc. of the EAAP Symposium of the Commission on Pig Production*, Helsinki, Finland, 1 July 1988, 98–102.
- Skjervold H, Gronseth K, Vangen O and Evensen A (1981), 'In vivo estimation of body composition by computerised tomography', *Zeitschrift für Tierzüchtung und Züchtungsbiologie*, 98, 77–79.
- Strzelecki J, Komender P, Borzuta K and Lisiak D (1998), 'Precision of the lean meat content estimation with the automatic grading equipment AUTOFOM on Polish pig carcasses', *Proc. 44th Int. Meat Sci. and Techn.*, 950–951.
- Suster D, Leury B J, Ostrowska E, Butler K L, Kerton D J, Wark J D and Dunshea F R (2003), 'Accuracy of dual energy X-ray absorptiometry (DXA), weight and P2 back fat to predict whole body and carcass composition in pigs within and across experiments', *Livest. Prod. Sci.*, 84, 231–242.
- Swatland H J (2001), 'Effect of connective tissue on the shape of reflectance spectra obtained with a fibre-optic fat-depth probe in beef', *Meat Sci.*, 57, 209–213.
- Szabo C, Babinszky L, Versteegen M W A, Vangen O, Jansman A J M and Kanis E (1999),

- ‘The application of digital imaging techniques in the in vivo estimation of the body composition of pigs: A review’, *Livest. Prod. Sci.*, 60, 1–11.
- Usborne W R, Menton D and McMillan I (1987), ‘Evaluation of the Destron PG-100 electronic probe for grading warm pork carcasses’, *Can. J. Anim. Sci.*, 67, 209–212.
- Vangen O (1984), ‘Evaluation of carcass composition of live pigs based on computed tomography’, *Proc. 35th Annual Meeting of the EAAP*, The Hague, August 6–9, 1984.
- Vangen O (1992), ‘Estimation of body composition of pigs using computer assisted tomography’ *Pig News and Information*, 13, 159–162.
- Vangen O and Standal N (1984), ‘Tissue deposition rate in genetically lean and fat pigs estimated by computed tomography’, *Proc. 35th Annual Meeting of the EAAP*. The Hague, August 6–9, 1984.
- Walstra P and Merkus G S M (1996), *Procedure for assessment of the lean meat percentage as a consequence of the EU reference dissection method in pig carcass classification*, Report ID-DLO 96.014, The Netherlands.
- Wang D and Doddrell D M (2001), ‘A segmentation-based and partial-volume-compensated method for an accurate measurement of lateral ventricular volumes on T1-weighted magnetic resonance images’, *Magnetic Resonance Imaging*, 19, 267–272.
- Whittaker A D, Park B, Thane B R, Miller R K and Savell J W (1992), ‘Principles of Ultrasound and Measurement of Intramuscular Fat’, *J. Anim. Sci.*, 70, 942–952.

New methods for analysis of factors affecting meat eating quality

V. H. Segtnan, K. I. Hildrum and J. P. Wold, Nofima Food, Norway

Abstract: This chapter focuses on novel spectroscopic techniques for the analysis of important quality attributes of meat. On-line spectroscopic technology is highlighted. Besides describing the various techniques, problems involved in the implementation of such techniques are discussed, e.g. pitfalls in the calibration and prediction procedures, as well as problems in sampling for both spectroscopic and reference analysis. Possible future developments in the field are then reviewed.

Key words: online spectroscopy, sampling, near infrared (NIR), fluorescence, Raman, microwave, X-ray.

22.1 Introduction

Analysis of the most important meat quality factors has already been dealt with in the previous chapters, i.e. in Chapters 3, 4, 5, 6 and 7 regarding tenderness, colour, flavour, water-holding capacity and nutritional quality, respectively. In this chapter the focus will be on novel spectroscopic methods for analysis of some of these quality factors, in particular techniques that lend themselves to on-line analysis in meat processing lines. This area has been highlighted to minimise possible overlap with other chapters.

It is not our intention to provide a comprehensive coverage of the considerable range of techniques and instruments on the market for these purposes. Focus will be on examples of the potential of implementing online spectroscopic techniques in the meat industry. Unfortunately, many instruments and devices on the market lack satisfactory documentation regarding their performance in practice. This makes it hard to evaluate their value in practical use and give meaningful advice in selecting between them. We will therefore mostly limit this chapter to techniques where we have practical experience. Besides describing the various techniques, we will highlight problems involved in the implementation of these, e.g. pitfalls in the

calibration and prediction procedures, as well as problems in sampling for both spectroscopic and reference analysis. Possible future developments in the field are also discussed.

22.2 Meat industry needs for on-line spectroscopic analysis

The large variability in meat raw materials is a consequence of the biological variation among individual animals, as well as differences in composition between different muscles and cuts in each carcass. Variability in fat, water and protein contents is important both for the economic value of meat raw materials and for the quality of comminuted meat products such as ground meat, sausages and hamburgers.

The meat processes must handle large variations in raw material composition and other properties.¹ Raw material costs account for a large share of the total production costs in meat processing. This stresses the need for optimal usage of raw materials, with stringent quality control procedures. Meat is likewise susceptible to deterioration, and rapid turnover is a prerequisite for safe and efficient processes.

What quality parameters of meat should be monitored? The proximal composition of raw materials and products are needed to be able to reduce variations in composition, to optimise quality, to comply with official food regulations and to minimise product costs. Estimation of collagen is required to secure use of high quality meat with low sinew content, while assessment of fat quality is needed to monitor nutritional and technological quality. Salt analysis is performed to control variation in texture and taste and to prevent excessive use of an additive that is nutritionally questionable for many consumers. Tenderness is assessed to prevent tough beef in the stores, while the presence of foreign materials in the products may be hazardous and detrimental to consumer confidence.

To be able to compensate efficiently for variations in raw material composition, analysis should be performed as soon as possible in the process.² This allows for early correction of meat blends through standardisation procedures. In processing lines for products based on ground meat, one early processing step is size reduction by grinding of meat raw materials. Assessment of the chemical composition of the meat raw materials should therefore be done before or during initial grinding or blending. Analytical data acquired later in the process can be used for real time control systems of intermediate or end products, but hardly for recipe correction. Corrective actions here will, in most cases, be considered as re-processing, and should be avoided due to high cost.

The use of spectroscopy in food science has increased tremendously in the last few decades. The development of improved instrumentation hardware is an important factor in this. Also the development of low-cost computers and software has been crucial, as they facilitate efficient extraction of relevant information from complicated spectra.

Spectroscopy can be defined as the study of electromagnetic interactions

between atoms and molecules. It has long been known that food constituents and their properties can be measured by absorbed or emitted radiation at different wavelengths. Techniques such as X-ray, ultraviolet, NIR and microwave spectroscopy derive their names from the use of a portion of the electromagnetic spectrum, and can be categorised according to the particular wavelength used, which also indicates the energy changes associated with each wavelength.³ Absorption spectroscopy has been used for the analysis of proteins, carbohydrates, minerals, vitamins and many additives. Emission spectroscopy is developing as a useful technique for analysis of fat oxidation, collagen and certain elements.

22.3 Selected on-line spectroscopic techniques for meat

Spectroscopic methods have, over the years, found many applications in food and agriculture. While the main emphasis has been on laboratory applications, on-line quantitative applications for industrial use are gaining in importance. In 1984, an attempt to assess the fat content of meat on-line was done by Newman,⁴ using video image analysis. The Continuous Fat Analyzer from Wolfking, Denmark, has been marketed since 1996.⁵ In the same period on-line applications for meat based on the MM 55/710 instrument from NDC, InfraRed Engineering, UK, have been implemented in processing plants.⁶

The transition of spectroscopic techniques from the laboratory to on-line use in industrial processes presents a number of challenges. On-line analysis does not permit sample pre-treatment such as grinding or homogenisation, and the instruments must be able to handle large differences in, e.g. homogeneity and particle size. The analysis must be continuous and very rapid. The instruments must be robust to withstand the variations in temperature, humidity, external vibrations and light conditions usually encountered under industrial conditions.

In recent years, a number of different systems for on-line analysis of meat have been developed, based on principles such as fluorescence,^{3,7} microwave,⁸ X-rays⁹ and NIR spectroscopy,^{5,6,10-12} electrical conductivity or impedance, ultrasonics and video image analysis. In the following sections, focus will be on those techniques that are within the experience of the authors, while techniques such as ultrasonics, video image analysis and bioimpedance will be discussed only briefly.

22.3.1 Near infrared (NIR) spectroscopy

During the last decade, a number of dedicated NIR on-line instruments have appeared on the market. Among these are reflectance filter instruments (e.g. MM710 and MG710, NDC-Infrared Engineering^{6,10}) and transmission instruments (CFA, Wolfking⁵), as well as reflectance instruments with diode array detectors^{11,12} (MSC 511 or Corona 45, Zeiss; DA-7000NIR/VIS analysis system, Perten Instruments; Springfield, Ill) .

Over the last two decades, numerous NIR applications on various foods have been published, of which a majority have never had practical implications. In

particular, during the first of these decades, many studies reported promising calibration results. Often these results did not rely on causal linkages between the variables, but were based on indirect relationships between NIR spectra and the variables to be estimated. Proper validation of the calibration results on new independent sets of data was often lacking. This resulted in unstable and non-robust calibrations of little practical value.

An example of such calibrations is the estimation of sensory odour and taste properties of meat products,¹³ which yielded interesting prediction results. However, in view of the low concentrations of aroma compounds in food products, it is unlikely that NIR spectroscopy would be sensitive to these. The correlation was probably indirect and based on relationships between aroma properties, as analysed by a sensory panel, and proximal composition (fat, water and protein) of the products. Co-correlation is generally a problem in sensory analysis, with high correlations between sensory scores of different attributes by each single assessor in the sensory panel. Another example of indirect relationships and NIR is aflatoxin assessment in walnuts. Here, the correlation was based on the relationship between the structural damage to the walnut, caused by the mould that produces aflatoxin, rather than the toxic agent itself. Although this calibration may work when such damage is caused by aflatoxin only, it will break down when the structure of walnuts is affected by causes other than mould growth.

Another example of possible non-robust calibrations in the meat field is the analysis of collagenous substances by NIR.^{14,15} However, the prediction of collagen in new, unknown samples proved unsatisfactory, indicating that the calibration was not robust. The reason was probably that there is generally a fairly high correlation between the abundant myofibrillar proteins and collagen in meat. The positive NIR prediction for collagen was probably based largely on the co-correlation between protein and collagen in the samples, and will most likely not be robust when analysing new samples with a different relationship between these compounds. However, the problem can be minimised by adding such samples into the calibration set.

The many incidences of non-robust NIR calibrations have damaged the reputation of the technique and made potential users reluctant to use it. In recent years, the attitude towards applications has been more realistic, and its use has been restricted to areas where the technique has true potential. The following summarisation of NIR on-line meat applications will focus on bulk compositional analysis.

Reflectance measurements

Reflectance measurements, i.e. instrumental set-ups where illumination and detection take place at more or less the same physical spot on the sample (Fig. 22.1), are still the most common in on-line NIR analysis of meat products. These setups provide chemical (and physical) information mainly from the surface of the meat sample, and thus require that the surface of the sample is representative of its interior in order to be robust and reliable. The fact that illumination and detection take place on the same side of the sample makes the set-up relatively flexible and

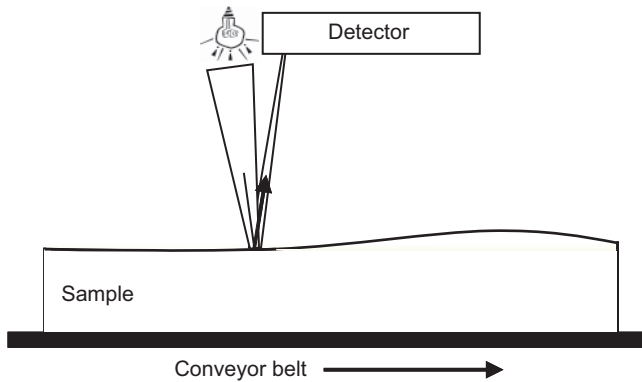


Fig. 22.1 Reflectance measurement on a conveyor belt.

easy to use. Typical locations for a reflectance instrument are above conveyor belts or at grinder or mixer outlets.

The first on-line meat application with a NIR instrument was reported by Isaksson *et al.*⁶ who used a non-contact reflectance filter instrument mounted at the outlet of an industrial meat grinder (MM55, NDC Infrared Engineering, Maldon, Essex, UK). The root mean square error of cross-validation (RMSECV) was calculated to be in the range 0.73–1.50% for fat, 0.75–1.33% for water and 0.23–0.32% of protein. It was found that the prediction error increased with increasing hole size in the grinder plate, which was probably mainly due to a higher NIR sampling error in the relatively small batches.

Using the same instrument, on-line NIR prediction of fat, water and protein in large industrial scale batches of beef and pork was further studied by Tøgersen *et al.*¹⁰ By scaling industrial batches up to 400–800 kg of ground beef (13 mm), the NIR samples were markedly increased in size due to the longer measurement time. To improve sampling, reference samples were taken from batches that were reground through 4 mm hole plates. The average distance from the meat surface to the sensing head was approx. 25 cm. The prediction errors were 1.09–1.33% and 1.30–1.49% for moisture and fat, respectively.

An upgraded version of the MM55 instrument (MM710) was tested in two other meat processing plants. The MM710 had a rotating filter wheel with eight filters, which opened up for more robust and precise calibrations. With instruments installed at the outlet of a grinder (4–13 mm hole plates), the prediction errors for analysis of fat content in ground beef for the two plants were 0.51% and 0.48% (unpublished results). The same type of instrument was also tested in a third processing plant on beef batches (60) ground to sizes of 18–40 mm (the last size refers to grinding without a grinder plate).¹² The prediction results were found to be satisfactory for fat, with a correlation coefficient of 0.98 and a standard error down to 0.72%. The lowest error was found for models with large batches (ca. 500 kg).

While filter instruments record discrete bands in the NIR spectrum, diode array instruments monitor the spectrum with higher resolution. The instrument used in the following experiment was an industrial reflectance head for measurement over a wide spectral range (Corona 45, Carl Zeiss Jena GmbH, Jena, Germany). The instrument measured in the 950–1700 nm range, with a band width of 6 nm per diode. The instrument was designed to measure at a distance of 3–5 cm above the sample, viz. the surface of the ground meat stream on a conveyor belt. The measurements were performed under industrial conditions on 60 batches of 150–500 kg.¹⁶ As the meat flow on the conveyor belt was frequently discontinuous, interfering spectral readings from the belt itself had to be identified and removed by principal component analysis (PCA) and SIMCA (Soft Independent Modelling of Class Analogy) classification.^{17,18} PLS (Partial Least Squares) calibration models for all samples at two different grinding sizes (40 and 18 mm) yielded correlation coefficients in the range 0.93–0.96, while the full cross-validated errors for fat and water were between 1.6 and 2.4%. The corresponding errors for protein were 0.5–0.8%. The predictions were generally best for the 18 mm grinding size. Before the implementation of these calibrations, they need to be re-evaluated under industry conditions on new independent batches.

Anderson and Walker,¹¹ using a DA-7000NIR/VIS analysis system (Perten Instruments, Springfield, Ill.), estimated fat content in ground beef in a continuous stream on a conveyor belt. The instrument made use of a fixed grating and a diode array. The batches were relatively small (27 kg), which gave a measurement time of only 1.94 s. The prediction errors for the validation set were in the range 2.15–2.28% for fat.

Interactance measurements

The principle of interactance is to transmit light into the sample at the surface and measure the transmitted light which resurfaces a distance away (Fig. 22.2). Directly reflected light is prevented from reaching the detector, meaning that only light that has traversed the interior of the product will be analysed. Interactance thus probes deeper into the material compared to reflectance, and suppresses surface effects. The method is therefore better suited for heterogeneous samples.

Interactance measurements have traditionally been measured with fibre optic-based contact probes, which is not a good solution for online food analysis. Recently (2004), an on-line non-contact NIR system based on interactance measurements was introduced to the food industry. The system is called Qmonitor and is manufactured by Qvision (Norway). Presently, the main applications delivered with this system are on-line fat, colour and pigment contents in salmon fillets, and water content in dried salted cod.¹⁹ The system is imaging, which enables detailed analysis of how different chemical constituents are distributed in the samples (Fig. 22.3). The system enables effective grading and sorting of every fillet in the production batch. The system is presently being developed and evaluated for different meat applications. Promising results have been obtained for fat determination in MAP packed ground beef, which would enable precise fat labelling on every package of beef. The method can also be used to determine fat and protein

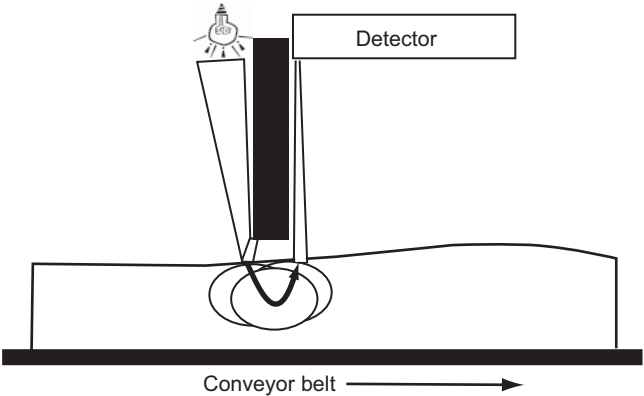


Fig. 22.2 Intertance measurements on a conveyor belt.

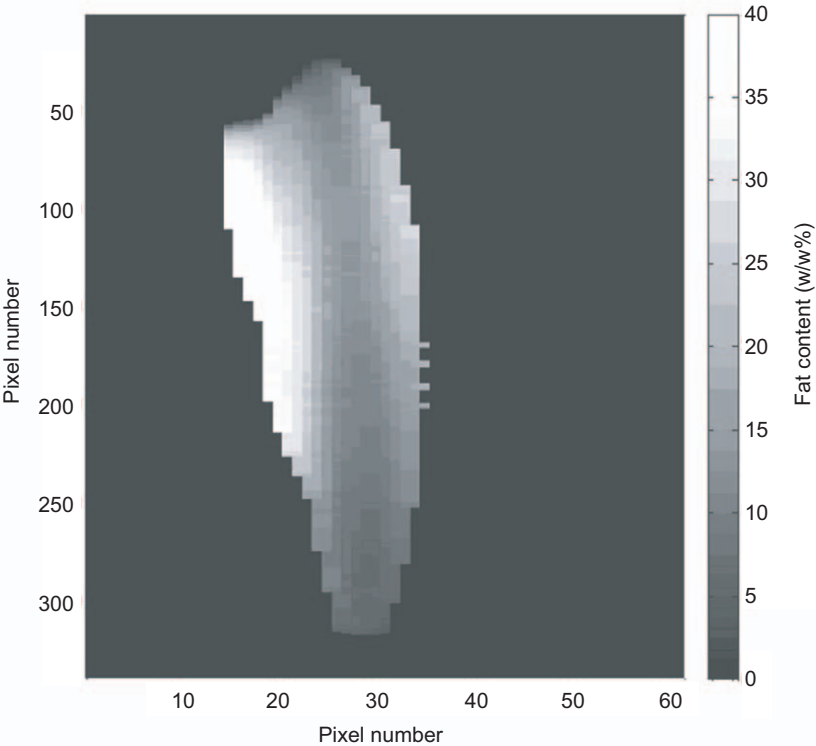


Fig. 22.3 Output image from on-line intertance NIR scanner shows pixel-wise prediction of fat content in a salmon fillet.

contents in intact meat trimmings on a conveyor belt, which gives the opportunity to, for instance, automatically sort meat into fractions of specified fat content.

22.3.2 Fluorescence spectroscopy

Fluorescence spectroscopy is a promising method for on-line quality measurements of meat. However, compared with NIR, on-line applications are still in their infancy. In the following, an update on two potential on-line meat applications is given.

Fluorescence is a very sensitive method, 100–1000 times more sensitive than other spectrophotometric techniques. It is rapid and, for most applications, a spectrum can be recorded within a second with a sensitive CCD camera. Meat contains many naturally occurring fluorophores, such as the aromatic amino acids tryptophan, tyrosine and phenylalanine, structural proteins such as elastin and collagen, the enzymes and coenzymes NADH, FAD and NADPH, the vitamins A, K and D, derivatives of pyridoxal, porphyrins and phospholipids, the lipid pigments lipofuscin and ceroids, as well as various stable oxidation products.²⁰

The requirements for at- or on-line measurement of fluorescence on solid samples such as meat, are essentially a powerful light source, excitation filters optimised for the constituents of interest, cut-off filters to block the excitation light from reaching the detector, a spectrometer covering the near UV and VIS regions, and a sensitive detector. Sensitivity is more important than spectral resolution, since most fluorescence spectra consist of broad peaks. A description of a suitable lab system can be found in Wold *et al.*²¹ Fluorescence spectra from intact meat can be complex and often require use of multivariate analysis to extract quantitative information.

For meat, there are, in particular, three applications which are ready for industrial evaluation: quantification of connective tissue; monitoring of lipid oxidation; and detection of fecal contamination. Collagenous connective tissue (CT) is an important parameter of meat quality, which relates to tenderness and texture. CT is beneficial due to its binding properties, but too high levels in ground meat products can have detrimental effects on the end quality. Knowledge of the amount of CT in ground meat and in different kinds of meat blends is of importance for monitoring raw materials and for optimising beef product recipes. Today's common technique for CT quantification is to determine hydroxyproline, a tedious, demanding, and not particularly precise chemical method.

Several lab studies based on realistic model systems or industrial samples have shown that it is possible to estimate the CT content in beef and pork meat with an accuracy of ± 0.37 – $\pm 0.55\%$ CT.^{3,22,23} The conclusion of these surveys is that autofluorescence spectroscopy might be well suited for rapid on-line determination of collagen in ground beef. Excitation at 380 nm is optimal for determination of CT, while excitation at 332 nm is feasible for simultaneous determination of fat and connective tissue. It is important to include expected biological variation in the calibration model. The precision obtained in the studies is relevant to the industry, but as far as we know, the method has not so far been implemented.

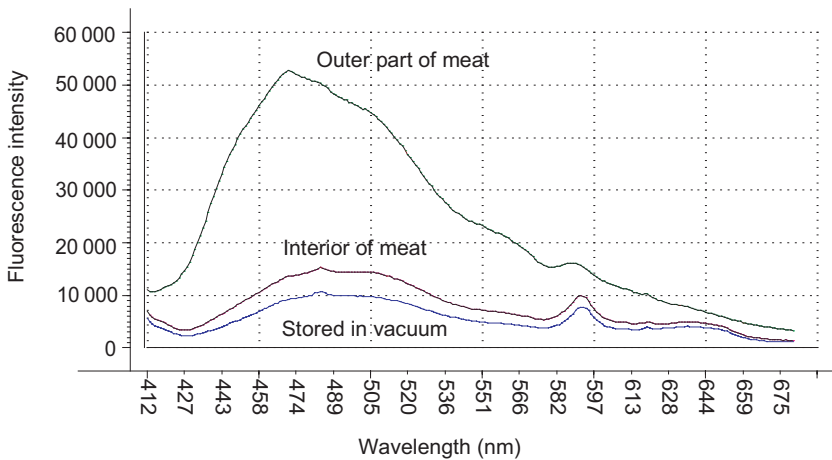


Fig. 22.4 Fluorescence spectra of ground turkey meat taken from the outer part (upper curve) and the interior part (middle curve) of sample stored in oxygen, and from sample stored in vacuum (lower curve).

In the food industry, the quality control of fat is achieved by a small set of methods for unspecific determination of oxidation products such as peroxides and aldehydes, conjugated dienes and other secondary oxidation compounds. One of the main industry needs is a reliable method, preferably rapid and non-invasive, to evaluate lipid oxidation at relevant levels. Autofluorescence is now recognised as a sensitive method for determining the level of lipid oxidation in complex foods such as fish and meat. The fluorescent compounds are formed from reactions of oxidising fatty acids or lipid oxidation breakdown products (hydroperoxides and aldehydes) with compounds containing primary amino groups (proteins, amino acids, DNA).²⁴ The method has many interesting properties when used on intact products: (i) It is rapid and non-destructive, i.e. it has potential for on-line/at-line use. (ii) The state of oxidation can be determined without any kind of extraction step, i.e. it might give a more 'correct' measure than other traditional measures such as TBARS (thiobarbituric acid-reactive substances) and peroxide value where extraction is required. (iii) It has potential for use on raw and cooked meats, as well as on raw materials and end products. (iv) For some products it has been reported to be at least as sensitive as sensory analysis.²⁵ (v) It is possible to create maps of lipid oxidation by spectral imaging of the fluorescence, enabling detailed studies of lipid oxidation distribution and progression in meat and meat products to be made (Fig. 22.4).²⁶

The concept of determining oxidative status by using solid sample spectroscopy in combination with chemometrics is promising. However, the approach is new, and needs to be evaluated for every new application/product. Specific calibration models have to be developed for each product category. Combining oxidation detection by fluorescence with image analysis offers new ways to study lipid oxidation and its progress in meat and meat products. It can be convenient and useful to actually see how fast the reaction is and where the oxidation starts and develops.

Fecal contamination on beef carcasses is one of the main sources of pathogens. An on-line method to detect fecal contamination would be important in assessing the safety of the carcass. Chlorophyll is ubiquitous in green plants and thus in livestock diets. During digestion, chlorophyll is only partially degraded and is therefore present in the feces as an excellent fluorescent marker. The fluorescent properties of chlorophyll have been exploited in the commercial Verifeye system to detect traces of fecal contamination in real time for both carcasses and smaller meat cuts. The system uses spectral imaging for detection of contaminated areas that may harbour potentially dangerous microbial pathogens.²⁷ The authors are not aware of how widespread the use of the Verifeye system is, but the principle of detection is convincing.

To summarise, there are some potential at-/on-line applications for fluorescence spectroscopy with meat. The method seems to be robust enough for on-line situations, as long as the relevant variability of the food system is taken into consideration. Interfering phenomena such as quenching, re-absorption, and spectral overlap should be modelled, and accounted for in multivariate calibrations. Since the instrumental requirements for fluorescence systems are rather modest, it is probably just a question of time before the first on-line systems for, e.g. rancidity screening are up and running in the meat industry.

22.3.3 Raman spectroscopy

Raman spectroscopy is excellent for biochemical analysis of tissue on both the macroscopic and microscopic scale. The technique can provide information about concentration, structure, and interaction of biochemical molecules within intact cells and tissues, nondestructively, without homogenisation or extraction. Raman spectroscopy has, until recently, received little attention, due to expensive instrumentation, cumbersome user interface, and some inherent problems associated with measurements of bio-materials. However, in recent years Raman systems have become much more affordable and easy to use due to the development of dedicated detectors, lasers and optics. Also, within different areas of food science, Raman spectroscopy has been recognised as a promising analytical tool,^{28,29} and one such area is in the rapid and non-destructive quality assessment of foods for in-line purposes.

Raman is a relatively specific spectroscopic technique that measures rocking, wagging, scissoring, and stretching fundamental vibrations of molecules containing bonds such as C–C, C–O, C–H, –S–S–, –C–S–, –C=C–. Raman is almost insensitive to water, enabling efficient measurement of samples such as meat and liquids.

Raman scattering is a relatively weak optical effect that requires laser light for efficient excitation. This method of excitation introduces two major challenges with regard to biomaterials, including meat. In most bio-materials, the incident laser light will produce autofluorescence, which is usually much more intense than the Raman scattered light. This fluorescence can make the Raman signals difficult or even impossible to measure. The problem can be partly overcome by using longer laser wavelengths, in the region 780 nm–850 nm, in combination with

algorithms specifically designed to remove the fluorescence background from Raman spectra. The other limitation is that the actual sampling area is very small, usually less than 0.2 mm², making representative sampling difficult for many applications. Lately, vendors have launched instruments with much larger laser diameters (up to 6 mm) to improve sampling of heterogeneous samples.

The potential of Raman spectroscopy for determination of meat quality has so far been little investigated. Some of the components contributing to the Raman scattering in muscle tissues are certain amino acids, collagen, elastin, carotenoids, fatty acids and cholesterol, all of which can be useful in describing meat quality. Raman spectroscopy is well known for its ability to determine the degree of saturation in fatty acids, and high correlations have been established with the Iodine number of oils.³⁰ Since there is increased interest in fat composition, especially on the ratios of saturated, monounsaturated and polyunsaturated fatty acids, it is of value to transfer this application to intact meat. Afseth *et al.*^{31,32} have shown that the above fatty acid features can be measured in meat model systems and salmon muscle. Another interesting feature of Raman spectroscopy is the ability to measure and describe changes in secondary protein structure. Certain Raman bands can be assigned to α -helix and β -sheet conformations, and the ratio can be measured. Beattie *et al.*³³ suggested that Raman spectroscopy can be useful for determination of such textural properties as tenderness and shear force, based on this ratio.

22.3.4 Microwave spectroscopy

On-line microwave spectrometry can be performed using two basically different instrumental principles: (i) Non-contact measurements using antennas mounted above the sample stream, i.e. reflectance measurements, and (ii) Guided microwave spectrometry (GMS), which utilises a waveguide chamber, i.e. transmittance measurements.

Microwave spectrometry is based on the orientation and relaxation of polar molecules in an electromagnetic field. Microwaves cover the frequency range 200 MHz–80 GHz. When a sample is irradiated with microwave energy, two basic molecular processes take place. The first is described by the dielectric constant (ϵ'), which reflects the field reduction due to the dielectric molecule. As an electromagnetic wave passes through a sample, it causes an alternating polarisation within the material. The material stores some of the energy, and releases it back to the wave slowly, thereby reducing the wave velocity. ϵ' has the value of one for a vacuum and greater than one for a dielectric substance. The small dipoles of water molecules can easily be oriented in a rapidly oscillating electromagnetic field, giving water a very high dielectric constant compared to almost all other molecules ($\epsilon' = 80.2$ at 20 °C). The second molecular process taking place is described by the dielectric loss (ϵ''). This is a heat energy loss caused by friction between the orienting molecules, resulting in a wave amplitude reduction. Measuring ϵ' or ϵ'' as a function of frequency provides a microwave spectrum.

Unlike waves in many other regions of the electromagnetic spectrum, micro-

waves can penetrate through large volumes of meat. Microwave spectrometry should thus be well suited for bulk on-line measurements of ground meat for standardisation purposes. It should be noted that microwave spectrometry has three main drawbacks that may affect the analysis of ground meat samples: (i) Frozen meat or ice will not give microwave signals, (ii) The presence of salt will disturb the measurements substantially, and (iii) Water molecules are highly polar and give very strong signals, proteins are semi-polar in the liquid state, and give weak signals, while fats and oils are non-polar, i.e. they will not be rotated, and give practically no signal. Thus, a microwave fat measurement relies on the internal correlation between fat, moisture and protein. In addition, microwave spectra are not as easy to interpret as those of most comparable techniques. The great benefit of microwaves is their penetration depth, which is of the order of several centimetres.

Knöchel *et al.*³⁴ reported that open microwave resonators were well suited for on-line moisture monitoring of cereal products. The same principle was tested by Kent *et al.*³⁵ for determination of water uptake and protein, fat, water, salt and phosphate contents in, e.g. pork and chicken. Also, the use of reflectance microwaves for analysing fat content in ground meat on a conveyer belt has been studied (unpublished results). This implies non-contact measurements, which have several advantages. A 'vector network analyser' from Rohde and Schwarz was used, where the frequency range 4–8 GHz was scanned, and the microwave signal was delivered to the meat sample by a horn antenna 40 cm over the conveyer belt. The measurements were performed on the same 60 batches (120–180 kg) of ground beef. Scans of the conveyer belt was used as reference. After belt correction and Fourier transform of the data, regression (CV) was performed against fat values. The explained variance of the model was 0.74, which was not impressive. However, by using similar physical waveguides to eliminate external interferences and freak reflections, the potential for improving the results could be considerable.

In on-line GMS analysis, the sample has to move through and fill a waveguide chamber. The main purpose of the waveguide chamber is to assure constant sample thickness, and to guide the microwaves towards the receiver. This is sought for by parallel horizontal metal plates. The metal plates reflect the microwave energy and restrict the wavelength range reaching the receiver. Only waves that can fit into the chamber can reach the detector. The spectroscopic response is an attenuation spectrum for the different microwave frequencies involved. The GMS utilises the lower frequency range of the microwave region, i.e. 200–3200 MHz.^{36,37}

GMS is suited for samples that can be pumped through the waveguide chamber, for example, ground meat. The instrument can be mounted on transportation pipes, or directly onto a meat grinder. Grinder mounting does not require any additional pumping of the meat. The main assets of GMS are that practically all the material is measured, and that it has a low sensitivity towards particle size and colour differences.

GMS is a relatively new technique, and few food applications have been reported. However, there are industrial GMS applications running, and one such implementation test will be presented shortly (unpublished results). A GMS

instrument from Thermo Electron Corp. (Round Rock, TX) was tested on a meat production site in Norway (Gilde Hed-Opp) in the fall of 2004. The equipment was mounted directly onto the meat grinder, and the target was to measure the total fat content in ground beef batches of up to 1000 kg. The calibration was performed on 47 samples in the range 3.1–77.3% fat. The calibration samples were not in motion while the spectra were taken, i.e. the calibration samples were obtained with the waveguide chamber filled with meat not in motion (~1 kg). The whole calibration sample was then removed from the chamber, homogenised and analysed using the fat reference method. The validation was based on spectra generated online from 19 full batches in the range 3.0–32.4% fat. The validation reference samples were collected after 10 minutes of mixing. Approximately 40 kg of sample were taken from each batch, homogenised and analysed using the same reference analysis as in the calibration step. This experiment gave an average prediction error (RMSEP) of 1.5% fat after a bias adjustment.

22.3.5 X-ray techniques

The measurement principle based on penetrating energy such as X-rays is that the various components of muscle have different properties when exposed to physical energy from this energy source. Relative density is the important property in analysis of muscle tissue as lean tissue has a different density to that of fat. Materials attenuate X-rays selectively, which gives rise to mono, dual (DEXA) or multiple (MEXA) energy X-ray absorptiometry. X-ray techniques have a long tradition in the meat industry, and at-line techniques (AnyIRay) appeared long ago for the analysis of fat content in raw, ground meat.³⁸ The instrument was based on measurement on one energy level, which meant that parameters such as sample size, packaging density and temperature had to be kept constant. On-line systems making use of the same systems have been developed (AVS Raytech), and are in use in the meat industry.

DEXA operates with dual energy systems, which allow determination of meat composition independently of the thickness of the samples. The meat industry has drawn heavily on medical applications in this field, and a method for determining the fat content in boneless meat has been developed in New Zealand.³⁹ DEXA has also been investigated for meat industry purposes in Denmark,⁹ where prediction errors were reported to be 0.3–0.6% for fat content.

22.3.6 Other techniques

As stated in the introduction of this chapter, the focus has been on spectroscopic techniques that have potential for on-line implementation in meat processes and also where the authors have had practical experience. Outside our own experience is nuclear magnetic resonance (NMR), which has had a spectacular development in medicine and science application. NMR techniques are highly sensitive, accurate and robust. Regarding meat applications, the estimation of fat and connective tissue contents and distribution would be particularly interesting.⁴⁰

However, at present the high cost of the instrumentation limits the implementation of NMR in the meat industry.³ Fourier transform infrared (FTIR) spectroscopy is another promising technique, which has found on-line applications for liquid foods. As for muscle foods, FTIR has been found very useful for analysing secondary and tertiary protein structures. However, no instrumentation for on-line analyses of solid samples has, to our knowledge, yet appeared.⁴¹ Bioimpedance is in widespread use in medicine, e.g. to assess muscle/fat ratio and fluid balances,⁴² but on-line applications for meat are not known. These spectroscopic techniques may also be added to the range of useful on-line techniques in due time.

22.4 Problems and pitfalls in on-line spectroscopic analysis

22.4.1 Sampling

The heterogeneity of meat raw materials often results in a large uncertainty in sampling for meat analysis. Sampling and subsequent preparation steps are usually found to be the greatest sources of errors in meat analysis, as is the case with many other food materials. A sample should be identical (within limits), in all of its intrinsic properties, with the bulk of the material from which it is taken. Very often the focus is on the analytical uncertainty of the instrument, while sampling problems are overlooked. As a rule of thumb, if the analytical uncertainty is less than one-third of the sampling uncertainty, additional reduction of the analytical uncertainty is of little value.⁴³

Sample heterogeneity

In order to achieve robust online measurement systems, it is important that the sample heterogeneity is considered. All solid foods are heterogeneous on one level or another. Minced meat or an intact piece of meat, for example, will have smaller or larger local regions that are almost pure fat, pure lean meat or pure connective tissue. For such heterogeneous foods, the distribution of the local differences is often approximately the same throughout the sample when considered on a large scale. In other samples, different layers (more or less homogeneous) will have different chemical compositions. An example could be an intact piece of meat with a surface layer consisting mainly of fat or connective tissue. Thus, the surface of the sample is not representative of the bulk, i.e. the interior. A third type of heterogeneity is found in foods that have compositional gradients. One example from this group could be a dried and salted ham, which normally has more salt and less water in the exterior parts than in the interior parts. In summary, these are the three main types of heterogeneity encountered in foods: (i) random region heterogeneity, (ii) layer heterogeneity, and (iii) gradient heterogeneity (Fig. 22.5).

Spectral sampling

The way in which the spectra are collected plays a crucial role for the quality of the measurement system. The most important basic principle of spectral sampling is to

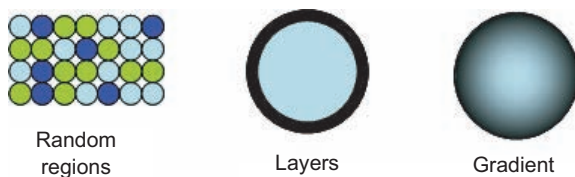


Fig. 22.5 Three main types of heterogeneity encountered in meat products.

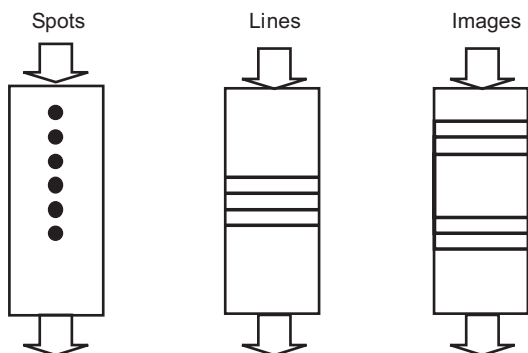


Fig. 22.6 Spectral sampling from meat on a conveyor belt.

assure spectral relevance, i.e. that the spectra contain the same chemical information as the samples themselves.

There are three principal ways in which spectroscopic data (i.e. for reflectance/interactance) can be obtained on-line (Fig. 22.6): (i) spot sampling, (ii) line scanning and (iii) imaging. Spot sampling will provide spectral data from a line on the sample surface, e.g. from the middle of the product stream. This spectral sampling is well suited for continuous streams of ground meat, where a central line normally will reflect the average composition of the whole batch. If the samples are discrete, heterogeneous objects, the spot sampling needs to be triggered such that the spots are obtained from the same regions of the objects each time. However, for such samples, spot sampling is not the best option. The alternative to spot sampling so far has been multispectral imaging in the NIR range. This technique assures that the whole sample is represented, but the number of wavelengths and the speed of sampling may be limited. An alternative that falls between these two sampling options is line scanning. Line scanning is a good option for continuous sample streams that have a gradient across the conveyor belt, and in particular for discrete, heterogeneous samples such as meat cuts or fish fillets. The output from a line scanner is the same as that of an imaging instrument, i.e. multispectral images.

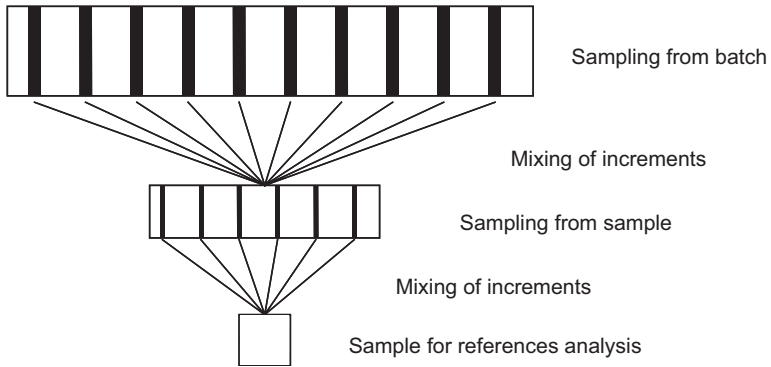


Fig. 22.7 Reference sampling from a meat stream on a conveyor belt.

Reference sampling

When collecting samples for reference analysis and subsequent use in calibration or validation, it is of the utmost importance that this is done in a way that ensures that the sample is representative of the whole batch. A theoretical framework for correct sampling was presented by Pierre Gy in the 1950s, and a summary of this framework was published in 2004.^{44–48} However, if a few practical rules from this framework are followed, representative sampling should be achieved. A fundamental principle is to take many small subsamples (increments) from the whole batch, mix them together, grind the mix if the particles are large, and then take new subsamples (increments). This principle is illustrated in Fig. 22.7.

How many times this should be done depends on how large the batch is to begin with, and how small the final reference sample should be. It can often be challenging to assure that the subsamples (increments) are taken from the whole batch, and not just from the top or from one side. The most practical and probably most correct way of collecting the subsamples would be during physical movement of the whole batch, e.g. during emptying of a grinder or mixer or during transportation of the batch through pipes or on a conveyor belt. The subsamples (increments) should be taken out as three-dimensional slices from the sample stream.

Replication of measurements is a key component in a good sampling and calibration/validation strategy. When replication is performed on each step of the sampling process, the average errors associated with each step or each analysis can be tracked.

22.4.2 Calibration, prediction and validation

Most of the spectroscopic methods that are described in this chapter rely on calibration against other instrumental measurements. Thus, the spectroscopic responses alone cannot directly provide the quantitative or qualitative information

that is needed. The simplest calibration case is when one single parameter (e.g. absorbance at one wavelength) can be used to assess the concentration or state of one specific compound. This is termed univariate calibration, and requires that no other compounds or phenomena affect the signal of the compound of interest. In other words, univariate calibration requires a selective analytical technique. Most spectroscopic techniques that can be used for on-line food analysis are non-selective. The combination of a series of non-selective variables may, however, compensate for the low selectivity of each variable. Modeling several spectral variables against one or a few reference variables is termed multivariate calibration.

Non-selective methods, like the ones described in this chapter, provide spectral variables that are more or less correlated or interdependent. In such cases, the calibration method must be able to compress the data in such a way that all important information is kept, and that specific information is related to as few variables as possible. The most frequently used multivariate calibration techniques are: (i) MLR (multiple linear regression). This method can be used when a few uncorrelated spectral variables are selected; (ii) PCR (principal component regression). In principal component analysis, the data are compressed into orthogonal scores (representing samples) and loadings (representing variables). The products of the scores and loadings are termed latent variables or principal components. The data compression is based on the spectral data only, and the regression is performed with the scores as input variables; (iii) PLSR (partial least squares regression). This technique resembles PCR, as it also decomposes the data into latent variables with scores and loadings. The main difference is that the data compression in PLSR is based on the covariance between the spectral variables and the response to be modeled, usually giving prediction models of lower complexity. In PLSR, the scores are orthogonal, but the loadings are not; (iv) ANN (artificial neural nets). This is the most complex of the four calibration methods mentioned here. A neural net consists of neurons or nodes that are organised in an input layer, an output layer and one or more hidden layers. The nodes in the hidden layer can be thought of as intermediate variables, similar to the latent variables in PCR and PLSR. ANNs have the benefit of handling non-linear relationships between spectral variables and response variables. The main drawbacks of the technique are the complexity and the poor interpretation abilities.

