Long-chain omega-3 polyunsaturated fatty acids in ruminant nutrition: benefits to animals and humans

Edward H. Clayton
Livestock Research Officer – Ruminant Nutrition
NSW Department of Primary Industries, Wagga Wagga Agricultural Institute
Pine Gully Rd, Wagga Wagga NSW 2650

Graham Centre Monograph No. 4

Edited by:
Toni Nugent and Catriona Nicholls

August 2014

Department of Primary Industries

Charles Sturt University

© State of New South Wales through Department of Trade and Investment, Regional Infrastructure and Services 2014

This publication is copyright. You may download, display, print and reproduce this material in an unaltered form only (retaining this notice) for your personal use or for non-commercial use within your organisation. To copy, adapt, publish, distribute or commercialise any of this publication you will need to seek permission from the NSW Department of Primary Industries.

Disclaimer: The information contained in this publication is based on knowledge and understanding at the time of writing (August 2014). However, because of advances in knowledge, users are reminded of the need to ensure that information upon which they rely is up to date and to check currency of the information with the appropriate officer of the NSW Department of Primary Industries or the user's independent advisor. All sources of information in the current publication are acknowledged in the text. No further reproduction should be made without first obtaining prior written approval of the copyright owner.

For updates to this publication, check www.grahamcentre.net/

Published by the **NSW Department of Primary Industries**.

First published August 2014

ISBN 978 1 74256 678 8

Cover design by: Sharon Kiss

Cover photo by: Toni Nugent, Graham Centre for Agricultural Innovation

Author's Contact:
Dr Edward Clayton, Livestock Research Officer, NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, Pine Gully Rd, Wagga Wagga NSW 2650
Email: edward.clayton@dpi.nsw.gov.au

Citation:
Clayton EH (2014). Graham Centre Monograph No. 4: Long-chain omega-3 polyunsaturated fatty acids in ruminant nutrition: benefits to animals and humans. Eds. T Nugent and C Nicholls, NSW Department of Primary Industries, Wagga Wagga, NSW. Available at: www.grahamcentre.net/

Acknowledgements:
The cost of printing this Monograph was funded by the Graham Centre.

FOREWORD

The Graham Centre Monograph series provides an in depth analysis of issues of importance to mixed farming systems. The aim is to make the information readily accessible to a broad audience including producers, students, researchers and funding organisations.

As the consumption of animal products increases globally, the demand for red meat will continue to grow. Along with this increased demand, the quality and health benefits of meat, for example, the possible role of omega-3 fatty acids in disease prevention, will be of greater importance to consumers.

This latest Monograph outlines the possible benefits of omega-3 fatty acids from beef and lamb, and the ability to maximise these levels by feeding forage diets. In particular, the role of forage conservation and the inclusion of conserved forage in production feeding rations to achieve this goal is examined.

This is the first Monograph focusing on animal nutrition and production. It provides an up-to-date summary of current research, and identifies gaps for future regional, national and international research opportunities.

Professor Deirdre Lemerle
Director, Graham Centre for Agricultural Innovation

Toni Nugent and Catriona Nicholls
Editors

ACKNOWLEDGEMENTS

I would sincerely like to thank Bill McKiernan (former manager, Livestock Production, NSW DPI) for his encouragement to pursue research into the effects of omega-3 fatty acids and the ability to manipulate the omega-3 content of meat. Much of the current review was conceived in the early days of our current research program. I would also like to thank the many researchers at the Wagga Wagga Agricultural Institute that have been involved with various aspects of our research, especially fatty acid analysis, including Richard Meyer, Jamie Ayton and Peter Hawkins.

I would like to thank the Graham Centre for Agricultural Innovation and Deirdre Lemerle for supporting our omega-3 research program and for providing preliminary funding for early research examining the effect of omega-3 on animal health and reproduction. I also thank Catherine Gulliver for her help initiating the research program examining the impact of omega-3 on reproduction in sheep. Catherine's input, hard work and dedication made much of our current research possible.

Thank you to the many people who have assisted with the collection of some of the data presented in the current Monograph, including Craig Lihou, Patricia O'Keeffe, the farm staff of the WWAI and a number of students from Charles Sturt University and Sydney University. I would also like to thank John Wilkins and John Piltz for many extremely helpful discussions about the effects of omega-3 fatty acids in sheep and cattle. I am particularly grateful to Robert Taylor and Robert Blake for teaching me a substantial amount about fatty acids and, in particular, fatty acid analysis. Their insights into all things to do with lipid and fatty acid extraction and analysis has helped me to form a solid base from which to start much of our current research.

EXECUTIVE SUMMARY

There is continued interest from researchers, health professionals and the general public in the potential health benefits of omega-3 fatty acids. Several theories link the consumption of long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA) to reducing the incidence of disease conditions including cardiovascular disease, inflammatory diseases, such as arthritis, and mental health disorders. Research efforts have focussed on the impact of two LCn-3PUFA found in oily fish: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). While EPA and DHA are only found in relatively low concentrations in red meat (including beef and lamb) compared with oily fish, the total LCn-3PUFA concentration in meat including docosapentaenoic acid (DPAn-3) is significant. The impact of different production systems on the LCn-3PUFA content of meat has gained significant attention during the past 10–15 years.

The current review summarises several possible health benefits of LCn-3PUFA in ruminants and humans and outlines the effects of different production systems on the content of beneficial fatty acids in meat. The LCn-3PUFA status of meat is significantly improved by incorporating fresh or conserved forage, compared with grain and concentrates, into the diet of ruminants. The ability of forages to maximise the amount of LCn-3PUFA in meat is dependent on the nature of forage, including the amount and type of lipid present and, is greater when animals consume fresh forage compared with hay or silage. Several research programs have examined the manipulation of lipid and fatty acid content of forages in order to maximise the amount of omega-3 available. There is scope to increase the amount of omega-3 available in silage by ensuring that it is produced under optimal conditions, including limiting wilting where possible and maximising the efficiency of fermentation.

The current Monograph also reviews the observed and recommended intakes of LCn-3PUFA in the Australian population and the contribution of red meat intake (beef and lamb) to this intake. Red meat contributes approximately 40% of the average daily intake of LCn-3PUFA for adult men and women in Australia and there is the potential to significantly increase the consumption of LCn-3PUFA from meat by manipulating the diet of ruminants by incorporating forage or feeding forages with improved omega-3 availability.

Several opportunities for future research programs are identified following the review of previous research, including the need for a survey of the amount of omega-3 available in pastures and forages grown in Australia and examination of the impact of ensiling forage on omega-3 concentrations under Australian conditions. There is a great need to determine measureable improvements in the health of those consuming meat enriched with LCn-3PUFA following the incorporation of forage into production diets. If the consumption of meat with enhanced LCn-3PUFA can decrease the incidence of disease, this would provide an incentive for producers to deliver this meat into the market.

KEYWORDS

Ruminant nutrition, omega-3 polyunsaturated fatty acids, health benefits, animal production.

TABLE OF CONTENTS

FOREWORD 3
ACKNOWLEDGEMENTS 4
EXECUTIVE SUMMARY 5
TABLE OF CONTENTS 6
TABLE OF FIGURES 8
TABLE OF TABLES 9
LIST OF ABBREVIATIONS 10
1. Introduction 11
2. Background and Importance of Omega-3 Fatty Acids 12
  2.1 Polyunsaturated fatty acids 12
  2.2 Long-Chain Omega-3 Polyunsaturated Fatty Acids 13
    2.2.1 Mechanisms of action of LCn-3PUFA 15
    2.2.2 Ratio of omega-6:omega-3 PUFA 17
  2.3 Structure of lipids important in ruminant nutrition 17
  2.4 Fatty acid metabolism in ruminants 18
    2.4.1 Lipolysis and release of free fatty acids 19
    2.4.2 Biohydrogenation of fatty acids in the rumen 19
3. Methodological Considerations with Lipid and Fatty Acid Analysis 21
  3.1 Lipid extraction and fatty acid analysis of feed 21
    3.1.1 Total lipid extraction 21
    3.1.2 Isolation of lipid fractions 23
    3.1.3 Methylation of fatty acids 25
    3.1.4 Analysis of fatty acids 25
  3.2 Lipid extraction and fatty acid analysis of meat 26
    3.2.1 Lipid extraction 26
    3.2.2 Interpretation of fatty acid analysis 26
  3.3 Lipid conversion factors (LCF) and recovery of fatty acids 28
    3.3.1 Lipid conversion factors 28
    3.3.2 Calculating the recovery of fatty acids 30
  3.4 Calculation of the ratio of Omega-6:Omega-3 fatty acids 32
4. Meat Quality 33
  4.1 Omega-3 and meat quality 33
  4.2 Influence of breed on fatness and omega-3 33
  4.3 Changes in fatty acid concentrations in meat following cooking 34
5. Altering LCn-3PUFA in Meat through Dietary Manipulation 35
  5.1 Manipulating LCn-3PUFA in meat by feeding LCn-3PUFA sources 35
    5.1.1 Beef 35
    5.1.2 Lamb 37
    5.1.3 Pork 38
  5.2 Manipulating LCn-3PUFA in meat by feeding linseed 41
    5.2.1 Cattle 41
  5.3 Manipulating omega-3 in beef by changing feed type or forage sources 43
    5.3.1 Surveys of feeding systems 43
    5.3.2 Forage versus grain feeding 43
    5.3.3 Feeding different types of forages 49
    5.3.4 Concentration of LCn-3PUFA in meat in the National Nutrition Database 49
  5.4 Manipulating omega-3 in lamb by changing feed type or forage sources 54
6. Potential Health Effects of LCn-3PUFA in Ruminants 56
  6.1 Inflammation 56
  6.2 Reproduction and behaviour 57
  6.3 Sex ratio of lambs 58
7. Manipulating the Concentration of LCn-3PUFA in Animal Feed 59
  7.1 Concentrations of α-linolenic acid and linoleic acid in feedstuffs 59
    7.1.1 Differences in fatty acid composition between forages and grains 59
    7.1.2 Effect of stage of growth on fatty acid composition 60

7.1.3 Differences in fatty acid composition between forage species 60
7.1.4 Change in fatty acid composition by conserving forage as silage 61
7.1.5 Potential future research 65
7.2 Factors affecting loss of fatty acids in the rumen 65
7.2.1 Lipolysis and release of free fatty acids.. 65
7.2.2 Biohydrogenation of fatty acids from different forages 66
7.2.3 Relationship between rumen pH and biohydrogenation of fatty acids 66
8. Meat Production Systems in Australia 68
9. Recommended and Reported Intakes of LCn-3PUFA in Humans 70
9.1 Recommended Intakes.. 70
9.1.1 Food sources of LCn-3PUFA 71
9.2 Reported intakes of LCn-3PUFA in Australia.. 72
9.3 Reported intake of meat and fish and contribution to LCn-3PUFA intake 75
9.3.1 Reported intake of meat and fish in Australia 75
9.3.2 Contribution of meat to LCn-3PUFA intake 78
9.4 Biological markers of LCn-3PUFA intake 79
10. Altering Consumer Intake of LCn-3PUFA by Enhancing Animal Products............................... 80
10.1 Increasing LCn-3PUFA intake by the consumption of meat other than fish..................... 82
11. Summary and Recommendations 83
12. References 84
APPENDIX I - Nomenclature and common names for fatty acids 98

TABLE OF FIGURES

Figure 1. The structure of omega-3 and omega-6 fatty acids. 12
Figure 2. Structure of linoleic acid (C18:2n-6) and CLA. 13
Figure 3. Summary of the metabolism of omega-3 and omega-6 fatty acids. 14
Figure 4. Summary of the metabolism of fatty acids to eicosanoids including prostaglandin. 15
Figure 5. Structure of phosphatidyl choline lipid. 18
Figure 6. Structure of triglyceride lipid. 18
Figure 7. Summary of the biohydrogenation of unsaturated to saturated fatty acids. 20
Figure 8. Total lipid concentration in *Lolium perenne* determined using an ether extract (EE) or an acid-hydrolysis ether extract method (AH-EE).. 22
Figure 9. Total non-esterified fatty acid concentration of *Lolium perenne* in silage compared with fresh forage.. 23
Figure 10. Correlation between the concentration of fatty acids in red blood cells analysed using two methods of lipid extraction. 27
Figure 11. Relationship between the proportion of total fatty acid in phospholipid and the analysis of fatty acids in red blood cells. 27
Figure 12. Increasing concentrations of omega-3 following supplementation with fish oil. 37
Figure 13. Relationship between intake and concentrations of omega-3 in meat. 38
Figure 14. Increasing concentrations of DHA in *Longissimus dorsi* of pigs fed increasing doses of DHA as fish meal. 39
Figure 15. Relationship between intramuscular fat concentration and concentration of DHA. 40
Figure 16. Change in concentrations of omega-3 following supplementation with linseed. 41
Figure 17. The ratio of n-6:n-3PUFA in meat with increasing length of time of grain feeding. 48
Figure 18. Concentration of omega-3 in meat following the consumption of forage or silage. 53
Figure 19. Correlation between the concentration of ALA in silage and the concentration of omega-3 in the plasma of sheep. 54
Figure 20. Change in plasma $PGF_{2\alpha}$ metabolite following an oxtyocin challenge in ewes fed omega-3 or omega-6. 57
Figure 21. Proportion of female lambs in X-Bred or Merino ewes fed silage high in omega-3 or oats/cottonseed meal high in omega-6. 58
Figure 22. Effect of rumen pH on biohydrogenation of omega-3 and omega-6 fatty acids. 67
Figure 23. Number of feedlot cattle on-feed in Australia per year. 68
Figure 24. Percentage of Australian lot-fed cattle for increasing days on feed. 69
Figure 25. Mean estimated intake of LCn-3PUFA by children and adolescents or adults in Australia compared with recommended intakes. 75
Figure 26. Apparent consumption of meat in Australia. 78
Figure 27. Change in fatty acids after consuming diets fortified with omega-3. 81