As soon as a proper calibration is established, the spectroscopic system needs to be tested on new and unknown samples. Validation is performed to estimate the analytical performance of the spectroscopic system, but it is also used to improve the calibration equation. Normally, calibration and initial validation are performed simultaneously, by splitting the data into one calibration set and one validation set (where the samples are treated as unknowns), or by performing cross-validation. The latter is often used when the data set is relatively small (approximately 50 samples or less). In cross-validation, all samples are left out of the calibration set once and used for prediction, giving an average calibration equation to be used for future prediction.

There are a few main validation criteria for prediction performance figures that

are commonly used, of which we describe two: (i) the correlation coefficient, R . This gives the correlation between the predicted values and the true or measured values, and should be as close to 1 as possible. The correlation may also be given as R^2 , which gives the amount of explained response variation; (ii) the root mean squared error of prediction (RMSEP). This expression represents the average prediction error, and is given by:

$$\text{RMSEP} = \left[\frac{\sum_{i=1}^N (y_i - \hat{y}_i)^2}{N} \right]^{1/2}$$

where y_i is the reference value, \hat{y}_i is the predicted value and N is the number of samples. For an introduction to multivariate calibration, see Næs *et al.*⁴⁹

22.5 Sources of further information and advice and future trends

A large number of references are identified in the literature on spectroscopic analysis of meat. However, restricting the search to on-line spectroscopic applications, the number of hits becomes very low (actually only our own publications). We believe this reflects the commercial interest in the field, and that implementation of successful calibrations is of high competitive value for the meat processors. The reluctance to publish also means that proper documentation regarding the precision and accuracy of different instrumentations is often lacking for on-line measuring equipment. Validation of the instrument is often up to the customer. For prospective customers, this creates problems in the choice of equipment. For us, it has been a problem in writing this chapter.

As stated before, modern processing plants are moving into the world of continuous processing lines with high throughput of products. As a consequence, processing lines need to be considered an integral parts of a quality system. This should include optimised usage of raw materials as well as optimised end product quality. On-line assessment of the raw material composition will hopefully be very useful in such systems. The authors believe that the instrumental development for online analysis is likely to move in two main directions. The first is multispectral imaging. Such instruments will facilitate the possibilities of measuring the distribution of different chemical components, which gives new opportunities in terms of sorting and optimised further processing of meat samples. The second main direction is multiplexing instruments for on-line spectroscopy. Such instruments give the opportunity to follow the production process with the same instrument, which may significantly reduce the time needed for quality control and process adjustment decisions.

22.6 References

1. K. I. Hildrum, B. N. Nilsson, F. Westad and N. M. Wahlgren (2004), *Journal of Near Infrared Spectroscopy*, 12 (6), 367.
2. G. Tøgersen, R. Rødbotten and K. I. Hildrum (2002), 'Recent advances in prediction of meat quality by NIR spectroscopy', in *Research Advances in the Quality of Meat and Meat Products*, F. Toldra, Ed. (Research Signpost, Physical Sciences/Technology, Trivandrum), p. 1.
3. K. I. Hildrum, J. P. Wold, V. H. Segtnan, J. P. Renou and E. Dufour (2006), 'New spectroscopic techniques for online monitoring of meat quality', in *Advanced Technologies for Meat Processing*, L. M. L. Nollet and F. Toldra, Eds. (CRC Press), p. 87.
4. P. B. Newman (1984), *Meat Sci.*, 10 (3), 161.
5. H. Schwarze (1997), *Process Control and Quality*, 9 (4), 133.
6. T. Isaksson, B. N. Nilsen, G. Tøgersen, R. P. Hammond and K. I. Hildrum (1996), *Meat Science*, 43 (3–4), 245.
7. B. Egelandsdal, J. P. Wold, A. Spönnich, S. Neegard and K. I. Hildrum (2002), *Meat Science*, 60 (2), 187.
8. C. Borggaard and L. Bager Christensen (2003), in *ICoMST* (Brasil).
9. P. W. Hansen, I. Tholl, C. Christensen, H. C. Jehg, J. Borg, O. Nielsen, B. Ostergaard, J. Nygaard and O. Andersen (2003), *Meat Science*, 64 (2), 141.
10. G. Tøgersen, T. Isaksson, B. N. Nilsen, E. A. Bakker, and K. I. Hildrum (1999), *Meat Science*, 51 (1), 97.
11. N. M. Anderson and P. N. Walker (2003), *Transactions of the ASAE*, 46 (4), 1135.
12. K. I. Hildrum, B. N. Nilsen and M. Wahlgren (2003), 'On-line assessment of the proximal composition of ground meat by NIR and other instrumental techniques', in *International Conference of Near Infrared Spectroscopy* (Cordoba, Spain).
13. M. R. Ellekjaer, T. Isaksson and R. Solheim (1994), *J. Food Sci.*, 59 (3), 456.
14. T. Isaksson and K. I. Hildrum (1990), 'Near infrared transmittance (NIT) analysis of meat products', in *International Conference on Near Infrared Spectroscopy* (Brussels), p. 202.
15. H. Berg and K. Kolar (1991), *Fleischwirtschaft*, 71 (7), 765.
16. K. I. Hildrum, B. N. Nilsen, F. Westad and M. Wahlgren (2004), *Journal of Near Infrared Spectroscopy*, 12 (6), 367–376.
17. J. P. Wold, F. Westad and K. Heia (2001), *Applied Spectroscopy*, 55 (8), 1025.
18. F. Westad and H. Martens (2000), *Journal of Near Infrared Spectroscopy*, 8 (2), 117.
19. J. P. Wold, I. R. Johansen, K. H. Haugholt, J. Tschudi, J. Thielemann, V. H. Segtnan, B. Narum and E. Wold (2006), *Journal of Near Infrared Spectroscopy*, 14 (1), 59.
20. N. Ramanujam (2000), 'Fluorescence Spectroscopy In Vivo', in *Encyclopedia of Analytical Chemistry*, R. A. Meyers, Ed. (John Wiley & Sons Ltd, Chichester), p. 20.
21. J. P. Wold, K. Kvaal and B. Egelandsdal (1999), *Appl. Spectrosc.*, 53 (4), 448.
22. B. Egelandsdal, G. Dingstad, G. Tøgersen, F. Lundby and Ø. Langsrud (2005), *Meat Science*, 69, 35.
23. J. P. Wold, F. Lundby and B. Egelandsdal (1999), *J. Food Sci.*, 64 (3), 377.
24. K. Kikugawa and M. Beppu (1987), *Chemistry and Physics of Lipids*, 44 (2–4), 277.
25. E. Olsen, G. Vogt, D. Ekeberg, M. Sandbakk, J. Pettersen and A. Nilsson (2005), *J. Agr. Food Chem.*
26. A. Veberg, O. Sorheim, J. Moan, V. Iani, P. Juzenas, A. N. Nilsen and J. P. Wold (2006), *Meat Science*, 73 (3), 511.
27. D. M. Kocak, F. M. Caimi, R. L. Flick and A. Elharti (2003), 'VerifEYE: A real-time meat inspection system for the beef processing industry', in *SPIE*, C. B. Johnson, D. Sinha and P. A. Laplante, Eds., p. 235.
28. Y. Ozaki, R. Cho, K. Ikegaya, S. Muraishi and K. Kawauchi (1992), *Applied Spectroscopy*, 46 (10), 1503.
29. E. C. Y. LiChan (1996), *Trends in Food Science & Technology*, 7 (11), 361.

30. H. Sadeghijorabchi, R. H. Wilson, P. S. Belton, J. D. Edwardswebb and D. T. Coxon (1991), *Spectrochimica Acta. Part a – Molecular and Biomolecular Spectroscopy*, 47 (9–10), 1449.
31. N. K. Afseth, V. H. Segtnan, B. J. Marquardt and J. P. Wold (2005), *Appl. Spectrosc.*, 59 (11), 1324.
32. N. K. Afseth, J. P. Wold and V. H. Segtnan (2006), *Analytica Chimica Acta*, 572 (1), 85.
33. R. J. Beattie, S. J. Bell, L. J. Farmer, B. W. Moss and P. D. Desmond (2004), *Meat Science*, 66 (4), 903.
34. R. Knöchel, F. Daschner and W. Taute (2001), *Food Control*, 12 (7), 447.
35. M. Kent, R. Knöchel, F. Daschner and U. K. Berger, *Food Control*, 12 (7), 467.
36. R. Wellock and A. D. Walmsley (2004), *Spectroscopy Europe*, 16 (4), 23.
37. A. D. Dane, G. J. Rea, A. D. Walmsley and S. J. Haswell (2001), *Analytica Chimica Acta*, 429 (2), 185.
38. A. Gordon (1973), *Food Pros. Ind.*, 42, 495.
39. C. M. Bartle, C. Kroger and J. G. West (2004), *Radiation Physics and Chemistry*, 71 (3–4), 843.
40. L. Foucat, J. P. Donnat, F. Humbert, G. Martin and J. P. Renou (1997), *J. Magn. Reson. Anal.*, 108.
41. U. Bocker, R. Ofstad, H. C. Bertram, B. Egelanddal and A. Kohler (2006), *J. Agr. Food Chem.*, 54 (18), 6733.
42. S. Grimnes and Ø. G. Martinsen (2000), *Bioimpedance and Bioelectricity Basics* (Academic Press, London).
43. Y. Pomeranz and C. E. Meloan (1994), *Food Analysis, Theory and Practice* (Chapman & Hall, New York).
44. P. Gy (2004), *Chemometr. Intell. Lab.*, 74 (1), 7.
45. P. Gy (2004), *Chemometr. Intell. Lab.*, 74 (1), 25.
46. P. Gy (2004), *Chemometr. Intell. Lab.*, 74 (1), 39.
47. P. Gy (2004), *Chemometr. Intell. Lab.*, 74 (1), 49.
48. P. Gy (2004), *Chemometr. Intell. Lab.*, 74 (1), 61.
49. T. Næs, T. Isaksson, T. Fearn and T. Davies (2002), *A User-friendly Guide to Multivariate Calibration and Classification* (NIR Publications, Chichester).

23

Chilling and freezing of meat and its effect on meat quality

S. J. James and C. James, University of Bristol, UK

Abstract: This chapter discusses the effects of chilling and freezing on the quality of meat. First discussed are the purpose of refrigerating meat, the primary differences between chilling and freezing, the principles of these methods, and the cold chain in general. The specific effects of chilling and freezing are then discussed in detail with regard to (i) meat tenderness and texture, (ii) drip production, and (iii) meat colour and appearance. Finally, future trends in meat refrigeration are discussed.

Key words: chilling, freezing, cold shortening, drip, colour.

23.1 Introduction

In a perfect world, red meat and poultry meat would be completely free of micro-organisms, both pathogenic (food poisoning) and spoilage, when produced. However, under current methods of production, sterile meat cannot be guaranteed. While the internal musculature of a healthy mammal or bird is essentially sterile after slaughter, all meat animals carry large numbers of different microorganisms on their skin/feathers and in their alimentary tract. Of these, only a few types of bacteria directly affect the safety and quality of the finished meat. The main purpose of refrigerating meat is to extend the storage life by either limiting (in the case of chilling), or preventing (in the case of freezing), the growth of spoilage organisms. In general, there is little difference in the microbial spoilage of beef, lamb, pork and other meat derived from mammals (Varnam and Sutherland, 1995). Differences can be accounted by differences in initial bacteria levels, tissue composition and pH (Blixt and Borch, 2002; Stern *et al.*, 1992). The spoilage bacteria of meats stored in air under chill conditions include species of *Pseudomonas*, *Brochothrix* and *Acinetobacter/Moraxella*. Different species

become important in the spoilage of vacuum packaged and modified atmosphere packaged meats.

Of particular concern to human health are pathogens such as *Campylobacter* spp., *Salmonella* spp., pathogenic serotypes of *Escherichia coli*, *Clostridium perfringens*, *Clostridium botulinum*, *Yersinia enterocolitica*, *Listeria monocytogenes* and, to a lesser extent, *Staphylococcus aureus* and *Bacillus cereus*. In general, the presence of small numbers of pathogens is not a problem because meat is normally cooked before consumption, and in the case of many ready meals, during production. Adequate cooking will substantially reduce the numbers of, if not completely eliminate, all the vegetative pathogenic organisms present on the meat. Most meat-based food poisoning is associated with inadequate cooking or subsequent contamination after cooking. One of the purposes of refrigeration is to reduce, or prevent, the growth of pathogens so that they do not reach levels that could cause problems. Few pathogens of major concern grow on meat at temperatures below 7 °C (POST, 1997).

The visual appearance of meat influences the consumers' willingness to buy it when raw. However, after cooking, the eating quality of meat is determined by tenderness, juiciness and flavour, as well as appearance (Aaslyng, 2002). Although the live animal determines some of these quality characteristics, how the animal is processed immediately after slaughter has a profound affect on the overall meat quality.

The prime purpose of a meat refrigeration process is to reduce the temperature of the meat to a value below which the rate of bacterial growth is either severely slowed (chilling) or stopped (freezing). A complete cold chain for meat or meat products will contain a number of temperature reduction processes, viz. primary chilling, secondary chilling, freezing, together with other processes where no change in average meat temperature is required, i.e. chilled and frozen storage, transport, retail and domestic storage. It may also contain processes such as thawing, tempering and cooking, where a controlled temperature rise is planned, and others such as cutting, boning and mincing, which can result in an uncontrolled temperature rise.

Where a controlled change in meat temperature is required, heat transfer can only occur by four basic mechanisms, viz. conduction, radiation, convection, and evaporation/condensation. Conduction requires a good physical contact between the meat to be cooled and the cooling medium, and this is difficult to achieve with carcasses and other irregular meat cuts. Radiation does not require any physical contact but a large temperature difference is required between the surface of the meat being cooled and that of surrounding surfaces to achieve significant heat flow. In primary chilling, radiation is important only in the initial stages of the process in a system where the carcass is not surrounded by other carcasses. Again, in the initial stages of the secondary chilling of cooked meat products (e.g. pies, pasties, joints), radiant heat loss can be substantial if the products are surrounded by cold surfaces. Evaporation from a meat surface reduces yield and is not desirable in most meat refrigeration operations. In poultry chilling, evaporative (spray) chilling systems are sometimes used (James *et al.*,



Fig. 23.1 Traditional primary air blast chilling of pig and lamb carcasses in a small multi-species abattoir.

2006). The principle of the process is to increase the rate of evaporative heat loss, and, by replacing the water lost, reduce the overall weight loss. Evaporative (vacuum) cooling is often used for the cooling of meat pie fillings and sauces (Burfoot *et al.*, 1987) and its application in the cooling of cooked meat joints has also been investigated (Burfoot *et al.*, 1990). Convection is by far the most important heat transfer mechanism employed in the majority of meat refrigeration systems. In most cases, refrigerated air is the transfer medium; however, in some cases water, brine, or a cryogenic gas can be used.

In processes where no change in meat temperature is required, the refrigeration system should be designed to minimise, if not eliminate, all the four methods of heat transfer already mentioned, from or into the surface of the meat.

Almost all carcass-chilling systems for red meat carcasses, and many poultry chilling systems, rely on refrigerated air as the cooling medium. Most red meat systems use large insulated rooms with hanging rails for the meat carcasses or sides (Fig. 23.1). One or more evaporator coils are located above the rails, and fans circulate the air over the warm carcasses. The rooms are loaded with freshly slaughtered carcasses over a variable time period, the doors closed and the meat chilled for a nominal period, which can range from as little as 8 hours for lamb

carcasses to over 2 days for beef sides (James and James, 2002). Some pork, and many poultry, carcasses are chilled in continuous air chillers where the carcasses are conveyed through a refrigerated room or tunnel with refrigerated air directed over the surfaces of the meat. Broiler (mainly for the frozen market), turkey, goose and duck carcasses are often immersion chilled (James *et al.*, 2006). In immersion chilling, carcasses are moved through a tank or series of tanks containing chilled water or a mixture of ice and water. Normally, counter-current immersion chilling systems are used with a maximum water inlet temperature of 4 °C. Dwell-time and degree of water agitation are controlled to limit water absorption by the carcasses.

In an air-based system, air temperature, air velocity, and to a limited extent, relative humidity, are the environmental factors that affect the cooling time of meat carcasses. Cooling rate will also be a function of the weight and fat cover of a given side. Data from studies carried out at Langford on the relationship between these variables and chilling times, and weight losses of beef sides (James and Bailey, 1989) and pork (Brown and James, 1992), lamb (James and James, 2002), goat (Gigiell and Creed, 1987) and chicken (James *et al.*, 2007) carcasses have been published. Chilling rates in immersion systems are a function of the cooling medium used, its temperature, the size of the carcass being chilled and whether it is wrapped or unwrapped (James *et al.*, 2006).

Modern commercial meat freezing has a surprisingly long history. It is believed that the first modern meat freezing works were established at Darling Harbour in Sydney, Australia in 1861 (Critchell and Raymond, 1912). Nineteen years later, the arrival of the *S.S. Strathleven* in London on December 8th 1880, with its cargo on 40 tons of frozen Australian beef and mutton, started the frozen meat trade. It was sold in the UK for up to three times its value in Australia and, as stated in *The Daily Telegraph*, 'It has been tested by the ordinary method of cooking, and found to be in such good condition that neither by its appearance in the butchers' shops, nor by any peculiarity of flavour when cooked for the table, could it be distinguished from freshly killed English meat' (Critchell and Raymond, 1912). However, for a long time, frozen beef has suffered from a reputation that its eating quality is not as 'good' as that of 'fresh' chilled meat. Certainly, in the eyes of the general public, frozen beef steak is considered inferior to chilled beef steak. In the middle 1950s, Swift and Company tried to introduce frozen red meat to the consumer but found that 'the consumer indicated she was not interested in purchasing frozen red meats' (Bernholdt, 1974). In Australia, in 1986, 24% of respondents in a survey of retail and consumer handling of beef considered that freezing 'definitely affected quality' and a further 13% felt it would 'under certain circumstances' (Walker and Mitchell, 1986). Today, some retailers and media still pedal this perception. For instance, one online store in the US quotes that 'When you are buying steaks online, you want to get a good value, and you want to get great quality, right? We do too! That means we want unfrozen steaks' (http://www.mybutcher.com/stores/xq/xfm/Store_id.634/page_id.61/qx/store.htm). However, contrary to this, consumers appear happy to purchase chilled meat and freeze it at home. A US survey found that approximately 80% of a major retailer's customers did this (Payne *et al.*, 2002). A similar New Zealand survey reported that, while the

majority of red meat (84.6%) and poultry (62.9%) purchased by consumers surveyed was fresh (rather than frozen), approximately 64% of fresh meat and poultry was frozen in the home (Gilbert *et al.*, 2007).

There is a general view that fast freezing offers some quality advantage with 'quick frozen' appearing on many meat products with the expectation that consumers will pay more for a 'quick frozen' product. However, there are little data in the literature to suggest that, in general, the method of freezing or the rate of freezing has any substantial influence on a meat's quality characteristics or final eating quality. Slightly superior chemical and sensory attributes have been found in meat cryogenically frozen in a few trials (Sebranek *et al.* 1978; Dobryzcki *et al.* 1977; Sebranek, 1980), but other trials have not shown any appreciable advantage (Lampitt and Moran, 1933) especially during short-term storage (Hill and Glew, 1973). In 2001, Sundsten and co-workers revealed some commercial advantages of fast freezing, but no quality advantages. The study compared three different freezing methods, spiral freezing (SF), cryogenic freezing (liquid nitrogen, LN) and impingement freezing (IF), of beef burgers. No significant difference could be seen in cooking losses or eating quality, even after 2 months' storage.

Air is by far the most widely used method of freezing meat, as it is economical, hygienic and relatively non-corrosive to equipment. Systems range from the most basic in which a fan draws air through a refrigerated coil and blows the cooled air around an insulated room, to purpose-built conveyerised blast freezing tunnels or spirals. Relatively low rates of heat transfer are attained from product surfaces in air systems. The big advantage of air systems is their versatility, especially when there is a requirement to freeze a variety of irregularly shaped products or individual products. In a continuous system, meat is conveyed through a freezing tunnel or refrigerated room, usually by an overhead conveyor or on a belt. This overcomes the problem of uneven air distribution since each item is subjected to the same velocity/time profile. Some meat products are frozen on racks of trays (2 m high), pulled or pushed through a freezing tunnel by mechanical means. For larger operations, it is more satisfactory to feed meat on a continuous belt through linear tunnels or spiral freezers.

Contact freezing methods are based on heat transfer by contact between products and metal surfaces (which in turn are cooled by either primary or secondary refrigerants) or direct immersion in a refrigerated liquid. An immersion freezer is made up of a tank with a cooled freezing liquid that can be any non-toxic salt, sugar or alcohol solution in water and a means of conveying the wrapped meat through the tank. Ice slurries are being considered as an alternative to conventional immersion liquids. Such binary systems are described in the scientific literature as flow ice, fluid ice, slush ice or liquid ice. Maria *et al.* (2005) reported that such systems achieve higher rates of heat transfer than the single state liquids. Contact freezing offers several advantages over air-cooling; for example, much better heat transfer and significant energy savings. However, disadvantages are the need for regularly shaped products with large flat surfaces with plate systems (Fig. 23.2), and the need to wrap and wash off the immersion liquid in immersion systems.

Cryogenic freezing is essentially a subset of immersion freezing, in that it uses



Fig. 23.2 Vertical plate freezer for freezing of blocks of boned meat.

cryogenic refrigerants, such as liquid nitrogen or solid carbon dioxide, directly. The method of cooling is essentially similar to water-based evaporative cooling, cooling being brought about by boiling off the refrigerant. The essential difference is the temperature required for boiling. As well as using the latent heat absorbed by the boiling liquid, sensible heat is absorbed by the resulting cold gas. Due to very low operating temperatures and high surface heat transfer coefficients between the product and medium, cooling rates of cryogenic systems are often substantially higher than other refrigeration systems.

23.2 Effect of chilling and freezing on meat tenderness and texture

To quote an Australia CSIRO report (1988) ‘Toughness (in meat) is caused by three major factors – advancing age of the animal, ‘cold shortening’ (the muscle fibre contraction that can occur during chilling) and unfavourable meat acidity (pH).’ There is general agreement on the importance of these factors, with many experts adding cooking as a fourth, equally important, influence.

Chilling can have serious effects on the texture of meat if it is carried out too rapidly when the meat is still in the pre-rigor condition; that is, before the meat pH has fallen below about 6.2 (Bendall, 1972). In this state, the muscles contain sufficient amounts of the contractile fuel, adenosine triphosphate (ATP), for

forcible shortening to set in as the temperature falls below 11 °C, the most severe effect occurring at about 3 °C. This is the so-called 'cold-shortening' phenomenon, first observed by Locker and Hagyard (1963), and its mechanism was described by Jeacocke in 1986. The meat 'sets' in the shortened state as rigor comes on, and this causes it to become extremely tough when it is subsequently cooked (Marsh and Leet, 1966). If no cooling is applied and the temperature of the meat is above 25 °C at completion of rigor, then another form of shortening, 'rigor'- or 'heat-shortening', will occur (Dransfield, 1994), also on cooking.

The severity of cold shortening is highly pH dependent, being much greater if meat temperatures are below 10 °C at pH 6.8 (i.e. exceptionally rapid chilling) than at pH 6.2 (i.e. at an easily attainable commercial rate of chilling). To allow a safety margin, and taking into account the fact that some carcasses will show high initial pH values in the eye muscle, it is recommended that any part of a beef or lamb carcass should not be chilled below 10 °C until at least 10 hours after slaughter. In pork, cold-shortening occurs if temperatures between 3 and 5 °C are reached before the onset of rigor (normally 3 to 8 hours), this will only occur in rapid pork chilling systems and is not common. Although poultry breast muscles are primarily composed of white fibres (Sams, 1999), which are less prone to cold shortening than the red fibres found in red meats, cold shortening has been shown to occur (Wood and Richards, 1974; Bilgili *et al.*, 1989). Avoiding cold-shortening in beef through the use of slow chilling rates can lead to problems of 'bone-taint' (James and James, 2002).

Electrical Stimulation (ES) of the carcass after slaughter can allow rapid chilling to be carried out without much of the toughening effect of cold-shortening. However, Buts *et al.* (1986) reported that, in veal, ES followed by moderate cooling affected tenderness in an unpredictable way and could result in tougher meat. ES will hasten rigor and cause tenderisation to start earlier at the prevailing higher temperature. In beef meat from carcasses given high or low voltage stimulation and slow cooling, adequate ageing can be obtained in about half the time of non stimulated beef. This will therefore reduce the requirement and cost of storage. In poultry processing, ES seeks to reduce the toughness of meat that is deboned prior to the normal ageing (or maturation) period (Li *et al.*, 1993; Sams, 2002).

When meat is stored at above freezing temperatures it becomes progressively more tender. This process, known as ageing (or alternatively as conditioning or maturation) is traditionally carried out by hanging meat carcasses for periods of 14 days or longer (in the case of beef) in a controlled environment (Fig. 23.3) at between -1 and 5 °C (so-called 'dry ageing'). Alternatively, the carcass may be divided into sub-primals and aged in vacuum packs (usually referred to as 'wet ageing'). The rate of ageing differs significantly among animal species (Dransfield, 1986) and necessitates different times for tenderisation. Beef, veal and rabbit age at about the same rate and take about 10 days at 1 °C to achieve 80% of ageing (Table 23.1). Lamb ages slightly faster than beef but more slowly than pork, and chicken is at least 14 times faster than pork. The ultimate tenderness will depend on the initial 'background' tenderness of the meat and the tenderisation that has occurred during chilling. The age of the animal is also important. In veal,



Fig. 23.3 Traditional dry-ageing of beef sides.

acceptable tenderness can be obtained after 5 d at 1 °C compared with 10 days for beef. The major increase in tenderness has been shown to occur in less than 14 days in beef. In a study by Martin *et al.* (1971), in which more than 500 animals were examined, it was concluded that, for beef carcasses, an ageing period of 6 days was sufficient for a consumer product of satisfactory tenderness. Buchter (1970) also showed that no significant increase in tenderness occurs after 4 to 5 days for calves and 8 to 10 days for young bulls at 4 °C. In the UK, ageing has seen a revival in recent years and UK supermarkets are currently marketing beef aged for up to 28 days, lamb aged for 14 days and pork aged for 10 days. In common with the accepted practice of hanging game birds, the hanging of turkeys is also receiving increased interest.

Although ageing is rapid in poultry meat, deboning before sufficient tenderisation has taken place can result in tough meat. Studies to determine the minimum amount of ageing required before deboning show that at least 2 and possibly 4 hours are required in chicken (Sams, 1999) and at least 6 and possibly 8 hours in turkeys (Fanatico, 2003).

The merits of 'dry' versus 'wet' ageing are an ongoing matter of debate. What is clear is that there is greater shrink, weight loss and trim loss associated with dry ageing and hence the process is more expensive than wet ageing. In a study by Parrish *et al.* (1991) comparing 21 day dry and wet aged loin and rib steaks, although differences could be detected by trained panellists, no differences were detected by consumer panellists.

Table 23.1 Time taken to achieve 50 and 80% ageing at 1 °C for different species

Species	Time (d) taken to achieve	
	50%	80%
Beef	4.3	10.0
Veal	4.1	9.5
Rabbit	4.1	9.5
Lamb	3.3	7.7
Pork	1.8	4.2
Chicken	0.1	0.3

The ageing process can be accelerated by raising the temperature of the meat, and the topic was well studied in the 1940s, 1950s and 1960s. Ewell (1940) found that the rate of tenderising more than doubled for each 10 °C rise. Meat from a 3-year-old steer requiring only 2 days at 23 °C to reach the same tenderness as reached after 10 days at 0 °C. Sleeth *et al.* (1958) showed that the tenderness, flavour, aroma and juiciness of beef quarters and ribs aged for 2 to 3 days at 20 °C were comparable to those aged 12 to 14 days at 2 °C. Busch *et al.* (1967) demonstrated that steaks from excised muscles held at 16 °C for 2 days were more tender than those stored at 2 °C for 13 days. The microbiological hazards of high-temperature ageing were also recognized and several investigators used antibiotics and/or irradiation to control bacterial growth during high-temperature ageing (Deatherage and Reiman, 1946; Wilson *et al.*, 1960). Although high-temperature ageing in conjunction with ultraviolet (UV) radiation was tried, its use has not expanded owing to its high cost and inability to cover all parts of the carcass (Marais, 1968). With irradiation gaining more acceptance in the USA, its use, together with modified atmosphere packaging, to prevent microbiological growth during accelerated high-temperature ageing has been investigated (Mooha Lee *et al.*, 1995). Irradiated steaks stored for 2 d at 30 °C were more tender than unirradiated controls stored at 2 °C for 14 days.

In red meat, there have been some reports that small changes to chilling practices alone may extend the subsequent storage-life by up to 50% (Gill, 1987); however, details, unfortunately are sketchy. There is little evidence of any relationship between chilling rates and subsequent frozen storage-life. However, there is evidence for a relationship between frozen storage-life and the length of chilled storage (ageing) prior to freezing. Chilled storage of lamb for one day at 0 °C prior to freezing can reduce the subsequent storage life by as much as 25% when compared to lamb which has undergone accelerated conditioning and only 2 hours storage at 0 °C (Winger, 1984). It has been shown that pork that has been held for 7 days prior to freezing deteriorates at a faster rate during subsequent frozen storage than carcasses chilled for 1 and 3 days prior to freezing (Harrison *et al.*, 1956). Ageing for periods greater than 7 days was found by Zeigler (1950) to produce meat with high peroxide and free fatty acid values when stored at -18 °C or -29 °C. Although shorter ageing times appear to have a beneficial effect on

storage-life, there is obviously a necessity for it to be coupled with accelerated conditioning to prevent any toughening effects.

Whether aged or unaged, chilled or frozen, it is in the cooked final product that tenderness and texture will be accessed by the consumer. Thus, the way the meat is cooked must always be considered. The consumers' environment or setting can also influence their appreciation of tenderness. In one study consumers were found to be more critical of the tenderness of beef steaks cooked in the home than those cooked in restaurants (Miller *et al.*, 1995). The Warner–Bratzler force transition level for acceptable steak tenderness was between 4.6 and 5.0 kg in the home and between 4.3 and 5.2 kg in the restaurants.

23.3 Effect of chilling and freezing on drip production

The presence of exudate or 'drip', which accumulates in the container of pre-packaged meat, or in trays or dishes of unwrapped meat, substantially reduces its sales appeal (Malton and James, 1983). Drip can be referred to by a number of different terms including 'purge loss', 'press loss' and 'thaw loss', depending on the method of measurement and when it is measured. The protein concentration of drip is about 140 mg ml⁻¹, about 70% of that of meat itself. The proteins in drip are the intracellular, soluble proteins of the muscle cells. The red colour is due to the protein myoglobin, the main pigment of meat. Drip loss occurs throughout the cold chain and represents a considerable economic loss to the red meat industry. The potential for drip loss is inherent in fresh meat and is related to the development of rigor mortis in the muscle after slaughter and its effect on pH. It is influenced by many factors. Some of these, including breed, diet and physiological history, are inherent in the live animal. Others, such as the rate of chilling, storage temperatures, freezing and thawing, occur during processing.

Drip potential clearly appears to be related to species. In general, beef tends to lose proportionately more drip than pork and lamb. Poultry meat is far less prone to drip. In pigs, especially, there are large differences in drip loss from meat from different breeds. Taylor (1972) showed that there was a substantial difference, up to 2.5 fold, in drip loss between four different breeds of pig. Conversely, breed does not appear to have an effect on water-holding capacity in chickens (Musa *et al.*, 2006), though meat from different chicken breeds may differ significantly in colour density, pH and tenderness.

There can be large differences in drip loss between different muscles. Taylor (1972) showed that there was a 1.7 to 2.8 fold difference in drip loss between muscle types in pigs. Since most of the exudate comes from the cut ends of muscle fibres, small pieces of meat also drip more than large intact carcasses, and the way that different muscles are cut will also have an influence on drip loss.

Rapid chilling reduces drip loss after subsequent cutting operations. The potential for drip loss is established in the first period of cooling; the temperature range conducive to drip is down to about 30 °C, or perhaps a little lower. Gigiel *et al.* (1985) removed cylindrical samples of muscle from freshly slaughtered beef.

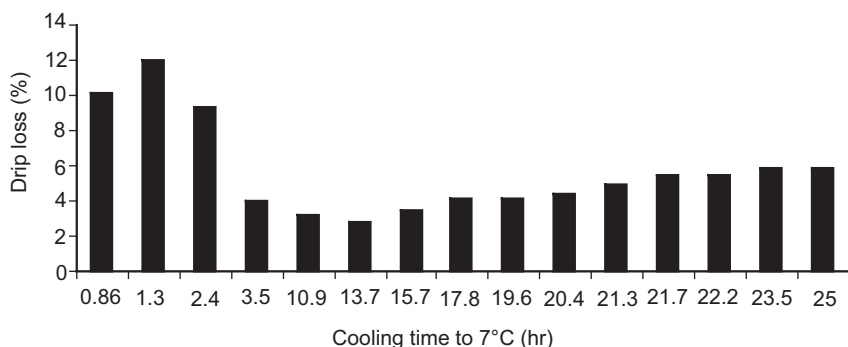


Fig. 23.4 Percentage drip loss from beef sample as a function of cooling time (hours) to 7 °C (source: Gigiel *et al.*, 1985).

The curved surface and one end of the cylinder were surrounded by insulation and the free end placed in contact with solid CO₂. Since heat was extracted only from one end, this produced a wide range of cooling rates through the length of the cylinder. After cooling and equalisation, the cylinder was cut into discs and the drip potential of each disc measured using a centrifuge technique. The resulting plot of drip loss against cooling rate is shown in Fig. 23.4. Close to the surface, in contact with the CO₂, the rate of cooling was highest, but freezing occurred and the drip loss was high. This was followed by a region which remained unfrozen but was 'cold shortened', which increased drip potential. Minimum drip potential was measured in the next region where high cooling rates were achieved without freezing or cold shortening. Drip loss then increased as cooling time to 7 °C increased. It has been recommended that deep muscle temperatures in turkeys should be reduced below 25 °C by 60 min post-mortem to reduce drip loss in this meat (Alvarado and Sams, 2002).

During chill storage, the rate of drip loss increases with storage temperature, and the amount of drip loss will increase with storage time. Low storage temperatures will reduce the amount of drip. Work by Lee *et al.* (1985) clearly showed the effect of these factors. Drip loss from pork cubes increased substantially during 21 days of storage at 0, 3 and 7 °C. The rate of increase was greater at the higher temperatures. During storage at 0 and 3 °C, no increase in drip loss with time was measured after 21 days. At 7 °C drip was still increasing between 21 and 28 days. In beef, Boakye and Mittal (1993) reported a different relationship between the length of time that *longissimus* was conditioned (aged) and drip loss. There was a small, but not significant increase in drip over the first 8 days of ageing. A marked increase in drip was measured on day 12 followed by a marked decrease on day 16. The importance of effective secondary cooling after cutting is shown by the data of Löndahl and Eek (1986). The amount of drip loss from pork rib after cutting when held at 10 °C was twice that of meat chilled and held at 2.5 °C.

Freezing always tends to decrease water-holding capacity and hence increase drip loss. When meat is frozen quickly, the water released by the fibrils as the meat

has gone into *rigor*, and that which is still held, are frozen simultaneously. Consequently, there is no change in their relative positions or amounts. At slower freezing rates, however, the water balance is altered, the extra-cellular water freezing first. As freezing continues, the existing ice crystals grow at the expense of water from the intra-fibrillar space.

A number of scientific investigations, which can be compared to commercial practice, have defined the effect of freezing rate on drip loss production. Petrovic *et al.* (1993) stated that the optimal conditions for freezing portioned meat are those that achieve freezing rates between 2 and 5 cm h⁻¹ to -7 °C. Grujic *et al.* (1993) suggest even tighter limits 3.33 to 3.95 cm h⁻¹. They found that 'slow freezing' (up to 0.39 cm h⁻¹) resulted in decreased solubility of myofibrillar proteins, increase in weight loss during, freezing thawing and cooking, lower water-binding capacity and tougher cooked meat. 'Very quickly frozen' meat (>4.9 cm h⁻¹) had a somewhat lower solubility of myofibrillar proteins, lower water-binding capacity and somewhat tougher and drier properties. The samples were thawed after storage times of 2 to 3 days at -20 °C, so the relationship between freezing rates and storage-life was not investigated. Sacks *et al.* (1993) found that, after 2.5 months, drip loss from mutton samples frozen using cryogenics was >2% less than in those using air freezing. These results are scientifically very interesting but in industrial practice, most meat is air frozen in the form of large individual pieces or cartons of smaller portions. In commercial situations freezing rates of 0.5 cm h⁻¹ in the deeper sections would be considered 'fast' and there would be considerable variation in freezing time within the meat. The samples frozen by Sacks *et al.* (1993) were much smaller (77.6 g in weight) than most commercial products. Even with such small samples, there was no significant difference in drip loss after 48 hours between cryogenic freezing at -90 °C and freezing in a walk-in freezer operating at -21 °C.

Excessive drip loss could have a small effect on the eating quality of meat. Perceived juiciness is one of the important sensory attributes of meat. Dryness is associated with a decrease in the other palatability attributes, especially with lack of flavour and increased toughness (Pearson, 1994). However, moisture losses during cooking are typically an order of magnitude higher than most drip losses during refrigeration. Consequently, small differences in drip loss will have little effect on eating quality.

23.4 Effect of chilling and freezing on meat colour and appearance

The appearance of meat at its point of sale is the most important quality attribute governing its purchase. Changes in colour of the muscle and blood pigments (myoglobin and haemoglobin, respectively) determine the attractiveness of fresh red meat, which in turn influences the consumers' acceptance of meat products (Pearson, 1994). Consumers prefer bright-red fresh meats, brown or grey-coloured cooked meats and pink cured meats (Cornforth, 1994). MacDougall comprehen-

sively reviewed the effect of chilling and freezing on the colour of meat in 1972 and 1974, respectively. Much of what he said then remains relevant today.

The pigment concentration in meat that governs its colour is certainly influenced by species. Beef and lamb contain substantially more myoglobin than pork and poultry meat. Thus accounting for the difference between 'red' (beef and lamb) and 'white' (pork and poultry) meats. Pigment concentration (myoglobin content) also increases with age; for example, veal is brownish pink versus beef from three-year old steers that is bright, cherry red (Miller, 2002). However, within a species, meat colour can be adversely affected by a variety of factors, including post-mortem handling, chilling, storage and packaging (Church and Wood, 1992; Miller, 2002).

The red colour (from oxymyoglobin) in meat is more stable at lower temperatures because the rate of oxidation of the pigment (to metmyoglobin) decreases. At low temperatures, the solubility of oxygen is greater and oxygen consuming reactions are slowed down. There is a greater penetration of oxygen into the meat and the meat is redder than at high temperatures. The rate of oxidation depends on the species. Thus the rate of cooling during chilling may have an effect on meat colour.

Different chilling methods can certainly affect the appearance of poultry meat. The scalding carcasses have received prior to plucking has a marked effect on the final appearance. Carcasses destined for air chilling can only be 'soft' scalded (i.e. at 50 to 53 °C), which retains the outer dermal layer, since higher scalding temperatures remove the outer dermal layer and makes the carcasses more susceptible to dehydration and discoloration during air chilling. The problem does not occur with immersion chilling. Hence carcasses chilled using this method can be 'hard' scalded at higher temperatures. While air chilling is preferred in most of Europe (Fig. 23.5), it is still very much a matter of debate as to the relative merits of the appearance of air or immersion chilled carcasses (James *et al.*, 2006).

Changes in colour have also been reported in red meat resulting from chilling treatment. Taylor *et al.* (1995) found that electrical stimulation of pork produced higher lightness (L), i.e. paler values than those measured in non-stimulated sides. Spray chilling of pork has some effect on its colour during the initial chilling period (Feldhusen *et al.*, 1995a). After 4 hours of chilling, the musculature of sprayed ham became lighter, and red and yellow values decreased. However, after 20 hours there was no significant difference in the colour values.

The muscle surface of fresh meat undergoes extensive oxygen penetration and oxygenation of myoglobin after short periods of exposure to air. The length of time meat is kept in chilled storage has an effect on the rate of colour change during retail display. Feldhusen *et al.* (1995b) showed that there were clear colour changes after exposure, in beef *longissimus dorsi* muscle stored for up to 5 days at 5 °C. The degree of lightness (L), percentage of red (a) and percentage of yellow (b) all increased by 3 to 4 units. The colour of meat stored for longer periods showed less intense colour changes during 5 hours of exposure.

The colour of frozen meat varies with the rate of freezing. Taylor (1930, 1931) reported that, as the speed of freezing diminished, the appearance of the product



Fig. 23.5 Continuous air blast chilling of chicken carcasses.

changed and at very low rates there was a marked development of translucence. Later experiments have demonstrated a direct relationship between freezing rate and muscle lightness; the faster the rate, the lighter the product. Guenther and Henrickson (1962) found that 2.5 cm thick steaks frozen at -9°C were dark. Those frozen at -34 to -40°C had the most desirable colour and those frozen at -73 to -87°C tended to be pale. Jakobsson and Bengtsson (1969, 1973) obtained similar results; very rapid freezing in liquid nitrogen spray at a freezing rate of about 13 cm hr^{-1} produced meat that was unnaturally pale. Air blast freezing at 2 cm hr^{-1} gave the best frozen appearance while very slow freezing at 0.04 cm hr^{-1} resulted in a darker colour and the formation of ice on the product surface. These differences in frozen meat lightness result from the dependence of ice crystal growth on the freezing rate. Small crystals formed by fast freezing scatter more light than large crystals formed by slow freezing and hence fast frozen meat is opaque and pale, and slow frozen meat is translucent and dark.

'Freezer burn' is the main appearance problem that traditionally affected the appearance of meat in frozen storage. Desiccation from the surface tissues produces a dry, spongy layer that is unattractive and does not recover after thawing. This is commonly called 'freezer-burn'. It occurs in unwrapped or poorly wrapped meat. The problem is accentuated in areas exposed to low humidity air at high velocities, and by poor temperature control. Since most meat is now wrapped and temperature control much improved, this is less of a problem than it once was, commercially. Provided problems of freezer burn can be eliminated, the major

appearance problem that affects frozen meat arises from oxidation of oxymyoglobin to metmyoglobin.

Both temperature and illumination level affect the rate of discoloration during frozen storage, but light is by far the more serious factor. Lentz (1971) reported the progress of discoloration in the light (160–220 decalux) and in the dark for frozen beef stored at a range of temperatures in terms of the Munsell colour notation. At -18°C , a temperature typical of good commercial display, the colour remained attractive for 3 months in the dark but for only 3 days in the light.

There is an interaction between the colour of meat after thawing and its freezing rate. Jakobsson and Bengtsson (1969, 1973) found that slowly frozen beef, which darkened on freezing also, showed considerable loss of redness after thawing. In contrast, meat frozen in liquid nitrogen and then defrosted was a light, bright red. Little difference was also found between thawed beef steaks which were frozen at 15 cm hr^{-1} in liquid nitrogen spray and those which were blast frozen at 4 cm hr^{-1} (Pap, 1972). In thawed meat, the rate of pigment oxidation is increased (Cutting, 1970) and therefore the colour will be less stable than in fresh. On prolonged frozen storage, a dark brown layer of metmyoglobin may form 1–2 mm beneath the surface so that on thawing, the surface colour will rapidly deteriorate. Meat, which has lost its attractiveness during frozen storage because of oxidation of oxymyoglobin on the surface, will remain brown after thawing.

Unwrapped meat thawed in high humidity air, water or in steam under vacuum appears very white and milky after thawing. However, if then stored in a chill room for 10 to 24 hours, it will be almost indistinguishable from fresh meat. Unwrapped meat thawed in air at high temperatures and low humidities will take on a dark, dry, tired appearance. It will not recover its appearance during chilled storage and will often require extensive trimming before sale (James and James, 2002).

In retail display, the colour stability of fresh wrapped meat is influenced to a marked degree by the temperature of display. Landrock and Wallace (1955) showed that meat held at 2°C in packaging films whose oxygen permeability was greater than $5000\text{ ml/m}^2\text{/atm/day}$ remained attractive for 4 days, while Heiss and Eichner (1969) showed that the rate of discoloration is roughly doubled for a 5°C rise in temperature. Similarly, MacDougall (1972) found that the rate of colour change is influenced by position in the display cabinet, and temperature differences of the order of 5°C have a large effect on the rate of colour change. For example, a change in redness, which takes 72 hours to 168 hours at 0°C , will occur in 24 hours to 48 hours at 5°C .

Changes in appearance are normally the criteria that limit the display life of unwrapped products, rather than microbiological considerations. Deterioration in the appearance of unwrapped meats has been related to the degree of dehydration, which makes the product unattractive to consumers (James and Swain, 1986). The rate of dehydration is a function of the temperature, velocity and especially the relative humidity of the air passing over the surface of the meat on display. Reducing the relative humidity from 95 to 40% can increase the rate of dehydration by a factor of 18. Evans and Russell (1994a,b) also showed that changing the type of illumination could change the rate of dehydration.

The major problem in retail marketing of frozen meat is its appearance. The freezing process causes changes in the structure and colour of the muscle, and the deterioration in appearance during frozen storage and display ultimately leads to rejection of the product by the consumer. Storage temperature, light intensity on the display area and method of packaging all affect the rate of deterioration. The appearance of fresh meat is a primary factor in acceptability at retail level and the same criteria of attractiveness will apply to frozen meat, retailed either frozen or after thawing. The poor colour of the frozen product, and the drip loss associated with it when it thaws, have in the past both contributed to consumer resistance. The appearance of frozen meat is markedly improved if retail-sized portions are first packed in film to exclude air between the meat surface and the film and then rapidly frozen. With this product, however, the price differential between fresh and frozen would necessarily be small and the consumer would have to be persuaded by the trade that such frozen meat was in no way inferior to fresh.

23.5 Future trends

The past few decades has produced a substantial reduction in the number of individual slaughterhouses within the UK and in most developed countries. Those that survive tend to be larger, modern developments, often supplying only a specific large retail operation. A small number of slaughterhouses do go against this trend and supply a niche market for locally reared, often organic, meat. Much of this meat is sold from farmers' markets or farm shops.

The widely publicised outbreaks of Bovine Spongiform Encephalopathy (BSE), Foot and Mouth disease, Highly Pathogenic Avian Influenza (HPAI) and food poisoning have driven the meat industry to concentrate on improvements in food safety and eating quality. Since, as already detailed, temperature control is a key element in improving the safety and eating quality of the meat, this has led to more emphasis being placed on the design and operation of meat refrigeration processes. One key development has been the reintroduction of ageing (maturing) rooms for beef, lamb and more recently pork and turkey. It is expected that aged meat will take up a higher percentage of the total market in the future.

In the past decade, the use of impingement technology to increase the surface heat transfer in freezing systems has received attention (Newman, 2001; Sundsten *et al.*, 2001; Everington, 2001). Impingement is the process of directing a jet or jets of fluid at a solid surface to effect a change. The very high velocity (20–30 m s⁻¹) impingement gas jets 'breakup' the static surface boundary layer of gas that surrounds a meat product. The resulting medium around the product is more turbulent and the heat exchange through this zone becomes much more effective.

In general, meat producers try to avoid surface freezing of the meat during chilling, and chilled storage and distribution. This is mainly related to concerns of its effect on drip loss. However, there is published evidence that crust freezing may have no affect on poultry meat quality (Vacinek and Toledo, 1973; Kennedy and Miller, 2004), and a process known as 'super' or 'deep' chilling has been

commonly utilised in the USA. Carcasses are water chilled, then put through an air freezer operating at -15°C for approximately 30 min (Jul, 1986). After packaging, they are again placed in an air freezer to achieve the required meat temperature. The carcasses are stored and distributed at -1 to -2°C . This product is seldom referred to as 'super-chilled' since, legally, in the USA poultry meat kept above -3.3°C (26°F) can be marketed as 'fresh' (US Poultry Products Inspection Regulations 9CFR381). Interestingly, prior to 1997, poultry in the US could be sold as 'fresh' even if it was frozen 'as solid as a block of ice' (http://www.fsis.usda.gov/Fact_Sheets/Poultry_Label_Says_Fresh/index.asp). However, in December 1997, the term 'fresh' was re-defined. The temperature of -3.3°C was apparently chosen because 'At 26°F , the product surface is still pliable and yields to the thumb when pressed. Most consumers consider a product to be fresh, as opposed to frozen, when it is pliable or when it is not hard to the touch.' (http://www.fsis.usda.gov/Fact_Sheets/Poultry_Label_Says_Fresh/index.asp).

There is very little published data on the freezing point of poultry meat. It is generally recognised to be between -1.5 and -2°C , though as yet unpublished studies by the authors of this chapter have measured a freezing point of approximately -1.3°C in the deep breast of standard UK carcasses. However, in the USA -2.2°C is used (Sams, 2001) and a value as low as -2.8°C has been quoted (Pflug, 1957).

There is increasing interest in the use of super (deep) chilling to increase the 'chilled' storage life of meat so that peaks and troughs in production can be overcome. The technique is also being considered for the transportation of 'chilled' meat and meat products from South America to markets in Europe.

23.6 Sources of further information and advice

Detailed information on all aspects of the refrigeration of red meat can be found in *Meat Refrigeration* by James, S.J. and James, C., Woodhead Publishing Limited, ISBN1 85573 442 7.

Poultry chilling has been comprehensively reviewed by James *et al.* (2006).

More general data on all aspects of meat science can be found in the *Encyclopedia of Meat Sciences*, edited by Jensen, W. K., Devine, C. and Dikeman, M., Academic Press, Elsevier Science Ltd. ISBN 0-12-464970-X and processing in the *Handbook of Food Science, Technology, and Engineering*, edited by Hui, Y.H., Taylor and Francis LLC, CRC.

Advice on all aspects of meat refrigeration and processing can be obtained from the Food Refrigeration and Process Engineering Research Centre (FRPERC) at the University of Bristol (www.frperc.bris.ac.uk).

23.7 References

Aaslyng M D (2002), 'Quality indicators for raw meat', in Kerry J, Kerry J and Ledward D, *Meat Processing: Improving Quality*, Cambridge, Woodhead Publishing Ltd, 8, 158–174.

- Alvarado C Z and Sams A R (2002), 'The role of carcass chilling rate in the development of pale, exudative turkey pectoralis', *Poultry Science*, 81, 1365–1370.
- Bendall J R (1972), 'The influence of rate of chilling on the development of rigor and cold shortening', *Meat Chilling – Why and How? Meat Research Institute Symposium No 2*, Meat Research Institute, Langford, UK, 3.1–3.6.
- Bernholdt H F (1974), 'Merchandising frozen meats and consumer attitudes in purchasing and preparation', *Meat Freezing – Why and How? Meat Research Institute Symposium No 3*, Meat Research Institute, Langford, UK, 4.1–4.7.
- Bilgili S F, Egbert W R and Huffman D L (1989), 'Research note: Effect of postmortem aging temperature on sarcomere length and tenderness of broiler *Pectoralis major*', *Poultry Science*, 68, 1588–1591.
- Blixt Y and Borch E (2002), 'Comparison of shelf-life of vacuum-packed pork and beef', *Meat Science*, 60, 371–378.
- Boakye K and Mittal G S (1993), 'Changes in pH and water holding properties of *longissimus dorsi* muscle during beef ageing', *Meat Science*, 34, 335–349.
- Brown T and James S J (1992), 'Process design data for pork chilling', *International Journal of Refrigeration*, 15 (5), 281–289.
- Buchter, L (1970), 'Development of a standardised procedure for the slaughter of experimental beef animals from the Danish Progeny Station 'Egtved'', *Proceedings of the 16th Meeting European Meat Research Workers*, Bulgaria.
- Burfoot D, Hayden R and Badran R (1987), 'Simulation of a pressure cook/water and vacuum cooled processing system', *Proc. Symp. Engineering Innovations in the Food Industry*, University of Bath, 13–15 April, 231–237.
- Burfoot D, Self K P, Wilkins T J and James S J (1990), 'Effect of cooking and cooling method on the processing times, mass losses and bacterial condition of large meat joints', *International Journal of Food Science and Technology*, 25, 657–667.
- Busch W A, Parrish F C Jr. and Goll, D E (1967), 'Molecular properties of post-mortem muscle. 4. Effect of temperature on adenosine triphosphate degradation, isometric tension parameters, and shear resistance of bovine muscle', *Journal of Food Science*, 32, 390.
- Buts B, Casteels M, Claeys E and Demeyer D (1986), 'Effects of electrical stimulation, followed by moderate cooling, on meat quality characteristics of veal *Longissimus dorsi*', *Meat Science*, 18, 271–279.
- Church P N and Wood J M (1992), *The Manual of Manufacturing Meat Quality*, London and New York, Elsevier Applied Science, ISBN 1 85166 628 1.
- Cornforth D (1994), 'Colour – Its basis and importance' Chapter 2, pp. 34–78. In *Quality Attributes and Their Measurement in Meat, Poultry and Fish Products* (Eds. A. M. Pearson and T. R. Dutson). Advances in Meat Research Series, Volume 9, Blakie Academic and Professional, UK.
- Critchell J T and Raymond J (1912), *A History of the Frozen Meat Trade*. 2nd edition Constable & Company Ltd, London.
- CSIRO (1988), 'Tender beef', *Meat Research News Letter*, 88/4.
- Cutting C L (1970), 'The influence of freezing practice on the quality of meat and fish' *Proceedings of the Institute of Refrigeration*, 66, 51.
- Deatherage F E and Reiman W (1946), 'Measurement of beef tenderness and tenderisation of beef by Tenderay process', *Food Research*, 11, 525.
- Dobryzcki J, Pietrzak E and Hoser A (1977), 'Rheological characteristics of fresh and frozen chicken muscles and their relation to tenderness measured by sensory methods', *Acta Alimentaria*, 6 (2), 107–111.
- Dransfield E (1986), 'Conditioning of meat', *Recent Advances and Developments in the Refrigeration of Meat Chilling*, Meeting of IIR Commission C2, Bristol (UK), Section 1, 61–68.
- Dransfield E (1994), 'Optimisation of tenderisation, ageing and tenderness', *Meat Science*, 36, 105–121.

- Evans J A and Russell S L (1994a), 'The influence of surface conditions on weight loss from delicatessen products', *FRPERC – Internal report*, August 1994.
- Evans J A and Russell S L (1994b), 'The influence of surface conditions on weight loss from delicatessen products', *FRPERC – Internal report*, November 1994.
- Everington D W (2001), 'Development of equipment for rapid freezing'. In *Rapid Cooling of Food*, Meeting of IIR Commission C2, Bristol (UK), Section 2, 173–180.
- Ewell A W (1940), 'The tenderising of beef', *Refrigeration Engineering*, 39, 237–240.
- Fanatico A (2003), *Small Scale Poultry Processing*, ATTRA–National Sustainable Agriculture Information Service, Fayetteville, US.
- Feldhusen F, Kirschner T, Koch R, Giese W and Wenzel S (1995a), 'Influence on meat colour of spray-chilling the surface of pig carcasses', *Meat Science*, 40, 245–251.
- Feldhusen F, Warnatz A, Erdmann R and Wenzel S (1995b), 'Influence of storage time on parameters of colour stability of beef', *Meat Science*, 40, 235–243.
- Gigiel A J and Creed P G (1987), 'Effect of air speed and carcass weight on the cooling rates and weight losses from goat carcasses', *International Journal of Refrigeration*, 10, 305–306.
- Gigiel A J (1985), 'Chilling of hot boned meat with carbon dioxide', *International Journal of Refrigeration*, 8, 91–96.
- Gilbert S E, Whyte R, Bayne G, Paulin S M, Lake R J and van der Logt P (2007), 'Survey of domestic food handling practices in New Zealand', *International Journal of Food Microbiology*, 117 (3), 306–311.
- Gill C O (1987), 'Prevention of microbial contamination in the lamb processing plant', in Smulders, F J M, *Elimination of Pathogenic Organisms from Meat and Poultry*, Amsterdam–New York–Oxford, Elsevier, 203–219.
- Grujic R, Petrovic L, Pikula B and Amidzic L (1993), 'Definition of the optimum freezing rate – 1, Investigation of structure and ultrastructure of beef *M. longissimus dorsi* frozen at different freezing rates', *Meat Science*, 33, 301–318.
- Guenther J J and Henrickson R L (1962), 'Temperatures, methods used in freezing determine tenderness, colour of meat', *Quick Frozen Foods*, 25, 115.
- Harrison D L, Hall J L, Mackintosh D L and Vail G E (1956), 'Effect of post mortem chilling on the keeping quality of frozen pork', *Food Technology*, 10, 104–108.
- Heiss R and Eichner K (1969), 'Research into the packaging of fresh meat. 1. Achieving optimum packaging for fresh meat', *Fleischwirtschaft*, 49, 757.
- Hill M A and Glew G (1973), 'Organoleptic assessment of products frozen by two methods: Liquid nitrogen and blast freezing', *Journal of Food Technology*, 8, 205–210.
- Jakobsson B and Bengtsson N E (1969), 'The influence of high freezing rates on the quality of frozen ground beef and small cuts of beef', *Proceedings of the 15th European Meeting of Meat Research Workers*, 482.
- Jakobsson B and Bengtsson N E (1973), 'Freezing of raw beef: Influence of ageing, freezing rate and cooking method on quality and yield', *Journal of Food Science*, 38, 560.
- James C, James S, Vincent C, de Andrade Lima T I and Foster A (2007), 'Air chilling of chicken carcasses', *The 22nd IIR International Congress of Refrigeration*, Beijing, China, 21–26 August 2007, ICR07-C2-1240.
- James C, Vincent C, de Andrade Lima T I and James S J (2006), 'The primary chilling of poultry carcasses – A review', *International Journal of Refrigeration*, 29 (6), 847–862.
- James S J and Bailey C (1989), 'Process design data for beef chilling', *International Journal of Refrigeration*, 12, 42–49.
- James S J and James C (2002), *Meat Refrigeration*, Cambridge, Woodhead Publishing Limited.
- James S J and Swain M V L (1986), 'Retail display conditions for unwrapped chilled foods', *The Proceedings of the Institute of Refrigeration*, 83, Session 1986–87, 3.1
- Jeacocke R E (1986), 'The mechanism of cold shortening'. In *Recent advances and developments in the refrigeration of meat chilling*. Meeting of IIR Commission C2, Bristol (UK), Section 4, 235–241.

- Jul M (1986), 'Chilling broiler chicken: An overview'. In *Recent advances and developments in the refrigeration of meat by chilling. Meeting of International Institute of Refrigeration Commission C2*, Bristol (UK), 133–43.
- Kennedy C and Miller J (2004), 'A new chilling technique for processing chicken', *Food Science and Technology*, 18, 30–33.
- Lampitt L H and Moran T (1933), 'The palatability of rapidly frozen meat', *Journal of the Society of Chemical Industry*, 52 (L11.21), 143–146.
- Landrock A H and Wallace G A (1955), 'Discoloration of fresh red meat and its relationship to film oxygen permeability', *Food Technology*, 9, 194.
- Lee B H, Simard R E and Laleye L C (1985), 'Effects of temperature and storage duration on the microflora, physicochemical and sensory changes of vacuum- or nitrogen-packed pork', *Meat Science*, 13, 99–112.
- Lentz C P (1971), 'Effect of light and temperature on colour and flavour of pre-packaged frozen beef', *Canadian Institute of Food Science and Technology Journal*, 4, 166.
- Li Y B, Siebenmorgen T J and Griffis C L (1993), 'Electrical-stimulation in poultry – A review and evaluation', *Poultry Science*, 72, 7–22.
- Locker R H and Hagyard C J (1963), 'Cold shortening in beef muscles', *Journal of the Science of Food and Agriculture*, 14, 787.
- Löndahl G and Eek L (1986), *Cooling of meat cuts*, AGA Frigoscandia publication.
- MacDougall D B (1972), 'The effect of time and storage temperature on consumer quality', *Meat Chilling: Why and How? Meat Research Institute Symposium No. 2* (Ed. C. L. Cutting), 8.1–8.11.
- MacDougall D B (1974), 'The appearance of frozen meat and its colour stability during storage', In *Meat Freezing: Why and How? Meat Research Institute Symposium No. 3* (Ed. C. L. Cutting), 10.1–10.
- Malton R and James S J (1983), 'Drip loss from wrapped meat on retail display', *Meat Industry*, 56 (5), 39–41.
- Marais G J K (1968), 'Aspects of the meat trade in the USA', *Meat Industry*, Pretoria, October–December 33.
- Maria G T, Abril J and Casp A (2005), 'Surface heat transfer coefficients for refrigeration and freezing of foods immersed in an ice slurry', *International Journal of Refrigeration*, 28, 1040–1047.
- Marsh B B and Leet N G (1966), 'Meat tenderness 3', *Journal of Food Science*, 31, 450–460.
- Martin A H, Fredeen H T and Weiss G M (1971), 'Tenderness of beef longissimus dorsi muscle from steers, heifers and bulls as influenced by source, post-mortem ageing and carcass characteristics', *Journal of Food Science*, 36, 619.
- Miller E, Hoover L C, Guerra A L, Huffman K L, Tinney K S, Ramsey C B, Brittin H C and Huffman L M (1995), 'Consumer acceptability of beef steak tenderness in the home and restaurant', *Journal of Food Science*, 60 (5), 963–965.
- Miller R K (2002), 'Factors affecting the quality of raw meat', in Kerry J, Kerry J and Ledward D, *Meat Processing: Improving Quality*, Cambridge, Woodhead Publishing Ltd, 3, 27–63.
- Mooha Lee, Sebranek J and Parrish Jr F C (1995), 'Accelerated post-mortem ageing of beef utilising electron-beam irradiation and modified atmosphere packaging', *Journal of Food Science*, 1 (5), 133–136.
- Musa H H, Chen G H, Cheng J H, Shuipe E S and Bao W B (2006), 'Breed and sex effect on meat quality of chicken', *International Journal of Poultry Science*, 5, 566–568.
- Newman M (2001), 'Cryogenic impingement freezing utilizing atomized liquid nitrogen for the rapid freezing of food products'. In *Rapid Cooling of Food, Meeting of IIR Commission C2*, Bristol (UK).
- Pap L (1972), 'Freezing with liquid nitrogen; Effect of freezing rate on the quality of sliced beef', *Acta Alimentaria*, 1, 371.
- Parliamentary Office of Science and Technology (POST) (1997), *Safer Eating – Microbio-*

- logical Food Poisoning and its Prevention*, Parliamentary Office of Science and Technology, London, ISBN 1 897941 56 0.
- Parrish F C, Boles, J A, Rust R E and Olson D G (1991), 'Dry and wet aging effects on palatability attributes of beef loin and rib steaks from three quality grades', *Journal of Food Science*, 56, 601–603.
- Payne K R, Brooks J C, Morgan J B and Ray F K (2002), *The effect of fresh and frozen storage on palatability, oxidative rancidity and colour of modified atmosphere packed beef steaks*, Animal Science Research Report, Oklahoma Agricultural Experimental Station, USA.
- Pearson A M (1994), 'Introduction to quality attributes and their measurement in meat, poultry and fish products'. In Pearson A M and Dutson T R, *Quality Attributes and their Measurement in Meat, Poultry and Fish Products*, Advances in Meat Research Series, Volume 9, Blakie Academic and Professional, 1, 1–33.
- Petrovic L, Grujic R and Petrovic M (1993), 'Definition of the optimal freezing rate – 2. Investigations of the physico-chemical properties of beef *M. longissimus dorsi* frozen at different freezing rates', *Meat Science*, 33, 319–331.
- Pflug I J (1957), 'Immersion freezing found to improve poultry appearance' *Frosted Food Field* 17. (Cited in *Ashrae Refrigeration Handbook*, 1994, 12.5).
- Sacks B, Casey N H, Boshof E and Vanzyl H (1993), 'Influence of freezing method on thaw drip and protein loss of low-voltage electrically stimulated and non-stimulated sheep muscle', *Meat Science*, 34 (2), 235–243.
- Sams A (2002), 'Post-mortem electrical stimulation of broilers', *Worlds Poultry Science Journal*, 58, 147–157.
- Sams A R (1999), 'Meat quality during processing', *Poultry Science*, 78, 798–803.
- Sams A R (2001), *Poultry Meat Processing*, New York: CRC Press.
- Sebranek J G (1980), 'Cryogenic freezing of ground beef patties shows superior organoleptic effects', *Quick Freezing*, August, 50–53.
- Sebranek J G, Sang P N, Rust R E, Topei D G and Kraft A A (1978), 'Influence of liquid nitrogen, liquid carbon dioxide and mechanical freezing on sensory properties of ground beef patties', *Journal of Food Science*, 43, 842–844.
- Sleeth R B, Kelley G G and Brady D E (1958), 'Shrinkage and organoleptic characteristics of beef aged in controlled environments', *Food Technology*, 12, 86.
- Stern N J, Lyon C E, Musgrove M T, Dickens J A and Wilson R L (1992), 'Comparison of spoilage rates in ground turkey and ground-beef', *Journal of Food Protection*, 55 (7), 518–521.
- Sundsten S, Andersson A and Tornberg E (2001), 'The effect of the freezing rate on the quality of hamburger'. In *Rapid Cooling of Food. Meeting of IIR Commission C2*, Bristol (UK), Section 2, 181–186.
- Taylor A A (1972), 'Influence of carcass chilling rate on drip in meat', *Meat Chilling: Why and How?* Meat Research Institute Symposium No. 2 (Ed. C. L. Cutting), 5.1–5.8.
- Taylor A A, Perry A M and Warkup C C (1995), 'Improving pork quality by electrical stimulation or pelvic suspension of carcasses', *Meat Science*, 39, 327–337.
- Taylor H F (1930), 'Solving problems of rapid freezing', *Food Industries*, 2, 146.
- Taylor H F (1931), 'What happens during quick freezing', *Food Industries*, 3, 205.
- Vacinek A A and Toledo R T (1973), 'Heat transfer, organoleptic quality changes and moisture exchange in air-blast chilled poultry carcasses', *Journal of Food Science*, 38, 924–928.
- Varnam A H and Sutherland J P (1995), *Meat and Meat Products*, Chapman and Hall, London, ISBN 0 412 49560 0.
- Walker C M and Mitchell G E (1986), 'Beef handling practices at the retail and household levels. The findings of two surveys', *Livestock and Meat Authority of Queensland, Research Series, Research Report No. 22*, Brisbane, Australia.
- Wilson G D, Brawn P D, Chesbro W R, Ginger B and Weir C E (1960), 'The use of antibiotics and gamma irradiation in the ageing of steaks at high temperatures', *Food Technology*, 14, 143.

- Winger R J (1984), 'Storage life and eating related quality of New Zealand frozen lamb: A compendium of irrepressible longevity', *Thermal Processing and Quality of Foods*, Elsevier Applied Science publishers, 541–552.
- Wood D F and Richards J F (1974), 'Isometric tension studies on chicken pectoralis major muscle', *Journal of Food Science*, 39, 525–529.
- Zeigler P T, Miller R C and Christian J A (1950), *Preservation of meat and meat products in frozen storage*, The Pennsylvania State College, School of Agriculture, Bulletin No. 530.

Carcass interventions and meat tenderness

M. M. Farouk, E. Wiklund and K. Rosenvold, AgResearch MIRINZ, New Zealand

Abstract: This chapter discusses interventions available for improving meat tenderness in whole carcasses or individual muscles/cuts. The chapter reviews well-established methods such as electrical stimulation, pelvic suspension and ageing, but also novel techniques such as whole-carcass enhancement, the use of ultrasound, hydrodynamic shock, pressure, and *pre-rigor* muscle stretching. It emphasises the applied rather than the fundamental aspects of these interventions as utilised for meat from cattle, sheep, deer and pigs.

Key words: carcass interventions, meat tenderization techniques, whole-carcass enhancement, *pre-rigor* muscle stretching, hydrodyne.

24.1 Introduction

Tenderness is one of the most important meat quality attributes affecting the acceptability of meat. One of the main goals of the meat industry worldwide is to produce meat of consistent tenderness to meet the requirements of today's more discerning consumer. For this reason, considerable time and resources are spent by the meat industry to come up with interventions to improve the tenderness of meat, with varying degrees of success. Some of the interventions/techniques developed over the years were aimed at improving tenderness in whole carcasses, while others were meant to be applied on individual muscles/cuts. The operational mechanisms in the interventions may be biochemical, biophysical, physical, or a combination of these. The success of any intervention is determined by how widely it is adopted by the meat industry. This, in turn, depends on a number of factors, including among others, and in no particular order: safety, potential impact of the technique on other meat quality attributes, capital and operating cost, demand for

a natural product, degree of invasiveness of the technique, and more recently its energy and environmental footprint.

This chapter discusses some of the techniques currently available or being used for improving meat tenderness, with particular emphasis on meat from some of the larger common animal species such as cattle, sheep, deer and pigs; and on the applied rather than the fundamental aspects of the processes.

24.2 Whole-car carcass interventions to improve tenderness

24.2.1 Electrical stimulation

In New Zealand, electrical stimulation was originally introduced to accelerate *rigor mortis* in both beef and sheep carcasses, before the meat was frozen, to avoid the toughness in meat caused by cold shortening (Davey and Chrystall, 1980; Devine *et al.*, 2004). The incidence of cold shortening was significantly increased when blast freezing was introduced as a processing technique in the slaughter industry after the Second World War (Locker *et al.*, 1975). Cold shortening may take place if the muscle temperature falls too quickly while the energy level in the muscle is still high (Locker and Hagyard, 1963). Susceptibility to cold shortening has been demonstrated to vary between different types of muscle fibres (Bendall, 1973) and consequently beef, lamb and deer meats (where most muscles are dominated by red, oxidative muscle fibres) were thought to be more sensitive to cold shortening than pork (Savell *et al.*, 2005). Electrical stimulation has been widely adopted in commercial slaughtering to improve meat tenderness in beef, lamb, goat and deer carcasses (Chrystall and Hagyard, 1976; Davey *et al.*, 1976; Savell *et al.*, 1977; Chrystall and Devine, 1983; Drew *et al.*, 1988; Geesink *et al.*, 1994). Although electrical stimulation improves pork tenderness, it is not applied commercially to pig carcasses due to the risk of developing pale, soft and exudative (PSE) pork, in spite of the fact that some studies have shown that electrical stimulation can be applied, together with accelerated chilling, without compromising the water-holding capacity of pork (Rosenvold and Andersen, 2003). Furthermore, the susceptibility to develop PSE is significantly reduced with the low incidence of the Halothane gene in today's pig population, indicating that electrical stimulation could have a future in enhancing the tenderness of pork.

Electrical stimulation resulted in significantly more tender beef and lamb (Smulders *et al.*, 1989; Polidori *et al.*, 1999) as well as accelerated tenderisation (Hwang *et al.*, 2003). Accelerated tenderisation in stimulated carcasses is attributed to more rapid proteolysis that follows from both earlier onset of *rigor mortis* and the higher carcass temperature in the early *post rigor* period, compared with non-stimulated controls (Dutson *et al.*, 1977; George *et al.*, 1980). Deer carcasses also show accelerated tenderisation following electrical stimulation (Chrystall and Devine, 1983; Wiklund *et al.*, 2001). It has been demonstrated that deer meat (venison) from electrically stimulated carcasses were more tender than venison from non-stimulated carcasses at 1 day, 7 days and 3 weeks post-slaughter (meat stored at -1.5°C), but this advantage disappeared after 6 and 12 weeks of

chilled storage as both treatments reached similar minimum shear force values (Wiklund *et al.*, 2001). Therefore, the accelerated tenderisation that follows from electrical stimulation offers advantages particularly for product intended to be frozen or likely to reach retail within a short time, but offers less of an advantage for chilled products that are exported to distant markets from, e.g. Australia, New Zealand and South America, and therefore have an extended ageing time during transport. The combination of electrical stimulation and a specified ageing period before freezing to achieve a desired tenderness level underpins the accelerated conditioning and ageing standards that currently apply in New Zealand.

The prevailing view is that the major effect of electrical stimulation is to ensure *rigor mortis* is achieved rapidly and that cold shortening is prevented. However, there is increasing evidence that electrical stimulation not only prevents cold shortening and induces accelerated tenderisation, but that it also protects against *rigor* shortening at high temperatures for both lamb and beef (Devine *et al.*, 2006; Rosenvold *et al.*, 2008). Further, the study on beef showed that stimulation did not affect drip but merely that *post rigor* drip appears earlier as a consequence of earlier ageing (Rosenvold *et al.*, 2008). The underlying mechanisms for the observed effects of electrical stimulation on tenderness are not yet completely clear. The fact that individual muscles exhibit different *pre rigor* behaviours was highlighted in a study of hot-boned, electrically stimulated beef (White *et al.*, 2006). In that study it was concluded that mechanisms other than the prevention of shortening could be involved in the tenderisation of meat induced by electrical stimulation and that a variation in fibre type composition and physical dimensions of the muscle might impact on the effects of electrical stimulation. In addition, it is also likely that the individual muscle fibres that enter *rigor mortis* soon after stimulation not only commence ageing early, but the adverse high temperature and low pH effects, which are known to affect protein denaturation, are significantly reduced once the individual fibre is in *rigor* (Offer, 1991).

Today, electrical stimulation is used world-wide; however, the stimulation setting and its duration vary significantly between different processing plants. Furthermore, the contribution of other electrical inputs such as electrical stunning, spinal discharge, immobilisation and back-stiffening during hide pulling are not controlled and their effects on tenderness and meat quality in general are poorly understood (Petch and Gilbert, 1997). Hence, it is likely that the optimum electrical inputs are only rarely used.

24.2.2 Pelvic suspension

From the time Locker (1960) established that muscles can enter *rigor mortis* with different degrees of contraction and that meat from relaxed muscles was more tender relative to that from cold shortened or contracted muscles, many techniques have been developed, with varying degrees of success, in an attempt to prevent *pre-rigor* muscles from contracting, or to stretch these muscles in order to make the resultant meat more tender. Some of these techniques have been reviewed by Sørheim and Hildrum (2002). Only a few of the techniques of stretching *pre-rigor*

muscles reported over the years are currently applied under commercial conditions.

The suspension of carcasses from the Achilles tendon is the established commercial practice of on-carcass stretching of some of the muscles in the hindquarter. A variant of the Achilles-tendon-stretch is the pelvic suspension or Tenderstretch method which was developed in the US in the early 1970s. The process involves hanging a carcass from the aitch bone (*Obsturator foramen*) soon after slaughter. This method has been shown to improve the tenderness of meat from some of the muscles in the middle and hindquarter such as *Mm. longissimus*, *semimembranosus*, and *gluteus medius* (Sørheim and Hildrum, 2002). Comparisons of these two hanging techniques were first carried out in beef (Bouton *et al.*, 1973; Hostetler *et al.*, 1970), where the variation in tenderness is considered to be the main reason for consumer dissatisfaction (Koochmaraie, 1996). Pelvic suspension has been demonstrated to increase tenderness in bull beef to a higher degree compared with beef from heifers (Fisher *et al.*, 1994; Lundesjö Ahnström *et al.*, 2003). Tenderness was improved in beef *M. longissimus* at cold shortening conditions using pelvic suspension (Sørheim and Hildrum, 2002). Also, tenderness of beef *M. semimembranosus* was increased and the variation in tenderness between animals decreased in the carcasses that were pelvic suspended, compared with the normal Achilles suspension (Ahnström *et al.*, 2006a). An additional positive effect of pelvic suspension (compared with Achilles suspension) demonstrated in beef was improved water-holding capacity by reducing moisture losses both during storage and cooking (Ahnström *et al.*, 2006a). The fact that pelvic suspension will alter the shape of various valuable cuts has been rendered less important as the demand from consumers has shifted to more uniform, small pre-packaged retail portions (Sørheim and Hildrum, 2002). Cuts from pelvic suspended carcasses have been judged superior in this regard (Wahlgren *et al.*, 2002).

In meat from other species, the positive effects of pelvic suspension on tenderness are very similar to the results from beef. Thompson *et al.* (2005) compared the effect of carcass suspension methods (pelvic suspension versus Achilles tendon) on the eating quality of sheep meat and found that the *M. longissimus* and *M. biceps femoris* of tenderstretched lamb and mutton carcasses produced meat that was more tender relative to those from carcasses suspended from the Achilles tendon. Similarly, tenderness of *Mm. longissimus*, *biceps femoris*, *semimembranosus*, *adductor femoris* and *vastus lateralis* from fallow deer (*Dama dama*) were improved using pelvic suspension (Sims *et al.*, 2004); the impact of the process was greatest on the muscles from young male fallow deer (18 months old), and to a lesser extent in older male deer (> 36 months old), with no impact at all on the tenderness of venison from female deer (Sims *et al.*, 2004). The specific muscles from deer carcasses that improved in tenderness due to pelvic suspension were similar to the ones reported in earlier studies on beef (Hostetler *et al.*, 1970; Bouton *et al.*, 1973). Further, the same positive effect of pelvic suspension on water-holding capacity mentioned for beef has been reported in lamb and venison during long-term chilled storage (up to 6 weeks at 2 °C)

(Wiklund *et al.*, 2004). Pelvic suspension of pig carcasses has also resulted in longer sarcomere lengths, more tender meat and reduced drip loss compared to samples from carcasses hung from the Achilles tendon; for review, see [Rosenvold and Andersen](#) (2003).

Another variation from the Achilles-tendon-stretch is the Tendercut process, developed in the US in the early 1990s (Wang *et al.*, 1994). The Tendercut procedure involves completely severing the 14th thoracic vertebrae of pig carcasses perpendicular to the vertebral column and ensuring the skin, adipose tissue, intercostal muscle and connective tissue are all severed at this site (Wang *et al.*, 1995). In a beef carcass, the 12th thoracic vertebrae is severed in a similar fashion, ensuring that only the *M. longissimus* muscle is left intact at the site, and the ischium of the pelvic bone junction between the 4th/5th sacral vertebrae and the connective tissues at the round/loin region are also severed, maximising the amount of stretch on the *M. longissimus* muscle (Claus *et al.*, 1997).

24.2.3 Whole-carcass enhancement

In the early 1960s, Beverly William was granted a United States patent (#3006.768) that involved the injection of one to three percent of body temperature water into a freshly slaughtered carcass at a pressure of 40–600 psi. It was claimed that the process improved tenderness by about 25%. The rationale for improved tenderness was that water injection activates the natural enzymes in meat. Also, the use of water at body temperature slows the rate of chilling of the carcass and therefore hastens *rigor* and the commencement of ageing. Karmas (1970) reported significant improvement in tenderness when water was injected into carcasses before the completion of *rigor*. The hypothesis for the improved tenderness was that water, when injected under pressure, will spread muscle fibres and get distributed uniformly throughout the meat. After the completion of *rigor*, the water will then set and be held as true hydration water with tenderness improving due to increased moisture content. Koohmaraie *et al.* (1988, 1989) reported a procedure for improving tenderness that involved low frequency electrical stimulation of ovine carcasses after death, followed by infusion with a solution containing calcium chloride prior to evisceration. Results of the studies showed this method significantly improved tenderness within 24 h *post mortem*. However, the study did not find any effect of infusing pure water on tenderness. Sears (1989) described a cardiovascular infusion process immediately after bleeding for whole beef carcass tenderisation using a pumping unit that displaced the remaining blood with glucose solution. MPSC, Inc. (Eden Prairie, MN) developed a post-exsanguination vascular infusion method to improve tenderness of a whole carcass. The process involved the infusion of ten percent of live weight of a solution containing maltose, glycerine, dextrose and sodium and potassium tripolyphosphates through the carotid artery (Farouk *et al.*, 1992a). The solution was pumped through the carotid artery immediately after the animal was bled at a pressure of 3.2 kg/cm². It was found that whole-carcass vascular infusion improved the tenderness of meat from both lamb and beef carcasses (Farouk and Price, 1994; Farouk 1992a,b). The

improved tenderness was ascribed to the acceleration of glycolysis and the tenderisation process early *post-mortem*. A more recent study by Yancey *et al.* (2002) did not find any improvement in tenderness due to vascular infusion of carcasses of steers. The difference in the effect of the vascular infusion between the two studies (Farouk *et al.*, 1992a; Yancey *et al.*, 2002) could be due to the age of the animals and/or the ageing time of the meat before tenderness was determined. Mature culled dairy cows were used by Farouk *et al.* (1992a) and the meat was aged at 2–4 °C for four to five days, while young A-maturity steers were used by Yancey *et al.* (2002) and the meat was aged for two weeks at 2–4 °C before tenderness determination, by which time the accelerated ageing effect of whole-carcass vascular infusion might have reached a plateau.

24.3 Ageing of meat to improve tenderness

Ageing or conditioning of meat to improve tenderness has been known since antiquity (Davey and Gilbert, 1976). The *post-mortem* tenderisation involves the breakdown of muscle structure due to proteolysis of myofibrillar and cytoskeletal proteins (Ouali, 1992; Sentandreu *et al.*, 2002). While it is recognised that meat tenderisation is enzymatic in nature, there is no agreement as to whether it is (i) calpains alone, (ii) calpains and cathepsins, or (iii) a multienzymatic process also including proteasomes and caspases, that is responsible for the tenderisation process.

Meat can be aged either on the carcass or after boning and packaging. There are two types of boned-meat ageing: (i) wet ageing involving the storage of vacuum packaged meat at refrigerated temperatures, and (ii) dry ageing involving unpackaged product being exposed to air at controlled temperatures. The two ageing types may be combined into one process such as dry-ageing the product for a specific time and thereafter vacuum packaging the meat for further ageing (Campbell *et al.*, 2001) or *vice versa*. Weight losses from dry ageing have been one of the main problems associated with this form of ageing, but recently Ahnström *et al.* (2006b) reported that weight losses due to dry-ageing could be reduced with no negative impact on quality by dry ageing in a highly moisture-permeable bag.

Meat that is tougher at the initiation of ageing, or from lower quality grade animals, will benefit more in terms of improved tenderness relative to a more tender meat or one from a higher-quality grade animal (Novakofski and Brewer, 2006).

Ageing rate and the extent of ageing are dependent both on intrinsic factors, such as animal species, muscle type, growth rate, and on extrinsic factors, such as pre-slaughter handling, electrical stimulation, chilling method, ageing time and temperature.

24.3.1 Animal species

The mechanism of ageing is likely to be the same for all species, but different

species have different ageing rates, even at the same temperature. The rate of ageing in meats is in the following order: pork > venison > lamb > beef, and it is correlated to the *post-mortem* rate of glycolysis (Smulders *et al.*, 1995). The ageing process typically takes 1–2 days in chicken, 3–6 days in pork and 10–20 days in beef (Smulders *et al.*, 1992). In a recent study (Farouk *et al.*, 2007), the tenderness of red deer (*Cervus elaphus*), venison and beef *M. semimembranosus* stored at -1.5°C for 4 weeks was compared. Within 24 h *post-mortem*, venison was already tender, while beef was very tough; after just 1 week of storage, venison samples were borderline to being considered ‘very tender’, while beef samples required 3 weeks storage to reach this point. Generally, the tenderness of meat from both species improved significantly over the ageing period with the difference in tenderness between venison and beef becoming less with time up to the 3rd and 4th week, at which point the meat from the two species did not differ in tenderness (Farouk *et al.*, 2007). Earlier studies comparing tenderness and ageing rate in reindeer (*Rangifer tarandus tarandus*) and beef showed that reindeer *longissimus* muscle was extremely tender; as early as 3 days *post-mortem* at 2°C , shear force values were only 2 to 3 kg/cm², which was very low compared with shear force values in beef of approximately 10 kg/cm² (Wiklund *et al.*, 1997). The increased ageing rate was ascribed to higher proteolytic enzyme activity in venison and reindeer compared with beef (Barnier *et al.*, 1999; Farouk *et al.*, 2007).

24.3.2 Muscle type

The variation in tenderness between different muscles is well documented, with the first coordinated study including 50 different beef muscles (Ramsbottom and Strandine, 1948). Tenderness is determined by three key factors: proteolysis, the amount and degree of cross-linking of connective tissue, and sarcomere length (Rhee *et al.*, 2004). The relative importance of these three factors to tenderness is muscle dependent. In a number of muscles all three factors contribute to tenderness, whereas for example, proteolysis is the major determinant of *M. longissimus* tenderness, while sarcomere length is most important for *M. psoas major* (Koochmaraie and Geesink, 2006). Stolowski *et al.* (2006) compared the effects of ageing on seven muscles from Angus and Brahman crosses and found that muscle type played the greatest role in determining tenderness, and on that basis they grouped the muscles into four ageing categories: (i) tender with slow but continued response to ageing up to 42 days *post-mortem* (*M. gluteus medius* and *M. longissimus dorsi*); (ii) slightly tender with ageing response up to 14 days *post-mortem* (*M. semimembranosus* and *M. triceps brachii*); (iii) slightly tough with slow ageing response after 28 days *post-mortem* (*M. semitendinosus* and *M. vastus lateralis*) and (iv) tough with no ageing response (*M. biceps femoris*). Gruber *et al.* (2006) determined the effect of *post-mortem* ageing at 2°C for up to 28 days *post mortem* on 17 muscles from 40 USDA select and choice beef carcasses and found that muscle-to-muscle tenderness differences depended on quality grade. Muscle fibre composition has been shown to determine the rate of tenderisation, as ageing seems to occur more rapidly in fast-twitch-glycolytic

bovine muscles than in slow-twitch-oxidative muscles (Gann and Merkel, 1978; Ouali *et al.*, 1983).

24.3.3 Growth rate

The growth rate of farm animals is related to muscle protein turnover – essentially the balance between protein synthesis and protein degradation. The proteolytic potential (the potential of muscle to tenderise) at the time of slaughter has long been regarded as an important factor in the tenderisation process of meat. Compensatory growth may occur after a period of feed restriction in broilers, cattle, pigs and sheep (Andersen *et al.*, 2005). During compensatory growth, both the rate of protein synthesis and degradation are elevated and the strategy has been shown to improve tenderness in beef from steers (Allingham *et al.*, 1998) and pork from female pigs, although not from castrates (Kristensen *et al.*, 2004). It appears that there is an optimal slaughter time during compensatory growth with respect to *post-mortem* tenderisation (Therkildsen, 2005).

In New Zealand, red deer is a farmed animal and the main product from the industry is venison. Deer are typically found in areas with marked seasonal variation and different species have adapted to this seasonality (Suttie and Webster, 1998). Therefore, deer have a seasonal pattern of growth, with maximum accretion of body tissue occurring in spring and summer, and weight may be lost during autumn with little or no weight gain taking place over the winter, i.e. a natural system of ‘built-in’ compensatory growth. Several studies have been performed to further understand the underlying physiological mechanisms responsible for the seasonal control of growth and how they might be practically manipulated for productive advantages (Suttie *et al.*, 1983), but no results are currently available on the possible differential effects of this seasonal variation on protein deposition or venison quality.