TABLE OF TABLES

Table 1. Eicosanoids derived from LCn-3PUFA or LCn-6PUFA. 16
Table 2. Published methods used for the analysis of lipid concentration in plant material. 24
Table 3. Examples of the range of gas chromatographs and capillary columns used in the analysis of fatty acids in forage and meat. 25
Table 4. Derivation of a Lipid Conversion Factor (LCF) for beef. 28
Table 5. Derivation of a Lipid Conversion Factor (LCF) for beef taking into account an in depth analysis of the phospholipid proportions. 29
Table 6. Lipid moieties and calculation of lipid conversion factor for wheat flour. 30
Table 7. Fatty acid recovery calculated for fresh forages, silages or concentrates. 31
Table 8. Calculation of the omega-6:omega-3 ratio in meat. 32
Table 9. Omega-3 concentrations in beef following supplementation with LCn-3PUFA. 36
Table 10. Omega-3 concentrations in beef following supplementation with α-linolenic acid. 42
Table 11. Fatty acid concentrations in beef from surveys of different feeding systems. 44
Table 12. Fatty acid concentrations in beef from cattle fed forage or grain-based diets. 46
Table 13. Fatty acid concentrations in beef from cattle fed different forage types. 50
Table 14. Omega-3 concentrations for beef in Australia used to construct the National Nutrition Database NUTTAB. 52
Table 15. Fatty acid concentrations in meat from sheep fed different diets. 55
Table 16. Concentrations of fatty acids in maize silage and grain concentrates compared with fresh grass and perennial ryegrass-white clover forages. 59
Table 17. Concentration of fatty acids in forage cut at different stages of development. 60
Table 18. Concentration of fatty acids in different forages at similar stages of development. 61
Table 19. Concentration of fatty acids in silages produced from ryegrass or clover. 61
Table 20. Change in fatty acid composition of perennial ryegrass following ensiling. 62
Table 21. Concentration of C18:3n-3 in perennial ryegrass. 62
Table 22. Change in fatty acid composition of fresh compared with ensiled lucerne. 63
Table 23. Effect of wilting prior to conserving forage as hay or silage on the concentration of fatty acids. 63
Table 24. Fatty acid concentration in silage ensiled without wilting compared with wilting. 64
Table 25. Concentration of fatty acids in fresh conventional or 'stay-green' perennial ryegrass and silage produced from these forages. 64
Table 26. Lipid classes in fresh oat/pea forage compared with silage. 65
Table 27. Proportion of fatty acids in the phospholipid, free fatty acid (NEFA) and total fatty acid classes prior to and following ensiling. 66
Table 28. Australian guidelines for recommended daily intakes of LCn-3PUFA. 70
Table 29. International guidelines for recommended daily intakes of LCn-3PUFA. 71
Table 30. Mean concentration of LCn-3PUFA in different meat sources in Australia. 72
Table 31. Mean reported daily intakes of omega-3 for different age groups in Australia. 73
Table 32. Reported concentrations of LCn-3PUFA in beef. 73
Table 33. Reported concentrations of DPAn-3 in different meat types. 73
Table 34. Mean reported daily intakes of LCn-3PUFA in a population of children and adolescents in Australia using a Food Frequency Questionnaire. 74
Table 35. Reported intakes of meat and fish for Australian adults. 76
Table 36. Reported intakes of meat and fish for Australian children and adolescents. 77
Table 37. Contribution of meat to the intake of LCn-3PUFA in Australia. 79
Table 38. Intake of LCn-3PUFA prior to and following the consumption of a range of food products enriched with LCn-3PUFA. 82
Table 39. Red blood cell membrane LCn-3PUFA prior to and following the consumption of a range of food products enriched with LCn-3PUFA. 82
Table 40. Reported intake of meat in Australia and estimated intake of LCn-3PUFA from meat if animals are fed predominantly grain or forage diets. 82

LIST OF COMMON ABBREVIATIONS USED IN THE MONOGRAPH

Abbreviation	Description
CLA	Conjugated linoleic acid
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DPAn-3	Docosapentaenoic acid – omega-3 configuration
DPAn-6	Docosapentaenoic acid – omega-6 configuration
EPA	Eicosapentaenoic acid
FAME[1]	Fatty acid methyl esters
LCn-3PUFA	Long-chain omega-3 polyunsaturated fatty acid
LCn-6PUFA	Long-chain omega-6 polyunsaturated fatty acid
LCF	Lipid conversion factor
LT	Leukotriene
LWt	Liveweight of animal
MUFA	Monounsaturated fatty acids
n-3	Omega-3 fatty acid
n-6	Omega-6 fatty acid
NEFA	Non-esterified fatty acid
NHMRC	National Health and Medical Research Council
NRV	Nutrient Reference Value
PG	Prostaglandin
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGI	Prostacyclin
RBC	Red blood cells
SFA	Saturated fatty acids
TG	Triclygeride (or Triacylglycerol)
TX	Thromboxane

[1]For a full list of abbreviations of fatty acids of interest in human and animal nutrition, see Appendix 1.

1. Introduction

There is continued interest from researchers, health professionals and the general public in the health benefits of omega-3 fatty acids. Several theories link the consumption of omega-3 fatty acids to reducing the incidence of conditions such as cardiovascular disease (CVD, Kris-Etherton et al., 2003), inflammatory diseases such as arthritis (Simopoulos, 1999) and mental health disorders (Parker et al., 2006; Clayton et al., 2007).

Research efforts have focussed on the impact of two main long-chain n-3 polyunsaturated fatty acids (LCn-3PUFA) found in oily fish: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Another LCn-3PUFA found in red meat, docosapentaenoic acid (DPA), may also have similar health benefits (Murphy et al., 2007). Red meat may, therefore contribute significantly to the overall intake of LCn-3PUFA in the Australian population and, hence, the impact of different production systems on the omega-3 content of meat has gained significant attention during recent years.

The concentration of LCn-3PUFA in meat is affected by a number of factors including breed, overall fatness and the type of feed consumed by the animal. In general, the amount of LCn-3PUFA available in meat is higher when animals consume fresh forage compared with grain and concentrates (Raes et al., 2004; McGee, 2005; Scollan et al., 2006; Scollan et al., 2014). The amount of omega-3 available in forage is also affected by a number of factors, including plant species and total lipid content, growing conditions and method of conservation (Khan et al., 2012; Glasser et al., 2013) as well as the extent of breakdown and loss in the rumen prior to absorption and metabolism (Van Ranst et al., 2009; Buccioni et al., 2012).

The ability to alter the concentration of LCn-3PUFA in meat through the incorporation of forage into Australian production systems has not been extensively studied. Likewise, the impact of increasing the amount of LCn-3PUFA in beef and lamb on the intake of these potentially healthy fatty acids by the Australian population and, the impact this change may have on measurable risk factors for disease, has not previously been examined.

The current review outlines the effects of different animal production systems on the content of potentially beneficial omega-3 fatty acids in meat products. The effects of maximising the omega-3 concentration in forage and increasing forage consumption by ruminants, in particular, on systemic LCn-3PUFA status, metabolism and incorporation of these fatty acids into animal products, is reviewed. While the focus of the current Monograph is on manipulating omega-3 in order to increase the amount of LCn-3PUFA for human consumption, the effects of altering systemic omega-3 status on animal health and production is also briefly assessed.

2. Background and Importance of Omega-3 Fatty Acids

Prior to examining the effects of forage feeding systems on the omega-3 status of ruminants and meat quality, it is important to review fatty acid nomenclature and some basic information on fatty acid metabolism. Fatty acids of importance in nutrition are categorised into three main classes: saturated fatty acids (SFA), which have no double bonds in their structure, monounsaturated fatty acids (MUFA), which have one double bond in their structure and polyunsaturated fatty acids (PUFA), which have more than one double bond. Saturated fatty acids in general are considered to be 'unhealthy' fats, while MUFA and PUFA are generally considered healthier. The following sections will review the structure and function of PUFA, with a focus on the fatty acids found in meat that have potential health benefits for humans.

2.1 Polyunsaturated fatty acids

Polyunsaturated fatty acids refer to fatty acids that contain two or more double-bonds in their structure. Meat contains several beneficial PUFA including omega-3 PUFA (n-3 PUFA) and conjugated linoleic acids (CLA). The first double bond in omega-3 fatty acids occurs 3 bonds from the methyl end of the fatty acid chain, whereas the first double bond in omega-6 fatty acids occurs 6 double bonds from the methyl end (Figure 1). It is important to note the international convention for the naming of fatty acids commences from the carboxyl end of the chain, however, the convention for naming omega fatty acids commences from the opposite, or methyl, end. A summary of scientific and common names of saturated and unsaturated fatty acids of importance in animal and human nutrition is shown in Appendix I.

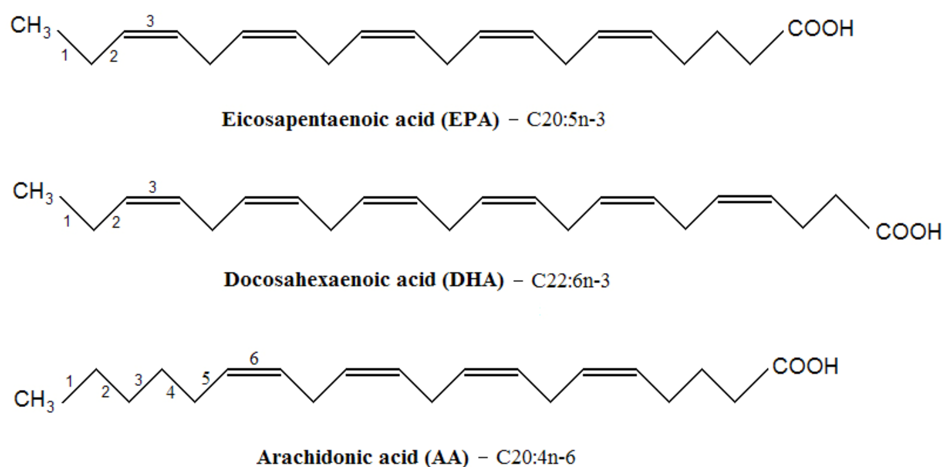


Figure 1. The structure of EPA, DHA LCn-3 PUFA and AA LCn-6PUFA showing the position of the first double bond either 3 or 6 bonds from the methyl end, respectively.

Conjugated linoleic acids are a group of isomeric *trans* fatty acids of the omega-6 fatty acid linoleic acid (Figure 2). The two main forms of CLA in meat and milk are *cis*-9-*trans*-11-CLA (rumenic acid) and *trans*-10-*cis*-12-CLA (Figure 2). A number of CLA may have health benefits for humans including reducing the incidence of cancer in a rat model (Ip et al., 1999) and reduced body fat accumulation (for review, see Eriksson and Pickova, 2007). The effects of CLA haven generally only been reported in animal models and human health benefits still remain to be confirmed.

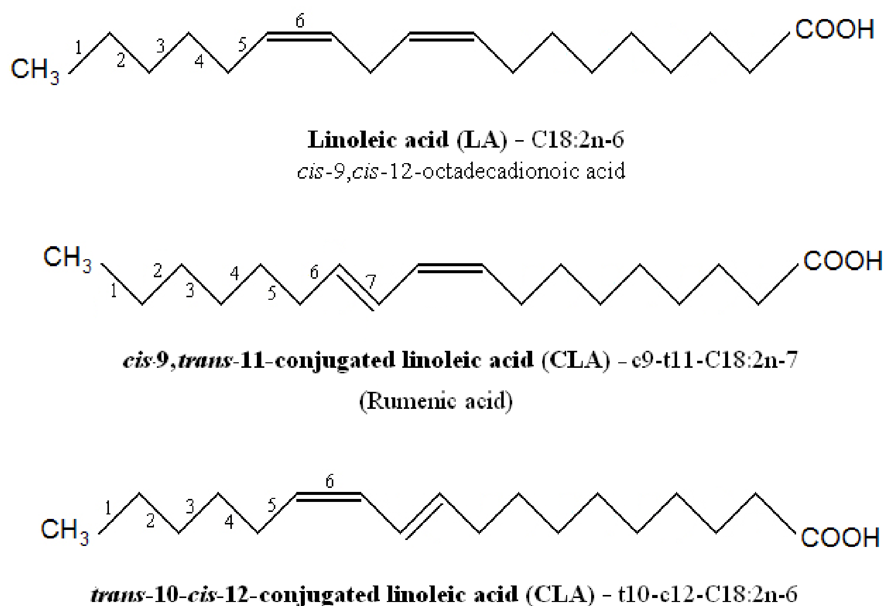


Figure 2. Structure of linoleic acid (C18:2n-6) and two conjugated linoleic acids (CLA) showing the position of the first double bond either 6 or 7 bonds from the methyl end. Note: naming convention commences from the carboxyl end.

2.2 Long-chain omega-3 polyunsaturated fatty acids

Fatty acids are named with reference to the number of carbons in their structure. Short chain fatty acids (or volatile fatty acids) are generally accepted as containing between 1 and 7 carbons in their structure, while medium chain fatty acids have between 8 and 18 carbons. The main medium chain omega-3 of importance is α-linolenic acid (C18:3n-3; octadecatrienoic acid; ALA), while the main medium chain omega-6 PUFA is linoleic acid (C18:2n-6, octadecadienoic acid, LA). Most interest in human health is in long-chain omega-3 PUFA (LCn-3PUFA) that have 20 or 22 carbons in their chain. The main LCn-3PUFA considered beneficial are: EPA (C20:5n-3), DPAn-3 (C22:5n-3) and DHA (C22:6n-3).

The shorter chain PUFA, ALA and LA, are important in ruminant and human nutrition because they are converted in the body to long-chain PUFA. The shorter chain ALA (found predominantly in fresh forages and plants such as linseed and canola) is converted to EPA, DPA and DHA, while LA (from grain or maize, for example) is converted to arachidonic acid (C20:4n-6, ARA Figure 3). LA and ALA are termed 'essential' fatty acids; that is they cannot be synthesised in the body and must be consumed in the diet (Lands, 1992). The long-chain fatty acids are essential components of cell membranes and eicosanoids and the current review will focus predominantly on LCn-3PUFA.

Human nutritionists are increasingly focussing on the importance of LCn-3PUFA, in terms of both absolute intake and the ratio of omega-6:omega-3 in human food sources. The major health benefits associated with meat intake are likely to be associated with an increase in the concentration of LCn-3PUFA or a reduction in LCn-6PUFA.

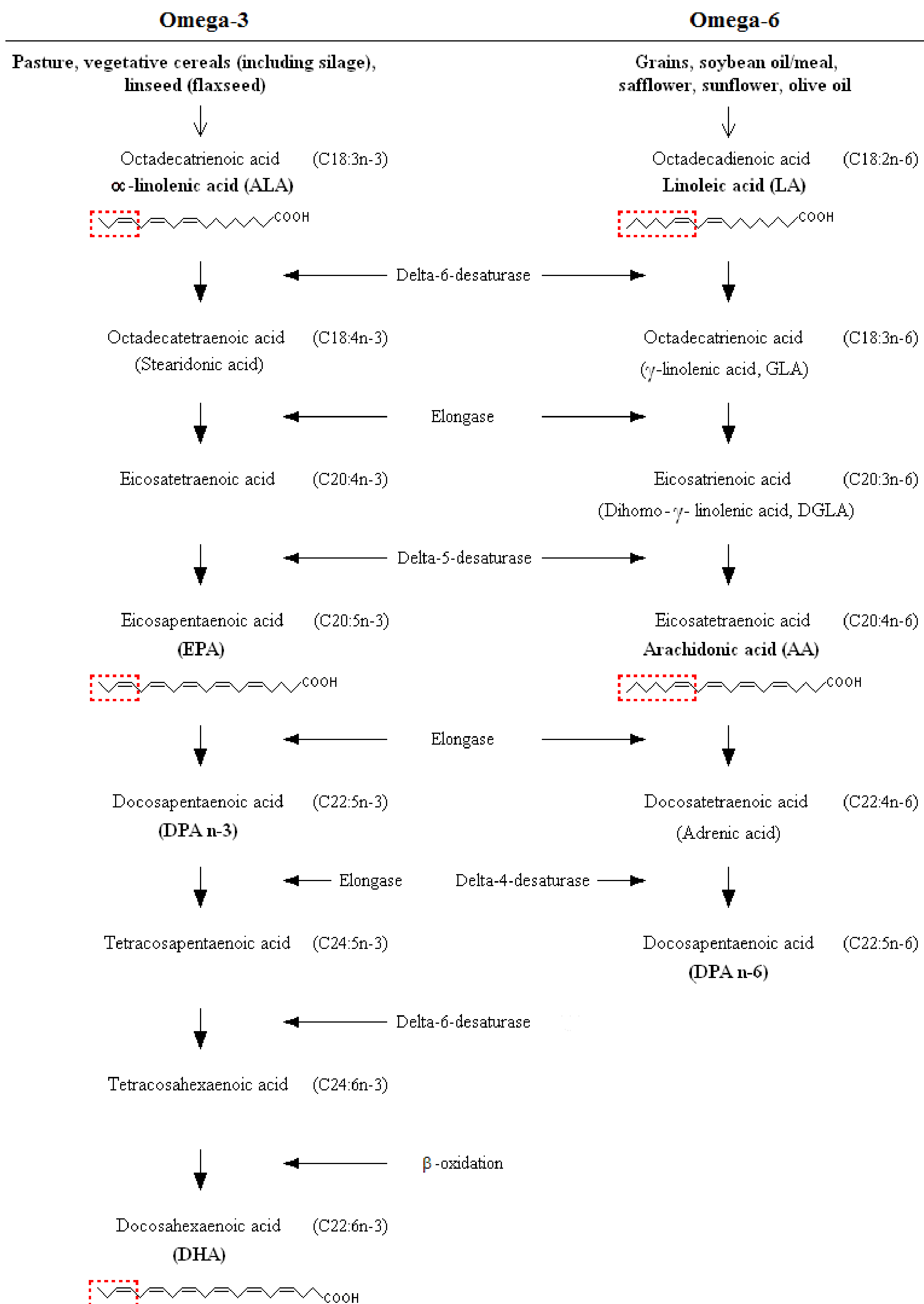


Figure 3. Summary of the metabolism of 18 carbon to 22 carbon omega-3 and omega-6 fatty acids. Adapted from: Moore et al. (1991); Wang and Anderson (1993); Parker et al. (2006); Clayton et al. (2007).

2.2.1 *Mechanisms of action of LCn-3PUFA*

LCn-3PUFA may have a number of beneficial effects in humans, including lowering fasting blood triglyceride and reducing platelet activation and clotting tendency facilitating a reduction in the risk of atherosclerosis and CVD (for review, see Howe et al., 2007). LC-PUFA are important for two main reasons: firstly, they are metabolised to prostaglandins that are either pro-inflammatory or anti-inflammatory and, secondly, they are integral components of cell membranes and cell function.

The long-chain PUFA EPA and ARA are the precursors for eicosanoids including prostaglandins (PG), prostacyclins (PGI), thromboxanes (TX) and leukotrienes (LT, Smith et al., 1991; Abayasekara and Wathes, 1999). The LC-PUFA are oxygenated and metabolised to eicosanoids by one of two pathways. The first pathway involves prostaglandin H synthase (PGHS, also called cyclooxygenase, COX) oxidation, which removes two double bonds, leading to the thromboxane (TX), prostaglandin (PG) and prostacyclin (PGI) series, while the second pathway involves lipoxygenase oxidation, which removes no double bonds and leads to the LT. The removal of two double bonds from ARA (20:4n-6) by prostaglandin H synthase leaves two double bonds and leads to the formation of series-2 eicosanoids, while the removal of two double bonds from EPA (20:5n-3) leads to the formation of series-3 eicosanoids (Figure 4).

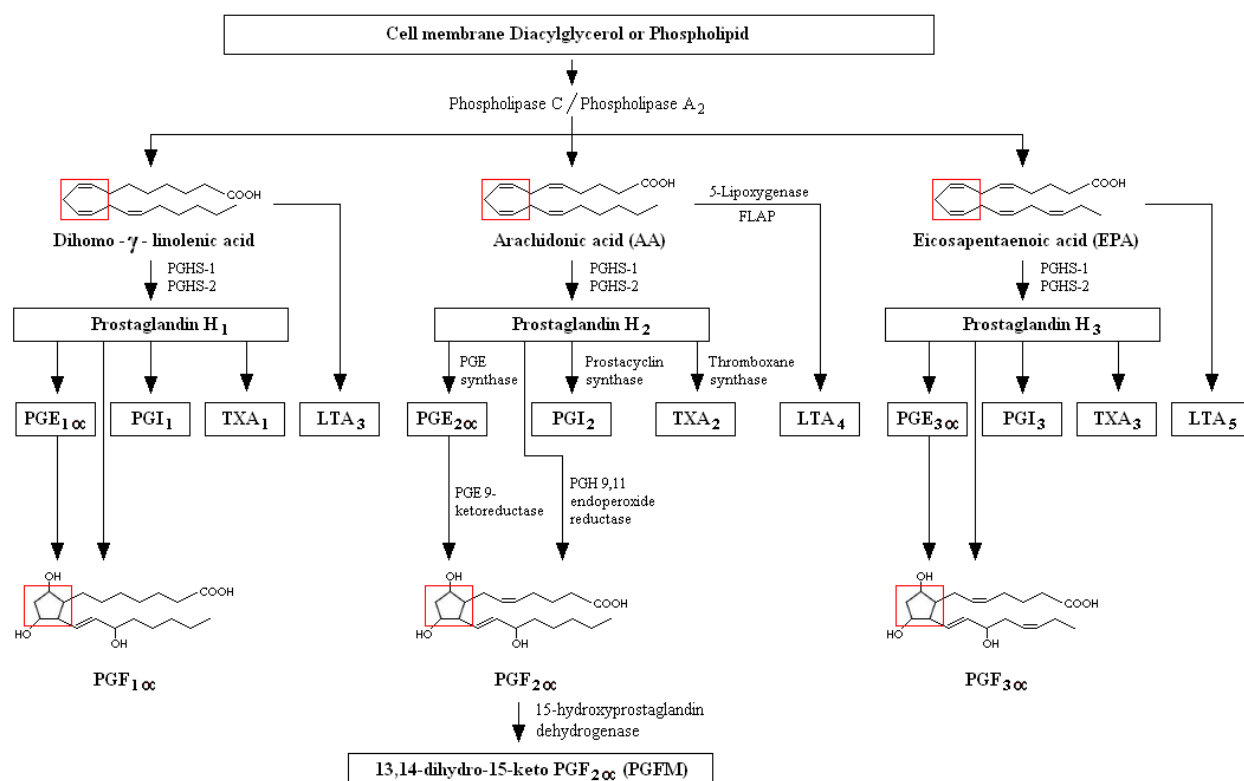


Figure 4. Summary of the metabolism of fatty acids to series 1, 2 or 3 eicosanoids including prostaglandin. PGHS = prostaglandin H synthase, PGF = prostaglandin F series, PGI = prostacyclin, TXA = thromboxane A, LTA = leukotriene A, FLAP = 5-lipoxygenase activating protein. Sources: Watanabe (2002); Cheng et al. (2005b); Murphy and Gijon (2007); Wathes et al. (2007); Dozier et al. (2008); Fortier et al. (2008).

LCn-3PUFA and inflammation

The eicosanoids are signalling molecules associated with a number of functions in the body including inflammation (Peet and Stokes, 2005). The series-1 and series-3 PG are less inflammatory, while the series-2 PG are more inflammatory (Table 1, Lands, 1992; Horrobin and Bennett, 1999). LCn-3PUFA and LCn-6PUFA, therefore, have opposing biological effects (Stoll et al., 1999) and, in general, the LCn-6PUFA ARA is proinflammatory and associated with up-regulation of inflammation, while the LCn-3PUFA EPA and DHA are anti-inflammatory (Horrobin and Bennett, 1999). The PG, in particular, series-2 PG including $PGF_{2\alpha}$, play an important role in several aspects of reproduction, including ovulation, oestrus, embryo survival and parturition (for review, see Abayasekara and Wathes, 1999), roles that are reviewed later.

Table 1. Eicosanoids derived from LCn-3PUFA or LCn-6PUFA.

Fatty Acid	Eicosanoid Product Series		Inflammation
	TX, PG or PGI	LT	
Arachidonic acid (C20:4n-6)	series-2	series-4	More inflammatory
Eicosapentaenoic acid (C20:5n-3)	series-3	series-5	Less inflammatory

TX = thromboxane, PG = prostaglandin, PGI = prostacyclin, LT = leukotriene.
Adapted from: Lands (1992).

LCn-3PUFA and cell membranes

The LCn-3PUFA DHA is a major structural component of cell membranes throughout the body, particularly in brain neurones (Horrobin et al., 1991). DHA is predominantly found in phospholipids, while EPA is found primarily in cholesterol esters, triglycerides and phospholipds. DHA is the most abundant unsaturated fatty acid found in the brain (Sastry, 1985), particularly in the cerebral cortex, accounting for approximately 14% of total fatty acids (McNamara and Carlson, 2006). DHA is also found in large concentrations in the retina, testes and sperm (Simopoulos, 1991). Normal brain growth and development in infants and children requires dietary intake of LCn-3PUFA as the major structural components of neural cell membranes (Farquharson et al., 1992; Innis, 2003) and DHA, in particular, appears to be involved in the development of cognition in infants (for review, see Willatts and Forsyth, 2000).

Membrane function is determined by its structure and, in particular, by the concentration of DHA in membrane phospholipids. LCn-3PUFA deficiencies may be involved in the aetiology of a number of psychiatric illnesses and symptoms of these illnesses may be ameliorated by an increase in the consumption of LCn-3PUFA (for review, see Clayton et al., 2007). LCn-3PUFA supplementation is associated with increased membrane fluidity in patients with bipolar disorder (Hirashima et al., 2004), which leads to a general dampening of signal transduction pathways (Tappia et al., 1997). Increased EPA and DHA concentrations in cell membranes also inhibit the activity of rat protein kinase C second messenger systems (Mirnikjoo et al., 2001; Seung Kim et al., 2001), again modulating signal transduction.

In summary, the LCn-3PUFA are associated with several possible health benefits and there is epidemiological evidence linking reduced LCn-3PUFA intake with several illnesses including asthma, CVD and several mental health disorders including depression and bipolar disorder (Clayton et al., 2009a). There is increasing awareness in both the actual intake of LCn-3PUFA and the ratio of n-6:n-3 PUFA in human diets.

2.2.2 Ratio of omega-6:omega-3 PUFA

The human diet in hunter-gatherer times is thought to have contained an omega-6:omega-3 (n-6:n-3) ratio of approximately 1:1 (Sinclair and O'Dea, 1993; Simopoulos, 1999; Cordain et al., 2002a; Cordain et al., 2002b) as reviewed previously (Vaneslow, 2007). Modern western European diets are largely deficient in LCn-3PUFA (Leaf and Weber, 1987) with the ratio of n-6:n-3 approximately 10:1 or 15:1 (Meyer et al., 2003; Howe et al., 2006). The change in the ratio of n-6:n-3 is largely attributable to two main factors (for reviews, see Mann, 2007; Ulijaszek et al., 2012). Firstly, during the 1960s and 1970s there was a large public health push to reduce the intake of SFAs and increase the intake of unsaturated fatty acids, as the incidence of CVD was seen to be linked to the consumption of SFA. These dietary recommendations lead to a decrease in the intake of meat and animal products and an increase in the intake of vegetable oils and spreads, which are extremely high in the omega-6 fatty acid LA. Secondly, food manufacturers have increased their reliance on cheap sources of vegetable oils in processed foods also high in LA.

Research in the area of LCn-3PUFA to date has predominantly concentrated on actual intakes of LCn-3PUFA (mg/day), with many guidelines for intakes now recommended (see Section 9). Future research will examine not only the total daily intake of LCn-3PUFA, but also the ratio of n-6:n-3 in the diet, as the ratio of these fatty acids determines the balance of pro and anti-inflammatory eicosanoids and the potential health effects of these fatty acids.

2.3 Structure of lipids important in ruminant nutrition

Fatty acids are contained in the lipid fraction of feed and meat. Lipid is not comprised entirely of fatty acids and different classes of lipids contain different non-lipid structural groups. Several different classes of lipids are found in feed and animal tissue and the way in which fatty acids are contained in these lipid classes influences their metabolism and availability to animals.

Total lipid in meat is comprised of several lipid classes that are broadly classified into neutral or polar lipids (Christie, 1982). The neutral lipid fraction contains several lipid classes, including: triacylglycerol (triglyceride, majority of lipid), cholesteryl esters, monoglycerides and diglycerides, free fatty acids and cholesterol. The polar lipid fraction of meat contains phospholipids, including glycerophospholipids and sphingolipids. Glycerophospholipids, in turn, contain several classes of lipids, including: phosphatidyl choline, lyso-phosphatidyl ethanolamine, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, diphosphatidyl glycerol and lyso-phospahtidyl choline. Plant material contains phospholipid, triglyceride and free fatty acid lipid classes, but not cholesteryl esters (Boufaied et al., 2003a). Most of the fatty acids contained in leaf tissue are found in the glycolipid and phospholipid fractions, which are often localised in leaf chloroplasts (Harfoot and Hazlewood, 1997).

Different lipid classes contain different concentrations of fatty acids as a proportion of total lipid. Phospholipids contain two fatty acid groups in conjunction with glycerol, phosphate and choline (for example, phosphatidyl choline, Figure 5) whereas triglycerides contain three fatty acid groups for each glycerol group (Figure 6). Polar lipids are of particular interest in omega-3 research as LCn-3PUFA are contained largely in phospholipids, while neutral lipids, in general, do not contain high concentrations of LCn-3PUFA (Scollan et al., 2001). Phospholipids represent approximately 68–78 % of polar lipids and approximately 12–25% of total lipid (Marmer et al., 1984; Duckett et al., 1993), however, this proportion is dependent on the muscle type (red muscle fibres have a higher phospholipid content), diet and fatness of the animal (Marmer et al., 1984).