24.3.4 Pre-slaughter handling – intermediate pH

Elevated ultimate pH values occur due to reduced glycogen stores at the time of slaughter induced by stress during pre-slaughter handling or from poor nutritional status of the animals. Normal pH values (~ 5.5–5.7) are required for optimal tenderness – the shear force will increase with increasing ultimate pH in most, but not all, muscles (Devine *et al.*, 2006). The mechanisms seem to arise from a decrease in the ageing rate (Watanabe *et al.*, 1996; Watanabe and Devine, 1996). Meat in the so called ‘intermediate pH’ range ($5.8 < \text{pH} < 6.3$) of tenderness becomes increasingly more variable with some samples being extremely tough. Recent studies (Pulford *et al.*, 2007, 2008) have shown that the presence of small heat shock proteins (sHSP) may contribute to the toughness of intermediate pH beef in the following way (Fig. 24.1): at the pH of muscle immediately *post-mortem*, most of the sHSP are in solution in the cell cytoplasm, offering little protection by way of chaperoning to the muscle structural proteins. As the intracellular pH falls, this pool of sHSP begins to interact with the denaturing

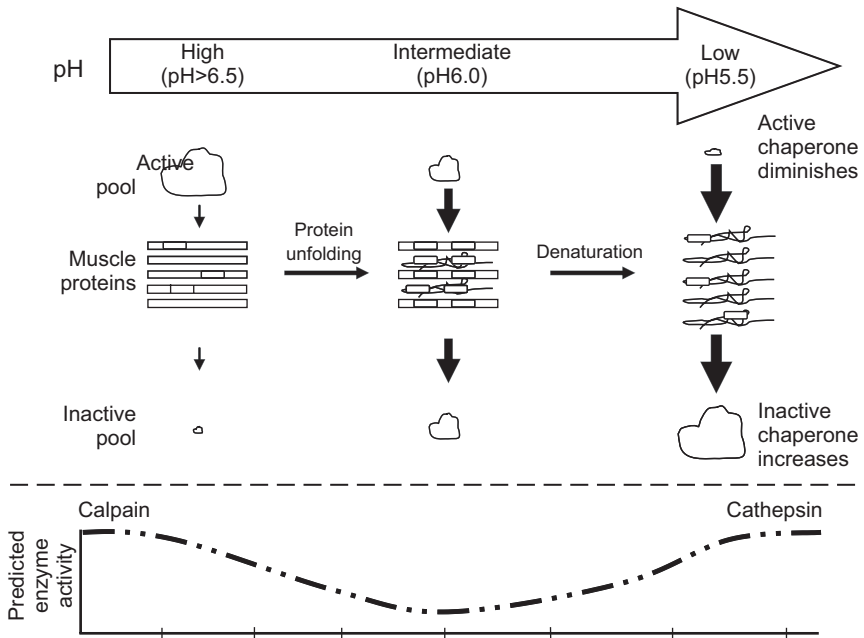


Fig. 24.1 Model of toughness in intermediate pH meat (D. Pulford, personal communication).

myofibrillar proteins by binding to them and thereby protecting them from proteolytic enzymes. At around pH 6.2 and lower, the sHSP also begin to precipitate out of solution and the pool of soluble sHSP capable of stabilising and shielding myofibrils is diminished. This precipitation continues so that, at pH 5.5, little soluble sHSP remains available to protect myofibrils, and myofibrillar-bound sHSP is also dissociated from the myofibril due to acid precipitation (Pulford *et al.*, 2008). In addition to the higher protection against proteolysis offered by the chaperoning activities of sHSP, it was also found that intermediate pH meat is neither optimal for calpain (physiological pH) or cathepsin (acid pH) activities (Pulford *et al.*, 2007). Therefore, it is hypothesised that the combination of a sHSP shield and low enzyme activity in intermediate pH meat maintains the integrity of the muscle structure and thereby reduces the rate of ageing of this meat.

24.3.5 Chilling

Chilling is an essential part of the slaughter process as it is critical to food safety, shelf-life, appearance and eating quality (essentially tenderness). With respect to tenderness, the chilling process should be optimised to minimise the degree of shortening in the *pre-rigor* muscle and maximise the activity of the enzymes involved in *post-mortem* proteolysis. Chilling rates need to be slow enough, particularly for small animals, to avoid cold shortening, yet rapid enough,

particularly in large animals, to avoid inhibition of ageing enzymes for non-stimulated carcasses (Devine *et al.*, 1999). However, following adequate stimulation, inferior tenderness induced by high *pre-rigor* temperatures is less of an issue (Rosenvold *et al.*, 2008). For beef and lamb it is generally accepted that the temperature/pH decline in the muscles should be controlled for optimal tenderness development. Several recommendations for chilling rates have been developed, most of these for beef: (i) beef carcasses not to be cooled below 12 °C in less than 15 h, i.e. before the completion of *rigor mortis* (Bendall, 1973), (ii) pH of *M. longissimus* must fall to values below 5.7 before/when the temperature reaches 7 °C (Hannula and Puolanne, 2004), and (iii) meat pH should not be lower than 6 if muscle temperature is greater than 35 °C and not higher than 6 if muscle temperature is lower than 12 °C (Meat Standard Australia pH/temperature window) (Thompson, 2002). For lamb, the following have been suggested: (i) an accelerated conditioning and ageing process, which involves electrical stimulation, holding the lamb carcasses above 6 °C until at least 8 h *post-mortem*, following which the temperature of the deep leg should not fall below -4 °C within 20 h *post-mortem* (Hagyard, 1979), and (ii) the temperature at pH 6 should be greater than 10 °C but lower than 30 °C (Thompson *et al.*, 2005).

While chilling of beef and lamb has been optimised for tenderness, the chilling process of pig carcasses has been optimised to reduce the risk of PSE caused by low pH and simultaneously high temperature early *post-mortem* and low pH (Briskey, 1964; Offer and Knight, 1988) by the application of chilling tunnels whereby the average carcass temperature can be reduced to 4 °C within two hours *post-mortem*. However, a recent study has shown that the chilling process can be optimised to significantly improve pork tenderness without compromising water-holding capacity (Rosenvold *et al.*, 2006a). These results were obtained in pigs which were group-wise transported, lairaged and stunned and were free of the Halothane gene. The group-wise pre-slaughter handling system reduces the temperature at the time of slaughter (Støier *et al.*, 2001).

24.3.6 Ageing time and temperature

While *pre-rigor* conditions dictate the extent of tenderisation, the major influence on the rate of ageing is the temperature that the meat is held at *post-rigor*, with higher temperatures increasing the ageing rate (Dransfield *et al.*, 1981). Whatever the ageing temperature, meat ages rapidly at the start and then more slowly over time, at an exponential falling rate. Hence, if tenderness was the only factor to consider, meat could be chilled to, e.g. 15 °C in order to minimise shortening (Locker and Hagyard, 1963; Tornberg, 1996) and then stored at high temperatures. For instance, when beef carcasses are chilled quickly to 15 °C and held at that temperature to optimise tenderisation, final tenderness level can be reached as early as 4 days *post-mortem* (Davey *et al.*, 1967). However, such conditions do not account for shelf-life (spoilage) or food safety, and meat is therefore cooled to, and stored, at much lower temperatures (normally -1.5 to 4 °C for chilled products and to -18 °C or below for frozen products).

Traditionally, meat has been stored at 2–4 °C for up to two weeks, which would give acceptable tenderness and shelf-life for consumption in local markets (e.g. within the EU). However, with advances in packaging technology and decreased storage temperatures (e.g. –1.5 °C), shelf-life can be extended for up to 16 weeks. These developments have enabled export of chilled products from South America, Australia and New Zealand to distant markets. Despite the low storage temperature, the meat will be fully tenderised when it reaches the consumer due to the extended ageing time.

For processors exporting meat to distant markets, over-tenderisation or loss of texture due to extended ageing time is of concern. In fact, loss of texture is one of the reasons the meat industry is not using exogenous plant and microbial proteolytic enzymes in the commercial tenderisation of meat for table uses. Although published data on over-tenderisation is scarce, feedback from markets indicates that over-tenderisation and consequent loss of ‘bite’ or texture occurs. Recently, the tenderness of vacuum-packed beef and venison aged for one month at –1.5 °C was compared (Farouk *et al.*, 2007). The authors suggested that care should be taken during processing to avoid over-tenderising venison to the point of losing its texture before it reaches the consumer in distant markets.

24.4 Novel technologies to improve tenderness

24.4.1 Pressure

The results of a number of studies indicate that pressure can be used to alter the properties of muscle proteins (Cheftel and Culioli, 1997). The effects of pressure on muscle proteins include the disruption of myofibrillar structures, accelerated proteolysis, and increased tenderness, solubility, aggregation and gelation (Ma and Ledward, 2004; Macfarlane, 1974; Macfarlane and McKenzie, 1976; Macfarlane *et al.*, 1984; Elgasim and Kennick, 1982). The use of *pre-rigor* pressurisation or hydrostatic pressure to improve tenderness was first reported by MacFarlane (1973). The author subjected *pre-rigor* ovine and bovine muscles to high pressure (103 MPa, 30–35 °C, 1–4 min), resulting in significant improvement in the tenderness of the cooked meat from these muscles. The improvement in tenderness according to MacFarlane (1973) was due to muscle contraction and the breakdown of muscle structure. Homma *et al.* (1994) showed that high pressure treatment of beef induced an increase in the release of cathepsins from lysosomes. In a follow-up study, Homma *et al.* (1995) demonstrated that calpain activity is unaffected in rabbit muscle pressurized up to 200 MPa but activity of calpastatin in the muscle was decreased and Ca²⁺ concentration increased, resulting in the total activities of calpains being increased by the pressure treatment. The increased cathepsin level and calpain activity in high-pressure treated meat will result in the tenderisation of meat and accelerated meat ageing. According to Cheftel and Culioli (1997), pressure processing is carried out by placing samples sealed in water impermeable flexible packaging in a liquid pressure-transmitting medium

such as water. A pump is then used to generate pressure and the pressurised liquid is maintained in a steel cylinder of adequate thickness and resistance.

24.4.2 Ultrasound

Whittaker *et al.* (1992) explained the principle of ultrasound use in tissues as follows. Ultrasonic waves in tissue are analogous to waves created by dropping a stone into a still pool of water. The 'stone', or excitation force, is normally provided by a piezoelectric element in acoustical contact with the tissue. The waves propagate through three dimensions away from the transducer and travel at a fixed speed. The velocity may be divided into three components: longitudinal velocity, horizontal shear velocity and vertical shear velocity. The speed at which the wavefront is moving across the pool away from the stone is the longitudinal velocity. Ultrasonic waves are high frequency acoustic waves with wavelengths varying from a few cm to few μm in water or biological tissues (Got *et al.*, 1999). A number of workers have investigated the use of ultrasound in meat tenderisation, with mixed outcomes (Jayasooriya *et al.*, 2007). Zayats and Orlova (1970) found that exposing beef to ultrasonic treatment carried out in a vat with a generator-fed magnetostriction vibrator for 3–15 min improved the tenderness of the meat relative to control samples. Lyng *et al.* (1997, 1998a,b) did not observe any improvement in tenderness by exposing *pre-* and *post-rigor* beef muscles and lamb to ultrasound using various high-intensity devices. Similarly, Got *et al.* (1999) saw no improvement in tenderness when they subjected *M. semimembranosus* muscles from cows to ultrasonic treatment (2.6 MHz; 10 W/cm²; 2 \times 15 s) *pre-* or *post-rigor*. Recently, Jayasooriya *et al.* (2007) subjected *M. longissimus lumborum et thoracis* and *M. semimembranosus* muscles from steers to high power ultrasound (24 kHz; 12 W/cm²) for up to 240 s and found that the treatment significantly reduced the shear force and hardness of the meat compared to untreated samples. The authors observed that the benefit of the ultrasound decreased with increasing ageing time of the meat and suggested that, with the availability of high power ultrasound generators, large-scale rapid tenderisation of meat is now feasible.

24.4.3 Hydrodyne

J.B. Long was granted US patents for the use of hydrodyne for tenderising meat in 1993 (5 273 766) and 1994 (5 328 403). The hydrodyne process uses a small amount of explosive to generate a hydrodynamic shock wave in a liquid medium (water), which increases the pressure inside a piece of meat submerged in the medium, resulting in the physical tenderisation of the meat. Solomon and colleagues (Claus *et al.*, 2001; Solomon *et al.*, 1997, 1998; Moeller *et al.*, 1999) have shown that hydrodyning can be used to improve the tenderness of various muscles of beef (up to 72%), lamb (33–67%), pork (17%) and broiler chicken breast (42%). The hydrodyne process used by Solomon and his colleagues essentially involves encapsulating meat twice, first in a polyolefin resin bag followed by encapsulation in a polymer of isoprene; the packaged samples were supported against the floor of

plastic containers, each fitted with a steel plate so that the ensuing wave was reflected back through the meat to intersect the incoming wave; the containers were situated below ground level and filled with water; explosives composed of liquid nitromethane and solid ammonium nitrate were submerged in water 30.5 cm away from the front surface of the meat and were wired to a detonating device, which triggered the explosive to create the hydrodynamic shock.

24.5 Processing techniques to improve tenderness of individual muscles/cuts

24.5.1 Meat stretching technologies

Hot boned muscles, because they are in a *pre-rigor* state, can be physically stretched to alter sarcomere length and tenderness in a manner analogous to the on-carcass Tenderstretch system of pelvic suspension. Manipulating hot boned meat also offers some unique advantages such as portion control, which allows otherwise irregular muscle shapes to be manipulated to produce a regular (usually cylindrical) shape. When stretching is applied in conjunction with the optimal *pre-rigor* temperature/pH conditions, improved eating quality benefits are realised. This principle has been used by a number of groups to develop processing technologies to stretch and restrain individual *pre-rigor* muscles from contracting during *rigor* formation. At least two systems have been developed for commercial use based on this principle, including the Pi-Vac packaging system and the Sarcostretch system developed at AgResearch MIRINZ (Farouk *et al.*, 2005; Simmons *et al.*, 2006).

The Pi-Vac system was first reported by Stiebing and Karnitzschky (1996). The system was developed and patented by Pi-Vac GmbH, Germany and is based on the principle of using a very elastic plastic film with low oxygen permeability and high degree of shrinkability in which the meat is extruded and moulded into a cylindrical or rectangular shape (Fig. 24.2). The Sarcostretch system uses a device made up of the following parts: (i) Three steel loading chambers that taper into three different sized parallel tubes; (ii) a mechanism that shuts and seals the loading chambers after *pre-rigor* muscle is loaded, (iii) a valve to allow air into the chambers to force the muscle down the taper and into a parallel tube, (iv) Sensors on the parallel tube that control the movement of the meat through the tube, and (v) unstretchable plastic film that the meat is pushed into as it exits the parallel tube (this plastic film holds the stretched meat during subsequent storage). The Sarcostretch was used by Farouk *et al.* (2005) to stretch *pre-rigor* bovine *Mm. semitendinosus*, *semimembranosus* and *biceps femoris* and the authors found that the muscles were lengthened by 43–97% (*M. semitendinosus* 97.1%, *M. semimembranosus* 77.9% and *M. biceps femoris* 43.7%), suggesting that more stretch was achieved with muscles that have fibres aligned parallel to the length of the muscle (*M. semitendinosus* and *M. semimembranosus*) compared with muscles with fibres that have numerous orientations (*M. biceps femoris*). Overall, stretching



Fig. 24.2 Pi-Vac device on trial at AgResearch MIRINZ Centre, New Zealand.

improved tenderness, uniformity, presentation, portion control of the meat, and reduced its drip loss.

24.5.2 Mechanical tenderisation

Mechanical tenderisation – also known as blade tenderisation or pinning – is the process of physically disrupting the muscle structure by penetrating the meat with closely spaced thin, sharp blades, which disrupt the fibres and sever the connective tissue. Mechanical tenderisation is applied either alone or is used to enhance the effect of tumbling, marinating or brine injection. The method has been applied to successfully improve the overall tenderness and tenderness variability of (especially) lower value cuts of beef (Pietrasik and Shand, 2004; Jeremiah *et al.*, 1999; Kolle *et al.*, 2004; Rosenvold *et al.*, 2006b). This agrees with the improved tenderness and reduced tenderness variability observed when methods such as chilling, stretching or wrapping are applied to beef and pork during the first 24 hours after slaughter (Thompson *et al.*, 2006; Rosenvold *et al.*, 2006a,b; Sørheim and Hildrum, 2002).

Mechanical tenderisation may have negative effects on the shelf-life, colour, drip loss and yield of fresh beef. The effect of mechanical tenderisation on these attributes of beef has not been sufficiently studied: see Jeremiah *et al.* (1999) for a comprehensive overview.

In the trials reported above, mechanical tenderisation was applied at different times after slaughter and the time at which the effect on tenderness was measured

also varied. Hence it is difficult to compare the different studies. The potential of using mechanical tenderisation as a means of reducing time to market has not been reported in the literature. Any reduction in the time to market could also balance the food safety and shelf-life issues caused by the blades potentially contaminating the otherwise sterile muscle.

24.5.3 Hot-boning

Hot-boning is defined as the removal of muscles from the carcass before chilling (West, 1983). The interest in hot-boning is a result of economic advantages due to savings in space, energy and labour, as well as a result of demonstrated improvements in functional properties of meat (Pisula and Tyburcy, 1996). Further, hot-boning has potential as a technique which allows each muscle/cut to be separated from the carcass early *post-mortem* and therefore be treated optimally according to its intrinsic properties (White *et al.*, 2006). However, excised muscles removed *pre-rigor* are more susceptible to shortening due to the loss of muscle-bone attachment, and rapid chilling/freezing would enhance the likelihood of cold shortening (Devine *et al.*, 2004; Savell *et al.*, 2005). In such cases, hot-boning would be a disadvantage in terms of tenderness; nevertheless, this can be prevented and minimised by the use of electrical stimulation, stretching and optimal chilling (Devine *et al.*, 1999, 2004).

Hot-boned beef muscles (*Mm. longissimus* and *semimembranosus*) without electrical stimulation had shorter sarcomere lengths, higher shear force values and lower sensory scores for tenderness compared with stimulated muscles (White *et al.*, 2006). In a study of electrically stimulated hot-boned *M. longissimus* from sheep, it was found that the shear force values in the meat from all the muscles were greater than the recommended tenderness threshold for acceptable table meat (Toohey and Hopkins, 2006). Hot-boning of pork has been slow to be adopted world-wide, largely due to the logistics of changing to this technique and also to the potential detrimental impact on tenderness due to the rapid drop in muscle temperature. Hot-boned pork loins chilled at 0 °C resulted in toughening and higher drip loss caused by cold shortening (Rees *et al.*, 2002). In a recent study, electrical stimulation was used in a field slaughter system for reindeer, and lower-quality cuts (shoulder meat) were hot-boned and frozen in the field. Three different products were prepared from the frozen shoulder meat (thin sliced, cubed and ground meat) and assessed in a consumer preference test. The consumers confirmed that electrical stimulation significantly increased tenderness in the hot-boned cubed and sliced meat products compared with non-stimulated control samples (Wiklund *et al.*, 2008).

The commercial application of hot-boning in processing beef has largely been pioneered in New Zealand where up to 20% of beef, mainly from bulls and cows, is hot-boned and exported for manufacturing practices. An increasing number of prime beef animals are hot-boned and the meat from these animals, given the right processing inputs, has been suggested to match the quality of meat produced by conventional cold-boning procedures (Simmons *et al.*, 2006).

24.5.4 Enhancement of individual muscles/cuts

The injection of meat with up to 20% marinade (mostly a solution of salt and phosphates) to enhance tenderness and palatability has become a common practice in many countries, particularly the USA and Australia (Maca *et al.*, 1997; Vote *et al.*, 2000; Robbins *et al.*, 2003a,b,c; Baublits *et al.*, 2006; Lennon *et al.*, 2006; Stephens *et al.*, 2006; Anon, 2003; Hoogenkamp, 2003). Vote *et al.* (2000) injected US choice and select strip loins with up to 15% solution containing phosphate, lactate and chlorides, and then subjected the steaks to sensory evaluation using a trained panel. It was found that injecting the loins with the solution improved the tenderness, juiciness, and cooked beef flavour. In a more recent study, Robbins *et al.* (2003a) injected strip loins and rounds with up to 10% of a solution containing sodium tripolyphosphates and sodium chloride, then evaluated the steaks using a consumer panel. They found that the injected beef steaks were more acceptable than the controls. Baublits *et al.* (2006) injected pork loins with a solution containing sodium tripolyphosphates and sodium chloride at two pump levels and found that the pumped loins had lower shear force values relative to controls.

The manufacture of enhanced meat involves the following steps: (i) Weigh an appropriate meat cut; (ii) inject the cut with a brine solution or marinade to the desired green weight; (iii) allow the excess marinade to drain; (iv) vacuum package and store chilled or frozen. These four steps can be adapted to suit different situations; the variation may include short-time tumbling of the injected cuts before or after packaging to improve brine distribution and to reduce cook loss, and pinning or some other kind of mechanical tenderisation to improve overall tenderness and eating quality (Rosenvold *et al.*, 2006b; Pietrasik and Shand, 2005). The brine could contain a number of additives or ingredients but generally is mostly kept simple for labelling purposes. Vote *et al.* (2000) injected a solution made up of sodium tripolyphosphate, sodium lactate and sodium chloride at 15% of the green weight; the brine was formulated to produce injected product concentrations of 0.25% sodium tripolyphosphate, 0.5% sodium chloride and 2.5% sodium lactate. Robbins *et al.* (2003a) injected a solution of only sodium tripolyphosphate and sodium chloride at the 10% level to achieve 0.4% concentration of both ingredients in the injected beef. The former study used a hand-held injector, while the later used a multi-injector system. Both studies reported improvement in tenderness and other palatability attributes.

A more complicated method for producing a pH-enhanced meat product was patented by Roth (2002a,b). The patent describes a procedure for manufacturing pH enhanced meat as follows: (i) Ammonia is placed in contact with a meat product; (ii) an intermediate meat product with a pH higher than the initial meat is produced; (iii) excess ammonia is then removed from the surface of the intermediate meat to avoid producing ammoniated meat; (iv) a mechanical action is employed to drive the ammonia on the surface of the intermediate meat into the meat; (v) the pH of the meat is then reduced to a desired final pH using a suitable material such as carbon dioxide and using mechanical action similar to when the pH was initially raised.

24.6 Future trends

The following are some of the future directions for research based on the review of the literature used in writing this chapter and the authors' understanding of the current and future needs of the beef, lamb, venison and pork industries:

- (i) The current practice in the meat industry is to produce meat using accepted processing inputs, age the meat appropriately, then supply it to markets hoping that its eating quality meets the needs of the consumer with little measure of its attributes in the market place. The use of non-invasive methods such as NIR and NMR to predict tenderness of meat early *post-mortem* so that processors can guarantee the tenderness of meat sold to consumers would add a new dimension to meat retailing and should be encouraged.
- (ii) The current practice of applying various electrical stimulation waveforms and durations without determining the appropriateness for the markets needs to be evaluated. Over-stimulation has been raised as an issue but that is hardly likely to be important as the animals physiologically cannot respond past a certain point. Additionally, multiple electrical inputs separated by significant intervals appears to toughen meat whatever the duration. A system that will customise the electrical input to each carcass for maximum quality and consistency was developed at AgResearch MIRINZ and work is continuing in New Zealand. This system has not yet been sufficiently reported on in the public domain to speculate on its viability.
- (iii) Ageing is fine for table meats but is of less importance or even detrimental to the functionality of manufacturing meats (Farouk and Wieliczko, 2003). Thus selective stimulation of muscles could be beneficial. Individual muscle stimulation can be combined with hot-boning and immersion chilling to produce cuts that are optimised for table or manufacturing purposes.
- (iv) Maintenance of muscles at rest length or even slight stretching can be achieved if the muscles are removed from the carcass and wrapped or squeezed into a tube or mould. Thus, methods to simply achieve stretching that are commercially attractive should be developed. Whole carcass rather than individual muscle *pre-rigor* stretching through mechanical and electrical manipulation of the carcasses also offers a means of accelerating the ageing of meat and should be investigated.
- (v) While high pressure, ultrasound and hydrodyne technologies can improve tenderness, methods need to be developed to commercialisation.

24.7 Sources of further information and advice

- Jeremiah, L. E., Gibson, L. L. and Cunningham, B. (1999), The influence of mechanical tenderization on the palatability of certain bovine muscles. *Food Research International*, 32, 585–591.
- Koohmaraie, M. and Geesink, G. H. (2006), Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science*, 74, 34–43.

- Ouali, A., Obled, A., Cottin, P., Merdaci, N., Ducastaing, A. and VALIN, C. (1983), Comparative effects of post-mortem storage and low-calcium-requiring neutral proteinase on bovine and rabbit myofibrillar proteins. *Journal of the Science of Food and Agriculture*, 34, 466–476.
- Rosenvold, K. and Andersen, H. J. (2003), Factors of significance for pork quality – A review. *Meat Science*, 64, 219–237.
- Savell, J. W., Mueller, S. L. and Baird, B. E. (2005), The chilling of carcasses. *Meat Science*, 70, 449–459.
- Sørheim, O. and Hildrum, K. I. (2002), Muscle stretching techniques for improving meat tenderness. *Trends in Food Science and Technology*, 13, 127–135.

24.8 References

- Ahnström, M. L., Enfalt, A.-C., Hansson, I. and Lundström, K. (2006a), Pelvic suspension improves quality characteristics in *M. semimembranosus* from Swedish dual purpose young bulls. *Meat Science*, 72, 555–559.
- Ahnström, M. L., Seyfert, M., Hunt, M. C. and Johnson, D. E. (2006b), Dry aging of beef in a bag highly permeable to water vapour. *Meat Science*, 73, 674–679.
- Allingham, P. G., Harper, G. S. and Hunter, R. A. (1998), Effect of growth path on the tenderness of the *semitendinosus* muscle of Brahman-cross steers. *Meat Science*, 48, 65–73.
- Andersen, H. J., Oksbjerg, N., Young, J. F. and Therkildsen, M. (2005), Feeding and meat quality – a future approach. *Meat Science*, 70, 543–554.
- Anon (2003), Can good beef have 20% added water? *Australian Meat News*, November.
- Barnier, V. M. H., Wiklund, E., Vandijk, A., Smulders, F. J. M. and Malmfors, G. (1999), Proteolytic enzyme and inhibitor levels in reindeer (*Rangifer tarandus tarandus*) vs. bovine longissimus muscle, as they relate to ageing rate and response. *Rangifer*, 19, 13–18.
- Baublits, R. T., Meullenet, J. F., Sawyer, J. T., Mehaffey, J. M. and Saha, A. (2006), Pump rate and cooked temperature effects on pork loin instrumental, sensory descriptive and consumer-rated characteristics. *Meat Science*, 72, 741–750.
- Bendall, J. R. (1973), The biochemistry of rigor mortis and cold-contraction. *Proceedings of the 19th European Meeting of Meat Research Workers*, 1–27.
- Bouton, P. E., Fisher, A. L., Harris, P. V. and Baxter, R. I. (1973), A comparison of the effects of some post-slaughter treatments on the tenderness of beef. *Journal of Food Technology*, 8, 39–49.
- Briskey, E. J. (1964), Etiological Status and Associated Studies of Pale, Soft, Exudative Porcine Musculature. *Advances in Food Research*, 13, 89–178.
- Campbell, R. E., Hunt, M. C., Levis, P. and Chambers IV, E. (2001), Dry-aging effects on palatability of beef longissimus muscle. *Journal of Food Science*, 66, 196–199.
- Cheftel, J. C. and Culioli, J. (1997), Effects of high pressure on meat: A review. *Meat Science*, 46, 211–236.
- Chrystall, B. B. and Devine, C. E. (1983), Electrical stimulation of deer carcasses. *New Zealand Journal of Agricultural Research*, 26, 89–92.
- Chrystall, B. B. and Hagyard, C. J. (1976), Electrical stimulation and lamb tenderness. *New Zealand Journal of Agricultural Research*, 19, 7–11.
- Claus, J. R., Schilling, J. K., Marriort, N. G., Duncan, S. E., Solomon, M. B. and Wang, H. (2001), Hydrodynamic shockwave tenderization effects using a cylinder processor on early deboned broiler breasts. *Meat Science*, 58, 287–292.
- Claus, J. R., Wang, H. and Marriort, N. G. (1997), Prerigor carcass muscle stretching effects on tenderness of grain-fed beef under commercial conditions. *Journal of Food Science*, 62, 1231–1234.
- Davey, C. L. and Chrystall, B. B. (1980), Conditions for an efficient post-mortem electrical stimulation. *Ann Technol Agric*, 29, 547–561.

- Davey, C. L. and Gilbert, K. V. (1976), The Temperature Coefficient of Beef Ageing. *Journal of Science of Food and Agriculture*, 27.
- Davey, C. L., Gilbert, K. V. and Carse, W. A. (1976), Carcass electrical stimulation to prevent cold shortening toughness in beef. *New Zealand Journal of Agricultural Research*, 19, 13–18.
- Davey, C. L., Kuttel, H. and Gilbert, K. V. (1967), Shortening as a factor of meat ageing. *Journal of Food Technology*, 2, 53–56.
- Devine, C. E., Hopkins, D. L., Hwang, I. H., Ferguson, D. M. and Richards, I. (2004), Electrical Stimulation. In W. J. Jensen, C. E. Devine and Dikeman, M. (Eds.) *Encyclopedia of Meat Science*. Oxford: Academic Press.
- Devine, C. E., Lowe, T. E., Wells, R. W., Edwards, N. J., Edwards, J. E. H., Starbuck, T. J. and Speck, P. A. (2006), Pre-slaughter stress arising from on-farm handling and its interactions with electrical stimulation on tenderness of lambs. *Meat Science*, 73, 304–312.
- Devine, C. E., Wahlgren, N. M. and Tornberg, E. (1999), Effect of rigor temperature on muscle shortening and tenderisation of restrained and unrestrained beef m. longissimus thoracicus et lumborum. *Meat Science*, 51, 61–72.
- Dransfield, E., Jones, R. C. D. and Macfie, H. J. H. (1981), Quantifying changes in tenderness during storage of beef. *Meat Science*, 5, 131–137.
- Drew, K. R., Crosbie, S. F., Forss, D. A., Manley, T. R. and Pearse, A. J. (1988), Electrical stimulation and ageing of carcasses from red, fallow and New Zealand wapiti-type male deer. *Journal of the Science of Food and Agriculture*, 43, 245–259.
- Dutson, T. R., Yates, L. D., Smith, G. C., Carpenter, Z. L. and Hostetler, R. L. (1977), Rigor onset before chilling. *Proceedings of the 30th Annual Reciprocal Meat Conference*, 30, 79–86.
- Elgasim, E. A. and Kennick, W. H. (1982), Effect of high hydrostatic pressure on meat microstructure. *Food Microstructure*, 1, 75–82.
- Farouk, M. M., Beggan, M., Hurst, S., Stuart, A., Dobbie, P. M. and Bekhit, A. E. D. (2007), Meat quality attributes of chilled venison and beef. *Journal of Food Quality*, 30, 1023–1039.
- Farouk, M. M., Graham, D. M., Wood, T., Hafegee, I. I., Collinson, M. M. and Simmons, N. J. (2005), Development of a muscle stretching device: Effect on meat quality. *MIRINZ report CR 1039*.
- Farouk, M. M. and Price, J. F. (1994), The effect of post-exsanguination infusion on the composition, exudation, color and post-mortem metabolic changes in lamb. *Meat Science*, 38, 477–496.
- Farouk, M. M., Price, J. F. and Salih, A. M. (1992a), Post-exsanguination infusion of ovine carcasses: Effect on tenderness indicators and muscle microstructure. *J. Food Sci.*, 57, 1311–1315.
- Farouk, M. M., Price, J. F., Salih, A. M. and Burnett, R. J. (1992b), The effect of postexsanguination infusion of beef on composition, tenderness, and functional properties. *Journal of Animal Science*, 70, 2773–2778.
- Farouk, M. M. and Wieliczko, K. J. (2003), Optimum Time for Using Chilled Beef in Gelled Products. *Journal of Food Science*, 68, 164–167.
- Fisher, A. V., Nute, G. R., Fursey, G. A. J. and Cook, G. (1994), Post mortem manipulation of beef quality. *Meat Focus International*, 3, 62–65.
- Gann, G. L. and Merkel, R. A. (1978), Ultrastructural changes in bovine *Longissimus* muscle during postmortem ageing. *Meat Science*, 2, 129–144.
- Geesink, G. H., Van Laack, R. L. J. M., Barnier, V. M. H. and Smulders, F. J. M. (1994), Does electrical stimulation affect the speed of ageing or ageing response? *Sciences des Aliments*, 14, 409–422.
- George, A. R., Bendall, J. R. and Jones, R. C. D. (1980), The tenderising effect of electrical stimulation of beef carcasses. *Meat Science*, 4, 51–68.
- Got, F., Culioli, J., Berge, P., Vignon, X., Astruc, T., Quideau, J. M. and Lethiecq, M. (1999),

- Effects of high-intensity high-frequency ultrasound on ageing rate, ultrastructure and some physico-chemical properties of beef. *Meat Science*, 51, 35–42.
- Gruber, S. L., Tatum, J. D., Scanga, J. A., Chapman, P. L., Smith, G. C. and Belk, K. E. (2006), Effects of postmortem aging and USDA quality grade on Warner–Bratzler shear force values of seventeen individual beef muscles. *Journal of Animal Science*, 84, 3387–3396.
- Hagyard, C. J. (1979), Aging regimes for electrically stimulated lamb. *MIRINZ Publication No. RM 88*.
- Hannula, T. and Puolanne, E. (2004), The effect of cooling rate on beef tenderness: The significance of pH at 7 degrees C. *Meat Science*, 67, 403–408.
- Homma, N., Ikeuchi, Y. and Suzuki, A. (1994), Effects of high pressure treatment on the proteolytic enzymes in meat. *Meat Science*, 38, 219–228.
- Homma, N., Ikeuchi, Y. and Suzuki, A. (1995), Levels of calpain and calpastatin in meat subjected to high pressure. *Meat Science*, 41, 251–260.
- Hoogenkamp, H. W. (2003), The case for enhancement. *Asia Pacific Food Industry*, 15.
- Hostetler, R. L., Landmann, W. A., Link, B. A. and Fitzhugh H.A. JR. (1970), Influence of carcass position during rigor mortis on tenderness of beef muscles: Comparison of two treatments. *Journal of Animal Science*, 31, 47–50.
- Hwang, I. H., Devine, C. E. and Hopkins, D. L. (2003), The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. *Meat Science*, 65, 677–691.
- Jayasooriya, S. D., Torley, P. J., D'Arcy, B. R. and Bhandari, B. R. (2007), Effect of high power ultrasound and ageing on the physical properties of bovine *Semitendinosus* and *Longissimus* muscles. *Meat Science*, 75, 628–639.
- Jeremiah, L. E., Gibson, L. L. and Cunningham, B. (1999), The influence of mechanical tenderization on the palatability of certain bovine muscles. *Food Research International*, 32, 585–591.
- Karmas, E. (1970), *Fresh Meat Processing*, Noyes Data Co., Parkridge, NJ, USA.
- Kolle, B. K., McKenna, D. R. and Savell, J. W. (2004), Methods to increase tenderness of individual muscles from beef rounds when cooked with dry or moist heat. *Meat Science*, 68, 145–154.
- Koohmaraie, M. (1996), Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Science*, 43, 193–201.
- Koohmaraie, M., Babiker, A. S., Schroeder, A. L., Merkel, R. A. and Dutson, T. R. (1988), Acceleration of Postmortem Tenderization in Ovine Carcasses Through Activation of Ca^{2+} -Dependent Proteases. *Journal of Food Science*, 53, 1638–1641.
- Koohmaraie, M., Crouse, J. D. and Mersmann, H. J. (1989), Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: effect of concentration and ionic strength. *Journal of Animal Science*, 67, 934–942.
- Koohmaraie, M. and Geesink, G. H. (2006), Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science*, 74, 34–43.
- Kristensen, L., Therkildsen, M., Aaslyng, M. D., Oksbjerg, N. and Ertbjerg, P. (2004), Compensatory growth improves meat tenderness in gilts but not in barrows. *Journal of Animal Science*, 82, 3617–3624.
- Lennon, A. M., Moon, S. S., Ward, P., O'Neill, E. E. and Kenny, T. (2006), Effects of enhancement procedures on whole and re-formed beef forequarter muscles. *Meat Science*, 72, 513–517.
- Locker, R. H. (1960), Degree of muscular contraction as a factor in tenderness of beef. *Food Research*, 25, 304–307.
- Locker, R. H., Davey, C. L., Nottingham, P. M., Haughey, D. P. and Law, N. H. (1975), New concepts in meat processing. *Advances in Food Research*, 21, 157–222.
- Locker, R. H. and Hagyard, C. J. (1963), A Cold Shortening Effect in Beef Muscles. *Journal of the Science of Food And Agriculture*, 14, 787–793.
- Lundesjö Ahnström, M., Enfält, L., Johansson, J., Virhammar, K., Hansson, I., Johansson,

- L. and Lundström, K. (2003), Effect of pelvic suspension on sensory and instrumental evaluation on four beef muscles in heifers and young bulls. *49th International Congress of Meat Science and Technology*. Sao Paulo, Brazil.
- Lyng, J. G., Allen, P. and McKenna, B. (1998a), The effects of pre- and post-rigor high-intensity ultrasound treatment on aspects of lamb tenderness. *LWT – Food Science and Technology*, 31, 334–338.
- Lyng, J. G., Allen, P. and McKenna, B. M. (1997), The influence of high intensity ultrasound baths on aspects of beef tenderness. *Journal of Muscle Foods*, 8, 237–249.
- Lyng, J. G., Allen, P. and McKenna, B. M. (1998b), The effect on aspects of beef tenderness of pre- and post-rigor exposure to a high intensity ultrasound probe. *Journal of the Science of Food and Agriculture*, 78, 308–314.
- Ma, H.-J. and Ledward, D. A. (2004), High pressure/thermal treatment effects on the texture of beef muscle. *Meat Science*, 68, 347–355.
- Maca, J. V., Miller, R. K., Maca, J. D. and Acuff, G. R. (1997), Microbiological, sensory and chemical characteristics of vacuum-packaged cooked beef top rounds treated with sodium lactate and sodium propionate. *Journal of Food Science*, 62, 586–590 + 596.
- MacFarlane, J. J. (1973), Pre-rigor pressurization of muscle: Effects on pH, shear value and taste panel assessment. *Journal of Food Science* 38, 294–298.
- MacFarlane, J. J. (1974), Pressure-induced solubilization of meat proteins in saline solution. *Journal of Food Science*, 39, 542–547.
- MacFarlane, J. J. and McKenzie, I. J. (1976), Pressure-induced solubilization of myofibrillar proteins. *Journal of Food Science*, 41, 1442–1446.
- MacFarlane, J. J., McKenzie, I. J., Turner, R. H. and Jones, P. N. (1984), Binding of comminuted meat: Effect of high pressure. *Meat Science*, 10, 307–320.
- Moeller, S., Wulf, D., Meeker, D., Ndife, M., Sundararajan, N. and Solomon, M. B. (1999), Impact of the Hydrodyne Process on Tenderness, Microbial Load, and Sensory Characteristics of Pork *Longissimus* Muscle. *Journal of Animal Science*, 77, 2119–2123.
- Novakofski, J. and Brewer, S. (2006), The paradox of toughening during the aging of tender steaks. *Journal of Food Science*, 71.
- Offer, G. (1991), Modelling of the formation of pale, soft and exudative meat: Effects of chilling regime and rate and extent of glycolysis. *Meat Science*, 30, 157–184.
- Offer, G. and Knight, P. (1988), The structural basis of water-holding in meat. In Lawrie, R. A. (Ed.) *Developments in Meat Science*. London, Elsevier.
- Ouali, A. (1992), Proteolytic and physicochemical mechanisms involved in meat texture development. *Biochimie*, 74, 251–265.
- Ouali, A., Obled, A., Cottin, P., Merdaci, N., Ducastaing, A. and Valin, C. (1983), Comparative effects of post-mortem storage and low-calcium-requiring neutral proteinase on bovine and rabbit myofibrillar proteins. *Journal of the Science of Food and Agriculture*, 34, 466–476.
- Petch, P. E. and Gilbert, K. V. (1997), Interaction of electrical processes applied during slaughter and dressing with stimulation requirements. *43rd International Conference of Meat Science and Technology*. Auckland, New Zealand.
- Pietrasik, Z. and Shand, P. J. (2004), Effect of blade tenderization and tumbling time on the processing characteristics and tenderness of injected cooked roast beef. *Meat Science*, 66, 871–879.
- Pietrasik, Z. and Shand, P. J. (2005), Effects of mechanical treatments and moisture enhancement on the processing characteristics and tenderness of beef *semimembranosus* roasts. *Meat Science*, 71, 498–505.
- Pisula, A. and Tyburcy, A. (1996), Hot processing of meat. *Meat Science*, 43, 125–134.
- Polidori, P., Lee, S., Kauffman, R. G. and Marsh, B. B. (1999), Low voltage electrical stimulation of lamb carcasses: Effects on meat quality. *Meat Science*, 53, 179–182.
- Pulford, D. J., Fraga Vazquez, S., Frost, D. F., Fraser-Smith, E., Dobbie, P. and Rosenvold, K. (2008), The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. *Meat Science*, 79, 623–630.

- Pulford, D. J., Rosenvold, K., Frost, D. F., Fraga Vazquez, S., Dobbie, P., Fraser-Smith, E., Stuart, A. and Farouk, M. M. (2007), The contribution of pH towards endopeptidase activity and small heat shock protein distribution in beef and its role with achieved tenderness. *53rd International Congress of Meat Science and Technology*. Beijing, China.
- Ramsbottom, J. M. and Strandine, E. J. (1948), Comparative tenderness and identification of muscles in wholesale beef cuts. *Food Research*, 13.
- Rees, M. P., Trout, G. R. and Warner, R. D. (2002), Tenderness, ageing rate and meat quality of pork *M. longissimus thoracis et lumborum* after accelerated boning. *Meat Science*, 60, 113–124.
- Rhee, M. S., Wheeler, T. L., Shackelford, S. D. and Koohmaraie, M. (2004), Variation in palatability and biochemical traits within and among eleven beef muscles. *Journal of Animal Science*, 82, 534–550.
- Robbins, K., Jensen, J., Ryan, K. J., Homco-Ryan, C., McKeith, F. K. and Brewer, M. S. (2003a), Consumer attitudes towards beef and acceptability of enhanced beef. *Meat Science*, 65, 721–729.
- Robbins, K., Jensen, J., Ryan, K. J., Homco-Ryan, C., McKeith, F. K. and Brewer, M. S. (2003b), Dietary vitamin E supplementation effects on the color and sensory characteristics of enhanced beef steaks. *Meat Science*, 64, 279–285.
- Robbins, K., Jensen, J., Ryan, K. J., Homco-Ryan, C., McKeith, F. K. and Brewer, M. S. (2003c), Effect of dietary vitamin E supplementation on textural and aroma attributes of enhanced beef clod roasts in a cook/hot-hold situation. *Meat Science*, 64, 317–322.
- Rosenvold, K. and Andersen, H. J. (2003), Factors of significance for pork quality – A review. *Meat Science*, 64, 219–237.
- Rosenvold, K., Borup, U. and Therkildsen, M. (2006a), Tender Pork Through Stepwise Chilling. *52nd International Conference of Meat Science and Technology*. Dublin, Ireland.
- Rosenvold, K., Clausen, I. and Madsen, N. T. (2006b), Mechanical tenderisation and enhancement improve eating quality of beef from dairy cows. *52nd International Conference of Meat Science and Technology*. Dublin, Ireland.
- Rosenvold, K., North, M., Devine, C. E., Micklander, E., Hansen, P. W., Dobbie, P. M. and Wells, R. (2008), The protective effect of electrical stimulation and wrapping on beef tenderness at high pre rigor temperatures. *Meat Science*, 79, 299–306.
- Roth, E. (2002a), Method for producing a pH enhanced meat product. *United States Patent Number US2002/0127314A1*.
- Roth, E. (2002b), pH enhanced meat composition and method for producing a pH enhanced meat composition. *United States Patent Number US2002/0150659A1*.
- Savell, J. W., Mueller, S. L. and Baird, B. E. (2005), The chilling of carcasses. *Meat Science*, 70, 449–459.
- Savell, J. W., Smith, G. C., Dutson, T. R., Carpenter, Z. L. and Suter, D. A. (1977), Effect of electrical stimulation on palatability of beef, lamb and goat meat. *J. Food Sci.*, 42, 702–706.
- Sears, N. A. (1989), New tenderizing process gets good reception. *The National Provisioner*, December 11.
- Sentandreu, M. A., Coulis, G. and Ouali, A. (2002), Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science and Technology*, 13, 398–419.
- Simmons, N. J., Daly, C. C., Mudford, C. R., Richards, I., Jarvis, G. and Pleiter, H. (2006), Integrated technologies to enhance meat quality – An Australasian perspective. *Meat Science*, 74, 172–179.
- Sims, K. L., Wicklund, E., Clhutchison, Mulley, R. C. and Littlejohn, R. P. (2004), Effects of pelvic suspension on the tenderness of meat from Fallow deer (*Dama dama*). *Abstracts 50th International Congress of Meat Science and Technology*, 119.
- Smulders, F. J. M., Barnier, V. M. H., Geesink, G. H. and van Laack, R. (1995), The muscle

- biological background of meat tenderness. In Lundström, K., Hannsson, I. and Wiklund, E. (Eds.) *Composition of Meat in Relationship to Processing, Nutritional and Sensory Quality. From Farm to Fork*. Utrecht, ECCEAMST.
- Smulders, F. J. M., Eikelenboom, G., Lambooy, E. and Van Logtestijn, J. G. (1989), Electrical stimulation during exsanguination: Effects on the prevalence of blood splash and on sensory quality characteristics in veal. *Meat Science*, 26, 89–99.
- Smulders, F. J. M., Toldra, F., Flores, J. and Prieto, M. (1992), *New Technologies for Meat and Meat Products*. Utrecht, The Netherlands: Audet Tijdschriften, 182, 186–188.
- Solomon, M. B., Carpenter, C. E., Snowden, G. D. and Cockett, N. B. (1998), Tenderizing callipyge lamb with the hydrodyne process and electrical stimulation. *Journal of Muscle Foods*, 9, 305–311.
- Solomon, M. B., Long, J. B. and Eastridge, J. S. (1997), The Hydrodyne: A New Process to Improve Beef Tenderness. *Journal of Animal Science*, 75, 1534–1537.
- Sørheim, O. and Hildrum, K. I. (2002), Muscle stretching techniques for improving meat tenderness. *Trends in Food Science and Technology*, 13, 127–135.
- Stephens, J. W., Dikeman, M. E., Unruh, J. A., Haub, M. D. and Tokach, M. D. (2006), Effects of pre-rigor injection of sodium citrate or acetate, or post-rigor injection of phosphate plus salt on post-mortem glycolysis, pH, and pork quality attributes. *Meat Science*, 74, 727–737.
- Stiebing, A. and Karnitzschky, I. (1996), A new type of packaging for fresh meat without using vacuum. *Neuartige frischfleischverpackung ohne vakuumanwendung, Fleischwirtschaft*, 76, 1087–1092.
- Støier, S., Aaslyng, M. D., Olsen, E. V. and Henckel, P. (2001), The effect of stress during lairage and stunning on muscle metabolism and drip loss in Danish pork. *Meat Science*, 59, 127–131.
- Stolowski, G. D., Baird, B. E., Miller, R. K., Savell, J. W., Sams, A. R., Taylor, J. F., Sanders, J. O. and Smith, S. B. (2006), Factors influencing the variation in tenderness of seven major beef muscles from three Angus and Brahman breed crosses. *Meat Science*, 73, 475–483.
- Suttie, J. M., Goodall, E. D., Pennie, K. and Kay, R. N. (1983), Winter food restriction and summer compensation in red deer stags (*Cervus elaphus*). *British Journal of Nutrition*, 50, 737–747.
- Suttie, J. M. and Webster, J. R. (1998), Are arctic ungulates physiologically unique? *Rangifer*, 18, 99–118.
- Therkildsen, M. (2005), Muscle protein degradation in bull calves with compensatory growth. *Livestock Production Science*, 98, 205–218.
- Thompson, J. (2002), Managing meat tenderness. *Meat Science*, 62, 295–308.
- Thompson, J. M., Hopkins, D. L., D'Souza, D. N., Walker, P. J., Baud, S. R. and Pethick, D. W. (2005), The impact of processing on sensory and objective measurements of sheep meat eating quality. *Australian Journal of Experimental Agriculture*, 45, 561–573.
- Thompson, J. M., Perry, D., Daly, B., Gardner, G. E., Johnston, D. J. and Pethick, D. W. (2006), Genetic and environmental effects on the muscle structure response post-mortem. *Meat Science*, 74, 59–65.
- Toohy, E. S. and Hopkins, D. L. (2006), Eating quality of commercially processed hot boned sheep meat. *Meat Science*, 72, 660–665.
- Tornberg, E. (1996), Biophysical Aspects of Meat Tenderness. *Meat Science*, 43, S175–S191.
- Vote, D. J., Platter, W. J., Tatum, J. D., Schmidt, G. R., Belk, K. E., Smith, G. C. and Speer, N. C. (2000), Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. *Journal of Animal Science*, 78, 952–957.
- Wahlgren, N. M., Goransson, M., Linden, H. and Willhammar, O. (2002), Reducing the influence of animal variation and ageing on beef tenderness. *Proceedings 48th International Congress of Meat Science and Technology*, Rome, Italy, 240–241.

- Wang, H., Claus, J. R. and Marriott, N. G. (1994), Selected skeletal alterations to improve tenderness of beef round muscles. *J. Muscle Foods*, 5, 137–147.
- Wand, H., Claus, J. R. and Marriott, N. G. (1995), A research note: Tenderness of prerigor stretched porcine longissimus muscle. *J. Muscle Foods*, 6, 75–82.
- Watanabe, A., Daly, C. C. and Devine, C. E. (1996), The effects of the ultimate pH of meat on tenderness changes during ageing. *Meat Science*, 42, 67–78.
- Watanabe, A. and Devine, C. (1996), Effect of meat ultimate pH on rate of titin and nebulin degradation. *Meat Science*, 42, 407–413.
- West, R. L. (1983), Functional characteristics of hot-boned meat. *Food Technol.*, 37, 57–66.
- White, A., O'Sullivan, A., Troy, D. J. and O'Neill, E. E. (2006), Effects of electrical stimulation, chilling temperature and hot-boning on the tenderness of bovine muscles. *Meat Science*, 73, 196–203.
- Whittaker, A. D., Park, B., Thane, B. R., Miller, R. K. and Savell, J. W. (1992), Principles of ultrasound and measurement of intramuscular fat. *Journal of Animal Science*, 70, 942–952.
- Wiklund, E., Barnier, V. M. H., Smulders, F. J. M., Lundstrom, K. and Malmfors, G. (1997), Proteolysis and tenderisation in reindeer (*Rangifer tarandus tarandus* L.) bull longissimus thoracis muscle of varying ultimate pH. *Meat Science*, 46, 33–43.
- Wiklund, E., Finstad, G., Johansson, L., Aguiar, G. and Bechtel, P. J. (2008), Carcass composition and yield of Alaskan reindeer (*Rangifer tarandus tarandus*) steers and effects of electrical stimulation applied during field slaughter on meat quality. *Meat Science*, 78, 185–193.
- Wiklund, E., Mulley, R. C., Hutchison, C. L. and Littlejohn, R. P. (2004), Effect of carcass suspension method on water holding capacity of *M. longissimus* from fallow deer (*Dama dama*) and lamb. *Proceedings 50th International Congress of Meat Science and Technology*. Helsinki, Finland.
- Wiklund, E., Stevenson-Barry, J. M., Duncan, S. J. and Littlejohn, R. P. (2001), Electrical stimulation of red deer (*Cervus elaphus*) carcasses – Effects on rate of pH-decline, meat tenderness, colour stability and water-holding capacity. *Meat Science*, 59, 211–220.
- Yancey, E. J., Dikeman, M. E., Addis, P. B., Katsandidis, E. and Pullen, M. (2002), Effects of vascular infusion with a solution of saccharides; sodium chloride; phosphates; and vitamins C, E, or both on carcass traits, Warner–Bratzler shear force, and palatability traits of steaks and ground beef. *Journal of Animal Science*, 80, 1904–1910.
- Zayats, Y. F. and Orlova, T. N. (1970), Ultrasonic vibrations for improving meat tenderness. *Izvestiya Vysshikh Uchebnykh Zavedenii, Pishchevaya Tekhnologiya*, 4.

Sensory and quality properties of packaged meat

M. G. O'Sullivan and J. P. Kerry, University College Cork, Ireland

Abstract: This chapter focuses on the sensory and quality properties of packaged meat. The chapter first reviews packaged meat systems, including modified atmosphere packaging (MAP). It then discusses packaged meat with respect to colour changes, lipid oxidation, catalysis of lipid oxidation and tenderness. Finally, some future trends are anticipated.

Key words: meat, modified atmosphere packaging, sensory, colour, lipid oxidation, tenderness.

25.1 Introduction

In order for any given food product to be commercially successful, consumer desires and demands must be addressed and met with respect to the sensory properties of such products, before other quality dimensions become relevant (Chambers and Bowers, 1993). Each food product category presents its own unique challenges in this regard and meat is no different. Unfortunately, fresh meat colour is short-lived and surface discoloration that occurs during chilled and frozen storage is considered a sign of un-wholesomeness and product deterioration (Faustman and Cassens, 1990). The bright, cherry-red colour of fresh beef is used by consumers as an indicator of meat quality (Cassens *et al.*, 1988; Kennedy *et al.*, 2004). In red meats, consumers relate the bright red colour to freshness, while discriminating against meat that has turned brown in colour (Hood and Riordan, 1973; Morrissey *et al.*, 1994). It is because of such sensory quality changes in fresh meat that so much attention has focused on developments within the area of packaging technologies, especially within the last 20–30 years. Colour perception plays a major role in consumer evaluation of meat quality (Lanari *et al.*, 1995; Risvik, 1994). In the case of beef, two important visual clues that determine

perceived quality are colour and packaging (Issanchou, 1996). MAP is defined as 'A form of packaging involving the removal of air from the pack and its replacement with a single gas or mixture of gasses' (Parry, 1993). Beef steaks are commonly displayed under high oxygen concentrations in modified atmosphere packs (MAP) in order to promote colour stability (Zakrys *et al.*, 2007). In the United States, there is also a high demand for fresh 'bone in' pork chops; however, colour deterioration of both muscle and bone limits marketing options (Lanari *et al.*, 1995). The colour of lamb may also be extended by storage under MAP conditions (Kerry *et al.*, 2000). The rate of discoloration of meat is believed to be related to the effectiveness of oxidation processes and enzymic reducing systems in controlling metmyoglobin levels in meat (Faustman and Cassens, 1989). Discoloration in retail meats during display conditions may occur as a combined function of muscle pigment oxidation (oxymyoglobin to metmyoglobin) and lipid oxidation in membrane phospholipids (Sherbeck *et al.*, 1995). The breakdown products of lipid oxidation have been associated with the development of off-flavours and off-odours, and loss of colour in meat (Faustman and Cassens, 1989). Many researchers believe transition metals, notably iron, play a role in initiating lipid oxidation either directly (Harel and Kanner, 1985) or indirectly by facilitating the generation of other initiating species.

The colour stability of meat products depends on variables such as muscle type, pH, storage temperature, oxygen availability and lighting type and intensity during display (Andersen and Skibstead, 1991). Additionally, different muscles exhibit different rates of oxymyoglobin oxidation under controlled conditions (Ledward, 1985). Furthermore, MAP meat products held in high oxygen (O_2) atmospheres may result in protein oxidation, which may have a negative effect on meat tenderness. Rowe and co-workers (2004) found that increased oxidation of muscle proteins early post-mortem could have negative effects on meat tenderness. Recent studies have indicated that storage under high O_2 atmospheres can result in a decrease in beef tenderness (Torngren, 2003). As such, the requirements for colour stability must be balanced against the deteriorative action of lipid oxidation.

Many attempts have been made to reduce pigment and lipid oxidation in meats through treatments with, for instance, antioxidants and MA packaging (O'Grady *et al.*, 2006; Carpenter *et al.*, 2007; O'Sullivan *et al.*, 1998). MAP is one of the principal methods of maintaining and prolonging meat colour sensory quality.

25.2 Packaged meat

The idea of MAP is by no means new. Killefer (1930), using 100% carbon dioxide (CO_2) at 4–7 °C, found that pork and lamb remained fresh for twice as long as equivalent products stored in air and held at similar temperatures. Subsequently, Callow worked on the shelf-life extension of bacon by packaging in CO_2 -enriched atmospheres (Callow, 1932). A further commercial application was used in the 1930s to transport refrigerated beef carcasses from Australia and New Zealand in a carbon dioxide-enriched environment (Floros and Matsos, 2005). MAP of retail-

sized meat packages was not introduced until the 1950s, in the form of vacuum packaging (Floros and Matsos, 2005). It was not until 1981 that gas-flushed fresh meat in plastic trays was introduced by Marks and Spencer (Inns, 1987). It is now used ubiquitously across the meat industry for many different meat products. Modified atmosphere (MA) packs usually contain mixtures of two or three gases: O₂ (to enhance colour stability), CO₂ (to inhibit microbiological growth), and N₂ (to maintain pack shape) (Sorheim *et al.*, 1999; Jakobsen and Bertelsen, 2000; Kerry *et al.*, 2006). The fact that these gases promote the overall quality of fresh red meat has been well established (Gill, 1996). High O₂ concentrations promote oxymyoglobin (OxyMb), the cherry-red form of myoglobin (O'Grady *et al.*, 2000). In order to optimise shelf-life, sensory quality and microbiological safety using MAP, the packaging system applied is highly product specific (Church and Parsons, 1995). By packaging beef in a MA and storing at low temperature, the shelf-life can be prolonged considerably (Young *et al.*, 1983; Gill and Penney, 1988). Beef and lamb are both red meats and share similar properties, but considerable differences in shelf-lives are apparent between them due to their relative susceptibility to chemical and microbial spoilage. In contrast to beef cuts, much of the surface of lamb is adipose tissue, which has a pH close to neutrality and has no significant respiratory activity (Robertson, 2006). The pH of beef is lower than that of lamb, thus making it less susceptible to microbial spoilage (Gill, 1989; Kerry *et al.*, 2000).

There are four categories of preservative packaging that can be used with raw muscle foods. These are vacuum packs (VP), high-oxygen modified atmosphere packs, (high O₂ MAP), low oxygen modified atmosphere packs, (low O₂ MAP) and controlled atmosphere packs (CAP) (Gill and Gill, 2005).

Vacuum packaging was one of the earliest forms of MAP methods developed commercially and still is extensively used for products such as primal cuts of fresh red meat and cured meats (Parry, 1993). Vacuum packs are composed of evacuated pouches or vacuum skin packs, in which a film of low gas permeability is closely applied to the surface of the product. Preservative effects are achieved by the development of an anaerobic environment within the pack (Gill and Gill, 2005). VP extends the storage life of chilled meats by maintaining an oxygen deficient environment within the pack (Bell *et al.*, 1996). Under good vacuum conditions, the oxygen level is reduced to less than 1%. Due to the barrier properties of the film used, entry of oxygen from the outside is restricted (Parry, 1993; Robertson, 2006). It is hoped that any residual O₂ in any remaining atmosphere, or dissolved in the product, will be removed by enzymatic reactions within the muscle tissue, or through other chemical reactions with tissue components (Gill and Gill, 2005). In the case of vacuum-packaged meat, respiration of the meat will quickly consume the vast majority of residual O₂, replacing it with CO₂, which eventually increases to 10–20% within the package (Taylor, 1985; Parry, 1993; Gill, 1996). As the capacity of the muscle tissue for removing O₂ is limited, the amount of O₂ remaining in the pack at the time of closure must be very small if the product is to be effectively preserved (Gill and Gill, 2005). Vacuum-packaged meat is unsuitable for the retail market because depletion of O₂ coupled with low O₂

permeability of the packaging film causes a change of meat colour from red to purple due to the conversion of oxymyoglobin to deoxymyoglobin. These are not acceptable meat colours to the consumer (Allen *et al.*, 1996; Parry, 1993). If fresh beef colour is not a bright cherry red, the meat may be considered undesirable or even spoiled. Bright red beef outsells discoloured beef (20% surface metmyoglobin) by a ratio of 2:1 (Hood and Riordan, 1973). Colour is perhaps the most important sensory attribute of a food because, if it is deemed unacceptable, the food will not be purchased and/or eaten, and consequently, all other sensory attributes lose significance (Clydesdale, 1978). American consumers have demonstrated a bias against the purchase of vacuum-packaged beef which displays the purple colour of deoxymyoglobin (Meischen *et al.*, 1987). A further disadvantage is the accumulation of drip during prolonged storage of meat in vacuum packs (Jeremiah *et al.*, 1992; Parry, 1993; Payne *et al.*, 1997). However, this can be partly overcome by vacuum skin packaging (VSP) using a film that fits very tightly to the meat surface, leaving little space for the accumulation of any fluid exudate (Hood and Mead, 1993). VSP involves production of a skin package in which the product is the forming mould. It was first introduced using an ionomer film, which softens on heating to such an extent that it can be draped over sharp objects without puncturing (Robertson, 2006). An advantage and disadvantage of this package is that it gives the product a unique look. The product shelf-life can be 15–22 days depending on the cut. Since the product is displayed in the myoglobin state, there is no loss of colour in the display case and oxidation issues are minimised with this type of package (Belcher, 2006).

High O₂ MAP packs contain atmospheres of O₂ and CO₂, and often N₂. O₂/CO₂ mixtures have been used commercially for a considerable time (Brody, 1970). A patent in 1970 specified a range of O₂ and CO₂ concentrations suitable for MAP beef (Georgala and Davidson, 1970). Results demonstrated that at least 60% O₂ is required to achieve a colour shelf-life of 9 days and the patent claims that a mixture of 80% O₂ plus 20% CO₂ keeps meat red for up to 15 days at 4 °C (Georgala and Davidson, 1970). Only O₂ and CO₂ have preservative effects (Gill and Gill, 2005). Okayama *et al.* (1995) observed that MAP using a high level of O₂ (70–80%), preserved the bright red colour of fresh meat. Typically, fresh red meats are stored in MAP containing 80% O₂:20% CO₂ (Georgala and Davidson, 1970) and cooked meats are stored in 70% N₂:30% CO₂ (Smiddy *et al.*, 2002). Beef steaks are commonly displayed under high oxygen concentrations in MAP in order to promote colour stability (Zakrys *et al.*, 2007). The major function of oxygen is to maintain the muscle pigment myoglobin in its oxygenated form, oxymyoglobin (Kerry *et al.*, 2006). However, CO₂ is highly soluble in both muscle and fat tissue and in the pack the O₂ will be respired by tissue and bacteria. MA packs usually contain mixtures of two or three gases: O₂, to enhance colour stability, CO₂ to inhibit growth of spoilage bacteria (Seideman and Durland, 1984) and the N₂ is used in MAP as an inert filler gas, either to reduce the proportions of the other gases or to maintain pack shape (Bell and Bourke, 1996; Sorheim *et al.*, 1999; Jakobsen and Bertelsen, 2000; Kerry *et al.*, 2006). High O₂ concentrations promote the development of oxymyoglobin (OxyMb), the cherry red form of myoglobin

(O'Grady *et al.*, 2000) but may impact negatively on the oxidative stability of muscle lipids and lead to development of undesirable flavours (Rhee and Ziprin, 1987; Estevez and Cava, 2004). Much work has been done on a distinctive off-flavour that develops rapidly in meat that has been precooked, chilled-stored and reheated. The term 'warmed over flavour' (WOF) has been adopted to identify this flavour deterioration (Renner and Labadie, 1993). High oxygen levels within MAP also promote oxidation of muscle lipids over time (O'Grady *et al.*, 1998). High O_2 -MAP increases lipid oxidation in meat; Jakobsen and Bertelsen (2000) in beef; Lund *et al.* (2007) in pork; Kerry *et al.* (2000) in lamb. Oxidation of polyunsaturated fatty acids not only causes the rapid development of meat rancidity, but also affects the colour, the nutritional quality and the texture of beef (Kanner, 1994). While high O_2 levels promote the oxidation of lipids, it is the membrane phospholipids that are particularly susceptible to oxidation processes, thereby causing the rapid development of meat rancidity (Renner, 1990).

Low O_2 MAP are generally packed with CO_2 , (usually enough to dissolve into the product) and also N_2 , while residual O_2 may be present or included during the packing process. Here again the CO_2 acts as the preservative and the N_2 maintains the pack shape. For low O_2 MAP, carbon monoxide (CO) may also be used as a gas for prolonging meat colour integrity. CO is a colourless, odourless and tasteless gas. It is produced mainly through incomplete combustion of carbon-containing materials (Sørheim *et al.*, 1997). Although a substantial increase in the shelf-life of meat can be obtained by using various MAs, it is often limited by discoloration due to the oxidation of oxymyoglobin to metmyoglobin. This discoloration can be prevented by the inclusion of a low level of CO in the gas mixture. Inclusion of 0.4% CO in conjunction with O_2 will not influence colour stability, metmyoglobin-reducing activity, or O_2 consumption. This is likely to be the result of greater formation of oxymyoglobin (oxyMb) in atmospheres containing 20–80% O_2 , which dominates or limits the ability of carboxymyoglobin (COMb) to form (Seyfert *et al.*, 2007). COMb is more resistant to oxidation than oxymyoglobin, owing to the stronger binding of CO to the iron–porphyrin site on the myoglobin molecule (Wolfe, 1980). However, one of the main consumer fears relating to the use of CO is the possible loss of quality due to a break in the cold chain causing deterioration in spite of its attractive appearance (Wilkinson *et al.*, 2006). CO also results in the development of off-odours, which may warn consumers of possible loss of quality (Knut and Nolet, 2006).

The storage life of chilled meat can be extended by packaging the product under CAP with N_2 or CO_2 (Gill and Molin, 1991). CAP do not change with respect to their atmospheres during storage and as such, have very low gas permeabilities. O_2 scavengers may also be incorporated to remove any residual O_2 that may have been included during the manufacturing process.

25.3 Colour changes and packaged meat

Most muscles are composed of a heterogeneous mixture of at least two or more

types of fibres. In pigs, the red muscle *M. psoas major* contains a higher percentage of Type IIA fibres and less Type IIB fibres when compared to the white muscle *M. longissimus dorsi* (Lauridsen *et al.*, 2000). O'Keefe and Hood (1982) reported that the colour stability of beef muscles follow the order: *longissimus* > *gluteus* > *psoas* and that the difference is due primarily to differences in O_2 consumption rate of these muscles.

Over time, the cherry red colour of oxymyoglobin is oxidised to the grey-brown pigment of metmyoglobin (Fig. 25.1). Oxymyoglobin is a heme protein in which iron exists in the ferrous form (Fe^{+2}), unlike metmyoglobin that possesses the ferric form (Fe^{+3}). The conversion of the ferrous to the ferric form is a result of oxidation (Liu *et al.*, 1995). Oxymyoglobin may be maintained in meat by delaying oxidation to metmyoglobin (Lynch *et al.*, 1999).

The formation of metmyoglobin from oxymyoglobin is positively correlated to lipid oxidation and appears to be dependent on antioxidant status (Yin *et al.*, 1993). Transition metals, notably iron, play a role in initiating lipid oxidation (Harel and Kanner, 1985). Free iron and copper accelerate the auto-oxidation of oxymyoglobin (Snyder and Skrydlant, 1966), the photo-oxidation of oxymyoglobin (Assef *et al.*, 1971), the oxidation of reducing agents, e.g. cysteine, glutathione, ascorbate, tocopherols reducing the antioxidative capacity of the muscle (Kanner *et al.*, 1988), and the propagation phase in lipid peroxidation (Harel and Kanner, 1985). Consequently, free iron, either directly or indirectly, stimulates discoloration of meat through acceleration of the oxidation of oxymyoglobin to metmyoglobin. After slaughter, the iron in meat is released from high molecular weight sources (e.g. hemoglobin, myoglobin, ferritin, hemosiderin) and made available to low molecular weight compounds such as amino acids, nucleotides and phosphates, with which it is believed to form chelates (Decker and Crum, 1993; Morrissey *et al.*, 1998). The colour stability of packaged pork chops can be improved by vitamin E supplementation in diets of slaughter pigs (Faustman and Cassens, 1990; Morrissey *et al.*, 1994; Lanari *et al.*, 1995). However, the principal method of improving the sensory colour quality of meat is through the use of MAP. Because consumers use meat colour as an indicator of freshness and wholesomeness, recent advances in MAP have focused on finding the correct blend of gases that maximises initial colour, colour stability, and shelf-life while also minimising microbial growth, lipid oxidation, and gaseous headspace (Mancini and Hunt, 2005). High-oxygen atmospheres (80% O_2) promote pigment oxygenation, and therefore prolong the time before metmyoglobin is visible on the muscle surface. The drawback to high O_2 MAP is that, although it maintains redness during storage, rancidity often develops in the meat while colour is still desirable (Jayasingh *et al.*, 2002). Beef steaks are commonly displayed under high O_2 concentrations in MAP in order to promote colour stability. However, such conditions may also cause quality deterioration through lipid oxidation and decreased tenderness (Zakrys *et al.*, 2007). It has been reported by Jakobsen and Bertelsen (2000) that while, O_2 levels higher than 20% were necessary to promote meat colour, package O_2 contents higher than 55% did not result in additional colour stabilising benefits. Ultra-low- O_2 atmospheres

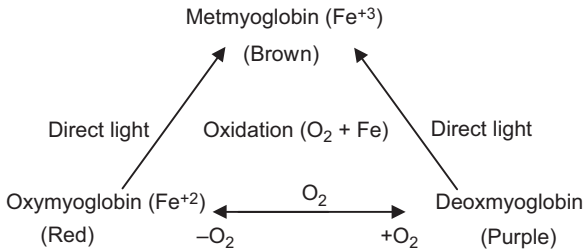


Fig. 25.1 Oxidation of oxymyoglobin and deoxymyoglobin to metmyoglobin.

minimise lipid oxidation and aerobic microbial growth; however, muscle reducing capacity coupled with poor blooming (deoxymyoglobin oxygenation) after prolonged storage can be major drawbacks to this system, especially if ultra-low levels of residual O₂ are not maintained. The levels of O₂ historically quoted (1–2%) are too high. Residual O₂ in meat packs needs to be less than 1% for pork and less than 0.05% for beef. If the meat lacks sufficient intrinsic O₂ consumption, O₂ scavengers may be required. CO has been added to packages to eliminate the disadvantages of commercial ultra-low O₂ MAP. It has a high affinity for myoglobin and forms a bright cherry red color on the surface of beef (Hunt *et al.*, 2004; Jayasingh *et al.*, 2001; Luno *et al.*, 2000; Sørheim *et al.*, 1999). Hunt *et al.* (2004) concluded that the use of 0.4% CO during storage in MAP improved beef colour without masking spoilage. Upon removal of product from CO packaging, meat colour (likely to be a combination of COMb and OMb) deteriorated during display in a manner not different from product exposed only to air.

Carpenter *et al.* (2001) showed that consumer preference for beef colour was sufficient to influence their likelihood to purchase, but was not enough to bias taste scores. It is likely that once a decision to purchase beef is made in the market, whether the beef is the red of fresh bloomed beef, the brown of discounted beef, or the purple of vacuum-packaged beef, consumer eating satisfaction at home will depend only on the beef quality attributes of tenderness, juiciness and flavour.

25.4 Lipid oxidation and packaged meat

Lipid and protein oxidation are closely associated deteriorative processes occurring in meat, although relatively little is known about the repercussions of the latter on the quality of meat products (Rhee and Ziprin, 1987; Estevez, and Cava, 2004). The products of fatty acid oxidation produce off-flavours and odours usually described as rancid (Gray and Pearson, 1994). Oxidation of polyunsaturated fatty acids causes not only the rapid development of meat rancidity, but also affects the colour, the nutritional quality and the texture of beef (Kanner, 1994). Lipid

oxidation in muscle systems is initiated at the membrane level in the phospholipid fractions as a free-radical autocatalytic chain mechanism (Labuza, 1971) in which pro-oxidants interact with unsaturated fatty acids resulting in the generation of free radicals and propagation of the oxidative chain (Asghar *et al.*, 1988). Tichivangana and Morrissey (1985) revealed that, in raw meat and model emulsions, ferric haematin pigments are powerful catalysts of lipid oxidation. The relationship of rancidity to flavour is unclear. As rancid flavours develop, there is a loss of desirable flavour notes (Campo *et al.*, 2006).

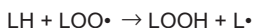
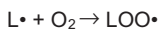
Rapid oxidation changes can also occur in raw meat when processed by modern-day technologies (St. Angelo and Bailey, 1987). Although raw meat is generally considered less susceptible to WOF than heated meat, after grinding and exposure to air it rapidly develops odours that are similar to those in oxidised cooked meats (Sato and Hegarty, 1971). This phenomenon is also referred to as WOF (Pearson *et al.*, 1977).

It is difficult to determine the limiting point at which beef can be rejected due to lipid oxidation, based on sensory perceptions (Campo *et al.*, 2006). Greene and Cumuze (1981) reported that oxidised flavour in beef was detected over a broad range of TBARS from 0.6–2.0 mg MDA/kg, indicating a big variation in the threshold of the panellists. The general population of meat consumers would not detect oxidation flavours until oxidation products reached levels of at least 2.0 mg/kg tissue. Perceptions will depend upon personal thresholds, which can vary due to experience, among other factors. But thresholds indicate the point from which stimuli can be perceived, not necessarily from which the stimuli may produce rejection of the product (Campo *et al.*, 2006). Zakrys *et al.* (2007) investigated the effects of O₂ concentration (0%, 10%, 20%, 50% and 80%) on the quality of MAP beef steaks (*M. longissimus dorsi*) stored at 4 °C for 15 days and tested the experimental samples for lipid oxidation, and sensory acceptability (up to day 12) by trained panellists ($n = 16$) of the resulting cooked meat. They reported that the quality of steaks was best promoted by packaging under atmospheres containing 50% O₂. These authors postulated that, because the measured TBARS levels were below 2 mg MDA/kg, it could be that the panellists found the higher O₂ samples more acceptable in their experiment due to a flavour adaptation effect within the population. The 50% O₂ samples produced mean levels of 1.0885 mg MDA/kg, whereas the 80% O₂ samples produced mean levels of 1.329 mg MDA/kg. Sensory panel results indicated that the 50% O₂ samples were more acceptable to panellists, even when offered samples held at 80% O₂, indicating that this group had reached a threshold limit with respect to the actual oxidative flavour levels at 50% O₂. These results are in agreement with the findings of Campo *et al.* (2006), who indicate that a TBARS value of 2 could be considered the limiting point from where rancid flavour overpowers beef flavour, and therefore should be considered as the maximum level for positive sensory perception of cooked beef. From that point onwards, we can expect beef to be rejected due to a strong sensory perception of lipid oxidation. Zakrys *et al.* (2008) also performed a consumer study ($n = 137$) using fresh beef MAP steaks stored at 4 °C for 12 days, packed in atmospheres which included 40%, 50%, 60%, 70% and 80% oxygen, with all packs containing

1. Initiation:



2. Propagation:



3. Termination:

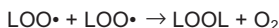


Fig. 25.2 Mechanism and stages of lipid oxidation.

20% CO₂ and the make up gas N₂. The results from these 134 consumers indicated that samples packed in 40% O₂ were the most acceptable and liked by panellists, followed by 80% O₂ packed samples. It appeared that here consumers did not find oxidised flavour objectionable in high oxygen-packed samples.

25.5 Catalysis of lipid oxidation

The free-radical chain mechanism involved in the oxidation of unsaturated fatty acids is thought to occur in three stages: (i) initiation, the formation of free-radicals; (ii) propagation, the free-radical chain reactions; (iii) termination, the formation of non-radical products (Tappel, 1962) (Fig. 25.2). This mechanism depends on the presence of preformed fatty acid hydroperoxides, which react with haem compounds and undergo homolytic decomposition. The alkoxy (LO•) radical formed in turn can propagate the peroxidation reaction. Lipid hydroperoxides may also be decomposed by ferrous iron (Fig. 25.3), and form very reactive alkoxy radicals. However, ferric iron produces the less reactive peroxy (LOO•) radicals from fatty acid hydroperoxides (Ingold, 1962).

The ability of haem pigments and non-haem iron to accelerate the propagation step of the free-radical chain mechanism can explain the rapid rate of oxidation in cooked meats (Pearson *et al.*, 1977). In uncooked muscle, pre-formed

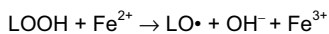


Fig. 25.3 Decomposition of lipid hydroperoxides by ferrous iron and the resultant formation of the very reactive alkoxy radicals.

lipid hydroperoxides are not present and the above mechanism of lipid peroxidation does not explain what initiates lipid oxidation in such systems. However, a number of mechanisms may be responsible. The spontaneous lipid radical formation or direct reaction of unsaturated fatty acids with molecular oxygen is thermodynamically unfavourable (Gray *et al.*, 1996). This is partly due to the principle of spin conservation, which applies to an addition reaction between ground state molecules (Chan, 1987). Molecular oxygen in the ground state contains two unpaired electrons and unsaturated fatty acids in the ground state contain no unpaired electrons. Thus, there is a spin barrier which prevents the direct addition of triplet O_2 to singlet-state unsaturated fatty acid molecules. This spin conservation principle may be overcome by several mechanisms including photo-oxidation, partially reduced or activated O_2 species such as hydrogen peroxide, superoxide anion, hydroxyl radical, active O_2 -iron complexes (ferryl radical) and iron-mediated homolytic cleavage of the hydroperoxides which generates organic free radicals (Hsieh and Kinsella, 1989). The area of lipid oxidation in biological systems has been well researched, but the evidence of proposed initiators seems more suggestive than conclusive. Transition metals, notably iron, are believed to be pivotal in the generation of species capable of abstracting a proton from an unsaturated fatty acid (Gutteridge and Halliwell, 1990; Kanner, 1994).

About two-thirds of body iron is found as haemoglobin and smaller amounts in myoglobin. A very small amount comprises components in various iron-containing enzymes and in the transport protein transferrin. The remainder is present in intracellular storage proteins such as ferritin and haemosiderine (Aisen and Listowsky, 1980). A small pool of non-protein, non-haem iron provides 'free' iron at micromolar concentrations in tissue. This small 'transit pool' of iron seems to be chelated to small molecules. The nature of these compounds has not been fully elucidated, but it may be that iron ions are attached to phosphate esters (ATP, ADP), organic acids (citrate), and perhaps to membrane lipids and DNA (Crichton and Charleaux-Wauters, 1987). These compounds are capable of decomposing H_2O_2 (hydrogen peroxide) or LOOH to form free radicals.

Ferritin is the main storage protein for iron in cells (Aisen and Listowsky, 1980). Iron can be released from ferritin and utilised by mitochondria for the synthesis of haemoproteins, e.g. myoglobin. Boyer and McCleary (1987) found that O_2 releases iron from ferritin and is the primary reductant in ascorbate-mediated ferritin iron release. Seman *et al.* (1991) suggested that ferritin may be responsible for catalysing lipid peroxidation in muscle foods. The relative contributions of different forms of iron, whether 'free' or protein bound, haem or non-haem, oxidised or reduced, in catalysing lipid oxidation in meats have not

been assigned definitively (Gray *et al.*, 1996). There is conflicting evidence about the roles of haem and non-haem iron (Sato and Hegarty, 1971; Johns *et al.*, 1989), and the adequacy of the muscle fibre model systems used in these studies to fully represent intact muscle has to be questioned. Kanner *et al.* (1991) contended that the aqueous extraction of muscle fibres might also extract several essential compounds such as enzymes, hydrogen peroxide, reducing compounds or chelating agents, which may affect the overall catalysis of muscle lipid oxidation *in situ*. Model systems with water-extracted muscle residues implied that myoglobin was not the principal pro-oxidant in meat and that non-haem iron was the main catalyst (Sato and Hegarty, 1971; Tichivangana and Morrissey, 1985). Similarly, model systems using linoleate contained pre-formed hydroperoxides, which produced erroneous results (Kanner, 1994). They could not simulate lipid peroxidation in muscle cells, which are mostly of a membranous nature, containing un-oxidised phospholipids. Most researchers using washed muscle tissues had omitted from the system several important compounds, which during exhaustive washing or dialysis were removed (Sato and Hegarty, 1971; Asghar *et al.*, 1988; Johns *et al.*, 1989). During incubation of minced muscle tissue H_2O_2 is generated (Harel and Kanner, 1985) and this could activate myoglobin or haemoglobin to feryl compounds, which initiate membrane lipid peroxidation. H_2O_2 is only one of the cytosolic compounds that could affect lipid peroxidation in muscle foods. They contain both pro- and anti-oxidants, which will affect *in situ* lipid peroxidation.

25.6 Tenderness and packaged meat

For many authors, tenderness is considered the most important qualitative characteristic of meat (Savell *et al.*, 1987, 1989; Smith *et al.*, 1987; Destefanis *et al.*, 2008). According to Miller *et al.* (2001), the consumer would be willing to pay a higher price in the marketplace for beef as long as it is guaranteed to be tender. Previous work by Decker *et al.* (1993) reported that protein oxidation can affect the quality of meat and meat products. Oxidation might play a role resulting in the loss of enzyme activity and solubility, and formation of protein complexes and non-enzymatic browning products (Mercier *et al.*, 2004), and could be linked to meat tenderness. Recent studies have indicated that storage under high O_2 atmospheres can result in a decrease in beef tenderness (Tørngren, 2003).

Currently, relatively little is known about the effects of protein oxidation on the quality of beef (Rowe *et al.*, 2004). In meat, protein oxidation may lead to decreased eating quality such as reduced tenderness and juiciness, flavour deterioration, and discoloration (Xiong, 2000). Seyfert *et al.* (2005) found that steaks stored in low O_2 MAP increased in flavour intensity, decreased in off-flavours, and increased in tenderness scores. Tørngren (2003) noted steaks packaged in high O_2 MAP had decreased tenderness and flavor, as well as increased off-flavour, compared with steaks packaged in low O_2 MAP. Seyfert *et al.* (2005) suggested that the increased tenderness in low O_2 MAP was due to protein oxidation. Rowe *et al.* (2004) found greater shear force values for beef LM steaks that had more

protein oxidation early post-mortem (<14 d). Even though protein oxidation was not measured in Seyfert's study (Seyfert *et al.*, 2005), it is plausible that the reduction in lipid oxidation in low O₂ MAP would also retard protein oxidation and promote tenderization, whereas high O₂ MAP could have the opposite effect. Zakrys *et al.* (2007) observed a directional increase in protein oxidation of samples of MAP beef *M. longissimus dorsi* muscle packed in increasing O₂ atmospheres over 15 days of refrigerated retail display. They also found that Warner–Bratzler shear force values had a positive correlation to O₂ levels in MA packed beef samples; all samples appeared to become less tender with increasing O₂ level during the 15-days storage. However, no significant differences were observed between experimental treatments. It appears that samples packed with 50% and 80% O₂ were tougher than low O₂ treated samples. This is in agreement with findings of Lund *et al.* (2007), who reported an effect of packaging atmosphere on protein oxidation showing significant increases of carbonyl content in beef patties stored under high O₂ atmosphere after 6 days of shelf-life, in comparison with packaging with 100% N₂. Lund *et al.* (2007) investigated the effect of MAP (70% O₂/30% CO₂) and skin packaging (no oxygen) on protein oxidation and texture of pork *M. longissimus dorsi* muscle during storage for 14 days at 4 °C and found that the high oxygen atmosphere resulted in reduced tenderness and juiciness of samples. Additionally, their SDS–PAGE data revealed cross-linking of myosin heavy chain through disulfide bonding, and the content of protein thiols was reduced, indicating protein oxidation. Zakrys *et al.* (2008) reported that the toughness evaluation by a consumer panel ($n = 134$) corresponded directionally to the MAP beef samples containing higher oxygen levels compared to those with lower oxygen levels.

Estevez and Cava (2004) reported a significant correlation between TBARS values and carbonyl contents during storage of refrigerated liver pâtés. Mercier *et al.* (2004) also reported a close relationship between lipid and protein oxidation in meat and liver products. Miller *et al.* (2001) reported that consumer perceptions of beef flavour and juiciness have a greater impact on consumer overall acceptability levels of New York strip steaks as the Warner–Bratzler shear force and toughness levels increase. In short, as beef steaks become tougher, flavour and juiciness have a greater effect on consumer satisfaction. Consumers can also segregate differences in beef tenderness and are willing to pay more for more-tender beef.

Physical and chemical changes in oxidised proteins include amino acid destruction, decreases in protein solubility due to protein polymerisation, loss of enzyme activity, and formation of amino acid derivatives, including carbonyls (Meucci *et al.*, 1991; Stadtman and Oliver, 1991; Starke-Reed and Oliver, 1989; Uchida *et al.*, 1992). According to Xiong (2000), oxidative changes in myofibrillar proteins during meat ageing may contribute to post-mortem proteolysis and tenderisation of meat as the peptide bonds of oxidised proteins become easily accessible by enzymes. Oxidative conditions can make proteins less susceptible to enzymatic proteolysis (Davies, 2001), thereby reducing development of tenderness by proteolysis in meat. A previous study by Rowe *et al.* (2004) reported that increased protein oxidation during the first 24 h post-mortem can substantially

decrease beef tenderness, even in steaks aged 14 days. It is possible that any influence of protein oxidation on beef tenderness occurs early post-mortem and that such effects may not be relevant to beef quality once the ageing process is complete. As such, the requirements for colour stability of high O₂ MAP meat must be balanced against the deteriorative action of lipid oxidation (Torngren, 2003; Zakrys *et al.*, 2007, 2008) and any reduction in meat tenderness. Several researchers have reported accelerated post-mortem tenderisation and increased tenderness in lamb primals injected with calcium chloride after slaughter (Clare *et al.*, 1997; Koohmaraie *et al.*, 1990). They concluded that calcium chloride enhanced the activity of the endogenous calcium-dependent proteases (m- and μ -calpain), ultimately increasing tenderness.

25.7 Future trends

Mize and Kelly (2004) reported the trends in fresh meat packaging at retail level in the United States. They found that in 2002, 69% of the linear footage of the self-service meat case was occupied by fresh meat and poultry. This figure declined to 63% in 2004, reflecting a growing conversion of meat items to products with greater consumer convenience, such as fully cooked entrées and marinated meats, as well as hams and sausages. They also reported an increase in packages that were case ready, from 49% in 2002 to 60% in 2004.

As stated earlier, high O₂ MAP is now used ubiquitously across the meat industry for many different meat products. Alternatively, low O₂ packaging systems have been readily available in the US, but not as widely implemented as their high oxygen counterparts. Vacuum packaging continues to be, in many cases, the most cost-effective packaging strategy. A relatively recent innovation in vacuum packaging has been the evolution of shrinkable films in use with horizontal form-fill-seal machinery (Salvage and Lipsky, 2004). This packaging format uses a styrene or polypropylene tray and a barrier film that can form around the product to reduce any amount of purge from coming out of the product. An additional web of film or a header can also be added for pre-pricing and pre-labelling (Belcher, 2006).