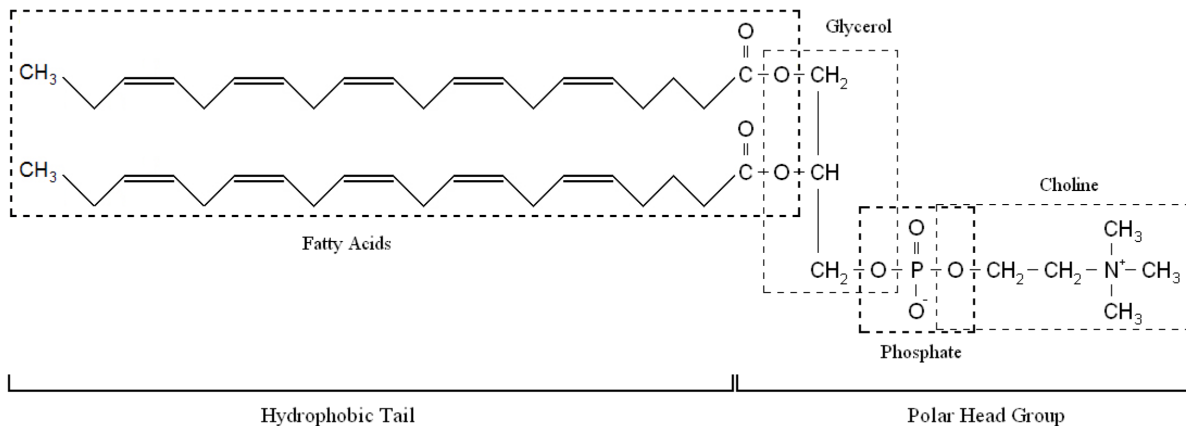


Figure 5. The interaction between two molecules of EPA with glycerol, phosphate and choline making up one phosphatidyl choline lipid molecule.

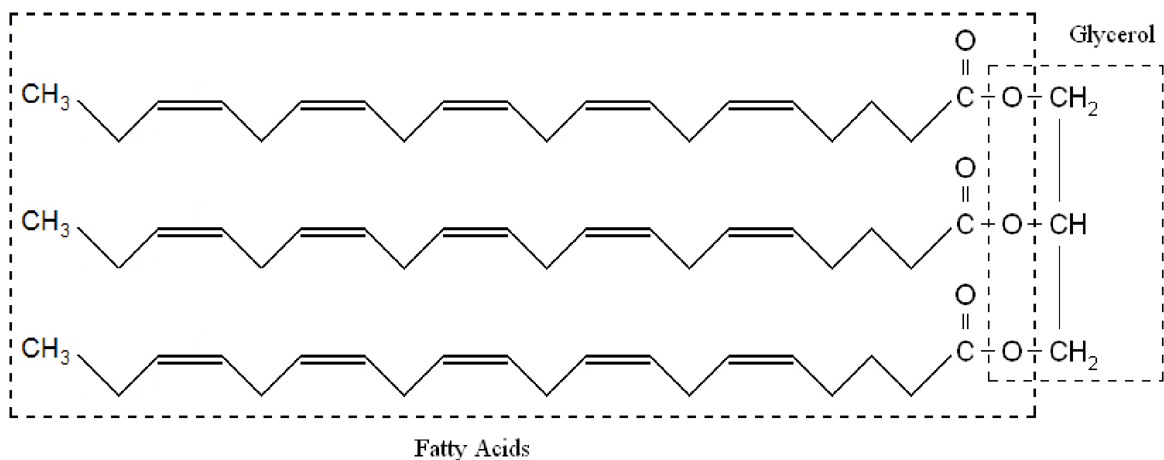


Figure 6. The interaction between three molecules of EPA with one molecule of glycerol making up one triglyceride lipid molecule.

2.4 Fatty acid metabolism in ruminants

Prior to being able to review the effects of different feed sources on the fatty acid status of ruminants and, subsequent availability of omega-3 for humans, it is necessary to review some basic concepts in fatty acid metabolism. The following section describes the metabolism and potential loss of n-3 and n-6 PUFA in ruminants, thereby providing background information relevant to manipulation of these factors.

Fatty acids from plant material are metabolised, to a large extent, in the rumen prior to absorption and incorporation into meat and milk. The class of lipid in which the fatty acid is contained affects the amount of metabolism and loss that will occur in the rumen. The majority of lipid in plant material is contained in leaf chloroplasts as phospholipid and there is little fatty acid in the form of non-esterified fatty acid (NEFA), also referred to as free fatty acid (Buccioni et al., 2012). To be metabolised and hydrogenated in the rumen, lipid must first undergo lipolysis to release NEFA and these NEFA are then biohydrogenated to less unsaturated fatty acids and eventually to saturated fatty acids.

2.4.1 Lipolysis and release of free fatty acids

The process of lipolysis of plant lipids occurs under the action of either plant or microbial lipases (Dewhurst et al., 2003c) and has been extensively reviewed previously (for example, see Lee et al., 2004; Buccioni et al., 2012). Plant lipases predominate in fresh plants following cutting (Dewhurst et al., 2003c), as this is a protective mechanism to provide energy to survive. Plant lipases are also active in the rumen (for review, see Lee et al., 2004) along with microbial lipases from rumen bacteria, which leads to the release of NEFA. The action of lipase enzymes will be greater for mono or diacylglycerols compared with triglycerides or phospholipids (Christie, 1982). During the process of complete metabolism of PUFA from lipid to SFA, lipolysis is the rate-limiting step (Chow et al., 2004).

2.4.2 Biohydrogenation of fatty acids in the rumen

Once fatty acids are released from lipids as NEFA following the action of lipase enzymes, unsaturated fatty acids can be hydrogenated to either MUFA or SFA by rumen microorganisms in a process termed biohydrogenation (Demeyer and Doreau, 1999; Dewhurst et al., 2003c). The biohydrogenation of ALA and LA involves several steps and the formation of several intermediate fatty acids also of importance in nutrition, including CLA. These fatty acids can also be completely hydrogenated to stearic acid (C18:0, Figure 7).

The biohydrogenation of PUFA is affected by several factors including:

- The type and amount of fatty acid (Noble et al., 1974)
- Dietary nitrogen content (Gerson et al., 1983)
- Forage to concentrate ratio (Gerson et al., 1985)
- The concentration of LA compared with ALA (Harfoot et al., 1973)
- pH of rumen fluid (van Nevel, 1996)

These factors are described in more detail, together with factors that affect the biohydrogenation of fatty acids found in plant material, in Section 7.

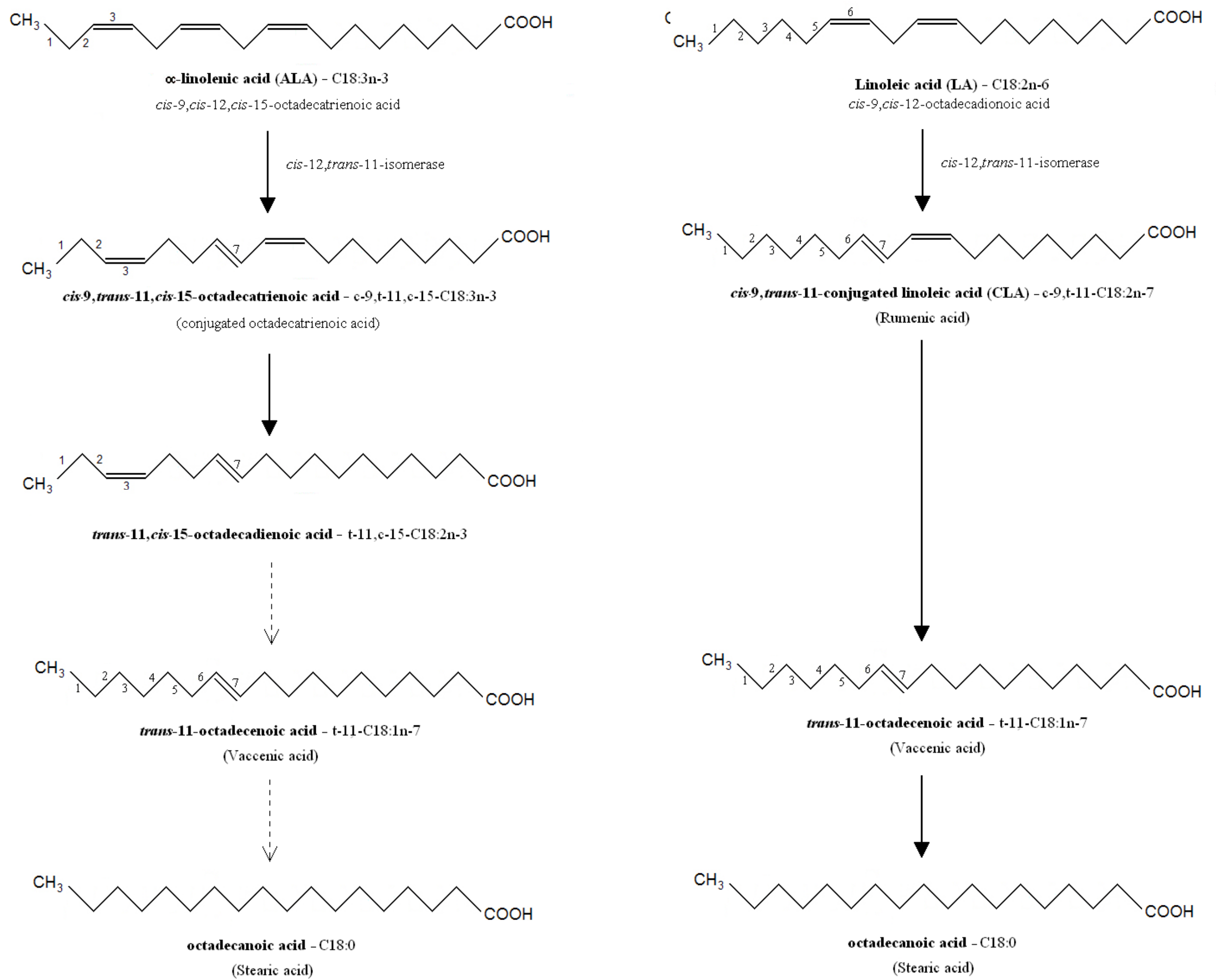


Figure 7. Summary of the biohydrogenation of ALA (C18:3n-3) and LA (C18:2n-6) in the rumen to stearic acid (C18:0). Data adapted from: Boufaied et al. (2003b); Buccioni et al. (2012). Broken lines indicate pathways that are less well defined.

3. Methodological Considerations with Lipid and Fatty Acid Analysis

To gain a greater understanding of studies examining the concentration of fatty acids in forages and the manipulation of fatty acids in ruminants, it is necessary to be able to review the methods used in published studies in detail. There are several different methods used in the analysis of fatty acids and many ways to present and interpret data, for example, presenting the total concentration or relative proportion of fatty acids. The following section reviews several aspects of fatty acid analysis and data reporting that is important for the interpretation of data presented in subsequent sections.

3.1 Lipid extraction and fatty acid analysis of feed

There are several different international conventions for lipid extraction and fatty acid analysis for forages and meat. The convention in Australia is to extract total lipid using a solvent such as ether, hence, the process is termed 'ether extract' (EE). Different methods of analysis are used across many countries (Table 2) and, even within Australia, many methods are employed for the analysis of lipid via EE, for example using diethyl-ether or petroleum ether. There are also several methods available for the analysis of fatty acids in feeds which are briefly reviewed in Section 3.1.4.

3.1.1 Total lipid extraction

The research group from the Institute of Biological Environmental and Rural Sciences (IBERS, Aberystwyth University, Wales, UK) report separate methods for extracting lipid from silage compared with concentrates (Choi et al., 2000; Scollan et al., 2001; Scollan et al., 2003). The total lipid concentration in silage was analysed using an EE method with diethyl ether (Bauchart et al., 1984), while total lipid in concentrates was analysed using acid hydrolysis diethyl ether extraction (Choi et al., 2000).

Further publications from the same group in Aberystwyth report different references for the acid hydrolysis method for lipid extraction and do not make a distinction between the analysis of lipid in forage, silage or concentrates (Thomas et al., 1988; Dewhurst et al., 2000; Dewhurst et al., 2003a; Dewhurst et al., 2003b). The same group at IBERS also report direct extraction and hydrolysis of fatty acids using an acid-hydrolysis EE procedure including methylation using potassium hydroxide in methanol and then sulphuric acid (Lee et al., 2003).

The research group in the Department of Plant Sciences at Wageningen University (the Netherlands) estimated 'crude fat' (CF) using a number of methods (Elgersma et al., 2003). Firstly, CF was extracted using two EE methods: petroleum ether (PE) at 40-60°C or extraction with PE preceded by HCl (3 mol/L) (AH-EE ISO, 1999). Secondly, total fat content was estimated from the sum of total fatty acids determined using a one-step extraction and methylation of fatty acids using ethanol and sodium sulphate (see Section 3.1.3 below).

There are a number of methodological issues surrounding lipid and fatty acid analysis highlighted in the analysis of perennial ryegrass (Elgersma et al., 2003), including the estimation of total lipid using the sum of fatty acids. The total lipid of plant material is only approximately 72.9% fatty acids (as described below), as there are a number of other components of lipids apart from fatty acids. Secondly, the total lipid concentration was significantly higher when total lipid was extracted from fresh forages using the AH-EE compared with the EE method (Figure 8). When the total lipid content of silage was analysed, however, the two methods did not differ significantly. The extraction of lipid from fresh forage using AH-EE may have resulted in the hydrolysis of non-lipid cell wall components,

thereby resulting in a higher concentration compared with EE alone (Elgersma et al., 2003). The ensiling process may also have led to a similar hydrolysis of cell wall components, which may account for the significantly higher total lipid fraction estimated from silage compared with fresh forage using the EE method.