As the meat industry moves towards central processing that employs MAP and Vacuum-Skin Packaging (VSP), processors may need to overcome consumer preference for fresh beef that is bright red in colour and packaged with the traditional PVC overwrap. Nevertheless, it is encouraging that the initial perceptions of quality will likely not bias eating satisfaction once a decision to purchase is made and the meat is taken home, thereby hastening the acceptance of the newer packaging technologies (Carpenter *et al.*, 2001). Additionally, meat processing and packaging technologies which are accepted by the market and adopted by the industry will have to become more efficient, consistent and leaner in activity if future global challenges are to be met.

In recent years there has been much attention paid to the shift from purchasing meat from the family butcher shop to purchasing it at the local supermarket. We

have seen more and more of the traditional butcher shops close because they cannot compete on price, they do not offer the same one-stop shop opportunity of the supermarket, but also they do not offer the extended shelf-life of MAP meat available on refrigerated supermarket shelves. This is the situation in most developed countries, particularly within the EU, where sales of fresh meat have increased in supermarkets at the expense of the specialised butchers' stores (Mannion, 1995). However, the consumer has become very much more discerning with respect to the origins of the food they consume. Poor labelling by the supermarkets has resulted in a swing back towards the local butcher where meat traceability is transparent and promoted as a selling point, in addition to green issues relating to product movement to markets (air miles) and support for local product producers. The impact of such developing trends on the pre-pack sales of meat at supermarket level remains to be seen.

25.8 References

- Aisen, P. and Listowsky, I. (1980), Iron transport and storage proteins. *Annual Review of Biochemistry*, 49, 357–396.
- Allen, P., Doherty, A. M., Buckley, D. J., Kerry, J., O'Grady, M. N. and Monahan, F. J. (1996), Effect of oxygen scavengers and vitamin E supplementation on colour stability of MAP beef. In *42nd International Congress of Meat Science and Technology*, 88–89.
- Andersen, H. J. and Skibsted, L. H. (1991), Oxidative stability of frozen pork patties – Effect of light and added salt. *Journal of Food Science*, 56, 1182–1184.
- Asghar, A., Gray, J. I., Buckley, D. J., Pearson A. M. and Booren, A. M. (1988), Perspectives on warmed-over flavor. *Food Technology*, 42, 102–108.
- Assef, S. A., Bratzler, L. J., Cameron, B. F. and Yunis, A.A. (1971), Photo-oxidation of bovine oxymyoglobin in frozen solutions. The effect of redox active inorganic elements in muscle extracts. *Comparative Biochemical Physiology*, 39B, 395–407.
- Belcher, J. N. (2006), Industrial packaging developments for the global meat market. *Meat Science*, 74, 143–148.
- Bell, R. G., Penney, N. and Moorhead, S. M. (1996), The retail display life of steaks prepared from chill stored vacuum and carbon dioxide-packed sub-primal beef cuts. *Meat Science*, 42 (2), 165–178.
- Bell, R. G. and Bourke, B. J. (1996), Recent developments in packaging of meat and meat products. In *Proceedings of the International Developments in Process Efficiency and Quality in the Meat Industry* (pp. 99–119), Dublin Castle, Ireland.
- Boyer, R. F. and McCleary, C. J. (1987), Superoxide ion as a primary reductant in ascorbate-mediated ferritin iron release. *Free Radical Biology and Medicine*, 3, 389–395.
- Brody, A. L. (1970), Shelf life of fresh meat. *Modern Packaging*, 1, 81.
- Callow, E. H. (1932), Gas storage of pork and bacon. Part 1. Preliminary experiments. *Journal of the Society of Chemical Industry*, 51, 116–119.
- Campo, M. M. Nute, G. R. Hughes, S. I. Enser, M. Wood J. D. and Richardson, R. I. (2006), Flavour perception of oxidation in beef. *Meat Science*, 72, 303–311.
- Carpenter, C. E., Cornforth, D. P. and Whittier, D. (2001), Consumer preferences for beef color and packaging did not effect eating satisfaction. *Meat Science*, 57, 359–363.
- Carpenter R., O'Grady M. N., O'Callaghan Y. C., O'Brien N. M. and Kerry J. P. (2007), Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Science*, 76, 604–610.
- Cassens, R. G., Faustman, C. and Jimenez-Colmenero, F. (1988), Modern developments in

- research on colour of meat. In B. Krol, P. Van Roon and J. Houben (Eds.), *Trends in Modern Meat Technology* 2 (p. 2), Wageningen, The Netherlands: Pudoc.
- Chambers IV, E and Bowers, J. (1993), Consumer perception of sensory quality in muscle foods: Sensory characteristics of meat influence consumer decisions. *Food Technology*, 47, 116–120.
- Chan, H. W. S. (1987), The mechanism of autoxidation. In: H. W. S. Chan, *Autoxidation of Unsaturated Lipids*, (pp 1–17). London: Academic Press.
- Church, I. J. and Parsons, A. L. (1995), Modified atmosphere packaging technology: Review. *Journal of the Science of Food and Agriculture*, 67, 143–152.
- Clare, T. L. Jackson, S. P. Miller, M. F. Elliot C. T. and Ramsey, C. B. (1997), Improving tenderness of normal and Callipyge lambs with calcium chloride. *Journal of Animal Science*, 75, 377–385.
- Clydesdale, F. M. (1978), Colorimetry – Methodology and applications. *CRC Critical Reviews in Food Science and Nutrition*, 10, 243–301.
- Crichton, R. R. and Charleatoux-Wauters, M. (1987), Iron transport and storage. *European Journal of Biochemistry*, 164, 485–506.
- Davies, K. J. A. (2001), Degradation of oxidized proteins by the 20S proteasome. *Biochimie*, 83, 301–310.
- Decker, E.A. and Crum, A.D. (1993), Antioxidant activity of carnosine in cooked ground pork. *Meat Science*, 2, 245–253.
- Decker, E. A., Xiong, Y. L., Calvert, J. T., Crum, A. D. and Blanchard, S. P. (1993), Chemical, physical, and functional properties of oxidized turkey white muscle myofibrillar proteins. *Journal of Agricultural and Food Chemistry*, 41, 186–189.
- Destefanis, G., Brugiapaglia, A., Barge, M. T. and Dal Molin, E. (2008), Relationship between beef consumer tenderness perception and Warner–Bratzler shear force, *Meat Science*, 78, 153–156.
- Estevez M. and Cava R. (2004), Lipid and protein oxidation, release of iron from heme molecule and colour deterioration during refrigerated storage of liver pâté. *Meat Science*, 68, 551–558.
- Faustman, C. and Cassens, R.G. (1989), Strategies for improving fresh meat colour. In *Proceedings, 35th International Congress of Meat Science and Technology*, (pp 446–453) Copenhagen, Denmark.
- Faustman, C. and Cassens, R.G. (1990), Influence of aerobic metmyoglobin reducing capacity on colour stability of beef. *Journal of Food Science*, 55, 1279–1283.
- Floros, J. D. and Matsos, H. I. (2005), CH10. Introduction to modified atmosphere packaging. In Han, J. H., editor. *Innovations in Food Packaging*, 159–171.
- Georgala, D. L. and Davidson, C. M. (1970), Food Package. *British Patent* 1 199 998.
- Gill, C. O. (1989), Packaging for prolonged chill storage: The Captech process. *British Food Journal*, 91 (7), 11–15.
- Gill, C. O. and Molin, G. (1991), Modified atmospheres and vacuum packaging. In *Food Preservatives*, ed. N. J. Ruse I and G. W. Gould, Blackie and Sons Ltd, Glasgow, p. 172.
- Gill, C. O. and Penney, N. (1988), The effect of the initial gas volume to meat weight ratio on the storage life of chilled beef packaged under carbon dioxide. *Meat Science*, 22 (1), 53–63.
- Gill C. O. (1996), Extending the storage life of raw chilled meats, *Meat Science*, 43 (Suppl.), S99–S109.
- Gill, A.O. and Gill, C.O. (2005), CH13. Preservative packaging for fresh meats, poultry and fin fish. In Han, J.H., editor. *Innovations in Food Packaging*, 204–220.
- Gray, J. L. and Pearson, A. M. (1994), Lipid-derived off-flavours in meat: Formation and inhibition. In F. Shahidi (Ed.), *Flavour of Meat and Meat Products* (pp. 116–143). London: Blackie Academic.
- Gray, J. I., Goma, E. A. and Buckley, D. J. (1996), Oxidative quality and shelf life of meats. *Meat Science*, 43, S111–S123.
- Greene, B. E. and Cumuze, T. H. (1981), Relationship between TBA numbers and

- inexperienced panellists' assessments of oxidized flavour in cooked beef. *Journal of Food Science*, 47, 52–58.
- Gutteridge, J. M. C. and Halliwell, B. (1990), The measurement and mechanism of lipid peroxidation in biological systems. *Trends in Biochemical Sciences*, 15, 129–135.
- Harel, S. and Kanner, J. (1985), Muscle membranous lipid peroxidation initiated by H_2O_2 -activated metmyoglobin. *Journal of Agriculture and Food Chemistry*, 33, 1188–1192.
- Hood, D. E. and Riordan, E. B. (1973), Discoloration in pre-packed beef. *Journal of Food Technology*, 8, 333–348.
- Hood, D. E. and Mead, G. C. (1993), Modified atmosphere storage of fresh meat and poultry, In R.T. Parry, *Principles and Applications of Modified Atmosphere Packing of Food* (pp 269–298), London: Blackie Academic and Professional.
- Hsieh, R. J. and Kinsella, J. E. (1989), Oxidation of polyunsaturated fatty acids: Mechanisms, products and inhibition with emphasis on fish. *Advances in Food and Nutrition Research*, 33, 233–341.
- Hunt, M. C., Mancini, R. A., Hachmeister, K. A., Kropf, D. H., Merriman, M., DelDuca, G. *et al.* (2004), Carbon monoxide in modified atmosphere packaging affects color, shelf life, and microorganisms of beef steaks and ground beef. *Journal of Food Science*, 69 (1), C45–C52.
- Ingold, K.O. (1962), Metal catalyses. In H.W. Schultz, E.A. Day and R.O. Sinnhuber, *Symposium on Foods: Lipids and their Oxidation*. Connecticut: AVI Publishing.
- Inns, R. (1987), Modified atmosphere packaging. In *Modern Processing, Packaging and Distribution Systems for Food*, Vol. 4 (F. A. Paine, ed.), Blackie, Glasgow, UK, 36–51.
- Issanchou, S. (1996), Consumer expectations and perceptions of meat and meat products. *Meat Science*, 43, S5–S19.
- Jakobsen M. and Bertelsen G. (2000), Colour stability and lipid oxidation of fresh beef. Development of a response surface model for predicting the effects of temperature, storage time, and modified atmosphere composition. *Meat Science*, 54, 49–57.
- Jayasingh, P., Cornforth, D. P., Brennand, C. P., Carpenter, C. E. and Whittier, D. R (2002), Sensory evaluation of ground beef stored in high-oxygen modified atmosphere packaging. *Journal of Food Science*, 67 (9), 3493–3496.
- Jayasingh, P., Cornforth, D. P., Carpenter, C. E. and Whittier, D. (2001), Evaluation of carbon monoxide treatment in modified atmosphere packaging or vacuum packaging to increase color stability of fresh beef. *Meat Science*, 59 (3), 317–324.
- Jeremiah, L. E., Gill, C. O. and Penney, N. (1992), The effect on pork storage life of oxygen contamination in nominally anoxic packagings. *Journal of Muscle Foods*, 3, 263–281.
- Johns, A. M., Birkinshaw, L. H. and Ledward, D. A. (1989), Catalysts of lipid oxidation in meat products. *Meat Science*, 25, 209–220.
- Kanner, J., Hazan, B. and Doll, L. (1988), Catalytic free iron in muscle foods. *Journal of Agriculture and Food Chemistry*, 36, 412–415.
- Kanner, J., Salan, M. A., Harel, S. and Shegalovich, I. (1991), Lipid peroxidation of muscle foods. The role of the cytosolic fraction. *Journal of Agriculture and Food Chemistry*, 39, 242–246.
- Kanner, J. (1994), Oxidative processes in meat and meat products: Quality implications. *Meat Science*, 36, 169–189.
- Kennedy, C., Buckley, D. J. and Kerry, J. P. (2004), Display life of sheep meats retail packaged under atmospheres of various volumes and compositions. *Meat Science*, 68, 649–658.
- Kerry, J. P., O'Grady, M. N. and Hogan, S. A. (2006), Past, current and potential utilisation of active and intelligent packaging systems for meat and muscle-based products: A review. *Meat Science*, 74, 113–130.
- Kerry, J.P., O'Sullivan, M.G., Buckley, D.J., Lynch, P.B. and Morrissey, P.A. (2000), The effects of dietary α -tocopheryl acetate supplementation and modified atmosphere packaging (MAP) on the quality of lamb patties. *Meat Science*, 56, 61–66.

- Killefer, D. H. (1930), Carbon dioxide preservation of meat and fish. *Industry Engineering Chemistry*, 22, 140–143.
- Knut, F. and Nolet, G. (2006), Envasado con CO: Una nueva tecnolog a de envasado sin ox geno para la industria carnica de la Union Europea. *Eurocarne*, 143, 195–199.
- Koohmaraie, M., Whipple G. and Crouse, J. D. (1990), Acceleration of postmortem tenderization in lamb and Brahman-cross beef carcasses through infusion of calcium chloride. *Journal of Animal Science*, 68, 1278–1283.
- Labuza, T. P. (1971), Kinetics of lipid oxidation in foods. *CRC Critical Reviews of Food Technology*, 2, 355–404.
- Lanari, M. C., Schaefer, D. M. and Scheller, K. K. (1995), Dietary vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. *Meat Science*, 41, 237–250.
- Lauridsen, C., Kr gh Jensen, S., Skibsted, L. H. and Bertelsen, G. (2000), Influence of supranutritional vitamin E on α -tocopherol deposition and susceptibility to lipid oxidation of porcine membranal fractions of *M. psoas major* and *M. longissimus dorsi*. *Meat Science*, 54, 377–384.
- Ledward, D. A. (1985), Post-slaughter influences on the formation of metmyoglobin in beef muscles. *Meat Science*, 15, 149–171.
- Liu, Q., Lanari, M. C. and Schaefer, D. M. (1995), A review of dietary vitamin E supplementation for improvement of beef quality. *Journal of Animal Science*, 73, 3131–3140.
- Lu, J., Tan, J., Shatadal, P. and Gerrard, D. E. (2000), Evaluation of pork color by using computer vision. *Meat Science*, 56, 57–60.
- Lund M. N., Hviid M. S. and Skibsted L. H. (2007), The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. *Meat Science*, 76, 226–233.
- Lund M. N., Lametsch R., Hviid M. S., Jensen, O. N. and Skibsted L. H. (2007), High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine *longissimus dorsi* during chill storage. *Meat Science*, 77, 295–303.
- Luno, M., Roncales, P., Djenane, D. and Beltran, J. A (2000), Beef shelf life in low O₂ and high CO₂ atmospheres containing different low CO concentrations. *Meat Science*, 55 (4), 413–419.
- Lynch, M. P., Kerry, J. P., Buckley, D. J., Faustman, C. and Morrissey, P. A. (1999), Effect of dietary vitamin E supplementation on the colour and lipid stability of fresh, frozen and vacuum-packed beef. *Meat Science*, 52, 95–99.
- Mancini, R. A. and Hunt, M. C. (2005), Current research in meat colour. *Meat Science*, 71, 100–121.
- Mannion, P. (1995), Meat retail: Major change. *Meat international*, 3 (4), 10–13.
- Meischen, H. W., Huffman, D. L. and Davis, G. W. (1987), Branded beef-product of tomorrow today. *Proceedings of the Reciprocal Meat Conference*, 40, 37–46.
- Mercier Y., Gatellier P. and Renere M. (2004), Lipid and protein oxidation in vitro, and antioxidant potential in meat from Charolais cows finished on pasture or mixed diet. *Meat Science*, 66, 467–473.
- Meucci, E., Mordente, A. and Martorana, G. E. (1991), Metal-catalyzed oxidation of human serum albumin: Conformational and functional changes. *Journal of Biological Chemistry*, 266, 4692–4699.
- Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L. and Hoover, L. C. (2001), Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science*, 79, 3062–3068.
- Mize, J. and Kelly, J. (2004), America's dynamic meat case. *Cryovac Retail Wrap-up*, December.
- Morrissey, P. A., Buckley, D. J., Sheehy, P. J. A. and Monaghan, F. J. (1994), Vitamin E and meat quality. *Proceedings of the Nutrition Society*, 53, 289–295.

- Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry J. P. and Buckley, D. J. (1998), Lipid stability in meat and meat products. *Meat Science*, 49, S73–S86.
- O'Grady, M. N., Monahan, F. J., Bailey, J., Allen, P., Buckley, D. J. and Keane, M. G. (1998), Colour-stabilising effect of muscle vitamin E in minced beef stored in high oxygen packs. *Meat Science*, 50, 73–80.
- O'Grady, M. N., Monahan, F. J., Burke, R. M. and Allen, P. (2000), The effect of oxygen level and exogenous α -tocopherol on the oxidative stability of minced beef in modified atmosphere packs. *Meat Science*, 55, 39–45.
- O'Grady, M. N., Maher, M., Troy, D. J., Moloney, A. P. and Kerry, J. P. (2006), An assessment of dietary supplementation with tea catechins and rosemary extract on the quality of fresh beef. *Meat Science*, 73, 132–143.
- Okayama, T., Muguruma, M., Murakami, S. and Yamada, H. (1995), Studies on modified atmosphere packaging of thin sliced beef. 1. Effect of two modified atmosphere packaging systems on pH value, microbial-growth, metmyoglobin formation and lipid oxidation of thin sliced beef. *Journal of the Japanese Society for Food Science and Technology-Nippon Shokuhin Kagaku Kogaku Kaishi*, 42, 498–504.
- O'Keefe, M. and Hood, D. E. (1982), Biochemical factors influencing metmyoglobin formation on beef from muscles of differing color stability. *Meat Science*, 3, 209–228.
- O'Sullivan, M. G., Kerry, J. P., Buckley, D. J., Lynch, P. B. and Morrissey, P. A. (1998), The effect of dietary vitamin E supplementation on quality aspects of porcine muscles. *Irish Journal of Agriculture and Food Research*, 37, 227–235.
- Parry, R. T. (1993), Introduction. In Parry, R. T., editor. *Principles and Applications of Modified Atmosphere Packaging of Food*. New York: Blackie Academic and Professional, p3.
- Payne, S. R., Durham, C. J., Scott, S. M. Penney, N., Bell, R. G. and Devine, C. E. (1997) The effects of rigor temperature, electrical stimulation, storage duration and packaging systems on drip loss in beef. *Proceedings of the 43rd International Congress of Meat Science and Technology*, Auckland, (GI-22) 592–593.
- Pearson, A. M., Love, J. D. and Shorland, F. B. (1977), 'Warmed-over' flavour in meat, poultry and fish. *Advances in Food Research*, 23, 1a.
- Renerre, M. (1990), Review: Factors involved in the discoloration of beef meat. *International Journal of Food Science and Technology*, 25, 613–630.
- Renerre, M. and Labadie, J. (1993), Fresh meat packaging and meat quality. *Proceedings of the 39th Int. Cong. of Meat Science and Tech.*, Calgary, Canada. pp 361.
- Rhee, K. I. and Ziprin, Y. A. (1987), Lipid oxidation in retail beef, pork and chicken muscles as affected by concentrations of heme pigments and nonheme iron and microsomal enzymic lipid peroxidation activity. *Journal of Food Biochemistry*, 11, 1–15.
- Risvik, E. (1994), Sensory properties and preferences. *Meat Science*, 36, 67–77.
- Robertson, G. L. (2006), Packaging of Flesh Foods. In *Food Packaging Principles and Practice*. Second edition. CRC press, Taylor and Francis Group, Boca Raton, FL, Chapter 16, 286–309.
- Rowe L. J., Maddock K. R., Lonergan S. M. and Huff-Lonergan E. (2004), Influence of early post-mortem protein oxidation on beef quality. *American Society of Animal Science*, 82, 785–793.
- Salvage, B. and Lipsky, J. (2004), Focus on packaging and process, *The National Provisioner*, 64–79.
- Sato, K. and Hegarty, G.R. (1971), Warmed-over flavour in cooked meat. *Journal of Food Science*, 36, 1098–1102.
- Savell, J. W., R. E. Branson, H. R. Cross, D. M. Stiffler, J. W. Wise, D. B. Griffin and G. C. Smith. (1987), National Consumer Retail Beef Study: Palatability evaluations of beef loin steaks that differed in marbling. *Journal of Food Science*, 52, 517–519, 532.
- Savell, J. W., H. R. Cross, J. J. Francis, J. W. Wise, D. S. Hale, D.L. Wilkes and G. C. Smith. (1989), National Consumer Retail Beef Study: Interaction of trim level, price and grade

- on consumer acceptance of beef steaks and roasts. *Journal of Food Quality*, 12, 251–274.
- Seideman, S. C. and Durland, P. R. (1984), The utilization of modified atmosphere packaging for fresh meat: A review. *Journal of Food Quality*, 6, 239–252.
- Seman, D. L., Decker, E. A. and Crum, A. D. (1991), Factors affecting catalysis of lipid oxidation by a ferritin containing extract of beef muscle. *Journal of Food Science*, 56, 356–358.
- Seyfert, M., Hunt, M. C., Mancini, R. A., Hachmeister, K. A., Kropf, D. H., Unruh, J. A., and Loughin T. M. (2005), Beef quadriceps hot boning and modified-atmosphere packaging influence properties of injection-enhanced beef round muscles. *Journal of Animal Science*, 83, 686–693.
- Seyfert, M., Mancini, R. A., Hunt, M. C., Tang J. and Faustman, C. (2007), Influence of carbon monoxide in package atmospheres containing oxygen on colour, reducing activity, and oxygen consumption of five bovine muscles. *Meat Science*, 75 (3), 432–442.
- Sherbeck, J. A. Wulf, D. M., Morgan, J. B., Tatum, J. D. Smith, G. C. and Williams, S. N. (1995), Dietary supplementation of vitamin E to feedlot cattle affects retail display properties. *Journal of Food Science*, 60, 250–252.
- Smiddy, M., Papkovskaia, N., Papkovsky, D. B. and Kerry, J. P. (2002), Use of oxygen sensors for the non-destructive measurement of the oxygen content in modified atmosphere and vacuum packs of cooked chicken patties: Impact of oxygen content on lipid oxidation. *Food Research International*, 35, 577–584.
- Smith, G. C., J.W. Savell, H. R. Cross, Z. L. Carpenter, C. E. Murphey, G. W. Davis, H. C. Abraham, F. C. Parrish and B. W. Berry. (1987), Relationship of USDA quality grades to palatability of cooked beef. *Journal of Food Quality*, 10, 269–287.
- Snyder, H. E. and Skrydlant, H. B. (1966), The influence of metallic ions on the autoxidation of oxymyoglobin. *Journal of Food Science*, 31, 468–479.
- Sorheim O., Aune, T. and Nesbakken T. (1997), Technological, hygienic and toxicological aspects of carbon monoxide used in modified-atmosphere packaging of meat. *Trends in Food Science and Technology*, 8, Issue 9, September 1997, 307–312.
- Sorheim O., Nissen H. and Nesbakken T. (1999), The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. *Meat Science*, 52, 157–164.
- Stadtman, E. R. and Oliver, C. N. (1991), Metal-catalyzed oxidation of proteins. *Journal of Biological Chemistry*, 266, 2005–2008.
- St. Angelo, A. J. and Bailey, M. E. (1987), *Warmed-over Flavor of Meat*. Florida: Academic Press, vii–viii.
- Starke-Reed, P. E. and Oliver, C. N. (1989), Protein oxidation and proteolysis during ageing and oxidative stress. *Archives of Biochemistry and Biophysics*, 275, 559–567.
- Tappel, A. L. (1962), Heme compounds and lipoxidase as biocatalysts. In: H.W. Schultz, E.A. Day and R.O. Sinnhuber, *Symposium on Foods: Lipids and their Oxidation* (pp122–126), Connecticut: AVI Publishing .
- Taylor, A. A. (1985), Packaging fresh meat, In *Developments in Meat Science*, Vol. 3, Lawrie, R. A., Ed., Elsevier Applied Science Publishers, Essex, England, 1985, Ch4.
- Tichivangana, A. J. and Morrissey, P. A. (1985), Metmyoglobin and inorganic metals as pro-oxidants in raw and cooked muscle systems. *Meat Science*, 15, 107–116.
- Torngren, M. A. (2003), Effect of packaging method on colour and eating quality of beef loin steaks. *49th International Congress of Meat Science and Technology*. Brazil, September, 495–496.
- Uchida, K., Kato, Y. and Kawakishi, S. (1992), Metal-catalyzed oxidative degradation of collagen. *Journal of Agricultural and Food Chemistry*, 40, 9–12.
- Wilkinson, B. H. P., Janz, J. A. M., Morel, P. C. H., Purchas, R. W. and Hendriks, W. H. (2006), The effect of modified atmosphere packaging with carbon monoxide on the storage quality of master packaged fresh pork. *Meat Science*, 75 (4), 605–610.
- Wolfe, S. K. (1980), Use of CO and CO₂ enriched atmospheres for meats, fish, and produce. *Food Technology*, 34 (3), 55–63.

- Xiong, X. L. (2000), *Protein Oxidation and Implications for Muscle Food Quality. Antioxidants in Muscle Foods*. Chapter 4, p: 85–111.
- Yin, M. C., Faustman, C., Riesen, J. W. and Williams, S. N. (1993), The effects of α -tocopherol and ascorbate upon oxymyoglobin and phospholipid oxidation. *Journal of Food Science*, 58, 1273–1276.
- Young, L. L., Reviere, R. D. and Cole, A. B. (1983), Fresh red meats: A place to apply modified atmospheres. *Food Technology*, 42, 65–69.
- Zakrys, P. I., Hogan, S. A., O'Sullivan, M. G., Allan, P and Kerry, J. P. (2008), Effects of oxygen concentration on the sensory evaluation and quality indicators of beef muscle packed under modified atmosphere. *Meat Science*, 79, 648–655.
- Zakrys, P. I., O'Sullivan, M. G., Allan, P and Kerry, J. P. (2008), Consumer acceptability and physiochemical characteristics of modified atmosphere packed beef steaks. *Meat Science* (submitted for publication).

Characterizing muscle properties to develop muscle-specific intervention strategies and improve meat cuts for the consumer

C. R. Calkins, University of Nebraska, USA and D. D. Johnson, University of Florida, USA

Abstract: This chapter summarizes beef muscle characterization research conducted in the US. Known as muscle profiling, this initiative has been applied to young, market-weight beef cattle to identify candidate muscles for upgrading in value. Chemical, physical and sensorial traits among bovine muscles (veal, young beef, beef cows, and dairy cows) are presented. In addition, the responses of beef muscles that are borderline in palatability to four different enhancement methods are discussed. Muscles were evaluated after cooking, frozen storage and reheating and results indicate that muscles respond differently to enhancement. This suggests muscle-specific protocols for enhancement are needed.

Key words: muscle profiling, muscle enhancement, marination, bovine myology.

26.1 Introduction

Sir Francis Bacon is widely credited with having said ‘Knowledge is power.’ One application of this basic tenet is in the development of new meat products. Without a thorough and complete understanding of the basic properties of a particular muscle, the nuances of product development will be lost. It is not enough to generalize across all muscles. Development of high-quality meat cuts for the consumer demands specialized approaches to muscles that differ in basic fundamental traits and thus differ in ultimate product characteristics.

It should be of no surprise that there is considerable variation among muscles for the various physical and biochemical properties. Muscles themselves differ in

their biological function and skeletal location, as well as in their basic biochemical makeup. This variation offers the opportunity to develop successful, consumer-friendly meat products. Failure to acknowledge and use this variation can lead to frustration and low-quality products with little chance for success in the marketplace.

Over the past ten years, scientists in the US have sought to characterize the properties of muscles that influence the character of the finished products. This initiative has become associated with the term 'muscle profiling.' Following the dictionary definition of a profile as an essay of a subject's most noteworthy characteristics, the muscle profiling research has sought to characterize and document the unique characteristics of individual muscles so that product development efforts may be focused upon the ideal raw materials for a given product. Doing so will help to assure that high-quality products are offered to discriminating consumers. Other countries, notably France, are recognized for cutting meat to optimize the use of individual muscles. This chapter is intended to describe the work that thus far has been conducted to characterize muscle properties so that muscle-specific intervention strategies may be adopted and improved meat cuts may be offered to the consumer.

26.2 Overview of US beef muscle profiling projects

The value of beef in the US experienced a decline of more than 20% from 1990 to 1998. The Cattlemen's Beef Board, through producer-funded market research studies, began to study the underlying causes for a reduction in consumer demand for beef products. Results revealed a differential response by primal cut. Wholesale cuts from the rib and loin had increased in value by three to four percent during the five-year time period of 1993 to 1998, whereas cuts from the chuck and round, in addition to trimmings, had experienced a 24 to 28% decline in value during that same time period (United States Department of Agriculture, USDA, 2005). This was especially troubling since these primal cuts make up about 69% of total beef carcass weight. Marketeers and meat scientists identified several reasons for this decline in value. These carcass portions often produced cuts that had tenderness problems because they were from areas of the carcass that were used for locomotion. Such muscles typically have increased amounts of both total connective tissue and a higher proportion of less soluble collagen, both surrounding and within the muscle. Not only were these cuts less tender but they were also quite variable in tenderness. Another issue that often surfaced as a consumer concern was the amount of intermuscular (seam) fat that was very objectionable to end users. This fat is difficult to remove without destroying the integrity of the cut. In addition, cuts from these portions of the carcass were not perceived as convenient to consumers. This was primarily due to the large size of the cuts often offered to the user and the need, many times, to utilize a moist cooking method that required long times and low cooking temperatures to achieve a satisfactory eating experience. This was antagonistic to the findings of current

market research that showed that household size was becoming smaller and most consumers wanted total preparation and cooking times to be less than 30 minutes to meet their lifestyle requirements. Researchers noted that the current style of carcass fabrication that produced multiple-muscle cuts often accentuated tenderness and convenience problems because muscles could not be cut across the grain to produce more tender retail cuts or remove large (heavy) connective tissue sheets.

Research funded by the US Beef Check-Off program was initiated by the Cattlemen's Beef Board to better understand the aforementioned issues and to suggest ways to overcome the problems. The first step was to characterize muscles from the beef round and chuck. Such information could be used to determine the best use for each muscle. The University of Florida and University of Nebraska – Lincoln were contracted to profile the muscles from the beef chuck and round to accomplish the task. The two institutions endeavored to design a study to fully describe the characteristic of the muscles as to tenderness, composition, processing tracts, physical dimensions, color, and yield. This would eliminate the unknown hurdles that surround these muscles and produce a body of work that packers and processors could use in product development and value enhancement. Ultimately, a series of studies were conducted, characterizing muscles from youthful market cattle, cow meat, veal, and even poultry. Additional research, reported here, attempted to characterize the response of selected muscles to muscle-specific enhancement strategies.

26.2.1 Young, market-weight beef

In the first study (Von Seggern *et al.*, 2005), 39 muscles (Table 26.1) from 142 chucks and rounds were removed from young (A-maturity) beef carcasses of various yield grades (one, two, three, and four/five combined), marbling scores (Moderate and Modest combined, Small, and Slight), and carcass weights (250 to 295 kg, 296 to 385 kg, and 386 to 431 kg). Muscles were weighed at three fat trim levels and physical dimensions were recorded for denuded muscles. Warner–Bratzler shear force values (WBS) and sensory panel ratings were obtained for the muscles from the middle carcass weight range (296 to 385 kg) for each of the selection categories (marbling score and USDA yield grade), using two cooking methods (moist and dry heat cookery).

For percentage of carcass weight represented by individual muscles, USDA Yield Grade had an effect on 19 out of 39 muscles studied, where generally, as yield grade numerically decreased, the percentage of carcass weight represented by each muscle increased. Warner–Bratzler shear force was measured on 37 muscles, of which 25 were found to be influenced by cooking method (Table 26.2). Seventeen of the moist cooked muscles had lower ($P < 0.05$) WBS values than the dry cooked muscles (Table 26.3). This was expected, as muscles in the chuck and round vary in their connective tissue content (Von Seggern *et al.*, 2005). Table 26.4 presents the sensory panel tenderness ranking of 22 muscles of the chuck and round by cooking method. Sensory panelists ranked muscles for tenderness similarly to the WBS scores. In general, chuck muscles tended to have higher

Table 26.1 Identification of muscles sampled in muscle profiling research

Muscle name	Sampled in				
	Muscle code	Youthful round	Youthful chuck	Beef cow	Dairy cow
<u>Chuck muscles</u>					
<i>Biceps brachii</i>	BIB		X		
<i>Brachialis</i>	BRA		X		
<i>Brachiocephalicus omo-transversarius</i>	BOT		X		
<i>Complexus</i>	COM		X	X	X
<i>Cutaneous omo-brachialis</i>	COB		X		
<i>Deep pectoral (pectoralis profundus)</i>	DEP		X	X	X
<i>Deltoideus</i>	DEL		X		
<i>Dorsalis oblique</i>	DSO		X		
<i>Infraspinatus</i>	INF		X	X	X
<i>Intertransversales</i>	INT		X		
<i>Latissimus dorsi</i>	LAD		X	X	
<i>Levatores costarum</i>	LVC		X		
<i>Longissimus capitus et Atlantis</i>	LCA		X		
<i>Longissimus costarum</i>	LGC		X		
<i>Longissimus dorsi</i>	LGD		X	X	
<i>Multifidus/Spinalis dorsi</i>	MSD		X	X	
<i>Rhomboideus</i>	RHM		X		X
<i>Scalenus dorsalis</i>	SCD		X		
<i>Serratus ventralis</i>	SEV		X	X	X
<i>Splenius</i>	SPL		X		X
<i>Subscapularis</i>	SUB		X		
<i>Superficial pectoral</i>	SPP		X		
<i>Supraspinatus</i>	SUP		X	X	X
<i>Tensor fascia antibrachii</i>	TFA		X	X	
<i>Teres major</i>	TEM		X	X	X
<i>Trapezius</i>	TRA		X		
<i>Triceps brachii</i>	TRB		X	X	X
<u>Round muscles</u>					
<i>Adductor</i>	ADD	X		X	X
<i>Biceps femoris</i>	BIF	X		X	X
<i>Gluteus medius</i>	GLM	X		X	X
<i>Gracilis</i>	GRA	X			
<i>Pectineus</i>	PEC	X			
<i>Rectus femoris</i>	REF	X		X	X
<i>Sartorius</i>	SAR	X			
<i>Semimembranosus</i>	SEM	X		X	X
<i>Semitendinosus</i>	SET	X		X	X
<i>Vastus intermedius</i>	VAI	X		X	X
<i>Vastus lateralis</i>	VAL	X		X	X
<i>Vastus medialis</i>	VAM	X		X	X

scores for juiciness and flavor intensity than muscles from the round (not shown in tabular form, Brickler, 2000). Neither marbling scores nor yield grades had a major or consistent effect on sensory panel traits or WBS values.

Results from this study indicate that several muscles from the chuck and round,

Table 26.2 Analysis of variance results and mean values for Warner–Bratzler shear force from muscles of the beef chuck and round

Muscle ^a	Mean ^b	Std dev ^c	Qual ^d	Yield ^e	Meth ^f	QxY ^g	QxM ^h	YxM ⁱ	QxYxM ^j
ADD	4.48	0.87	— ^k	—	—	—	—	—	0.03
BIB	3.28	0.63	—	—	—	—	—	—	—
BIF	4.66	1.48	<0.01	—	—	—	0.03	—	—
BOT	6.37	1.24	—	—	<0.01	—	—	—	—
BRA	4.85	0.70	—	—	—	—	—	—	—
COM	4.48	0.92	—	—	—	—	—	—	—
DEL	5.02	0.79	<0.01	0.03	0.01	—	0.04	—	—
DEP	5.52	1.31	—	—	—	—	—	—	—
DSO	4.68	0.97	—	—	0.02	—	—	—	—
GLM	5.58	1.29	—	—	0.02	—	—	—	—
GRA	3.90	0.75	—	—	0.03	—	—	—	—
INF	3.13	0.77	<0.01	—	<0.01	—	—	—	—
INT	4.21	1.15	—	—	<0.01	—	—	—	—
LAD	5.05	0.75	—	—	—	—	—	—	—
LCA	3.60	0.67	—	—	<0.01	—	—	—	—
LGC	4.59	1.38	—	—	<0.01	—	—	—	—
LGD	4.62	1.10	—	—	0.01	—	—	—	—
LVC	2.79	0.62	0.04	—	<0.01	—	—	—	—
MSD	3.49	0.76	—	—	—	—	—	0.04	—
PEC	3.99	0.74	<0.01	—	<0.01	—	—	—	—
REF	3.73	0.70	—	—	—	—	—	—	—
RHM	5.60	1.22	—	—	<0.01	—	—	—	—
SAR	4.54	0.48	—	—	—	—	<0.01	0.02	0.04
SCD	4.50	0.85	—	—	<0.01	—	—	—	—
SEE	4.19	1.00	—	—	—	—	—	—	—
SET	4.86	0.79	—	—	—	—	—	—	—
SEV	4.00	0.91	<0.01	—	—	<0.01	0.05	—	—
SPL	4.60	0.79	—	—	0.01	—	—	—	—
SPP	4.57	0.68	—	—	0.05	—	—	—	—
SUB	3.97	1.09	—	—	<0.01	—	—	—	—
SUP	4.30	1.22	0.05	<0.01	<0.01	0.02	—	—	—
TEM	3.72	0.66	—	—	—	—	<0.01	—	—
TFA	5.46	1.27	—	—	<0.01	—	—	<0.01	—
TRB	4.33	0.61	0.02	—	—	—	—	—	—
VAI	3.77	0.65	—	—	0.01	—	—	—	—
VAL	5.05	0.91	—	—	—	—	—	—	—
VAM	3.81	0.69	—	—	—	—	—	—	—

^aRefer to Table 26.1. ^bKilograms of force required to shear a 1.27 cm core. ^cStandard deviation. ^dQuality grade category effect. ^eYield grade category effect. ^fCooking method effect. ^gInteraction of quality and yield grade. ^hInteraction of quality grade and cookery method. ⁱInteraction of yield grade and cookery method. ^jInteraction of quality grade, yield grade, and cookery method. ^kNot significantly different ($P > 0.05$).

especially *infraspinatus*, *teres major*, *multifidus/spinalis dorsi*, *serratus ventralis* and *triceps brachii* in the chuck and *gracilis* and *vastus intermedius* in the round, have very desirable WBS values and sensory panel scores if removed intact and cut perpendicular to the muscle fibers (Figs 26.1 and 26.2). Thus, opportunities exist

Table 26.3 Rank of 37 chuck and round muscles by Warner–Bratzler shear force and based on cookery method

Dry cookery method			Moist cookery method		
Musc ^a	Mean ^b	Std dev ^c	Musc	Mean	Std dev
LVC	3.02 ^d	0.69	LVC	2.56 ^d	0.44
BIB	3.28 ^{de}	0.71	INF	2.82 ^{de}	0.64
MSD	3.37 ^{def}	0.78	LCA	3.22 ^{def}	0.53
SUB	3.39 ^{def}	0.80	BIB	3.28 ^{defg}	0.54
INF	3.45 ^{def}	0.78	SUP	3.42 ^{defgh}	0.89
REF	3.65 ^{defg}	0.75	VAI	3.53 ^{efghi}	0.62
PEC	3.70 ^{defg}	0.65	MSD	3.61 ^{efghij}	0.73
VAM	3.73 ^{defgh}	0.74	GRA	3.67 ^{efghijk}	0.61
TEM	3.74 ^{defgh}	0.79	INT	3.68 ^{efghijk}	0.54
SEV	3.82 ^{defghi}	1.00	TEM	3.70 ^{efghijk}	0.51
LCA	3.99 ^{defghij}	0.58	REF	3.81 ^{fghijkl}	0.65
VAI	4.02 ^{defghijk}	0.60	VAM	3.88 ^{fghijkl}	0.63
GRA	4.12 ^{efghijkl}	0.81	SCD	3.90 ^{fghijklm}	0.53
TRB	4.22 ^{efghijklm}	0.70	LGC	4.00 ^{fghijklmn}	1.13
SEE	4.30 ^{fghijklmn}	1.23	SEE	4.10 ^{fghijklmn}	0.70
SPP	4.36 ^{fghijklmn}	0.67	SEV	4.17 ^{ghijklmno}	0.79
SAR	4.45 ^{ghijklmn}	0.47	LGD	4.22 ^{hijklmnop}	0.96
ADD	4.48 ^{ghijklmn}	1.10	COM	4.24 ^{hijklmnop}	0.87
BIF	4.51 ^{ghijklmn}	1.33	PEC	4.27 ^{hijklmnop}	0.73
COM	4.71 ^{hijklmno}	0.93	SPL	4.31 ^{hijklmnopq}	0.77
SET	4.72 ^{hijklmno}	0.84	DSO	4.35 ^{ijklmnopq}	0.94
INT	4.73 ^{hijklmno}	1.36	TRB	4.44 ^{jklmnopq}	0.50
BRA	4.81 ^{ijklmno}	0.45	ADD	4.47 ^{jklmnopqr}	0.59
SPL	4.89 ^{ijklmno}	0.71	TFA	4.55 ^{klmnopqr}	0.77
LAD	4.91 ^{ijklmno}	0.77	SUB	4.56 ^{klmnopqr}	1.03
DSO	5.00 ^{klmno}	0.91	SAR	4.63 ^{lmnopqr}	0.48
LGD	5.02 ^{klmno}	1.11	SPP	4.78 ^{mnopqr}	0.63
SCD	5.08 ^{lmnop}	0.69	DEL	4.79 ^{mnopqr}	0.82
LGC	5.17 ^{mno pq}	1.38	VAL	4.82 ^{no pqr}	0.73
SUP	5.19 ^{mno pq}	0.79	BIF	4.82 ^{no pqr}	1.64
DEL	5.26 ^{no pq}	0.70	BRA	4.89 ^{no p qrs}	0.89
VAL	5.28 ^{no pq}	1.03	SET	5.02 ^{o p qrs}	0.72
DEP	5.69 ^{o p q r}	1.19	RHM	5.11 ^{p qrs}	0.85
GLM	6.04 ^{p qrs}	1.14	GLM	5.12 ^{p qrs}	1.28
RHM	6.09 ^{qrs}	1.35	LAD	5.19 ^{qrs}	0.71
TFA	6.36 ^{rs}	1.00	DEP	5.35 ^{rs}	1.42
BOT	7.03 ^s	1.07	BOT	5.73 ^s	1.06
SEM	0.18		SEM	0.16	

^a Refer to Table 26.1.
^b Kilograms of force required to shear a 1.27 cm core.
^c Standard deviation.
^{d–s} Means in the same column with a common superscript are not significantly different ($P > 0.05$).

to increase the value of these muscles either through innovative merchandising or by muscle-specific intervention strategies.