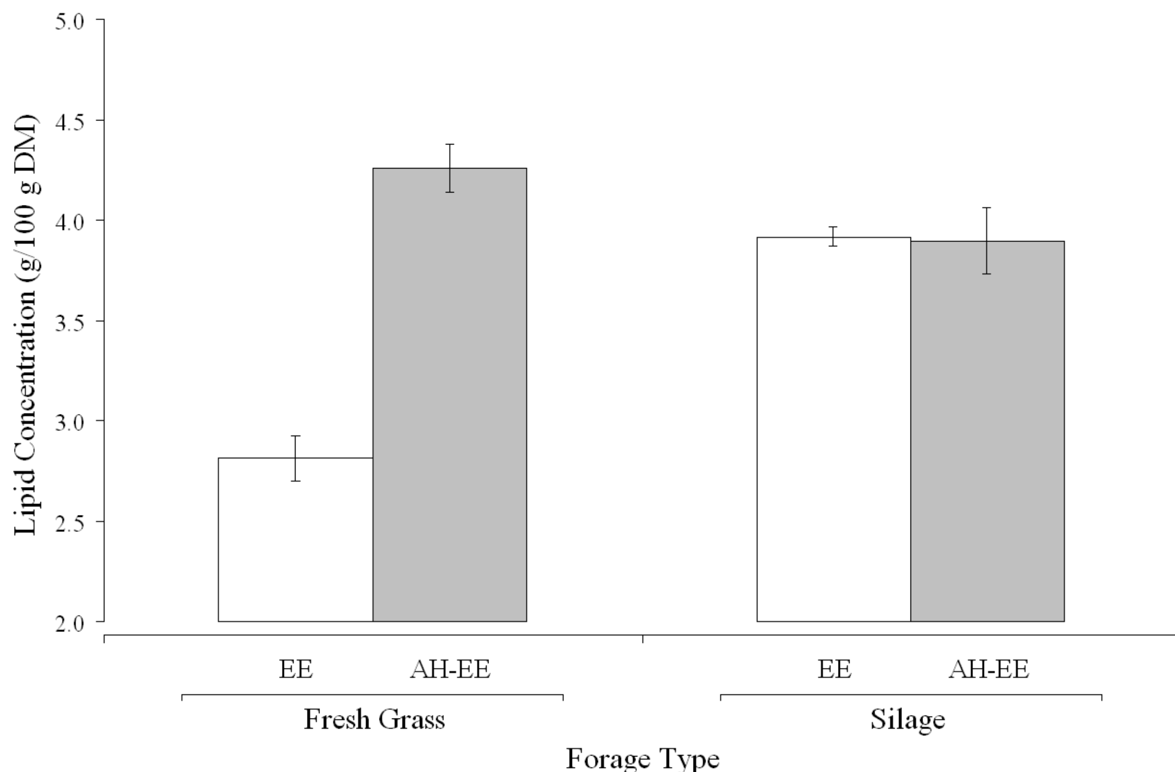


Figure 8. Total lipid concentration in fresh samples of six cultivars of *Lolium perenne* determined using an ether extract (EE, unshaded bars) or an acid-hydrolysis ether extract (AH-EE, shaded bars) method. Data adapted from: Elgersma et al. (2003).

Total fatty acid concentration estimated from the one-step extraction and methylation procedure using ethanol and sodium sulphate was significantly lower when fatty acids were extracted from silages (1949.3 ± 57.9 mg/100 g DM) compared with fresh forage (2913.0 ± 66.8 mg/100 g DM). The free fatty acid proportion of fresh forage was approximately 2%, while the free fatty acid proportion of silage was approximately 27–73% (Figure 9). The lower fat concentration may be due to oxidation of lipid (Elgersma et al., 2003) or may be due to the loss of volatile free fatty acids during the analysis.

The Teagasc research group at the Grange Research Centre, Co. Meath, Ireland (Noci et al., 2005; Moloney, 2007; Noci et al., 2007) report the analysis of lipid in concentrates and do not report using a separate method for the analysis of lipid in silage. Researchers at the Department of Animal and Poultry Science, University of Guelph, Ontario, Canada report the analysis of feed for CF using method 920.39 in the Association of Official Analytical Chemists (AOAC, 1990) which uses a Soxhlet extraction procedure (Whiting et al., 2004).

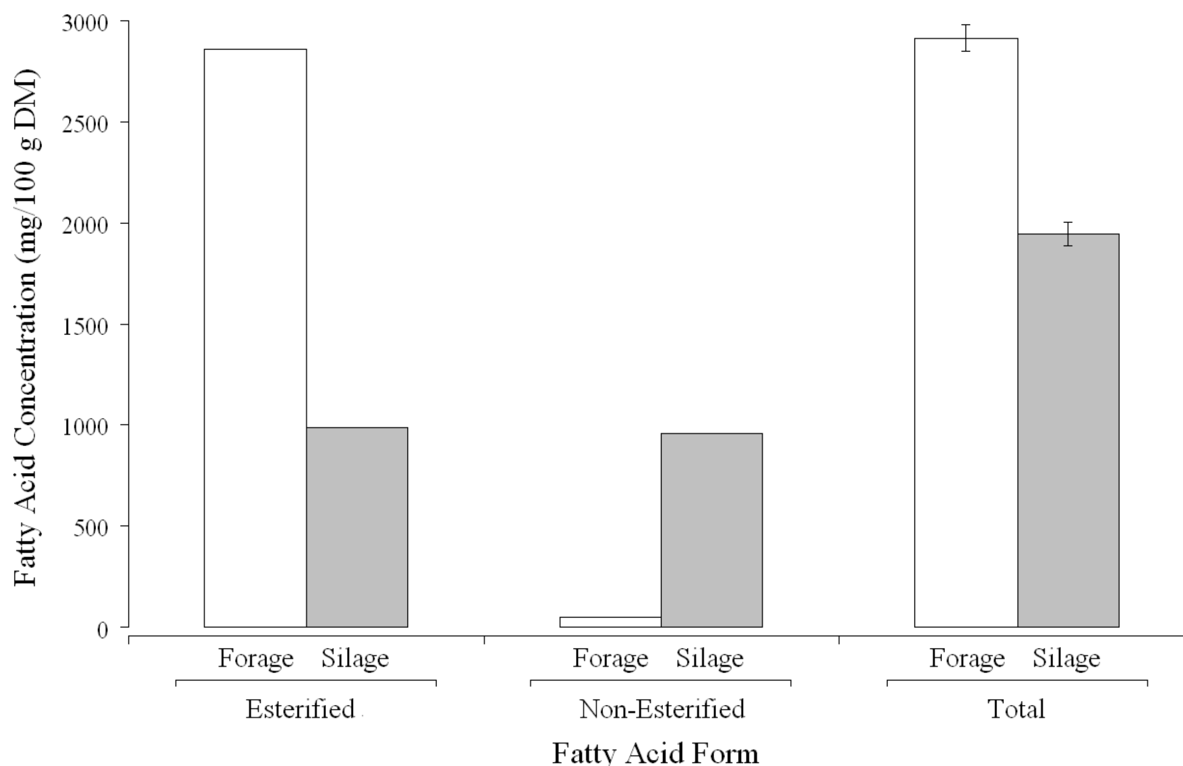


Figure 9. Total non-esterified fatty acid concentration of six cultivars of *Lolium perenne* in fresh forage (unshaded bars) and silage (shaded bars). Standard errors could only be calculated from data presented for total fatty acid concentrations. Data adapted from: Elgersma et al. (2003).

The acid hydrolysis method for baked goods and pet food is reported to be AOAC Official Method 954.02. A further AOAC method (No. 945.16, Oil in Cereal Adjuncts; Petroleum Ether) is also available. The Department of Food Science at the Swedish University of Agricultural Science report a modified EE method of Folch et al. (1957) for the extraction of feed lipid (Eriksson and Pickova, 2007). Researchers at the Research Institute for Biology of Farm Animals in Dummerstorf, Germany report using HCl hydrolysis CF determination according to the method of Kuhla (1983) (referred to by, Nuernberg et al., 2002; Dannenberger et al., 2004; Nuernberg et al., 2005; Dannenberger et al., 2006). The original reference quoted is in German, so it is difficult to determine the details of the analysis.

In Australia the recommended method for analysing CF is by EE without acid hydrolysis (AFIA, 2006). This method recommends using diethyl ether as the solvent in the analysis, however, most laboratories use petroleum ether and, recently, have also altered the method to use hexane.

3.1.2 Isolation of lipid fractions

Commonly, the isolation of lipid fractions is undertaken using thin layer chromatography (TLC, Sukhija and Palmquist, 1988). Lipid classes are also separated using solid phase extraction (SPE) with Strata aminopropyl (NH_2) sorbent cartridges according to the methods of Kaluzny et al. (1985) with the modification of Kim and Salem (1990).

Table 2. Published methods for the analysis of lipid concentration in plant material.

Analysis[1] / Organisation[2]	Method	References
Ether Extract (EE)		
AFIA	Method 3.1D - Determination of crude fat (ether extract)	(AFIA, 2006)
AOCS	AM 5-04 - Rapid determination of oil/fat utilizing high temperature solvent extraction	(AOCS, 1998)
AOAC	AOCS Method 920.39 - Fat (crude) or ether extract in animal feed	(AOAC, 1990)
FOSS Soxtec	FOSS Application Note AN 3004 - Hexane extraction of fat in feed, cereal grain and forage	(Randall, 1974; Thiex et al., 2003)
Acid Hydrolysis (AH)		
AOCS	AOCS Method Ce2c-11	(AOCS, 1998)
AOAC	AOAC Method 954.02 - Fat (crude) or ether extract in pet food	(AOAC, 2003)
Acid Hydrolysis Ether Extract		
Silage		
NI	NI	(Bauchart et al., 1984; Scollan et al., 2001)
Concentrates		
NI	Perstorp Analytical Ltd, Maidenhead, Berkshire	(Choi et al., 2000)
MAFF	Prediction of energy value of compound feeding stuffs for farm animals	(Choi et al., 2000)
European Community - Teagasc	Marketing of Feedstuffs, Regulation Statutory Instruments SI No. 200	(Noci et al., 2005; Moloney, 2007)
International Organisation for Standardization	Animal Feeding Stuffs - Determination of Fat Content (ISO 6492)	(ISO, 1999; Elgersma et al., 2003)

[1]EE = Ether Extract, AH = acid hydrolysis, NI = not indicated.
[2]AFIA = Australian Fodder Industry Association, AOCS = American Oil Chemists' Society, AOAC = Association of Official Analytical Chemists, FOSS = FOSS Industries, Hoganas, Sweden, MAFF = Ministry of Agriculture, Forestry and Fisheries (UK).

3.1.3 Methylation of fatty acids

There are several methods available for methylation of fatty acids prior to analysis using gas chromatography (GC). The method used for methylation is determined by the type of lipid extracted and the fatty acids analysed. Acid-catalysed methylation is used to identify a number of common fatty acids in feed and meat, whereas base-catalysed methylation or a combination of acid and base methylation is used to identify a broader range of fatty acids, in particular, isomers of C18:1 and CLA (Christie, 2003).

3.1.4 Analysis of fatty acids

Fatty acid analysis is generally carried out by GC using a flame ionisation detector (FID). Several different columns and methods are reported (Table 3). The column used is determined by the type of fatty acids being analysed and the precision required. The BPX70 column provides good separation for a range of fatty acids commonly found in blood and meat (Gulliver et al., 2013a), while the CP Sil 88 column is commonly used where an in-depth analysis of trans fatty acids and CLA is required (Choi et al., 2000).

Table 3. Examples of the range of gas chromatographs and capillary columns used in the analysis of fatty acids in forage and meat.

Gas Chromatograph	Column[1] (Length)	Carrier Gas	Reference
NI	CP Sil 88 (x 50 m)	Helium	(Choi et al., 2000)
NI	CP Sil 88 (x 100 m)	NI	(Enser et al., 1998; Lee et al., 2003)
Varian	CP Sil Select CB (x 100 m)	Helium	(Dewhurst et al., 2003b)
Varian 3800	J&W DB-23 (x 30 m)	NI	(Whiting et al., 2004)
NI	HP Innowax (x 60 m)	NI	(Ponnampalam et al., 2010)
Varian 3500	Supelcowax-10 (x 60 m)	Helium	(French et al., 2000)
Thermo Trace GC Ultra™	SP-2560 (x 100 m)	Hydrogen	(Khan et al., 2012)
NI	ZB-Wax (x 30 m)	Nitrogen	(Dewhurst et al., 2003b)
Agilent 6890	SGE BPX70 (x 30 m)	Helium	(Gulliver et al., 2013a)

[1]CP = Chromopack UK Ltd (London, UK), J&W = Agilent (Santa Clara, CA, USA), HP = Agilent (Santa Clara, CA, USA), (ZB = Phenomenex (Macclesfield, UK), SP = Supelco (Bellefonte, PA, USA), SGE = SGE Ltd (Ringwood, Vic, Australia).
NI = Not indicated.

3.2 Lipid extraction and fatty acid analysis of meat

3.2.1 Lipid extraction

Several procedures are available to extract the total lipid in meat and many of these are similar to those reported for forage. Ether extract following acid hydrolysis is often used to analyse the total lipid content of beef (AOAC, 2006; Mach et al., 2006). Lipids are also extracted for the analysis of fatty acids (Scollan et al., 2001) using the Folch method (Folch et al., 1957) with chloroform:methanol (2:1 v/v) or a modified chloroform:methanol procedure (Bligh and Dyer, 1959).

The method of extraction of lipid and the solvents used can significantly affect the results obtained (Clayton et al., 2012a). For example, the efficiency of extraction of saturated fatty acids from red blood cells (RBC) was improved when fatty acids were extracted using methanol:toluene (Lepage and Roy, 1986) compared with chloroform:methanol (Folch et al., 1957).

The differential recovery of fatty acid methyl esters (FAME) from RBC (Figure 10) is due to the different polarity of solvents used for extraction and the recovery of fatty acids from different lipid fractions. The extraction of fatty acids from non-polar lipids, such as cholesterol and triglycerides, is greater when a lower polarity solvent mixture is used, such as that used in the Folch extraction (chloroform:methanol (2:1 v/v), dielectric constant = 14.2), compared with a higher polarity solvent mixture used in the Lepage and Roy extraction (methanol:toluene (4:1 v/v), dielectric constant = 26.9, Christie, 2003). Conversely, extraction of fatty acids from polar lipids, such as phospholipid, is expected to be greater when a higher polarity solvent is used. The improved extraction of FAME with methanol:toluene compared with chloroform:methanol was significantly positively correlated ($r^2 = 0.65$, $p < 0.01$) with the estimated proportion of each fatty acid contained in phospholipid (Figure 11). An understanding of the likely lipid components in the samples being analysed is, therefore, required in order to obtain accurate results.

3.2.2 Interpretation of fatty acid analysis

A number of other factors must also be considered when interpreting the results from fatty acid analysis. As shown previously, fatty acid data may be presented as a proportion of total fatty acids or as a concentration. When examining concentration, it is also important to consider whether the results are presented on a wet or dry matter basis. Concentration is usually presented as mg/100 g of meat wet weight. Data is often presented on a dry matter basis and care should be taken to accurately convert results to a wet-weight basis by using the correct dry matter content of meat (for example, *Longissimus dorsi* contains approximately 72% water).

The standard serving size of food is also important when interpreting results for fatty acid analyses. Although data for the concentration of fatty acids in meat is usually presented in mg/100 g edible portion, concentration can also be presented in mg/serve. A standard serving size varies for different food types and the standard serving size for beef and lamb is 135 g (Smith et al., 1998; NHMRC and Health, 2006) and knowledge of these is critical when assessing the actual concentration of fatty acid contained in food (Shrapnel and Baghurst, 2007).