Table 26.4 Tenderness ranking of 22 muscles of the chuck and round by cooking method

Dry cookery method			Moist cookery method		
Muscle ^a	Mean ^b	RMSE ^c	Muscle	Mean	RMSE
BOT	2.62 ^d	0.51	BOT	2.20 ^d	0.73
SPP	3.81 ^e	0.60	SPP	4.25 ^e	0.87
RHM	4.16 ^{ef}	0.79	RHM	4.91 ^{ef}	1.13
DEP	4.27 ^{efg}	0.82	ADD	5.24 ^{fg}	1.00
SPL	4.63 ^{fgh}	0.49	SET	5.30 ^{fg}	0.72
SEE	4.80 ^{ghi}	0.46	DEP	5.39 ^{fgh}	0.97
BIF	4.92 ^{hij}	0.67	SUO	5.42 ^{fgh}	1.43
ADD	5.02 ^{hijk}	0.54	SEE	5.44 ^{fghi}	0.67
SET	5.08 ^{hijk}	0.41	VAL	5.56 ^{fghij}	0.74
LAD	5.10 ^{hijk}	0.39	BIF	5.67 ^{fghijk}	0.78
SUP	5.23 ^{ijkl}	0.43	COM	5.76 ^{fghijk}	0.98
VAL	5.27 ^{ijkl}	0.73	LAD	5.86 ^{ghijkl}	0.59
COM	5.38 ^{ijkl}	0.49	SPL	5.96 ^{ghijkl}	0.81
GRA	5.38 ^{ijkl}	0.32	REF	6.03 ^{ghijkl}	0.55
VAI	5.47 ^{ijklm}	0.65	VAM	6.06 ^{ghijkl}	0.71
VAM	5.49 ^{klm}	0.47	SUB	6.19 ^{hijkl}	0.76
TRB	5.49 ^{klm}	0.29	VAI	6.28 ^{ijkl}	0.76
MSD	5.56 ^{klm}	0.69	GRA	6.38 ^{ijkl}	0.76
REF	5.74 ^{lm}	0.33	TRB	6.38 ^{ijkl}	0.42
SUB	5.76 ^{lm}	0.41	MSD	6.43 ^{kl}	0.75
SEV	6.00 ^{mn}	0.56	SEV	6.47 ^{kl}	0.79
INF	6.54 ⁿ	0.35	INF	6.67 ^l	0.37
SEM	0.11		SEM	0.17	

^aRefer to Table 26.1.^b8 = extremely tender to 1 = extremely tough.^cSquare root of the mean square error.^{d-f}Means in the same column with a common superscript are not significantly different ($P > 0.05$).

26.2.2 Cow meat

Although culled market cows are primarily a by-product of the beef industry dedicated to producing grain-fed A-maturity beef, they are still a valuable and significant contributor to the beef market. Approximately 5.1 million head of mature (C maturity and older) cows are harvested annually, accounting for almost 13% of domestically produced beef (USDA, 2005). Little is known about muscles, other than the *longissimus*, from older mature cow carcasses and how production practices may influence palatability traits. Therefore, a follow-up study was conducted (Calkins *et al.*, 2003) to evaluate fat thickness, muscling, maturity and carcass weight effect on muscle characteristics, quality and sensory traits (Buford, 2003; Stelzlinski, 2006). The study was conducted to evaluate fat thickness, muscling, maturity, and carcass weight effects on muscle characteristic, quality and sensory traits for both mature dairy and cow beef carcasses (Figs 26.3 and 26.4). Fat thickness, muscling, maturity, and carcass weights had only limited

Muscles	Shear force value – moist	Shear force value – dry	Fat%	Total collagen	Water- holding capacity	pH	Myoglobin	L*	a*
<i>Biceps brachii</i>									
<i>Brachiocephalicus omot.</i>									
<i>Brachialis</i>									
<i>Cutaneous omo brachialis</i>	-----	-----							
<i>Complexus</i>									
<i>Deep pectoral</i>									
<i>Deltoideus</i>									
<i>Dorsalis oblique</i>									
<i>Infraspinatus</i>									
<i>Intertransversales</i>									
<i>Latissimus dorsi</i>									
<i>Longissimus capitus et Atlantis</i>									
<i>Longissimus costarum</i>									
<i>Longissimus dorsi</i>									
<i>Levatores costarum</i>									
<i>Multifidus and spinalis dorsi</i>									

Scalenius dorsalis

Serratus ventralis

Splenius

Superficial pectoral

Subscapularis

Supraspinatus

Tensor fascia antibrachii

Teres major

Trapezius

Triceps brachii

^aThe white cells represent shear force values < 3.86 kg, fat content < 5%, total collagen content < 10 mg/g, water-holding capacity (expressible moisture values) < 36%, pH > 5.8, myoglobin content (heme iron) < 20 mg/kg, L* values > 45 and a* values < 28. The black cells represent shear force values > 4.54 kg, fat content > 10%, total collagen content < 15 mg/g, water-holding capacity (expressible moisture values) > 38%, pH < 5.7, myoglobin content (heme iron) > 32 mg/kg, L* values < 35 and a* values > 29. The grey cells are intermediate.

Fig. 26.1 Youthful beef chuck muscles by trait^a.

Muscles	Shear force value – moist	Shear force value – dry	Fat%	Total collagen	Water- holding capacity	pH	Myoglobin	L*	a*
<i>Adductor</i>									
<i>Biceps femoris</i>									
<i>Gluteus medius</i>									
<i>Gracilis</i>									
<i>Pactineus</i>									
<i>Rectus femoris</i>									
<i>Sartoris</i>									
<i>Semimembranosus</i>									
<i>Semitendinosus</i>									
<i>Vastus intermedius</i>									
<i>Vastus lateralis</i>									
<i>Vastus medialis</i>									

^aSee Fig. 26.1 for definition of cell categories.

Fig. 26.2 Youthful beef round muscles by trait^a.

Muscles	Shear force value	Fat%	Total collagen	Water-holding capacity	pH	Myoglobin	L*	a*
<i>Adductor</i>								
<i>Biceps femoris</i>								
<i>Complexus</i>								
<i>Deep pectoral</i>								
<i>Gluteus medius</i>								
<i>Infraspinatus</i>								
<i>Latissimus dorsi</i>								
<i>Longissimus dorsi, loin</i>								
<i>Multifidus/S pinalis dorsi</i>								
<i>Psoas major</i>								
<i>Rectus femoris</i>								
<i>Semimembranosus</i>								
<i>Semitendinosus</i>								
<i>Serratus ventralis</i>								
<i>Supraspinatus</i>								
<i>Tensor fascia latae</i>								
<i>Teres major</i>								
<i>Triceps brachi</i>								
<i>Vastus intermedius</i>								
<i>Vastus lateralis</i>								
<i>Vastus medialis</i>								

^aSee Fig. 26.1 for definition of cell categories.

Fig. 26.3 Beef cow muscles by trait^a.

Muscles	Shear force value	Fat%	Total collagen	Water-holding capacity	pH	Myoglobin	L *	a *
<i>Adductor</i>								
<i>Biceps femoris</i>								
<i>Complexus</i>								
<i>Deep pectoral</i>								
<i>Gluteus medius</i>								
<i>Infraspinatus</i>								
<i>Latissimus dorsi</i>								
<i>Longissimus dorsi, loin</i>								
<i>Multifidus/Spinalis dorsi</i>								
<i>Psoas major</i>								
<i>Rectus femoris</i>								
<i>Semimembranosus</i>								
<i>Semitendinosus</i>								
<i>Serratus ventralis</i>								
<i>Supraspinatus</i>								
<i>Tensor fascia latae</i>								
<i>Teres major</i>								
<i>Triceps brachii</i>								
<i>Vastus intermedius</i>								
<i>Vastus lateralis</i>								
<i>Vastus medialis</i>								

^aSee Fig. 26.1 for definition of cell categories.

Fig. 26.4 Dairy cow muscles by trait^a.

effects on sensory panel tenderness, juiciness, and flavor intensity. Muscles from dairy cow carcasses were lower in WBS values than corresponding muscles from beef cow carcasses for six of 22 muscles studied.

26.2.3 Cow meat versus USDA Select

In a third study (Mink, 2004; Patten *et al.*, 2007; Stelzlini *et al.*, 2007), commercially identified beef non-fed cows (B-NF), beef fed cows (B-F), dairy fed cows (D-F), and dairy non-fed cows (D-NF) were compared to A-maturity USDA Select grade (Slight degree of marbling) steer and heifer carcass (SEL) to 'benchmark' compositional characteristics, quality and sensory attributes. Instrumental and sensory panel tenderness measurements showed B-NFs to be the least tender group; and SEL the most tender, with the other groups being intermediate and not statistically different (Stelzlini *et al.*, 2007). All cow groups exhibited increased beef flavor intensity sensory panel ratings and increased off-flavor when compared to SEL. The *psoas major* was the most tender muscle studied with the *teres major* and *infraspinatus* second and third. However, the *psoas major* also had one of the highest levels of off-flavor, along with the *gluteus medius* and *triceps brachii*-lateral head. Sensory off-flavor scores were unrelated to fatty acid profiles in any group (Stelzlini, 2006).

26.2.4 Pork meat

The muscle profiling initiative continues. Recently, pork muscles have been characterized (Buege *et al.*, 2003; Doumit *et al.*, 2004).

26.2.5 Veal meat

The authors have just recently finished a similar project on veal muscles. Results of this work can be found in Fig. 25.5.

In the US, the benefits of muscle characterization have been substantial.

26.3 Methods

There were similarities among the four major beef muscle profiling studies that were conducted (young beef, cow beef, cow beef versus USDA Select, and veal). Notably, all samples were aged 14 days prior to determination of the Warner–Bratzler shear force and trained sensory panel evaluation. Although there were slight deviations in sample handling, the basic laboratory procedures were relatively constant to the first study on young beef (Von Seggern *et al.*, 2005). The procedures used to conduct this research will be summarized from this first project.

Our initial study (Brickler, 2000; Von Seggern, 2000; Von Seggern *et al.*, 2005) was designated to profile the muscles of the chuck and round of young (A-maturity) beef carcasses from a commercial harvesting operation from three

Muscles	Shear force value – dry	Fat%	Water- holding capacity	pH	L*	a*
<i>Complexus</i>						
<i>Deep pectoral</i>						
<i>Infraspinatus</i>						
<i>Rhomboideus</i>	-----					
<i>Serratus ventralis</i>						
<i>Splenius</i>	-----					
<i>Supraspinatus</i>						
<i>Teres major</i>	-----					
<i>Triceps brachii</i>						

^a

See Fig. 26.1 for definition of cell categories.

Fig. 26.5 Veal muscles by trait^a.

marbling categories (Modest and Moderate, Small, and Slight), four USDA Yield Grade categories (1, 2, 3, and 4/5 together) and three weight classes (250 to 295 kg, 296 to 385 kg, and 386 to 431 kg). One hundred and forty-two carcasses were selected representing four chucks and rounds for each marbling by yield grade by weight cell for muscle boning and subsequent analysis. Chuck and rounds from the 296 to 385 kg weight category were analyzed for Warner–Bratzler shear force and sensory panel evaluation.

Muscles for Warner–Bratzler shear analysis were selected from 48 chucks and rounds from carcasses with a weight of 296 to 385 kg. They were selected to obtain four samples (carcasses) per each quality, yield, and weight cell. Twenty-five muscles from the chuck and twelve muscles from the round were sampled for shear force determination (Table 26.1).

The muscles from two of the four animals of the four yield grade categories and three quality grades, for a total of 24 samples, were cooked utilizing a dry heat cookery method on a Farberware Open-hearth Grille (Yonkers, NY). The samples were cooked to an internal temperature of 71 °C and turned after the internal temperature reached 35 °C. The temperature was monitored using a thermocouple (Omega Engineering, Inc., Stamford, CT) and recorded using a 1100 Labtech Notebook for Windows 1995 (Computer Boards, Inc., Middleboro, MA). The muscles from the remaining animals were cooked by a moist cookery method. The cuts were placed in a preheated (204 °C) non-stick electric frying pan (Westbend Company, West Bend, WI) that was lightly sprayed with a non-stick cooking spray (Great Value, Wal-Mart Stores, Inc., Bentonville, AR). Cuts were browned for one to 1.5 minutes on each side depending on the cut size (small cuts less, larger cuts more). After browning, a thermocouple was placed into the geometric center. The cut was then placed on a wire rack in an oven-safe Pyrex Dutch oven with 50 ml of added water. The product was covered and cooked to an internal temperature of 71 °C at a 135 °C oven temperature. The temperature was monitored using a thermocouple (Omega Engineering, Inc., Stamford, CT) and recorded using a 1100 Labtech Notebook for Windows 1995 (Computer Boards, Inc., Middleboro, MA).

Thaw loss, cook loss, and total cooking time were measured for all samples. After cooking, the muscles or muscle portions were allowed to cool at 4 °C for 24 hours. After cooling, cores were removed for Warner–Bratzler shear force determination according to American Meat Science Association (AMSA 1995) guidelines. The cores, 1.27 cm in diameter, were removed parallel to the longitudinal orientation of the muscle fibers. The cores were sheared perpendicular to the longitudinal orientation of the muscle fibers with a Warner–Bratzler attachment (crosshead speed = 200 mm/min) on an Instron Universal Testing machine (Instron Corporation, Canton, MA).

Muscles for sensory panel analysis were selected from 48 chucks and rounds whose carcass weight was between 296 to 385 kg. Four samples (carcasses) per each of the selection cells were used.

One half of the muscles (i.e. two observations per cell) per each of the four yield grade categories and three quality grade categories, for a total of 24 samples, were

cooked utilizing a dry heat cookery method on a Farberware Open-hearth Grille (Yonkers, NY). The samples were cooked to an internal temperature of 71 °C and turned after the internal temperature reached 35 °C.

The remaining half of the muscles (observations per cell) was cooked by a moist cookery method by the Sensory Analysis Center of Kansas State University. The method was identical to the moist cookery method that was used in the Warner–Bratzler shear force analysis. Sensory panels were conducted according to AMSA (1995) guidelines.

Sensory panel evaluation was conducted on all muscles removed from the chuck and the round that were over 0.5 kg. This included 13 muscles from the chuck and nine muscles from the round for a total of 22 muscles. Sensory panel traits included juiciness (eight = extremely juicy; one = extremely dry), flavor (eight = extremely intense; one = extremely bland), tenderness (eight = extremely tender; one = extremely tough), connective tissue (eight = none detected; one = abundant amount), and off-flavor (six = none detected; one = extreme off-flavor).

Fresh (unfrozen) muscles were opened and a slice (about 6 mm thick) from the center of each muscle was obtained. Approximately 3 g of muscle was retained for determination of expressible moisture – termed water-holding capacity in the figures.

Color was objectively assessed on the external surface of the muscles that had been allowed to bloom (oxygenate) for 60 minutes. A Hunter Lab Mini-Scan XE Plus (Reston, VA) that had a 2.54 cm port was used with illuminant A and a 10 degree standard observer. Triplicate readings were averaged for L* (a measure of lightness, where larger numbers indicate more lightness), a* (a measure of redness, where larger numbers indicate more red color), and b* (a measure of blueness, where larger numbers indicate more blue).

Water-holding capacity (expressible moisture) was determined through centrifugation (Jauregui *et al.*, 1981). A 0.3 g sample was folded into three pieces of filter paper and centrifuged at $32\,566 \times g$ for 15 min at 4 °C. Water-holding capacity (expressible moisture) was reported as the percentage of weight lost from the original sample weight.

Composite samples were created by combining two identical muscles from separate carcasses within the selection cells that were used. This was done to minimize sample numbers. Muscles were ground together using a 0.95 cm plate on a Toledo Chopper (Toledo Scale Co., Toledo, OH). Subsamples were obtained from the ground, composite sample. One was frozen at –80 °C in an ultralow freezer and later homogenized (powdered) in liquid nitrogen using a Waring blender (Waring Products Division, New Hartford, CT) for determination of total collagen content, total heme iron content, and proximate composition. The other sample was held at 4 °C for emulsion capacity measurement and was subsequently frozen for determination of muscle pH. Each analytical procedure was conducted in duplicate.

Muscle pH was measured on a 10 g sample of meat that was homogenized in 100 ml of distilled, deionized water. A spear tip electrode (Corning Model 476580,

Corning Inc., Corning, NY) was attached to an Orion SA 720 pH meter (Orion Research, Inc., Boston, MA).

A LECO Thermogravimetric Analyzer-601 (Model 604-100-400, LECO Corp., St. Joseph, MI) was used to determine moisture and ash. This is an automated system that determines moisture loss and then ramps up the temperature to determine ash. Fat content was determined by ether extraction using the procedure of the Association of Official Analytical Chemists (AOAC, 1990).

A subsample of muscles (yield grade two for the high and low weight ranges) was evaluated for collagen content (Hill, 1966). The spectrophotometric procedure of Bergman and Loxley (1963), as modified by Kolar (1990) to increase the pH in the oxidant solution, was used to quantify hydroxyproline. The hydroxyproline concentration was multiplied by 7.25 to calculate total collagen (Goll *et al.*, 1963).

Total heme iron concentration was determined through extraction (Hornsey, 1956, as modified by Lee *et al.*, 1998). These values were converted to total pigment using the procedures of Lee *et al.* (1998).

26.4 Optimization

The initial Muscle Profiling project identified a group of muscles that were very desirable in palatability when evaluated individually and cut across the muscle grain. In addition to these high-quality muscles from the chuck and round, the muscle profiling study also identified another group of muscles that were intermediate in palatability, having shear force values between 3.5 and 5.0 kg and with sensory panel tenderness scores between slightly tender and slightly tough. Tenderness was not affected by carcass yield grade or by cooking method for this group of muscles (Jones *et al.*, 2004).

The rationale for our next project was to determine if eight individual muscles from the beef chuck, identified as borderline in palatability, could be enhanced by adding water, salt, and phosphate. If palatability, especially tenderness, of these eight muscles could be improved by enhancement, the muscles could offer additional alternatives to higher value cuts. These individual muscles from the beef chuck could be a good source of raw material for fresh or precooked, easy-to-prepare, beef products. Consumers could have a more convenient beef alternative and the value of the primal chuck could be increased.

The objective was to evaluate four techniques for incorporation of ingredients into muscles and evaluate their influence on palatability traits after cooking and during frozen storage and reheating (Molina *et al.*, 2005). Eight individual muscles from the beef chuck, characterized as having no major amounts of connective tissue, having shear force values in between 3.5 and 5.0 kg, and having sensory scores for tenderness between slightly tough and slightly tender were selected for analysis.

The study was replicated three times. For each replicate, 12 USDA Select two-piece beef chucks were purchased from a national supplier. From each two-piece chuck, the *complexus* (COM), *latissimus dorsi* (LAD), *rhomboideus* (RHB),

serratus ventralis (SEV), *splenius* (SPL), *subscapularis* (SUB), *supraspinatus* (SUP), and *triceps brachii* (TRB) muscles were extracted for evaluation. For each replicate of the study, three individual muscles were selected randomly from each muscle group and assigned to one of four treatments. At the end of the experiment, a total of nine representatives of each muscle group were used for each treatment.

Brine was formulated to have, after a ten percent brine pick-up, an end product with 0.5% sodium chloride (salt) and 0.4% sodium tripolyphosphate (STPP). The four treatments evaluated were: control with no added ingredients, marinated, needle pumped, or vacuum tumbled. A Dorit Injectomat PSM -10 (Dorit Machinery, Balmhotstr, Germany) was used to inject chilled brine ($2 \pm 2^\circ\text{C}$) at 0.7 bar pressure to reach the prescribed 10% pick-up for injected samples. A Lyco Model 40 (Columbus, WI) vacuum tumbler set at 28 rpm was utilized for the tumbled samples. Marinated samples were soaked as a group in excess brine at $2 \pm 2^\circ\text{C}$ without agitation until a 10% added weight was achieved, after a 30 s drip period. After injection or tumbling, muscles were weighed then individually vacuum-packed in Cryovac B620 bags (three to six cc/m² 24 h, one atm @ 4.5°C , 0% RH) held at 2°C until the marinated muscle treatment reached 10% pickup. The control muscles were packaged at the same time as the injected and tumbling treatments. Samples were allowed to equilibrate in the vacuum package for 24 h at 2°C after the marinate treatment reached its target pickup level, then all treatments were blast frozen (-40°C) until palatability analysis could be conducted. All muscles were of the same postmortem age when frozen. Large muscles (SUP, SEV, and TRB) were cut into 2.54 cm thick steaks and cooked on Farberware Open-Hearth Broilers (Farberware Products, Nashville, TN). The rest of the muscles were cooked whole in gas ovens (GE Model JGRS14, Louisville, KY) preheated to 135°C . Temperatures were monitored using copper-constantan thermocouples (Omega Engineering, Inc., Stamford, CT) and recorded using a 1100 Labtech Notebook for Windows 1998 (Computer Boards, Inc., Middleboro, MA). Steaks were turned after the internal temperature reached 35°C . Roasts were cooked in an open roasting pan and were not turned during cooking. Steaks and roasts were both cooked to an internal temperature of 71°C (AMSA, 1995).

Two sensory evaluations and two WBS determinations were conducted for each muscle. The first was conducted immediately after being cooked and the second after being cooked and stored frozen for 60 days. After the 60-day period, the samples were reheated to an internal temperature of 60°C using a microwave oven (75% power; Panasonic, Genius 1300). Samples were microwaved for 45 s per 100 g of sample weight and temperature was monitored with a thermometer. Cooked samples were packaged in vacuum bags (Foodsaver, Tilia, Inc. Patent #34,929) and stored at $-10 \pm 2^\circ\text{C}$.

The added ingredients did not have a significant effect on WBS with the exception of three muscles (Table 26.5). When needle pumped, the COM and TRB muscles had lower shear force values than the untreated control. All three enhanced treatments were shown to reduce shear force values in the SUB. There were no significant differences in any muscles among treatment means for application method. Numerically, the average WBS value reduction of the enhanced

Table 26.5 Effects of enhancement on Warner–Bratzler shear force (WBS) determination of oven-roasted (OR) *complexus*, *latissimus dorsi*, *rhomboideus*, *splenius*, *subscapularis* and grilled steaks (GR) from the *serratus ventralis*, *supraspinatus*, and *triceps brachii* muscles

Treatment	WBS-OR ^a					WBS-GR ^b		
	Complexus	Latissimus dorsi	Rhomboideus	Splenius	Subscapularis	Serratus ventralis	Supraspinatus	Triceps brachii
Control	3.5	4.7	4.1	3.2	3.5	3.3	3.7	3.6
Marinated	2.8	4.0	3.3	2.9	2.5*	2.8	3.1	2.8
Needle-pumped	2.3*	4.1	3.4	2.9	2.2*	2.5	2.9	2.6*
Vacuum-tumbled	2.7	4.4	3.8	2.7	2.7*	2.7	3.2	2.8
SEM	0.26	0.30	0.29	0.22	0.11	0.17	0.20	0.19
Day of evaluation								
Day-1	2.3	3.9	3.0	2.4	2.5	2.3	2.5	2.5
Day-60	3.4	4.7	4.2	3.4	3.0	3.5	3.9	3.04
<i>P</i> value	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
SEM	0.11	0.18	0.14	0.11	0.06	0.15	0.12	0.17

* Shows means that differed from the untreated control ($P < 0.05$) according to the Dunnett's test.

^a Warner–Bratzler shear values in kilograms for muscles roasted in a gas oven at 135 °C until the internal temperature reaches 71 °C.

^b Warner–Bratzler shear values in kilograms for grilled steaks (2.54 cm thickness) cooked to 71 °C internal temperature.

Table 26.6 Effects of enhancement on sensory panel overall tenderness of the oven-roasted (OR) *complexus*, *latissimus dorsi*, *rhomboideus*, *splenius*, *subscapularis*, and grilled steaks (GR) from the *serratus ventralis*, *supraspinatus*, and *triceps brachii* muscles

Treatment	Overall Tenderness-OR ^a					Overall Tenderness-GR ^a		
	Complexus	Latissimus dorsi	Rhomboideus	Splenius	Subscapularis	Serratus ventralis	Supraspinatus	Triceps brachii
Control	6.2	5.2	5.1	5.8	6.1	5.2	5.0	5.6
Marinated	6.5	5.9	5.9	6.3	6.68	6.1 ^{*xy}	6.0 ^{*y}	6.4 [*]
Needle-pumped	6.8	5.9	5.6	6.2	6.72 [*]	6.5 ^{*y}	5.9 ^{*xy}	6.9 [*]
Vacuum-tumbled	6.6	5.5	5.7	6.4	6.6	5.9 ^{*x}	5.5 ^x	6.4 [*]
SEM	0.2	0.18	0.33	0.17	0.13	0.11	0.18	0.16
Day of evaluation								
Day-1	6.7	5.9	5.9	6.4	6.7	6.2	5.9	6.5
Day-60	6.4	5.3	5.3	5.9	6.3	5.6	5.4	6.1
<i>P</i> value	0.04	<0.01	<0.01	<0.01	0.03	<0.01	<0.01	<0.01
SEM	0.09	0.06	0.04	0.05	0.11	0.08	0.07	0.07

* Shows means that differed from the untreated control ($P < 0.05$) according to the Dunnett's test.

^a Overall tenderness scores (5 = slightly tender, 6 = moderately tender) for muscles oven roasted in a gas oven at 135 °C until the internal temperature reached 71 °C.

^b Overall tenderness scores for grilled steaks cooked to 71 °C internal temperature.

^{x,y} Means with different superscript illustrate differences among treatments ($P < 0.05$) according to the Duncan's test. The untreated control was excluded from this test.

muscles was almost 20%, showing a trend for increased tenderness due to the addition of water, salt, and STPP. Prestat *et al.* (2002) showed that injecting pork loin chops with a solution of salt and phosphates reduced shear force measurements, even when cooked to a higher endpoint temperature, showing that added ingredients offered protection from the detrimental effects of over-cooking.

Similar to the tendency shown for shear force measurements, a trend for increased tenderness by the addition of water, salt, and STPP was noted in the sensory tenderness scores (Table 26.6). The average numeric score change due to enhancement was 0.7 of a sensory unit, an increase in tenderness of almost 13%. This improvement was significant in only four of the eight muscles measured. The SEV and TRB muscles were improved by added ingredients regardless of the application technique used. The SUP muscle tenderness improved when marinated and when needle-pumped, while the SUB muscle was improved only when needle-pumped. Differences between application techniques were detected in only two muscles. The SEV appeared to be more tender when needle-pumped than when vacuum-tumbled. When marinated, the SEV was intermediate with no differences from the other two techniques. The marinated SUP muscle was more tender than the vacuum-tumbled treatment but was not different from the needle-pumped treatment.

It was also noted that the marinated treatment appeared to have the greatest influence on juiciness and was different from the control in seven of eight muscles (data not presented in tabular form, Molina *et al.*, 2005). Brine treatment reduced sensory detected connective tissue in only two of eight muscles. More off-flavors were detected by the panel for marinated samples from six of eight muscles. All muscles had lower values for WBS, less connective tissue and off-flavors, and higher juiciness, overall tenderness, and beef flavor intensity on day one than day 60. The needle-pumped method was slightly superior to the marinated and vacuum-tumbled treatments, even though the differences were not always consistent. But regardless of the application method, palatability traits were generally enhanced by brine treatments.

26.5 Future trends

Indeed, knowledge is power. Knowledge of the average and variation for the myriad of muscle traits for each individual muscle can be used to develop new, muscle-specific, consumer-friendly products. Ultimately, it will be up to the meat industry to put this information to work. One thing is certain. Without these data, it would not be possible to do so. In 2006, the US sold over 42 million kg of flat iron steaks. These steaks are derived from the *infraspinatus*. Prior to the muscle profiling research, nearly all of them were being sold as ground beef or as an economically priced beef roast. This is but one example of how value-adding can occur.

There are some relationships between these biochemical and physical traits and meat properties. Muscles with higher pH have better water-holding capacity,

though they tend to be darker in color. A higher amount of connective tissue is usually associated with less tender beef and often responds to long time–low temperature cooking or other processing interventions. Through the application of science-based intervention strategies to specific muscles, new, high-quality consumer products can be developed.

26.6 Sources of further information and advice

Additional resources can be obtained from the bovine and porcine myology web sites maintained at the University of Nebraska. They can be found at <http://bovine.unl.edu> and <http://porcine.unl.edu>. Readers are encouraged to access these information-rich web sites and explore the variety of data and educational strategies that have been presented. Like all web sites, these continue to be developed. A three-dimensional presentation for muscle identification and fabrication is under development.

The National Cattlemen's Beef Association (Centennial, CO) has the *Bovine Myology and Muscle Profiling* (Jones *et al.*, 2004) book available at minimal cost. This, too, is a powerful tool. Additionally, the National Pork Board (Des Moines, IA) has muscle profiling booklets for pork.

26.7 References

- AMSA (1995), *Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat*. American Meat Science Association, Savoy, IL, USA.
- AOAC (1990), *Official Methods of Analysis*, 15th ed. Association of Official Analytical Chemists, Washington, DC.
- Bergman, I. and Loxley, R. (1963), Two improved and simplified methods for spectrophotometric determination of hydroxyproline. *Analytical Chemistry*, 35, p.1961–1965.
- Brickler, J. E. (2000), *The Effect of Carcass Weight, Yield Grade, Quality Grade, and Cooking Method on Physical Attributes, Warner–Bratzler Shear Force, and Sensory Panel Characteristics on Muscles of the Beef Chuck and Round*. M. S., University of Florida, USA.
- Buege, D., Sebranek, J., Doumit, M., Marple, D., Ahn, D., Huff-Lonergan, E., Lonergan, S., Fedler, C., Prusa, K., Helman, E. and Meisinger, D. (2003), *Pork Muscle Profiling*. [Online]. Available at: http://www.meatscience.org/pubs/rmcarchv/2003/presentations/rmc_2003_056_1_0000_all.pdf [accessed 29 September 2007]
- Buford, M. L. (2003), *Cow Muscle Profiling: Chemical and Physical Properties of 21 Muscles from Beef and Dairy Cow Carcasses*. M. S. Thesis, University of Nebraska, Lincoln, NE.
- Calkins, C. R., Johnson, D. D. and Gwartney, B. L. (2003), *Cow Muscle Profiling*. [Online]. Available at: http://www.meatscience.org/pubs/rmcarchv/2003/presentations/rmc_2003_056_1_0000_all.pdf [accessed 29 September 2007].
- Doumit, M. E., Ahn, D., Fedler, C., Huff-Lonergan, E., Huisinga, B., Larson, E., Lonergan, S., Marple, D., Petersohn, R., Prusa, K., Sebranek, J. and Buege, D. (2004), *Pork Muscle Profiling 2002 - NP#02-190*. [Online]. Available at: <http://www.pork.org/PorkScience/Research/Documents/02-190-DOUMIT.2-13-04.pdf>. [accessed 20 September 2007].

- Goll, D. E., Bray, R. W. and Huekstra, W. G. (1963), Age associated changes in muscle composition. The isolation and properties of collagenous residue from bovine muscle. *Journal of Food Science*, 28, 503–509.
- Hill, F. (1966), The solubility of intramuscular collagen in meat animals of various ages. *Journal of Food Science*, 31, 161–166.
- Hornsey, H. C. (1956), The color of cooked cured pork. I. Estimation of nitric oxide–haem pigments. *Journal of the Science of Food and Agriculture*, 7, 534–540.
- Jauregui, C. A., Regenstien, J. M. and Baker, R. C. (1981), A simple centrifugal method for measuring expressible moisture, a water-binding property of muscle foods. *Journal of Food Science*, 46, 1271, 1273.
- Jones, S. J., Calkins, C. R., Carpenter, B., Gwartney, B. L. and Johnson, D. D. (2004), *Bovine Myology and Muscle Profiling*. National Cattlemen's Beef Association, Centennial, CO, USA.
- Kolar, K. (1990), Colorimetric determination of hydroxyproline as a measure of collagen content in meat and meat products: NMKL collaborative study. *Journal of the Association of Official Analytical Chemists*, 73, 54–57.
- Lee, B. J., Hendricks, D. G. and Cornforth, D. P. (1998), Antioxidant effects of carnosine and phytic acid in a model beef system. *Journal of Food Science*, 63, 394–398.
- Mink, L. E. (2004), *Properties of Cow and Beef Muscles: Benchmarking the Differences and Similarities*. M.S., University of Nebraska – Lincoln, USA.
- Molina, M. E., Johnson, D. D. and Gwartney, B. L. (2005), Enhancing palatability traits in beef chuck muscles. *Meat Science*, 71, 52–61.
- Patten, L. E., Hodgen, J. M., Stelzl, A. M., Calkins, C. R., Johnson, D. D. and Gwartney, B. L. (2007), Properties of cow and beef muscles: Benchmarking the differences and similarities. *Journal of Animal Science*. Published online March 28, as doi 10.2527/jas.2007–0478.
- Prestat, C., Jensen, J., McKeith, F. K. and Brewer, M. S. (2002), Cooking method and endpoint temperature effects on sensory and color characteristics of pumped pork loin chops. *Meat Science*, 60, 395–400.
- Stelzl, A. M. (2006), *Feeding and Aging Effects on Carcass Composition, Fatty Acid Profiles and Sensory Attributes of Muscles from Cull Cow Carcasses*. Ph. D., University of Florida, USA.
- Stelzl, A. M., Patten, L. E., Johnson, D. D., Calkins, C. R. and Gwartney, B. L. (2007), Benchmarking carcass characteristics and muscles from commercially identified beef and dairy cull cow carcasses for Warner–Bratzler shear force and sensory attributes. *Journal of Animal Science*, 85, 2631–2638.
- USDA (2005), *Market News Report: National Weekly Boxed Beef Cut Out and Boxed Beef Cuts. LM XB 459*. Agricultural Marketing Service, United States Department of Agriculture, Des Moines, IA.
- Von Seggern, D. D. (2000), *Physical and Chemical Properties of 39 Muscles from the Beef Chuck and Round*. M. S., University of Nebraska – Lincoln, USA.
- Von Seggern, D. D., Calkins, C. R., Johnson, D. D., Brickler, J. E. and Gwartney, B. L. (2005), Muscle profiling: Characterizing the muscles of the beef chuck and round. *Meat Science*, 71, 39–51.

Animal welfare and meat quality

J. Hartung, B. Nowak and A. C. Springorum, University of Veterinary Medicine, Hanover, Germany

Abstract: Meat-producing animals such as cattle, pig and poultry are today usually raised in modern intensive indoor farming systems and, after finishing, transported to an abattoir where they are stunned and slaughtered. There is increasing concern among consumers about welfare of these animals, and that the poor welfare an animal suffers in the course of its life may have a negative influence on the quality of the meat deriving from this animal. Housing systems can have a high influence on animal welfare but there is little evidence that meat quality is impaired under usual management conditions. Transport stress can cause poor carcass quality, too low pH values and possibly PSE (pigs, turkey) or DFD meat (cattle) when the animals are transported for too long or under inappropriate conditions. Stunning and slaughter seem to have the highest impact on both animal welfare and meat quality. In order to protect the animals and meat quality, a critical review of all common housing systems, allowed transport and currently applied stunning methods should be carried out. 'Animal-friendliness' should become an additional and important quality trait for meat. This will meet consumer demands and will influence their purchasing decision. It is therefore necessary to further promote labelling systems, which give the consumer the choice between different product lines. Successful meat production in future has to be based on transparency of the food chain, displaying the safety of the product and the health and the welfare of the animals that provide the meat.

Key words: meat quality, animal welfare, housing conditions, transport and lairage, stunning and slaughter.

27.1 Introduction

Meat-producing animals such as cattle, pig and poultry are usually raised today in modern, intensive, indoor farming systems in order to make best use of their selected genetic qualities which enable them, under appropriate housing, feeding, hygiene, management and veterinary control, to reach high growth rates.

At the same time, the welfare of farm animals is of increasing public concern

(Bennett, 1996; Miele and Parisi, 2001). In recent polls, EU citizens rank it highly, giving it 8 out of 10 on average in terms of importance. Moreover, 62% would change shopping habits in order to access more animal welfare friendly goods (Eurobarometer, 2007), inferring that welfare is of such concern they would choose a product that had been reared to good welfare standards and avoid purchasing products they perceived as not being welfare friendly. Although animal welfare is now a major issue on the public agenda, and people refer to their role as consumers in public debates, these concerns are not reflected in the actual market shares of animal friendly products across Europe. The reasons for this discrepancy in the behaviour of the consumer are manifold. Firstly, there is a considerable lack of knowledge and understanding of modern animal production, which is characterised by the terms intensification, specialisation and regional concentration. Large farms are keeping high numbers of animals of one animal species only and which are often concentrated in certain rural areas where they can create environmental pollution of soil, water and air by odours, heavy metals, drug residues or a surplus of nutrients (Hartung and Wathes, 2001; Hamscher *et al.*, 2003). Secondly, the consumer wants to have food of the highest quality and safety available at any time for the lowest possible price. Thirdly, the consumer is confused about the scarcity of data that clearly demonstrate the influence of poor welfare an animal suffers in the course of its life on the quality of the meat deriving from that animal.

The hazards faced by meat-producing animals during their life cycle, possibly leading to poor welfare and consecutively to poor meat quality, may be derived from the husbandry system and its management, transport, stunning and the slaughter procedure. The animals are exposed to these hazards over longer or shorter periods with quite different intensities. After hatching, broilers and turkeys spend their whole life-span, which is about five weeks for broilers and about 20 weeks for tom turkeys, in the same barn. Following weaning, pigs remain in the same intensive production rearing systems for several months, while veal calves change after being kept in single crates for the first few weeks to systems consisting of small group holdings for final fattening. Meat-producing animals are usually transported twice during their lives. These transports can last between periods of a few minutes to the adjacent farm and for several hours or even days from the breeding farm to the fattening unit or, after finishing, to a slaughter house in another region or country. In the lairage of the abattoir and during stunning and slaughter, the animals are exposed to short but intensive stress situations.

This chapter will elaborate upon whether there is scientific evidence that poor animal welfare which typical meat-delivering animals such as cattle, pig, broiler and turkey may suffer while being raised in a particular keeping system and during transport, stunning and slaughter, has a negative effect on meat quality and whether this can be demonstrated by an orientating risk assessment approach.

27.2 Definition of animal welfare

There exists a variety of descriptions and definitions of the term animal welfare,

which range from very general physiological expressions such as ‘the animal lives in harmony with the environment’ (Hughes, 1976) to the more active and behavioural approach of an animal, which should be able ‘to cope with the environment’ (Broom, 1986). While there is a close relationship between health and well-being, ‘welfare’ can also be seen as part of mental health. The health of an animal encompasses both physical and mental health; this includes physical fitness, freedom from infectious and non-infectious diseases, absence of abnormal behaviour, pain and suffering and the ability to carry out essential life-maintaining tasks.

Many infectious (e.g. Foot and Mouth disease, Swine Fever, Bluetongue, Avian Influenza), as well as non-infectious (e.g. injuries, dystocia) diseases, cause severe welfare problems. Mental ill-health in animals is often not easy to recognise by typical clinical measures, but can be identified by behavioural indicators which are described as unusual in form, frequency and duration. Such pathological behaviours are often induced by the animals’ environmental conditions and can cause physical harm and disease. These animals are less fit, both for their breeding purpose and in an evolutionary sense. Examples would be animals kept in confined conditions (small cages) or that are permanently tethered.

The two main areas in which welfare can be judged stem from measures of their physiological responses and from their behaviour. For example, immunosuppression as a result of prolonged corticosteroid release due to prolonged poor welfare will make an animal less fit to resist disease, and may even jeopardise food safety and quality. Environmental stressors may lead to distress – where an animal loses its ability to adapt to its environment (Broom, 1988). Prolonged exposure to stressors can result in pituitary malfunction that will affect many areas of zootechnical performance, such as reproduction (e.g. prolonged calving intervals), growth rate, feed conversion, as well as other aspects of the endocrine system. Even with less of a stress response but still causing the animal poor welfare, productivity can still be adversely affected.

The second area of recognising poor welfare is using observations of their abnormal behaviour, posture and responses, as well as when they show overt clinical signs of abnormality such as lameness, diarrhoea, and respiratory distress. Such signs may reflect some intercurrent disease (mastitis) or a failure to adapt to the method of husbandry (sham chewing, repeated licking, pacing, repeated escape movements, feather pecking, and cannibalism). Only animals that are ‘sentient’ can ‘suffer’, and so, only when an animal has developed to a stage when it has the necessary organs and neural connections, will it be able to experience poor welfare due to pain, distress, frustration, boredom, etc. The Treaty of Amsterdam refers for the first time in European legislation to animals, including farm animals, as sentient beings which have to be protected: ‘The high contracting parties, desiring to ensure improved protection and respect for the welfare of animals as *sentient beings*, have agreed upon the following provision which shall be annexed to the Treaty establishing the European Community’ (Protocol on protection and welfare, 1.5.99).

There is increasing concern among consumers that the quality of the meat

Table 27.1 Indicators for poor animal welfare

	Indicators
Clinical/pathological	<ul style="list-style-type: none"> – disease (e.g. fever, immobility, dysentery) – injuries (e.g. lesions, fractures)
Ethological	<ul style="list-style-type: none"> – abnormal behaviour (e.g. stereotypes) – fear – aggression – flight
Physiological	<ul style="list-style-type: none"> – changed heart rate (e.g. tachycardia) – changed body temperature – release of stress hormones (e.g. adrenaline, cortisol) – abnormal posture

deriving from these sentient animals may be affected by the stress the animals suffer from the intensive keeping conditions and during transport and slaughter, leading to poor animal welfare. An intensive debate started recently on the consumption of meat deriving from cloned animals (FDA, 2008) because a large majority of the live born cloned piglets and calves are suffering from various diseases and only 58 to 67% of calves survive the first six months after birth, raising questions on welfare and on meat quality (EFSA, 2008).

The most important welfare problems are related to insufficient feeding and malnutrition, poor housing, shelter and management, pain, injuries and disease, treatment and the situations which cause fear, stress and mental suffering, and to conditions which do not allow the display of normal behaviour. These factors also formed the basis for the so called ‘five freedoms’ of farm animals (FAWC, 2004). The response of the animals to harmful conditions and situations can only be assessed indirectly by clinical, pathological, ethological and physiological indicators (Table 27.1).

27.3 Meat quality traits

Meat quality is a broad term and covers a variety of characteristics. The traits can be divided into four groups of parameters (Fig. 27.1), one covering components which are important for human nutrition – ‘animal nutrition is important for creating foods closer to the optimum composition for long-term human health’ (Givens and Shingfield, 2004); a second one referring to hygiene and toxicological aspects such as bacterial contamination and residues of toxic and pharmaceutical compounds; a third series of parameters concerning meat processing; and a fourth listing important sensory parameters which have the highest influence on the consumers’ purchase decision (Schutz *et al.*, 1986; Fishbein and Ajzen, 1975).

Consumer quality preferences (and therefore market value) for meat are generally placed upon colour, marbling, texture and juiciness. The colour of meat is usually measured by the $L^*a^*b^*$ colour space system, which was developed by

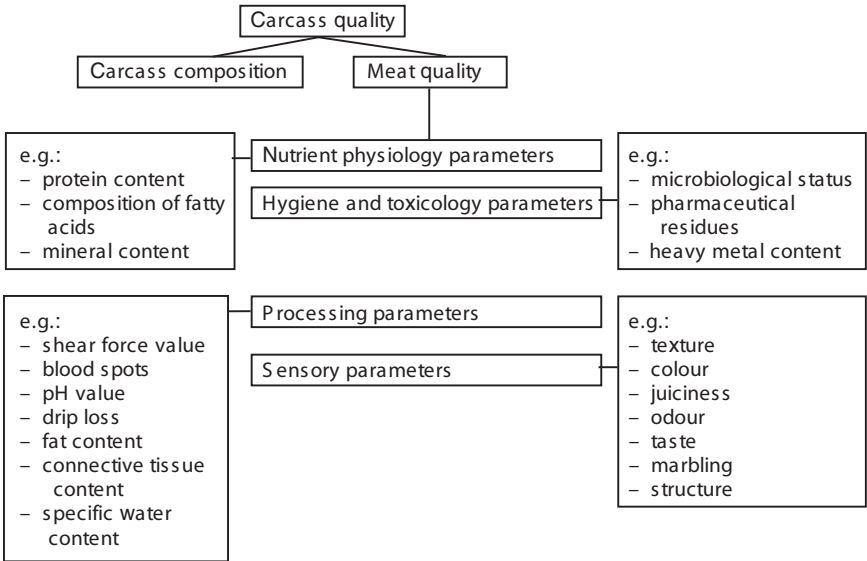


Fig. 27.1 Parameters of carcass and meat quality for meat-producing animals.

the International Illumination Commission (CIE; Commission Internationale de l'Eclairage) in the year 1976 and is designed according to human colour perception. The L^* value is a measurement for brightness, ranging from 0 (black) to 100 (white). In meat classification this means that the higher the L^* value, the paler the meat. The a^* value displays the colour range from green to red (–150 to +100). Negative values stand for the green share, positives for the red share. A high and positive a^* value in meat classification means an intensive red colouring. The b^* value is a scale unit for the colour range from blue to yellow (–100 to +150). Negative values stand for the blue share, positives for the yellow share. A high and positive b^* value indicates an intensive yellow colouring.