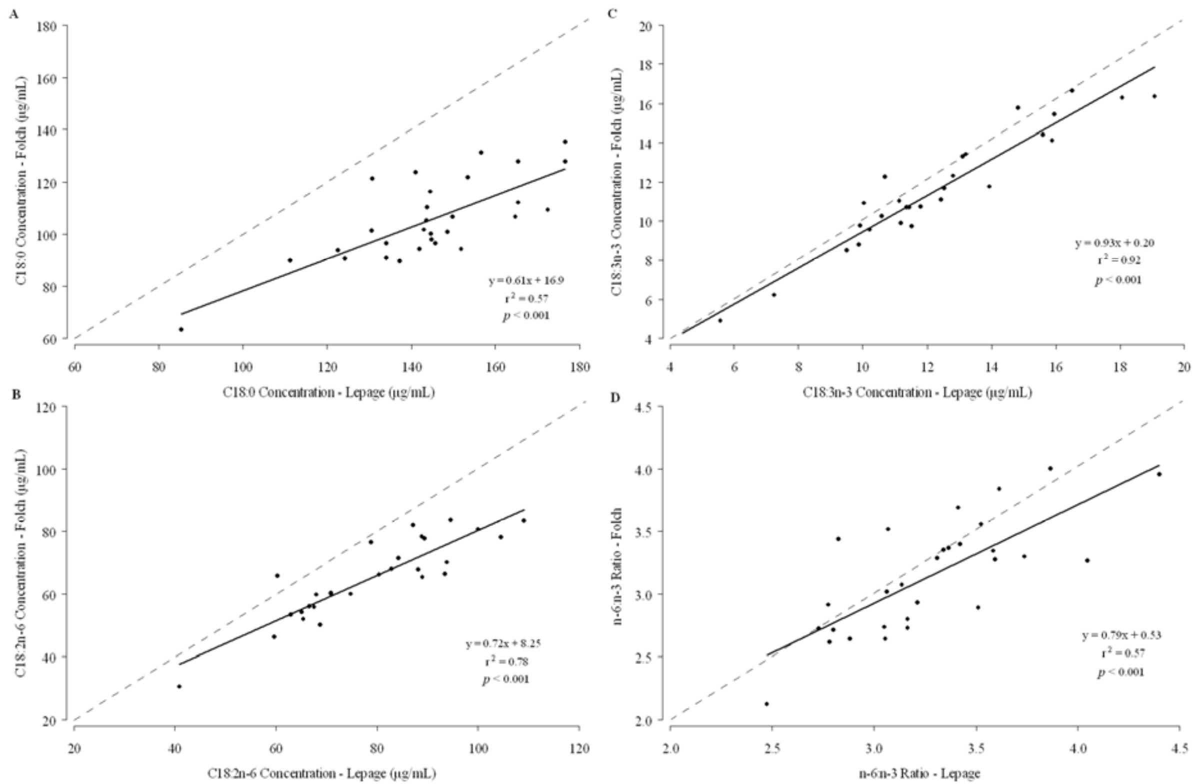


Figure 10. Correlation between the concentration of A) C18:0, B) C18:2n-6, C) C18:3n-3 (µg/mL) and D) the ratio of omega-6:omega-3 polyunsaturated fatty acids (n-6:n-3 ratio) in the red blood cells (RBC) of sheep following analysis of fatty acids using a one-step extraction and methylation procedure (Lepage and Roy, 1986) or a two-step procedure including a Folch extraction (Folch et al., 1957). Adapted from: Clayton et al. (2012a).

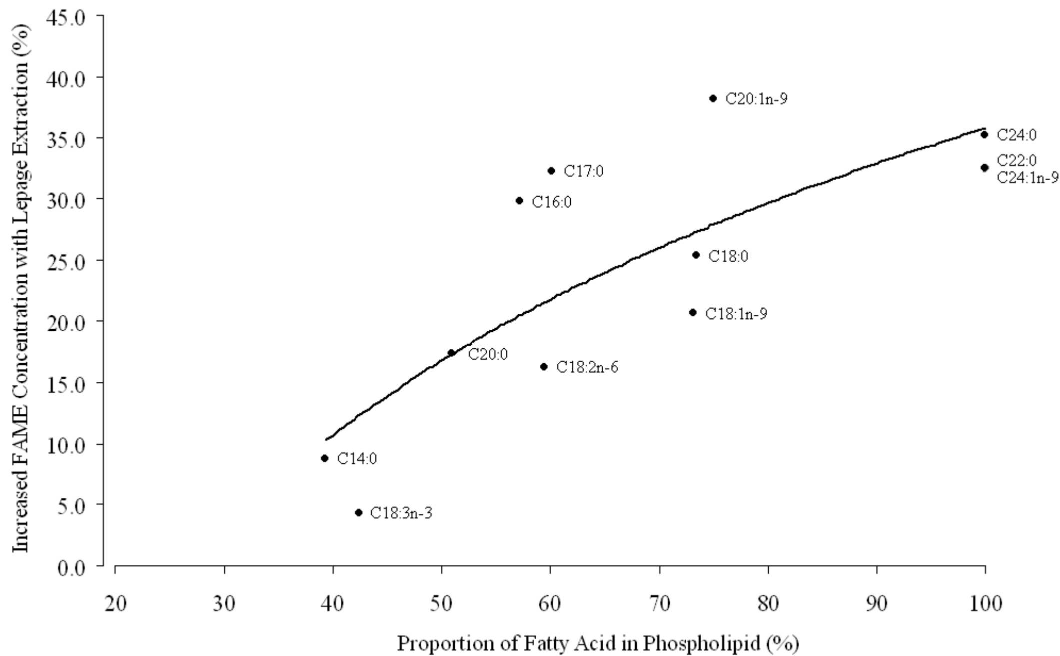


Figure 11. Relationship between the proportion of total fatty acid in phospholipid and the percentage increase in fatty acid methyl ester (FAME) concentration using a one-step extraction and methylation procedure (Lepage and Roy, 1986) compared with extraction of lipid using the Folch procedure (Folch et al., 1957). Adapted from: Clayton et al. (2012a).

3.3 Lipid conversion factors (LCF) and recovery of fatty acids

3.3.1 Lipid conversion factors

Measuring the likely impact of research programs examining more efficient production of beef, increasing production or increasing the content of fatty acids such as LCn-3PUFA requires knowledge of the concentration of LCn-3PUFA in meat. Nutritional guidelines for the intake of LCn-3PUFA also recommend daily intakes in mg/day (NHMRC and Health, 2006). In order to calculate the likely consumption of fatty acids by consumers from individual meat sources, the concentration of fatty acids in mg/100 g edible portion must, therefore, be known.

Data for LCn-3PUFA in meat is often presented as the proportion of total fatty acid and conversion to concentrations in mg/100 g edible portion is required to calculate total intake. As described in Section 2.3, total lipid is comprised of several components including fatty acids, glycerol and phosphate and the fatty acid concentration in total lipid is dependent on the type of lipid and type of fatty acid present. The percentage of fatty acid moieties in the total lipid fraction forms the basis for the lipid conversion factor (LCF). The concentration of fatty acids in meat can, therefore, be estimated from the concentration of total lipid, a LCF and the amount of each fatty acid as a proportion of total fatty acid.

The following example shows the calculation for the concentration of lipid as a proportion of total triglyceride for EPA (C20:5n-3, $C_{22} H_{34} 0_2$).

One mole of EPA FAME	= 317.49 g.
Total weight of 1 mole of triglyceride of EPA	= 948.42 g (2 x EPA + 1 x glycerol)
Proportion of EPA in triglyceride	= 317.49 / 948.42
	= **0.956 g fatty acid/g lipid.**

A LCF for beef can be calculated from the proportion of each lipid class in beef total lipid multiplied by the concentration of fatty acid in each lipid class. The weighted fatty acid proportion is then summed to give the LCF (g fatty acid/g total lipid). The original calculation of a LCF for beef (Anderson et al., 1975) was based on the criteria that beef lipid is approximately 87% triglyceride, 12% phospholipid and 1% cholesterol and cholesterol does not contain any fatty acids (Table 4). A LCF was also derived for pork using similar criteria (Anderson, 1976).

Table 4. Derivation of a lipid conversion factor (LCF) for beef from the proportion of lipid classes and the concentration of fatty acid in each lipid class (g fatty acid/g total lipid).

Lipid Component[1]	Proportion of Total lipid	Fatty Acid Concentration (g fatty acid/g lipid)	Total (g fatty acid/g total lipid)
Triglyceride	0.87	0.956	0.831
Phospholipid	0.12	0.707	0.085
Cholesterol	0.01	0.000	0.000
Total	**1.00**		**0.916**

[1]Calculation based on the assumption that beef lipid contains approximately 12% phospholipid and 1% cholesterol and that cholesterol does not contain any fatty acids.
Adapted from: Anderson et al. (1975).

The calculation of the LCF for beef was modified by taking into account a more in depth analysis of the proportions of different classes of phospholipid in total lipid, each with varying concentrations of fatty acids per mole of lipid (Table 5, Weihrauch et al., 1977). There are a number of limitations to these estimations, however, as the calculations do not take into account free fatty acids and assume that total lipid is only comprised of phospholipid and triglyceride.

Table 5. Derivation of a lipid conversion factor (LCF) for beef from the proportion of lipid classes and the concentration of fatty acid in each lipid class (g fatty acid/g total lipid) taking into account an in depth analysis of the phospholipid proportions in beef.

Lipid Component[1]	Proportion of Total lipid	Fatty Acid Concentration (g fatty acid/g lipid)	Total (g fatty acid/g total lipid)
Triglyceride	0.870	0.956	0.832
Phosphatidyl ethanolamine	0.038	0.750	0.029
Phosphatidyl choline	0.071	0.719	0.051
Phosphatidyl serine	0.006	0.718	0.004
Sphingomyelin	0.005	0.717	0.004
Cholesterol	0.010	0.000	0.000
Total	0.990		**0.919**

[1]Calculation based on the assumption beef lipid contains approximately 12% phospholipid and 1% cholesterol and that cholesterol does not contain any fatty acids (Anderson et al., 1975).
Adapted from: Weihrauch et al. (1977).

When fatty acid data in original publications are presented as percentage of total fatty acids, the concentration of fatty acid (mg/100 g edible portion) can be estimated in order to calculate the total intake of fatty acid from each food source. The concentration of fatty acid is calculated from total lipid content using a LCF according to the following formula (Meyer et al., 1999):

Concentration of fatty acid (mg/100 g edible portion) = Total lipid content (g/100 g) x LCF x (% fatty acid / 100) x 1000

For example, the concentration of DPAn-3 (C22:5n-3) in beef can be calculated from the total fat content and proportion of DPA as a percentage of total fatty acid (Sinclair et al., 1982) using a LCF for beef of 0.919 (Weihrauch et al., 1977):

Total lipid content = 2.48 g/100 g
DPAn-3 content = 1.0 % of total fatty acid

Concentration of DPAn-3 = 2.48 x 0.919 x (1.0 / 100) x 1000
= 22.79 mg/100 g edible portion

A LCF can also be determined for plant material if the proportion of each lipid class is known. A detailed analysis of the fatty acid concentration in different lipid fractions of wheat flour provides a good database of lipid classes (Table 6).

Table 6. Lipid moieties and calculation of lipid conversion factor for wheat flour.

Lipid Fraction	% of Total Lipid	Number of Fatty Acids	g fatty acid/g lipid class	g fatty acid/g total lipid
Non-polar lipids				
Cholesteryl esters	7.5	1	0.397	0.030
Triglyceride	20.8	3	0.956	0.199
Monoglyceride	1.3	1	0.788	0.010
Diglyceride	12.2	2	0.908	0.111
Free sterol	2.1	0	0.000	0.000
Free fatty acid	7.0	1	1.000	0.070
Glycolipids				
6-0 Acyl-MGDG	3.6	3	0.804	0.029
6-0 Acyl steryl glucoside	1.6	1	0.320	0.005
MGDG	4.9	2	0.719	0.035
Steryl glucoside	1.8	0	0.000	0.000
MGMG	0.4	1	0.505	0.002
DGDG	13.5	2	0.593	0.080
Cerimade diglycoside	0.03	0	0.000	0.000
DGMG	0.6	1	0.410	0.002
Phospholipids				
N-acyl phosphatidyl ethanolamine	4.9	3	0.839	0.041
N-acyl L phosphatidyl ethanolamine	2.9	3	0.756	0.022
Phosphatidyl ethanolamine	0.8	2	0.754	0.006
Phosphatidyl choline	5.8	2	0.712	0.041
L phosphatidyl ethanolamine	0.9	1	0.580	0.005
L phosphatidyl choline	7.1	1	0.530	0.038
Phosphatidyl inositol	0.1	2	0.649	0.001
Phosphatidyl serine	0.2	2	0.712	0.001
Total Lipid Conversion Factor				**0.729**

M = mono; G = galactosyl; D = di; G = glyceride; L = Lyso
Data adapted from: Morrison et al. (1975); Weihrauch et al. (1977).

3.3.2 Calculating the recovery of fatty acids

Using published values for total fatty acid (mg/100 g) and total lipid concentration (g/100 g), it is possible to estimate the recovery of fatty acids from total lipid. The presented total fatty acid concentrations vary from approximately 50% of total lipid to nearly 90% of total lipid (Table 7).

Table 7. Fatty acid recovery calculated for fresh forages, silages or concentrates.

Study	Major species	Details	Total Lipid (g/100 g)	Total FA (mg/100 g DM)	Estimated Fatty Acid Recovery
Fresh Forage					
(Whiting et al., 2004)	*Medicago sativa*	-	1.80	1399	0.777
(Elgersma et al., 2003)	*Lolium perenne*	Agri	4.41	2975	0.675
	cut after 25 days	AberGold	4.30	2862	0.665
	re-growth	Respect	4.69	2758	0.588
		Herbie	3.78	2722	0.719
		Barezane	4.17	3154	0.756
		Barnhem	4.21	3007	0.714
(Dewhurst et al., 2002)	*Lolium perenne*	Normal	1.74	930	0.534
		Stay-green	1.48	850	0.574
Silage					
(Noci et al., 2007)	*Lolium perenne*	Unwilted	4.05	2630	0.649
		32 hr wilt	3.26	2412	0.740
(Whiting et al., 2004)	*Medicago sativa*	Wilted	1.70	1299	0.764
(Al-Mabruk et al., 2004)	*Lolium perenne + L. multiflorum*	-	4.62	1390	0.301
(Dewhurst et al., 2003a)	*Trifolium pratense*	-	4.32	1400	0.324
(Lee et al., 2003)	*Lolium perenne*	-	3.25	1820	0.560
	Trifolium pratense	-	2.42	2200	0.909
	Trifolium repense	-	2.89	2410	0.834
(Elgersma et al., 2003)	*Lolium perenne*	Agri	5.32	1964	0.453
	cut after 25 days	AberGold	5.08	1808	0.554
	re-growth	Respect	5.55	2098	0.589
		Herbie	5.22	1757	0.440
		Barezane	5.25	2093	0.516
		Barnhem	5.29	1976	0.475
(Scollan et al., 2003)	*Lolium perenne*	-	3.70	1270	0.343
(Choi et al., 2000)	*Lolium perenne*	24 hr wilt	3.51	1880	0.536
Concentrates					
(Noci et al., 2007)	Barley + SBP[1] + soyabean oil	-	1.31	1140	0.870
(Scollan et al., 2003)	Barley + SBP + Megalac[2]	-	10.8	7150	0.662
(Choi et al., 2000)	Barley + SBP + Megalac	-	7.32	5980	0.817

[1]SBP = Sugarbeet pulp.
[2]Megalac = High palmitic acid (C16:0) supplement.