Marbling has been related to flavour, juiciness, and tenderness and is directly dependent on intramuscular fat content (IMF). Meat with a higher marbling score (1 = low to 10 = high) would be expected to have better eating quality. Nevertheless, consumers differ in their preferences for marbling based on attitudes towards eating quality versus increased calories associated with increased marbling.

Drip loss is associated with the firmness and water-holding capacity of the meat. Not only is high drip loss unattractive, it can result in excessive cooking losses and drying of meat during cooking.

Meat-solidity is a quality characteristic of importance for meat processing. It is measured by meat texture (e.g. shear force or compression force), which affects the performance at distortion.

The most important meat quality indicator is the pH value, which is highly correlated to colour, drip loss, and eating quality traits. As pH declines below the ideal range (e.g. 5.8 to 6.2 for pork), meat becomes paler, softer and higher in drip

loss. In addition, meat characteristics such as pale, soft and exudative (PSE) or dark, firm and dry (DFD) meat, skin injuries, broken bones and blood spots in muscle tissues can indicate that the animals suffered from significant stress at some stage during their life on the farm, during transport or at slaughter.

27.4 Impact of housing and management on meat quality

The growing demand for meat in Europe since the 1950s has led to intensive production systems for pork, beef and poultry, with all-year indoor keeping. Such confined and specialised buildings allow high animal densities and highly efficient phase feeding, resulting in fast growth, shorter fattening periods and increased production. With the evolution of confinement management strategies, reductions of air quality within facilities may occur, resulting in reductions in animals respiratory performance. At meat inspection, the lungs of approximately 30% of slaughter pigs from confined indoor keeping systems display younger or older lung tissue alterations deriving from earlier respiratory affections (Elbers, 1991; Blaha and Blaha, 1995). Meat-producing animals with reduced lung capacity may be more likely to experience acute acidosis under stress, which may result in blood pH reductions of 0.5 pH units and body temperature increases of 1–2.5 °C. Depending on the time before slaughter, this condition may lead to PSE or DFD meat (Carr *et al.*, 2005).

Pigs raised in outdoor housing systems showed distinctly lower lung affections at slaughter (Christiaens, 1987), lower morbidity and mortality during all raising periods, more active behaviour, higher live weight gain in weaned and in fattening pigs, but higher feed consumption as compared to indoor-housed pigs (Lahrmann *et al.*, 2004; Lebret *et al.*, 2006). Meat from free-range pigs resulted less often in meat classification E (lean meat percentage >55%). Results from studies regarding the impact of outdoor housing on pH value of pork differ from no effect (Lahrmann *et al.*, 2004; Warriss *et al.*, 1983) to slightly decreased pH in meat from pigs with outdoor access (Lebret *et al.*, 2006; Enfält *et al.*, 1996). Similarly, results gained for alternative systems (deep litter or more space with separated areas) compared with conventional housing range from lower pH (pigs from deep litter system, Morrison *et al.*, 2007) through no effect (feeding and resting areas, Barlocher, 2006), to higher pH values (extra space, Liorancas *et al.*, 2006). Also, temporary straw beddings did not show an effect on pH value (Peeters *et al.*, 2006).

Nevertheless, it has to be considered that different meat qualities in housing systems may also be affected by different dietary components, since outdoor access as well as several beddings can increase the fibre intake of the reared pigs. Dietary enrichment, with raw materials rich in linolenic acid, increased not only linolenic acid content, but also that of its derivatives EPA, DPA and DHA (Corino *et al.*, 2008). But no clear evidence has been given so far for any dietary effect on pH at 24 h or on colour coordinates (L^* , a^* and b^*) (Corino *et al.*, 2002; Riley *et al.*, 2000), although van Oeckel *et al.* (1996) observed a significant difference in colour coordinate L^* in light pigs fed linseed.

Anyhow, colour coordinates in pork could be influenced by housing and management. This was shown by an increased b^* value in the LM for pigs with outdoor access compared with conventionally reared pigs (Lebret *et al.*, 2006), whereas Hamilton *et al.* (2000) found higher L^* values for pigs that had extra floor space allowance (an important factor in animal welfare) compared with pigs from a crowded environment, in contrast to Warriss *et al.* (1983), who found lower L^* values for pigs kept outdoors compared with confinement reared pigs.

Fat score, IMF and marbling are closely linked and considered to be influenced by the housing system, as Lebret *et al.* (2006) found higher IMF and thicker backfat in pigs that had outdoor access, and Barlocher (2006) found a better fat score for pigs reared in alternative systems with more space compared to conventional systems. On the other hand, Enfalt *et al.* (1996) found that pigs reared outdoors had lower marbling scores than pigs reared in a traditional confinement system.

Drip loss and water-holding capacity (WHC) can also possibly be affected by housing and management: While there was no measurable effect of temporary straw bedding on WHC (Peeters *et al.*, 2006), housing with sawdust bedding and outdoor access resulted in higher drip loss (compared with conventional housing) (Lebret *et al.*, 2006; Enfalt *et al.*, 1996), and pigs from crowded environments had higher WHC than pigs reared under conventional conditions (Liorancas *et al.*, 2006).

Sensory parameters for meat quality were non-significant in most cases throughout the various studies dealing with the impact of housing and management on pork quality. Only Lebret *et al.* (2006) found an improved meat juiciness for rearing outdoors during summer and winter and Liorancas *et al.* (2006) stated that the meat from conventionally raised pigs was less tender (and also had a higher shear force) than meat from pigs reared in spacious pens.

For cattle, there is less knowledge on the impact of housing systems on meat quality traits. Gottardo *et al.* (2003) found no effects of housing conditions for young bulls (slatted floor v straw bedding) on growth performance, carcass traits and meat quality. Likewise, a study on veal calves comparing both traditional rearing in individual stalls with group rearing in collective pens and exclusive milk feeding with maize grain supplementation showed that neither the type of housing nor the feeding system significantly modified meat quality traits (Xiccato *et al.*, 2002). On the other hand, Andrighetto *et al.* (1999) found better growth performance and lower IMF and L^* value but better tenderness and flavour for calves reared in group pens than for calves reared in traditional individual crates.

A study on the effect of introducing hay in addition to milk feeding on different meat quality traits (Egger, 1995) revealed that lean meat content and fat content were not affected, but meat colour was influenced. Offering hay allows the veal calves to develop a rumen and rumination as in normal digestive physiology (Morisse *et al.*, 2000) – an important welfare aspect – but the resulting meat showed a more intense red colouring when fed with hay (Egger, 1995), which does not match the consumers traditional preferences for ‘white’ veal.

The main share of meat-producing poultry is held by broiler chicken. As there are only two commonly used breeds in the western world (*Cobb* and *Ross*), broiler performance has been thoroughly studied under various conditions. There have been several attempts to improve meat quality by food additions. For example, Mourão *et al.* (2008) found that feedstuffs containing higher levels of fibre can contribute to improve breast skin yellowness and fatty acid composition of broiler meat. Chicken meat with a higher share of yellow colouring is preferred by European consumers (similar to brown eggs compared to white, it implies more 'natural' properties), whereas a more reddish colouring is preferred in Asia, especially by Japanese consumers (Komai, 1997).

In common broiler production, the birds have only about five weeks to achieve slaughter weight so there is a high turnover in mass production, but Fanatico *et al.* (2007) revealed that growing performance (genotype dependent, 'fast'-growing/'slow'-growing) has an impact on broiler meat quality: The breast meat of the slow birds had a higher protein content than that of the fast birds and a lower fat content. The meat from slow birds had poorer WHC but showed more tenderness than meat from fast birds. The same study (Fanatico *et al.*, 2007) also investigated the impact of outdoor access on meat quality and it was shown that the meat of the outdoor birds had more protein than the indoor birds. The meat and skin of the slow birds became more yellow when the birds had outdoor access. However, this did not occur when the fast birds had outdoor access.

Modern turkey hybrids exceed most other poultry species in their fattening and carcass performances. However, the high performance is accompanied by inadequate adaptation of the muscle physiology, which particularly affects the pectoralis muscle and thus the most valuable cut. The changes in muscle physiology often lead to PSE-like meat which increases losses in yield. The basis of the problem is the increased diameter of the muscle fibres, indicating muscle hypertrophy and resulting in alterations of cell metabolism. Apart from the genetically determined susceptibility for quality alterations, exogenous factors play an important role (Branschield *et al.*, 2004) and the incidence of PSE-like meat in a commercial plant for turkeys is up to 50% (Owens *et al.*, 2000). The fast weight gain also leads to deformations of the long leg bones and to joint injuries. With increasing age and body mass, the birds are resting over longer periods on the litter which becomes in the course of the fattening period a mixture of wood shavings and faeces, causing foot pad lesions breast blisters which impair carcass quality because these 'buttons' have to be removed during meat inspection. A recent survey is given by Spindler (2007).

27.5 Impact of transport and lairage on meat quality

Several studies indicate that ante-mortem stress factors can influence the development of PSE meat in swine as well as in turkeys. Such ante-mortem factors can include environmental temperatures, relative humidity, pre-slaughter handling practices and transportation. Influencing factors on pork quality can be stocking

density during transport, the handling during offloading the pigs from the truck, stocking density and air temperature during lairage (Lammens *et al.*, 2007).

In order to lower stress during and after transport, it could be beneficial to provide enriched transport compartments and lairage pens. Meat from pigs that were offered plastic balls with corn filling during lairage showed a slower pH decline 45 min post-mortem and the pigs transported in enriched compartments had fewer shoulder lesions (Peeters and Geers, 2006).

Meat-producing animals suffer high stress levels during transport as it usually is the first or second time they have to face procedures such as being caught, rounded up, loaded on a transport vessel, being moved, etc. This is supported by an increase of stress hormone levels: cortisol tripled, catecholamines and lactate doubled in a study on pigs, compared to the control during transport (Marahrens *et al.*, 1997). After the lairage time, all three parameters were still 100% above the control values. The heart frequency rose from 80 beats/min (control without activity) to approximately 130 beats/min after lairage time to more than 200 beats/min during access to stunning. In the meat of animals showing adrenalin concentrations above 100 ng/l and heart frequencies higher than 120 beats/min during the lairage time, lower pH values were found compared to pigs with 'normal' adrenalin concentrations (60 ng/l) and heart beat rates (80 beats/min).

For turkeys, it was also shown that transport for three hours prior to processing had an impact on some meat quality traits: the breast muscle from transported turkeys had higher pH values and lower L* values compared with turkeys that were processed without transportation (Owens and Sams, 2000).

Duration of transport seems to influence the redness of broiler meat, as Bianchi *et al.* (2006) revealed higher a* values for breast fillets from broiler chicken transported over short distances compared with long distance transports. The a* values increase further with elongation of lairage time whereas L* values decrease. Also the temperature at lairage can have an impact on colour coordinates: While a* values of broiler breast meat decrease with increasing temperature, L* values become higher (Bianchi *et al.*, 2006).

For pigs, investigations of Pérez *et al.* (2002) showed that increasing lairage duration increased pH value after 24 hours. Additionally, percentage of PSE decreased while DFD increased with elongation of lairage time.

Fernandez *et al.* (1996) found no effect of transport duration on pH value, cooking loss, subjective colour score, juiciness or flavour of the muscles, although veal from calves transported for one hour was more tender than that from calves transported for 11 hours. In contrast, Grigor *et al.* (2004) observed an effect of transport on the b* value: Veal of transported calves was less yellow than that of calves slaughtered on farm, while redness, shear force, tenderness, juiciness and flavour were unaffected. Drip loss could also be affected by lengthy transport, as Honkavaara *et al.* (2003) found lower drip loss in beef from heifers and bulls transported over short distances compared with long distance transports. For rabbits it was found that pH value, a* value and shear force value increased with longer transport time while L* values decreased (Liste *et al.*, 2006).

27.6 Impact of stunning on animals and meat condition

After transport and handling in the abattoir, stunning is the last step in the slaughter process where the welfare of an animal can be severely impaired. In order to avoid fear, anxiety, pain, suffering and distress, stunning before killing by de-bleeding is a statutory requirement throughout the EU (with exceptions in some Member States for religious slaughter). The procedures applied should induce immediate unconsciousness and insensibility in the animals.

In modern slaughterhouses different methods are used on livestock, such as captive bolt stunning, electrical stunning and gas stunning.

A comparison of the nine different stunning methods (five electrical, two carbon dioxide gas and two captive bolt methods) by Nowak (2002) revealed differences in respect to animal welfare and meat quality. Table 27.2 gives a simplified ranking of the different stunning methods. Electrical stunning methods using a permanently adjusted current were more animal-friendly and resulted in better meat quality than other electrical stunning methods. The constant voltage method was the most favourable one. Probably the amount of voltage and duration of current flow induce a strong epileptic seizure in the animal which is trapped in the restrainer. The application of carbon dioxide (CO₂) concentrations of 80% during 73 sec proved to be not suitable to ensure a successful stun with a deep and long lasting unconsciousness: at the same time meat quality is negatively influenced. Using 90% CO₂ over a period of 73 s clearly gave better results. A

Table 27.2 Comparison of different stunning methods in relation to animal welfare and meat quality (based on Nowak, 2002). + and ++ mean positive and very positive for the welfare of the animal or for meat quality, ± means neutral or no observed effects on animal welfare and meat quality and – means negative for the welfare of the animal or for meat quality

Stunning method		Animal welfare	Meat quality
Electrical stunning – two-cycle method (head/chest, 3-point-electrode):	constant current (1.3 A 2 s 300 Hz/ 0,9 A 4 s 100 Hz)	±	+
	constant current (1.3 A 3 s 500 Hz/ 0,9 A 3 s 100 Hz)	–	–
	constant voltage (325 V 2 s 50 Hz/ 115 V 2 s 50 Hz)	+	±
Electrical stunning – head-only method (2-point-electrode):	constant voltage (250 V 12 s 100 Hz)	–	–
	constant total current (15 C 8 s 50 Hz)	+	++
	constant total current (25 C 14 s 50 Hz)	±	±
Gas stunning (carbon dioxide):	80% by volume CO ₂ : 70 s	–	–
	90% by volume CO ₂ : 70 s	+	++
Captive bolt stunning:	conventional captive bolt	+	+
	air jet supported captive bolt (40 bar, 3 s)	++	++

disadvantage is that a large number of the pigs used were already dead at the point of bleeding, which may cause hygienic concerns, as there is a risk of translocation of bacteria from the gut into the blood vessels and the meat if the time between the death of the animal and sticking is too long. Nowak *et al.* (2007a) found that the meat quality of pigs stunned with 80% CO₂ was superior (according to the pH values) to those stunned with 90% CO₂. It seems that the use of at least 85% CO₂ over 90 s provides an effective stun of slaughter pigs. Nevertheless, this cannot prevent the very unpleasant situation the animals are exposed to in the first 10 to 12 sec after entering in the gas chambers, when they are suffering from suffocation and the acidic effect of CO₂ on the mucous membranes of the respiratory tract.

In order to prevent suffocation, stress-controlled atmosphere stunning (CAS) was developed, where oxygen (O₂) in the gas chamber is – at least in the initial gas stunning phase – replaced by argon (Ar) or nitrogen (N₂). Positive effects of CAS were found in broiler chickens stunned by a mixture of CO₂ and O₂ compared with CO₂ and Ar. The animals exhibited less aversive behaviour to the gas mixture and their meat showed slightly higher pH values post-mortem and a lower shear force (McKeegan *et al.*, 2007); but compared to electrical stunning, both gas stunning and CAS displayed a slightly lighter fillet colour (Abeyesinghe *et al.*, 2007). Earlier studies revealed that the incidence of muscle haemorrhages in geese was lower with CAS compared with electrical stunning; however, other meat quality parameters remained unchanged (Turcsán *et al.*, 2001).

Captive bolt stunning, usually used for cattle, and in some cases for pigs (e.g. when slaughtered in small abattoirs, in casualty slaughter situations or as a backup method in case of failure of another stunning method), seems to be a very effective method, with least welfare implications when the instrument is correctly placed on the forehead of the animals while shooting. The brain is severely hit, causing concussion, and the animal is immediately unconscious. However, when the concussion is incomplete or the brain is not sufficiently destroyed, heavy convulsions can occur leading to muscle damage by exhaustion (decrease in pH value, blood spots). Recent studies show that an air pressure-aided captive bolt can improve meat quality. A high-pressure air stream is directed into the brain cavity immediately after the bolt has penetrated into the brain and causes not only a contusion but destruction of the brain stem (Nowak *et al.*, in preparation). The beneficial effects for welfare and meat quality are demonstrated by the facts that almost no clinical reactions occurred and the blood constituents remained almost unchanged. However, until now there have been no captive bolt systems for automatic slaughter routine available to stun larger numbers of animals, and the operation of the appliances requires experienced personnel.

Application of electrical stunning on pigs often results in severe muscle contractions leading to broken vertebral bones and haemorrhages in muscles. In a field study in a large commercial slaughter house in Germany using a three-point electrical high constant voltage stunning method (325 V head electrodes, 115 V heart electrode, 50 Hz), combined with an automatic backup restrainer system, Nowak (1998) found hemorrhages in 73% of the pig shoulders. Table 27.3 summarises the findings of the study. One reason seems to be that, under the impact

Table 26.3 Haemorrhages and fractures in the carcasses of 80 pigs stunned by three-point electrical high voltage constant stunning method (325 V head electrodes, 115 V heart electrode, 50 Hz) combined with an automatic backup restrainer system (Nowak, 1997)

Observed impairment of carcass	% observed
Bone fractures	
Vertebra compression fractures	51
Scapula avulsion fractures	3
No fractures observed	46
Haemorrhages	
Shoulder	
<i>M. supraspinatus/subclavius</i>	61
<i>M. biceps brachii</i>	28
<i>M. latissimus dorsi</i>	28
<i>M. triceps brachii</i>	24
<i>M. brachiocephalicus</i>	23
<i>M. tricipitis brachii</i>	15
<i>M. teres major</i>	13
Other tissues	16
Total	73
Back	
<i>M. longissimus dorsi</i>	23

of the electric current, muscles and muscle fibres contract simultaneously (Kranen *et al.*, 2000). Antagonist muscles will exert a non-physiological, severe strain on each other and on the skeleton. The strongest of the antagonist muscles will shorten, forcefully lengthening and damaging the weakest one while exerting maximal force; the weaker muscle is the part in which tissue damage and haemorrhage occurs. There are clear indications that a relationship exists between the total electrical current applied, the adrenalin concentration in the blood, and the area of blood spots in the muscles of pigs after head-only stunning (Fig. 27.2). When the total electrical current exceeds 6.4 A*s, blood spots are observed in the shoulder muscles of all investigated 80 animals. At the same time, the adrenalin concentrations in the sticking blood of these pigs are reduced (Fig. 27.3). When the total electrical current amounts increase, there is an increase of the areas of blood spots in the muscles while the concentration of adrenalin in the blood of the pig decreases. The same relationship holds true for noradrenalin (Nowak *et al.*, 1997). It seems that a too low total electrical current does not provide a sufficiently deep unconsciousness, which may lead to poor welfare (higher catecholamine release) in pigs but results in better meat quality (less blood spots). This may cause conflicts between welfare demands and meat quality traits.

In poultry, predominantly water-bath stunning is applied, using 50 Hz AC. Welfare and meat quality can be affected. When current levels greater than 105 mA per chicken and 150 mA per turkey are used, a significant increase in the incidence of haemorrhaging in the breast and leg muscles, as well as bone fractures, is

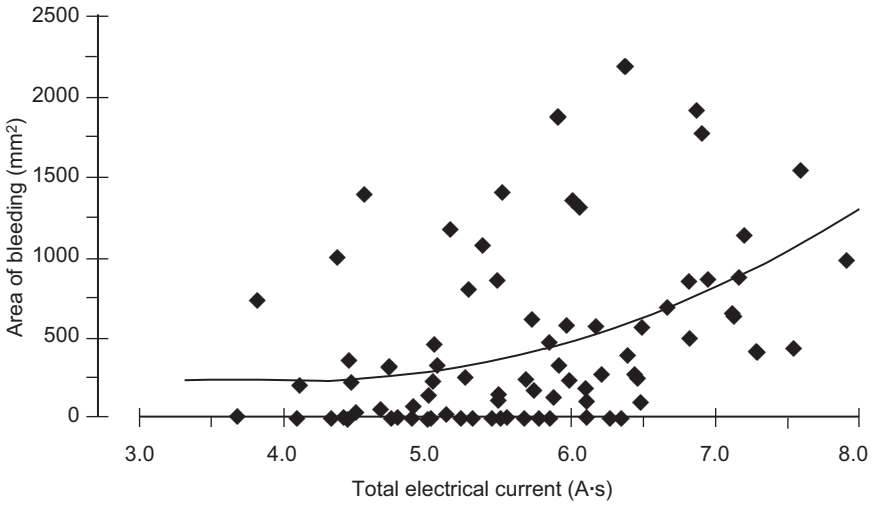


Fig. 27.2 Relationship between electrical current (A·s) and the area of bleedings (mm²) in the muscles of the shoulder of 80 slaughter pigs.

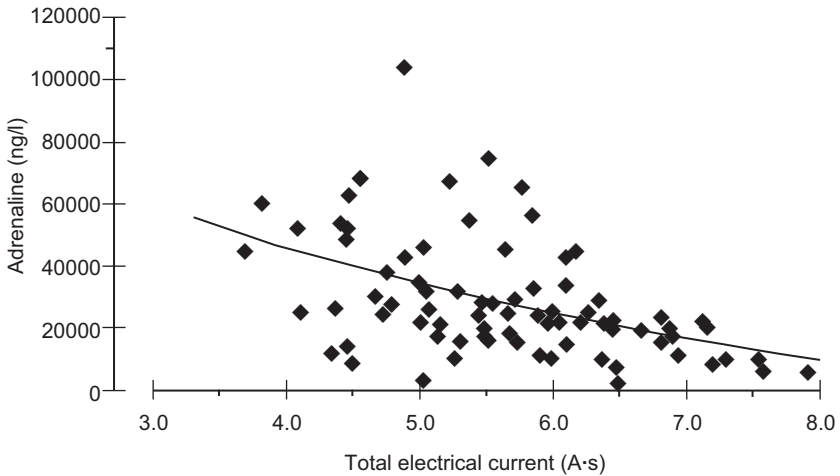


Fig. 27.3 Adrenaline concentration (ng/l) in the sticking blood of 80 slaughter pigs in relation to the applied total electrical current.

observed and can lead to regular downgrading of the carcasses (Gregory and Wilkins, 1989a,b). For an effective stun, the electric current should flow as much as possible through the brain of the animal. Due to the differences in the electrical resistance of various tissues of the birds and variations between individuals, it can happen that only a small proportion (10 to 28%) of the current applied in a water

bath will flow through the brain. The other part of the current flows through the carcass (Wooley *et al.*, 1986a,b), probably contributing to carcass and meat quality defects such as hemorrhages and broken bones. In addition, technical conditions can contribute to a poor stun and reduced meat quality. If adjacent birds hanging in the stunning line have physical contact, or if they do not dip deep enough into the water bath because of different body size, an effective amount of the applied current does not sufficiently reach the brain and the onset of immediate unconsciousness is reduced, which may lead to suffering. It has been shown that the time to onset of brain death in chicken is quicker with the induction of cardiac arrest at stunning, decapitation, and severance of two carotid arteries supplying oxygenated blood to the brain, than other neck-cutting procedures (Gregory and Wotton, 1986). Head-only stunning in chickens, unlike water-bath stunning, does not adversely affect carcass and meat quality provided that wing flapping is restricted. Convulsions or wing flapping in poultry may result in blood spots and electrically stunned birds show a higher incidence of broken bones, coracoid and furculum bone haemorrhages, and non-bone haemorrhages than birds stunned by concussion (Göksoy *et al.*, 1999). However, the incidence of red wing tips and shoulder haemorrhages is still lower in electrically stunned birds than concussion-stunned birds. The pH value of breast fillets is higher in electrically stunned birds but the fillets are less tender than those from concussion-stunned birds (Göksoy *et al.*, 1999).

27.7 A risk assessment approach for animal welfare and meat quality in slaughter animals

Risk assessment is the process of identifying hazards and evaluating the probability of an adverse event happening, in a population, as a consequence of exposure to certain hazards. It includes hazard identification, hazard characterization, exposure assessment and risk assessment. A hazard is defined as any event or factor that potentially can produce harm or cause an adverse effect. In the case of meat-producing animals, hazards for poor animal welfare are possible in every stage of the animal's life, from genetic predisposition and birth or hatching conditions, through rearing and fattening on the farm to finally transport, lairage, stunning and slaughter. Hazard characterisation is a qualitative evaluation of the nature of the adverse effect associated with the hazard in terms of likelihood, intensity and duration. Thus, housing conditions, for example, can form a hazard as they may lead to diseases (e.g. respiratory diseases through bad ventilation), high stress levels (e.g. social stress through overcrowding or heat stress through high temperatures) or even behavioural disorders (e.g. if housing conditions do not meet the types appropriate for the need to exercise). Exposure assessment means the qualitative evaluation of the frequency of exposure to the hazard in the animal population. Exposure to certain hazards for animal welfare in the meat producing sector varies widely in duration. For example, housing conditions affect an animal for nearly its whole life, whereas stunning lasts a matter of minutes or seconds.

Table 27.4 Possible hazards in farm animal production and their impact score (low, medium, high) on animal welfare and meat quality

Hazard source	Impact on animal welfare	Impact on meat quality
Housing conditions	Medium	Low
Transport	Medium to high	Medium
Lairage	Medium	Low to medium
Stunning/slaughter	High	Medium to high

Risk characterisation is the process of determining the qualitative estimation of the probability of occurrence, intensity and duration of poor animal welfare in a population. A risk for animal welfare or meat quality is a function of the probability of a negative animal welfare or meat quality effect and the severity and duration of that effect, consequential to the exposure to one or several hazards. Currently, there are no common standards for the risk assessment for animal welfare in relation to meat quality. Nevertheless, Table 27.4 can be a first approach to give a ranking for the impact of housing conditions on both animal welfare and meat quality.

27.8 Conclusions

Housing systems can have a considerable influence on the welfare of animals. However, there are only very few examples showing that poor housing conditions impair the quality of the meat, except when animals are not fed or conditions are so poor that they develop skeletal or metabolic diseases or suffer from injuries.

A higher impact can be expected by stressful transport. PSE meat in pigs and PSE-like conditions in turkeys can occur when the animals are transported for too long or under inappropriate conditions. Too high densities, lengthy transport and high temperatures have a negative influence on the well-being of animals and can lead to suffering and injuries, fatigue and exhaustion, resulting in poor carcass quality, too low pH values (due to glucose depletion) and possibly PSE (pigs, turkey) or DFD meat (cattle). Stressing transports can also impair the immunity status of animals and can cause the outbreak of infectious diseases or support the shedding of infectious agents contaminating the carcasses, which is known from *Salmonella* strains infecting fattening pigs when transported from the farm to the slaughter house (Nowak *et al.*, 2007b).

Stunning and slaughter can develop the highest impact on both animal welfare and meat quality. Stunning methods which do not stun immediately (e.g. ineffective electrical stunning, CO₂ stunning) or not deep enough can cause suffering in the animals and lead to broken bones and blood spots in valuable meat cuts.

27.9 Future trends

There is an urgent need for a review of all common housing systems, allowed transport and currently applied stunning methods relating to both animal welfare and meat quality. Methods are in place to carry out the necessary assessments. Governments and industry should have an interest to allow and apply animal-friendly and effective procedures.

Besides these legal and technical aspects, it is also important to include consumers and retailers in future developments. Whereas in the past decades, price, availability and safety were the main factors influencing meat purchase, ethical issues relating to the food chain are now of increasing concern, including environmental aspects of animal production, developments in biotechnology, occupational health at the work place of farm workers, and animal welfare (Mephram, 1996). In particular, 'animal-friendliness' becomes an additional and important quality trait for meat, that influences the consumers' purchase decision, at least in Europe and other developed countries. It is therefore necessary to further promote labelling systems that give the consumer the choice between different product lines. A successful example is the labelling of eggs that is now in place in the European Union, which provides information on the country of origin, the husbandry system in which the birds were kept and the date of production. Similar systems should be developed for meat, displaying the system in which the pig or beef was reared, the transport type and length, and the stunning method before slaughter.

Successful meat production in future has to be based on transparency of the food chain, displaying the safety of the product and the health and welfare of the animals which provide the meat.

27.10 References

- Abeyesinghe S M, McKeegan D E F, McLeman M A, Lowe J C, Demmers T G M, White R P, Kranen R W, van Bommel H, Lankhaar J A C and Wathes C M (2007), 'Controlled atmosphere stunning of broiler chickens. I. Effects on behaviour, physiology and meat quality in a pilot scale system at a processing plant', *British Poultry Science*, 48 (4), 406–423.
- Andrighetto I, Gottardo F, Andreoli D and Cozzi G (1999), 'Effect of type of housing on veal calf growth performance, behaviour and meat quality', *Livestock Production Science*, 57, 137–145.
- Barlocher H U (2006), 'Influence of alternative semi-outdoor housing systems in comparison with the conventional indoor housing on carcass composition and meat and fat quality of finishing pigs', *FAT scientific series*, 125.
- Bennett R M (1996), 'Willingness to Pay Measures of Public Support for farm animal welfare legislation', *The Veterinary Record*, 139, 320–321.
- Bianchi M, Petracci M and Cavani C (2006), 'The influence of genotype, market live weight, transportation, and holding conditions prior to slaughter on broiler breast meat colour', *Poultry Science*, 85, 123–128.
- Blaha T and Blaha M L (1995), *Qualitätssicherung in der Schweinefleischerzeugung. Tierärztliche Bestandsbetreuung – Tiergesundheit – Tierschutz*, Gustav Fischer Verlag, Jena, Stuttgart.

- Branscheid W, Hahn G and Wicke M (2004), 'Turkey meat quality: Problems and solutions', *Fleischwirtschaft*, 84 (11), 109–112.
- Broom D M (1986), 'Indicators of poor welfare', *Br. Vet. J.*, 142, 524–526.
- Broom D M (1988), 'The scientific assessment of animal welfare', *Appl. Anim. Behav. Sci.*, 20, 5–19.
- Carr S N, Gooding J P, Rincker P J, Hamilton D N, Ellis M, Killefer J and McKeith F K (2005), 'A survey of pork quality of downer pigs', *Journal of Muscle Foods*, 16, 298–305.
- Christiaens J P A (1987), 'Gas concentrations and thermal features of the animal environment with respect to respiratory diseases in pig and poultry', in: Bruce J M and Sommer M (eds), *Agriculture: Environmental Aspects of Respiratory Disease in Intensive Pig and Poultry Houses, Including the Implications for Human Health*, Proceedings of a meeting of the Commission of the European Communities October 1986, EUR 1020 EN, 29–43.
- Corino C, Musella M and Mourot J (2008), 'Influence of extruded linseed on growth, carcass composition and meat quality of slaughtered pigs at 110 and 160 kg liveweight', *J Anim Sc.* Published Online first on April 25, 2008 as doi:10.2527/jas.2007–0155
- Corino C, Magni S, Pagliarini E, Rossi R, Pastorelli G and Chiesa L M (2002), 'Effects of dietary fats on meat quality and sensory characteristics of heavy pig loins', *Meat Sci.*, 60, 1–8.
- EFSA Scientific Committee (2008), 'Food safety, animal health and welfare and environmental impact of animals derived from cloning by somatic cell nucleus transfer (SCNT) and their offspring, and products obtained from those animals', *The EFSA Journal*, 767, 1–49.
- Egger I (1995), 'Faut-il distribuer du foin aux veaux à l'engrais', *Revue Suisse Agric.*, 27, 177–181.
- Elbers A R W (1991), *The use of slaughterhouse information in monitoring systems for herd health control in pigs*, Dissertation, Rijksuniv Utrecht.
- Enfält A C, Lundström K, Hansson I, Lundeheim N and Nystrom P E (1996), 'Effects of outdoor rearing and sire breed (Duroc and Yorkshire) on carcass composition and sensory and technological meat quality', *Meat Science*, 45, 1–15.
- Eurobarometer (2007), 'Attitudes of EU citizens to animal welfare', http://ec.europa.eu/public_opinion/archives/ebs/ebs_270_en.pdf, 20.08.2007
- Fanatico A C, Pillai P B, Emmert J L and Owens C M (2007), 'Meat quality of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access', *Poultry Science*, 86, 2245–2255.
- FAWC (2004), *Report on the Welfare Implications of Animal Breeding and Breeding Technologies in Commercial Agriculture*, <http://www.fawc.org.uk/pdf/breedingreport.pdf>
- FDA (2008), *Animal Cloning: A Risk Assessment*, Center for Veterinary Medicine, U.S. Food and Drug Administration, Department of Health and Human Services, 1–968, http://www.fda.gov/cvm/CloneRiskAssessment_Final.htm.
- Fernandez X, Monin G, Culioli J, Legrand I and Quilichini Y (1996), 'Effect of duration of feed withdrawal and transportation time on muscle characteristics and quality in Friesian–Holstein calves', *J. Anim. Sci.*, 74, 1576.
- Fishbein M and Ajzen I (1975), *Belief, Attitude, Intention and Behavior: An Introduction to Theory and Research*. Massachusetts: Addison-Wesley.
- Givens D I and Shingfield K J (2004), 'Foods derived from animals: The impact of animal nutrition on their nutritive value and ability to sustain long-term health', *Nutrition Bulletin*, 29 (4), 325–332.
- Göksoy E O, McKinsty L J, Wilkins L J, Parkman I, Phillips A, Richardson R I and Anil M H (1999), 'Broiler stunning and meat quality', *Poultry Science*, 78, 1796–1800.
- Gottardo F, Ricci R, Preciso S, Ravarotto L and Cozzi G (2003), 'Effect of the manger space on welfare and meat quality of beef cattle', *Livestock Production Science*, 89 (2–3), 277–285.
- Gregory N G and Wilkins L J (1989a), 'Effect of slaughter on bleeding efficiency in chickens', *Journal of Science of Food and Agriculture*, 47, 13–20.
- Gregory N G and Wilkins L J (1989b), 'Effect of stunning current on carcass quality in chickens', *Vet. Rec.*, 124, 530–532.

- Gregory N G and Wotton S B (1986), 'Effect of slaughter on the spontaneous and evoked activity of the brain', *British Poultry Science*, 27, 195–205.
- Grigor P N, Cockram M S, Steel W B, McIntyre J, Williams C L and Leushuis I E (2004), 'A comparison of the welfare and meat quality of veal calves slaughtered on the farm with those subjected to transportation and lairage', *Livestock Production Science*, 91, 219–228.
- Hamilton D N, Ellis M B, Wolter F, Augspurger N R, McKeith F K and Wilson E R (2000), 'The growth performance of the progeny of two sire lines reared under differing environmental conditions', *J. Anim. Science*, 78, 239.
- Hamscher G, Pawelzick H T, Sczesny S, Nau H and Hartung J (2003), 'Antibiotics in Dust Originating from a Pig-fattening Farm: A New Source of Health Hazard for Farmers?', *Environmental Health Perspectives*, 111, 1590–1594.
- Hartung J and Wathes C M (2001), *Livestock Farming and the Environment*, Sonderheft 226, Landbauforschung Völknerode, Braunschweig.
- Honkavaara M, Rintasalo E, Ylonen J and Pudas T (2003), 'Meat quality and transport stress of cattle', *Dtsch. Tierärztl. Wochenschr.*, 110, 125–128.
- Hughes B O (1976), *Behaviour as an Index of Welfare*, Vth European Poultry Conf., Malta.
- Komai T (1997), 'Branded quality chicken booms', *Japan. Poult. Int.*, 36 (3), 34–35.
- Kranen R W, Lambooy B, Veerkamp C H, van Kuppevelt T H and Veerkamp J H (2000), 'Histological Characterization of Hemorrhages in Muscles of Broiler Chickens', *Poultry Sci.*, 78, 110–116.
- Lahrman H K, Bremermann N, Kaufmann O and Dahms S (2004), 'Health, growing performance and meat quality of pigs in indoor and outdoor housing – a controlled field trial', *Dtsch. Tierärztl. Wochenschr.*, 111 (5), 205–8.
- Lammens V, Peeters E, Maere H D, Mey E D, Paelinck H, Leyten J and Geers R (2007), 'A survey of pork quality in relation to pre-slaughter conditions, slaughterhouse facilities, and quality assurance', *Meat Science*, 75, 381–387.
- Lebret B, Meunier-Salaün M C, Foury A, Mormède P, Dransfield E and Dourmad J Y (2006), 'Influence of rearing conditions on performance, behavioral, and physiological responses of pigs to preslaughter handling, carcass traits, and meat quality', *J. Anim. Sci.*, 84, 2436–2447.
- Liorancas V, Bakutis B and Januskeviciene G (2006), 'Influence of rearing space on the behaviour, performance, carcass and meat quality of pigs', *Medycyna Weterynaryjna*, 62 (3), 274–277.
- Liste G, María G A, Buil T, Garcia-Belenguer S, Chacon G, Olleta J L, Sanudo C and Villarreal M (2006), 'Journey length and high temperatures: Effects on rabbit welfare and meat quality', *DTW. Dtsch. Tierärztl. Wochenschr.*, 113, 59–64.
- Marahrens M, Nowak B, Feldhusen F and Hartung J (1997), 'Stress response of pigs during transport, lairage time and slaughter and its relationship to meat quality', *Fleischwirtschaft*, 77 (8), 717–720.
- McKeegan D E F, Abeyesinghe S M, McLeman M A, Lowe J C, Demmers T G M, White R P, Kranen R W, van Bommel H, Lankhaar J A C and Wathes C M (2007), 'Controlled atmosphere stunning of broiler chickens. II. Effects on behaviour, physiology and meat quality in a commercial processing plant', *British Poultry Science*, 48 (4), 430–442.
- Mepharm B. (ed.) (1996), *Food Ethics*, Routledge, London, New York.
- Miele M and Parisi V (2001), *The level of consumer concern about animal welfare – The Italian Survey Report*. University of Pisa, Italy. EU FAIR CT98–3678.
- Morisse J P, Huonnic D, Cotte J P and Martrenchar A (2000), 'The effect of four fibrous feed supplementations on different welfare traits in veal calves', *Anim. Feed Sci. Technol.*, 84, 129–136.
- Morrison R S, Johnston L J and Hilbrands A M (2007), 'The behaviour, welfare, growth performance and meat quality of pigs housed in a deep-litter, large group housing system compared to a conventional confinement system', *Applied Animal Behaviour Science*, 103, 12–24.
- Mourão J L, Pinheiro V M, Prates J A M, Bessa R J B, Ferreira L M A, Fontes C M G A and

- Ponte P I P (2008), 'Effect of dietary dehydrated pasture and citrus pulp on the performance and meat quality of broiler chickens', *Poultry Science*, 87, 733–743.
- Nowak B, Hartung J, Marahrens M and Feldhusen F (1997), *Relationship between electrical stunning and blood spots in slaughter pigs*, Proceedings of the World Congress on Food Hygiene, 24., 29.08.1997, 166.
- Nowak B (2002), *Influence of three different stunning procedures on the stress reaction and the meat quality of slaughter pigs*, Dissertation. University of Veterinary Medicine Hannover.
- Nowak B, Mueffling v. T, Hartung J (2007a), 'Effect of different carbon dioxide concentrations and exposure times in stunning of slaughter pigs: Impact on animal welfare and meat quality', *Meat Science*, 75 (2), 290–298.
- Nowak B, Mueffling v. T, Chaunhom S, Hartung J. (2007b), 'Salmonella contamination in pigs at slaughter and on the farm: A field study using an antibody ELISA test and a PCR technique', *International Journal of Food Microbiology*, 115, 259–267.
- Nowak B, Mueffling v. T, Hartung J, 'Air pressure supported captive bolt stunning influences animal welfare and meat quality of slaughter pigs', (in preparation).
- Owens C M, Hirschler E M, McKee S R, Martinez-Dawson R and Sams A R (2000), 'The characterization and incidence of pale, soft, exudative turkey meat in a commercial plant', *Poultry Science*, 79, 553–558.
- Owens C M and Sams A R (2000), 'The influence of transportation on turkey meat quality', *Poultry Science*, 79 (8), 1204–1207.
- Peeters, E, Driessen, B, Moons, C P H, Odberg, F O and Geers, R (2006), 'Effect of temporary straw bedding on pigs' behaviour, performance, cortisol and meat quality', *Applied Animal Behaviour Science*, 98, 3–4.
- Peeters E and Geers R (2006), 'Influence of provision of toys during transport and lairage on stress responses and meat quality of pigs', *Animal Science*, 82, 591–595
- Pérez MP, Palacio J, Santolaria MP, Aceña MC, Chacón G, Gascón M, Calvo JH, Zaragoza P, Beltrán J A and García-Belenguer S (2002), 'Effect of transport time on welfare and meat quality in pigs', *Meat Science*, 61, 425–433.
- Riley P A, Enser M, Nute G R and Wood J D (2000), 'Effects of dietary linseed on nutritional value and other quality aspects of pig muscle and adipose tissue', *J. Anim. Sci.*, 71, 483–500.
- Schutz H G, Judge D S and Gentry J (1986), 'The importance of nutrition, brand, cost and sensory attributes to food purchase and consumption.' *Food Technology*, 40, 79–82.
- Spindler B (2007), *Pathological and histological investigations of joints, legs and foot pads of male B.U.T. Big 6 turkeys kept in a conventional barn and a barn with an attached outdoor scratching area*, Diss. Dr. vet med., University of Veterinary Medicine, Hannover.
- Turcsán Z, Szigeti J, Varga L, Farkas L, Birkás E and Turcsán J (2001), 'The effects of electrical and controlled atmosphere stunning methods on meat and liver quality of geese', *Poultry Science*, 80, 1647–1651.
- van Oeckel M J, Casteels M, Warnants N, van Damme L and Boucqué C V (1996), 'Omega-3 fatty acids in pig nutrition: Implications for the intrinsic and sensory quality of the meat', *Meat Sci.*, 44, 55–63.
- Wariss P D, Kestin S C and Robinson J M (1983), 'A note on the influence of rearing environment on meat quality in pigs', *Meat Science*, 9, 271–279.
- Wooley S A, Brothwick F J W and Gentle M J (1986a), 'Flow routes of electric currents in domestic hens during pre-slaughter stunning', *British Poultry Science*, 27, 403–408.
- Wooley S A, Brothwick F J W and Gentle M J (1986b), 'Tissue resistivities and current pathways and their importance in pre-slaughter stunning of chickens', *British Poultry Science*, 27, 301–306.
- Xiccato G, Trocino A, Queaque P I, Sartori A and Carazzolo A (2002), 'Rearing veal calves with respect to animal welfare: Effects of group housing and solid feed supplementation on growth performance and meat quality', *Livestock Production Science*, 75, 269–280.