3.4 Calculation of the ratio of omega-6:omega-3 fatty acids

One of the main focuses of LCn-3PUFA research in recent times has been the ratio of n-6:n-3 PUFA. There are some differences in the calculation of the ratio of n-6:n-3 depending mainly on the type of LCn-6PUFA measured in meat samples (Table 8). The ratio of n-6:n-3 is altered by incorporating different fatty acids into the ratio, however, this change is usually minor compared with differences observed following dietary treatment.

Table 8. Calculation of the omega-6:omega-3 ratio in meat is affected by the fatty acid groups included in the calculations.

Reference	n-6 Fatty Acids	n-3 Fatty Acids
(Choi et al., 2000)	C18:2, C20:3, C20:4	C18:3, C20:4, C20:5, C22:5, C22:6
(Scollan et al., 2001)	C18:2, C20:3, C20:4	C18:3, C20:4, C20:5, C22:5, C22:6
(Scollan et al., 2003)	C18:2, C20:3, C20:4, C22:6	C18:3, C20:4, C20:5, C22:5, C22:6
(Noci et al., 2007)	C18:2, C18:3, C20:2, C20:3, C20:4, C22:6	C18:3, C20:3, C20:5, C22:5, C22:6
(Mach et al., 2006)	C18:2, C18:3, C20:2, C20:3, C20:4, C22:6	C18:3, C20:3, C20:5, C22:5

The concentration of C18:4n-3 (stearidonic acid, SDA) in meat is often not reported, which may be due to the lack of suitable standards worldwide for this fatty acid. Stearidonic acid is an important intermediate in the conversion of ALA to LCn-3PUFA (Figure 3) and its quantification is required in order to gain a greater understanding of omega-3 metabolism. The concentration of SDA in plasma, RBC and meat can be significantly increased if animals are fed oil from *Echium plantagenium* (Patterson's curse, Kitessa and Young, 2009), fish oil or algae (Clayton et al., 2014).

4. Meat Quality

There are a number of factors that determine meat quality, including consumer satisfaction (tenderness, taste, juiciness), storage and shelf-life and health attributes (SFA and LCn-3PUFA content). Many of these attributes are strongly influenced by the fat content of meat (total fat percentage) and the composition of lipid, such as the concentrations of individual fatty acids. The current section of the Monograph focuses on the relationship between diet and meat concentrations of LCn-3PUFA as well as the ratio of n-6:n-3 fatty acids, outlining, in particular, opportunities for research and development in the area of utilising forages and silages to improve the health status of beef for human consumption.

4.1 Omega-3 and meat quality

The potential to alter the quality of meat through manipulation of fatty acid concentrations has been extensively reviewed (Doreau and Chilliard, 1997; Wood and Enser, 1997; Demeyer and Doreau, 1999; Scollan et al., 2006). The current section of the Monograph focuses on the potential to manipulate LCn-3PUFA content of meat through the use of conserved forages and highlights recent advances in the field of omega-3 nutrition.

There are a number of important points to note when interpreting research results examining the concentration of LCn-3PUFA in meat. Of particular importance is the potential for negative effects of increased LCn-3PUFA in meat, including the risk of increased lipid peroxidation, especially in pigs (Nilzen et al., 2001) and lamb (Hopkins et al., 2014). Although this peroxidation can be minimised by feeding Vitamin E, it is an important consideration for overall meat quality. In addition, the overall body structure and animal weight is important when examining the fatty acid composition of meat. When comparing forage versus grain feeding for example, different energy intakes need to be considered, as cattle fed concentrates are usually heavier and fatter than those fed grass or silage (Muir et al., 1998; McGee, 2005). Alternatively, animals fed concentrates may be younger than animals fed forage if grown to a specific bodyweight (French et al., 2000). The impact of these factors is discussed further in sections examining the manipulation of LCn-3PUFA in meat.

4.2 Influence of breed on fatness and omega-3

As animals mature and the total lipid content of meat increases, the concentration of SFA and MUFA in meat increase, but the concentration of PUFA does not increase in relative terms (for review, see De Smet et al., 2004). As a result, the proportions of fatty acids change and, as the total lipid content of meat increases, the amount of PUFA as a proportion of total fatty acid decreases (Knight et al., 2003). Breeds that are fatter, therefore, tend to have higher proportions of SFA and lower proportions of PUFA in their meat.

Changing concentrations of PUFA in meat with changing fatness also highlights the importance of comparing similar units of measures of fatty acids in meat. The standard convention is to report the measurement of fatty acids in mg/100 g edible tissue instead of % total fatty acid. In this way, the total 'dose' of LCn-3PUFA can be calculated from the consumption of a specified weight of meat. While the amount of LCn-3PUFA as a proportion of total fatty acid in meat decreases as total lipid content of meat increases, the total amount of LCn-3PUFA (mg/100 g edible tissue) may increase. The total intake of SFA and the ratio of PUFA:SFA should also be examined when considering the potential influences of meat consumption on risk factors for diseases including CVD.

The majority of PUFA (including LCn-3PUFA) are contained in the phospholipid fraction of meat (approximately 90% of total PUFA) while only 8% is contained in triglycerides. The lipid fraction analysed needs to be considered, therefore, when interpreting fatty acid concentrations. The concentration of fatty acids in the phospholipid fraction is commonly reported, as phospholipids have a significant impact on the sensory properties of meat through lipid oxidation (Buckley et al., 1989; Ponnampalam et al., 2002a), however, the lipid composition of the total lipid of meat may be different.

4.3 Changes in fatty acid concentrations in meat following cooking

There are often concerns as to whether the concentrations of fatty acids, particularly LCn-3PUFA, are altered following the preparation and cooking of meat compared with fresh meat. During the cooking process there is usually only a loss of water from meat, with little loss of lipid (Kronberg et al., 2006), however, a decrease in the concentration of EPA and DHA (as a proportion of fresh weight) was observed following grilling of *Longissimus lumborum* (rump steak, Sinclair et al., 1994). The loss of water associated with cooking is approximately 32% of wet weight (Varela et al., 2004) and the total dose of LCn-3PUFA per serve of meat usually remains unchanged, although the amount of fatty acid as a proportion of cooked meat may increase if the loss of water is taken into account.

5. Altering LCn-3PUFA in Meat through Dietary Manipulation

The concentration of LCn-3PUFA in meat can be altered by manipulating the diet of animals in a variety of ways. Animals can be fed diets containing LCn-3PUFA supplements (high in EPA and DHA, for example algae or fish meal), concentrated sources of ALA (such as linseed) or forages high in ALA compared with grains that are higher in LA. The current section of the Monograph outlines methods of manipulating LCn-3PUFA in beef and lamb primarily by altering forage intake, however, a brief review of other omega-3 supplements, including LCn-3PUFA and linseed, is included.

5.1 Manipulating LCn-3PUFA in meat by feeding LCn-3PUFA sources

The most common LCn-3PUFA sources for animal diets are fish meals which are naturally high in EPA and DHA. Marine algae is also high in EPA and DHA (Woods et al., 2005) and may also be a better source of LCn-3PUFA in animal feeds, as the feeding of animal products including fish meal to ruminants is banned in many countries including Australia. The following sections will outline the changes in meat LCn-3PUFA possible following the feeding of fish oil and fish meals.

5.1.1 Beef

The concentration of EPA and DHA in the *Longissimus thoracis* (ribeye muscle) of Holstein-Friesian or Welsh Black steers was significantly higher when cattle were fed fish oil from South American herring containing approximately 11.5% EPA and 5.7% DHA in addition to perennial ryegrass silage for 90 days compared with cattle receiving a SFA supplement (Table 9, Choi et al., 2000). The increase in all LCn-3PUFA, including EPA, DPA and DHA, was greater for Welsh Black compared with Holstein-Friesian steers (Choi et al., 2000). The mature body weight of Welsh Black steers is lower than Holstein-Friesian steers, therefore, if steers were slaughtered at the same carcase weight instead of at the same point in time, the Welsh Black steers would have been fatter and would likely have had a lower proportion of LCn-3PUFA in meat compared with Holstein-Friesian steers.

The concentration of EPA and DHA in the *L. thoracis* of Charolais steers was also higher when steers were fed fish oil in addition to a basal diet of silage, barley and sugarbeet pulp for 120 days compared with steers fed the basal diet with a high palmitic acid (C16:0) supplement, Megalac (Scollan et al., 2001). Although fish oil was included in the diet at approximately 59 g/kg DM, there are no details provided for the source or the concentrations of EPA and DHA in the fish oil, therefore, the actual intake of LCn-3PUFA cannot be determined for comparison with concentrations reported in meat.

The concentration of EPA and DHA in the *L. dorsi* of Friesian steers was also significantly higher when cattle were fed fish oil from mackerel and herring containing approximately 6.95% EPA and 11.76% DHA in addition to a basal diet of ryegrass silage, barley and sugarbeet pulp for 108 days compared with cattle receiving the basal diet alone (Table 9, Noci et al., 2007). The concentration of EPA and DHA in meat increased with increasing doses of fish oil received by the steers (Figure 12).

Table 9. Omega-3 concentrations in beef following supplementation with LCn-3PUFA sources.

					Fatty Acid Concentration (mg/100 g beef)[1]								
Study	Breed	Basal Diet	Intervention	Muscle Type	Total	Palmitic	ALA	EPA	DPA	DHA	LCn-3PUFA	n-6:n-3 Ratio
(Choi et al., 2000)	Holstein-Friesian	Perennial rye silage + Barley and SBP[3]	Megalac[2] control	*Longissimus thoracis*	3808	1043.9	26.03	13.21	23.06	2.65	38.92	3.43
			Linseed + FO		3857	1023.8	33.03	16.75	23.08	6.10	45.93	2.05
	Welsh Blacks	Perennial rye silage + Barley and SBP[3]	Megalac control	*L. thoracis*	3094	837.2	26.06	14.33	22.5	2.77	39.60	2.71
			Linseed + FO		2385	621.7	29.67	21.15	24.48	6.01	51.64	1.70
(Scollan et al., 2001)	Charolais	Barley + SBP[3] silage	Megalac Control	*L. thoracis*	3359	953	19.5	10	19	2.5	31.50	2.00
			Fish oil		4400	1336	27	24	24	5.3	53.30	0.91
(Noci et al., 2007)	Friesian	Concentrate (4.7 kg/hd)	Unwilted silage	*Longissimus dorsi*	6521	1519	28.16	8.43	16.3	3.16	27.89	3.89
			Wilted silage		7078	1708	31.13	7.69	16.3	2.09	26.08	3.72
			Unwilted + Fish oil		7802	1983	33.55	14.66	18.4	4.78	37.88	2.95

[1]Palmitic = Palmitic acid (C16:0), ALA = α-linolenic acid (C18:3n-3), EPA = eicosapentaenoic acid (C20:5n-3), DPA = docosahexaenoic acid (C22:5n-3), DHA = docosahexaenoic acid (C22:6n-3). Fatty acids analysed in total lipid unless otherwise indicated.
[2]Megalac = palm oil supplement high in C16:0.
[3]SBP = sugar beet pulp.
Significance for differences within study: $^{*}p < 0.05$; $^{†}p < 0.01$; $^{‡}p < 0.001$. p-value not always indicated for total LCn-3PUFA or n-6:n3 ratio.
All studies involved steers and reported neutral lipid + polar lipid fractions. Total fat (g/100 g) not reported in any studies.

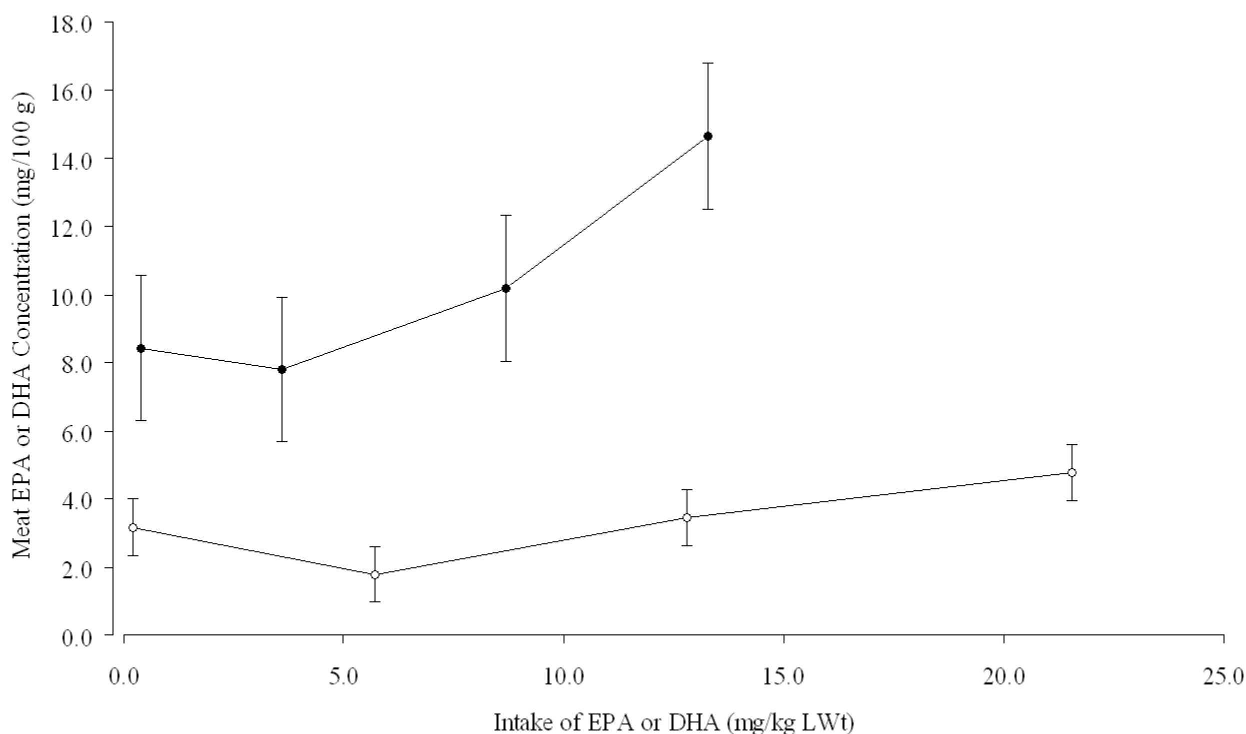


Figure 12. Increasing concentrations of EPA (●) and DHA (○) in *Longissimus dorsi* of Friesian steers fed increasing doses of EPA and DHA as fish oil. Values are least squares means ± pooled sem. Data adapted from: Noci et al. (2007).

5.1.2 Lamb

A series of studies examining fish meal and fish oil supplementation of lambs was conducted by the Victoria Department of Primary Industries between 2000 and 2002 (Ponnampalam et al., 2001a; Ponnampalam et al., 2001b; Ponnampalam et al., 2002a; Ponnampalam et al., 2002b). The concentration of EPA and DHA in the *L. thoracis* of Merino x Border Leicester x Poll Dorset or Dorset Horn x Merino wether lambs was significantly higher when lambs were fed Australian produced fish meal or fish oil in addition to a basal diet of oaten:lucerne chaff (30:70) compared with lambs fed the basal diet alone (Ponnampalam et al., 2001b).

The increase in EPA and DHA concentration in meat was related to the total dose of LCn-3PUFA consumed in the diet (mg/kg liveweight, Figure 13). The proportionate increase in total LCn-3PUFA was greater following the consumption of fish meal compared with fish oil, however, the greatest increase in LCn-3PUFA was when lambs received a higher total dose of LCn-3PUFA from fish oil. The rate of accumulation of EPA and DHA in meat declined as the intake of LCn-3PUFA increased, indicating a reduced efficiency of absorption or metabolism of LCn-3PUFA from the diet to meat (Figure 13).

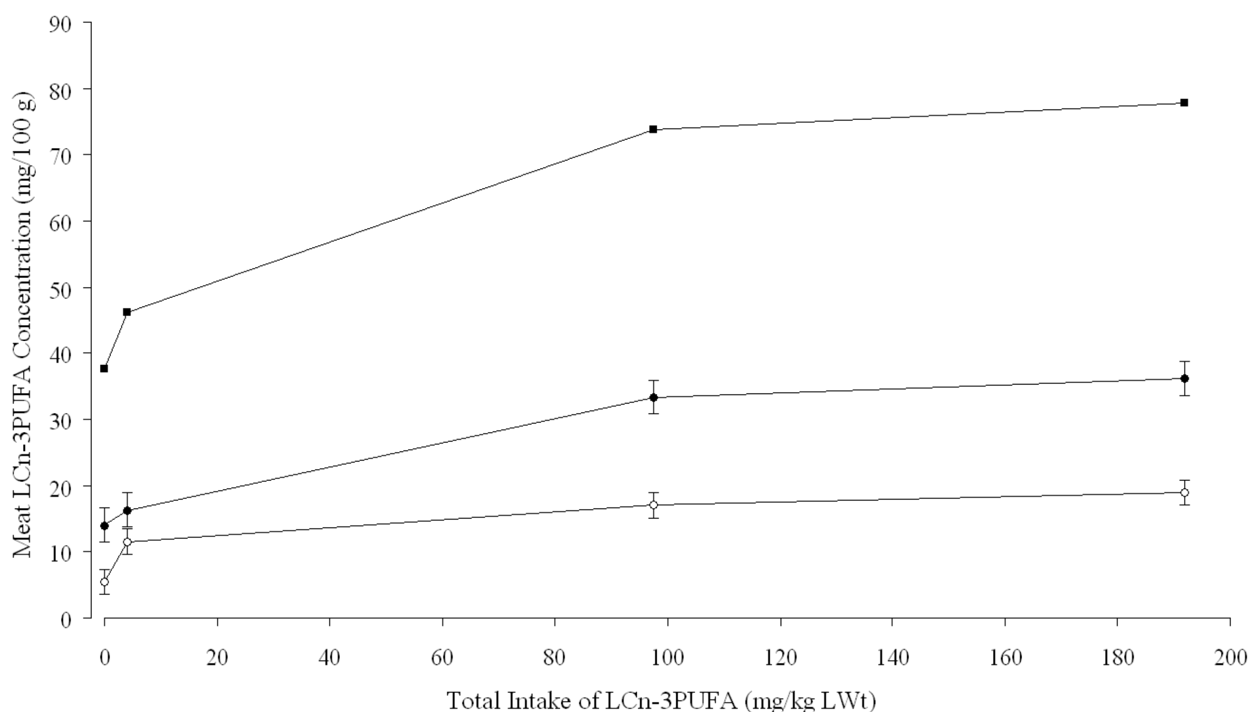


Figure 13. Relationship between intake and concentrations of EPA (●), DHA (○) and total LCn-3PUFA (■) in *Longissimus thoracis* of lambs fed increasing doses of EPA and DHA as fish meal or fish oil from two experiments. Values are least squares means of phospholipid + triglyceride meat concentrations with pooled sem. Data adapted from: Ponnampalam et al. (2001b); Ponnampalam et al. (2002b).

5.1.3 Pork

Fish meal and fish oil can also be added to pig diets to increase the concentration of EPA and DHA in muscle, highlighting important aspects of metabolism of fatty acids. The concentration of DHA in *L. dorsi* of Hampshire x Swedish Landrace x Swedish Yorkshire pigs was significantly higher when pigs were fed increased amounts of fish meal containing 1.53% DHA in addition to a basal diet of barley, wheat and soybean meal (Hertzman et al., 1988). The increase in concentration of DHA in meat was approximately linear with increasing doses of DHA in the diet (Figure 14). Within the treatment group receiving the highest amount of DHA (29.05 mg/kg DM) the proportion of DHA in intramuscular fat decreased (r^2 = 0.71) over time of feeding as total intramuscular fat increased (Figure 15A). The total concentration of DHA in muscle (mg/100 g) was, however, not significantly related to intramuscular fat content (Figure 15B) indicating the total intake of DHA from consuming muscle from these animals would not have been altered with an increased length of time of feeding and higher intramuscular fat.

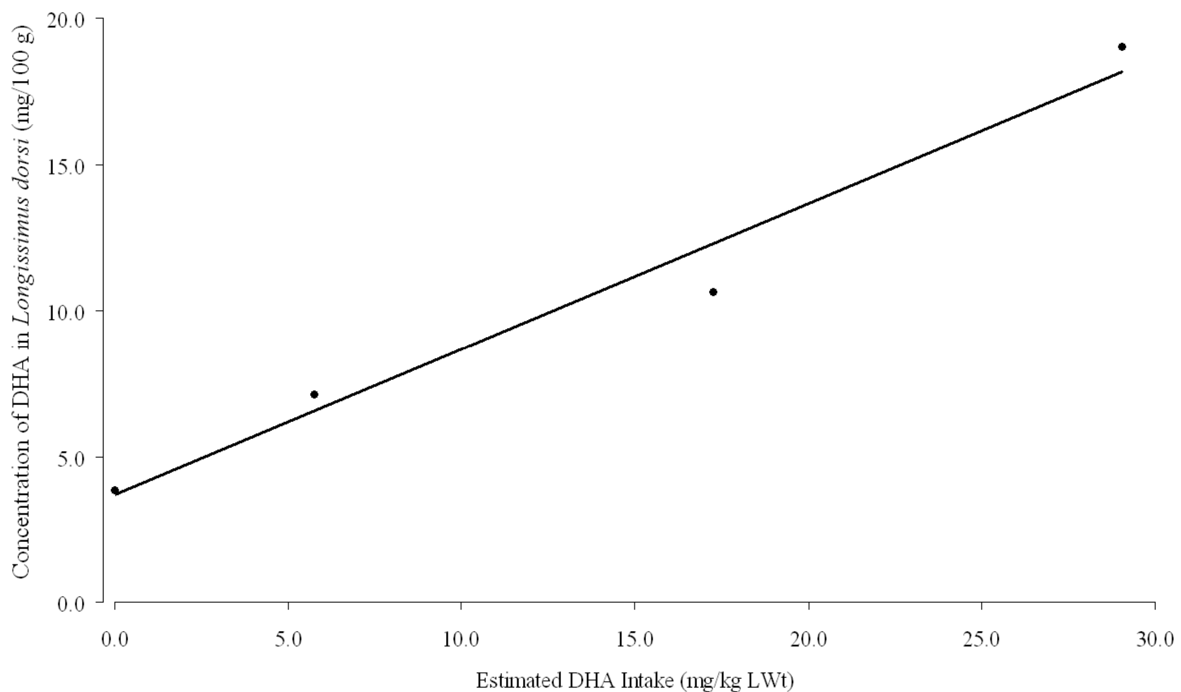


Figure 14. Concentration of DHA in *Longissimus dorsi* of pigs fed increasing doses of DHA as fish meal (DHA concentration in *L. dorsi* = 0.499 x intake + 3.669, $r^2 = 0.97$, $p < 0.01$). Fatty acid intake estimated from concentration of DHA in fish meal, estimated intake per day and average body weight. DHA concentration (mg/100 g) estimated from lipid concentration in *L. dorsi* (g/100 g) and % DHA in total lipid using a LCF of 0.918. Data adapted from: Hertzman et al. (1988).

A research group based at the University of Sydney fed a trademarked brand of stabilised tuna fish meal 'PorcOmega™' to pigs (breed unspecified) for 4 or 6 weeks prior to slaughter (Howe et al., 2002). Pigs were fed PorcOmega™ at 15% of the diet for 42 days or at 20% of the diet for 28 days prior to slaughter. The concentrations of EPA, DPAn-3 and DHA were higher following supplementation with PorcOmega™ at 15% of the diet compared with the basal diet. There are several limitations in the publication, however, that make interpretation of the results difficult. Firstly, the concentrations of EPA and DHA in the proprietary formulation are not presented and, therefore, the intake of EPA and DHA cannot be determined. Secondly, only the proportion of fatty acids in forequarter chops and not in loin or leg chops are presented, whereas the authors state that the leg chops were more likely to be considered 'healthy' due to a lower total fat content. Concentrations of all fatty acids in mg/100 g or mg/serve of meat are required to indicate possible health benefits of consumption of meat. Finally, even though data showing an increase in the proportion of EPA and DHA in forequarter chops is presented (Figure 5 in the publication), interpretation of this data is difficult due to the lack of presentation of error bars and statistical significances.

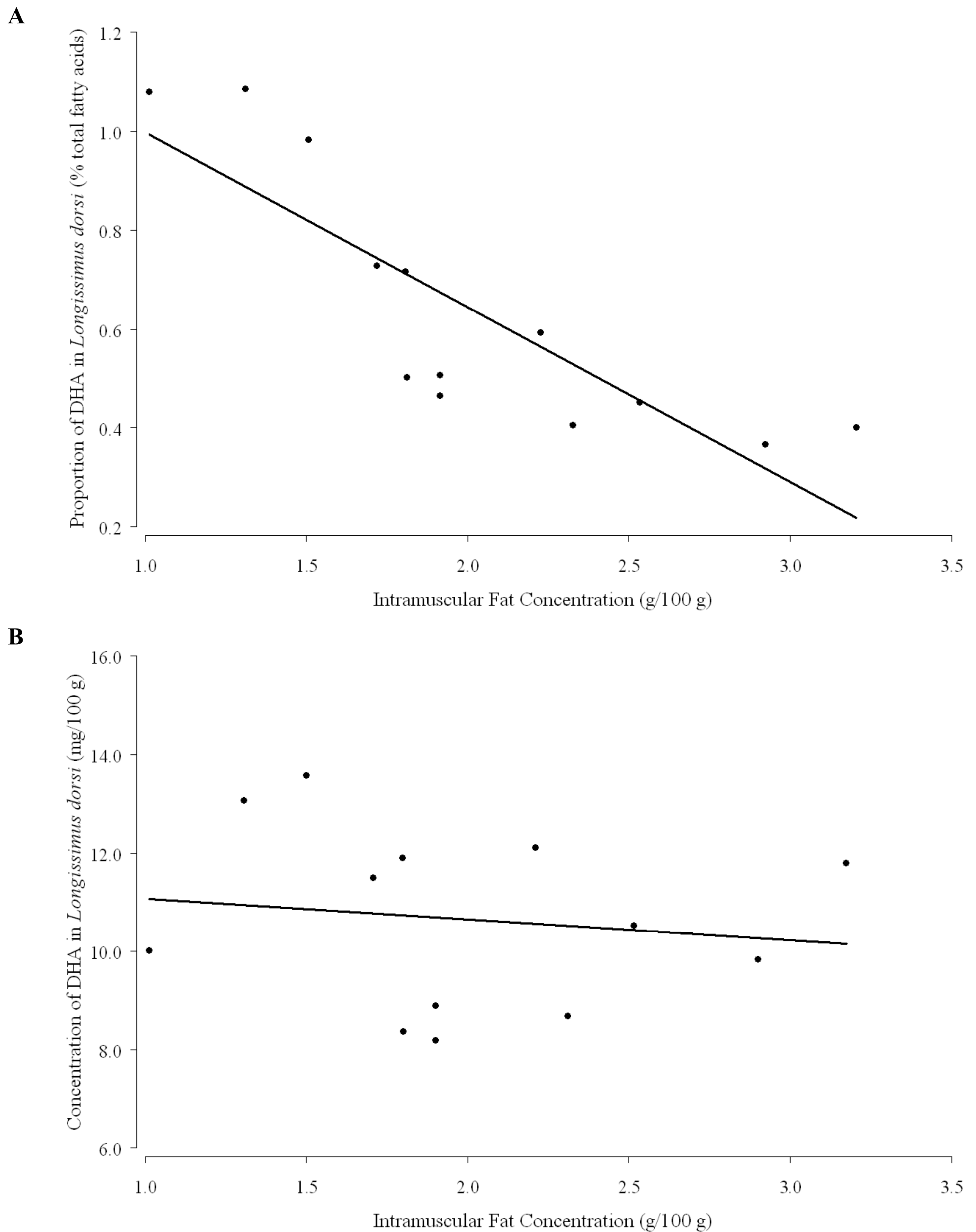


Figure 15. Relationship between intramuscular fat concentration (g/100 g) and *Longissimus dorsi* concentration of DHA estimated as either A) % of total fatty acids ($r^2 = 0.84$) or B) mg/100 g ($r^2 = 0.143$) in pigs fed approximately 17.2 mg/kg liveweight DHA as fish meal. DHA concentration (mg/100 g) estimated from total lipid concentration in *L. dorsi* (g/100 g) and % DHA in total lipid using a LCF of 0.918. Data adapted from: Hertzman et al. (1988).

5.2 Manipulating LCn-3PUFA in meat by feeding linseed

5.2.1 Cattle

The effect of incorporating linseed into beef production diets has been reviewed previously (Raes et al., 2004) and is summarised in Table 10. A number of studies highlight important aspects of metabolism of ALA from linseed oil. The concentration of ALA, but not EPA or DHA, in *L. thoracis* of Holstein-Friesian and Welsh Black steers was higher when animals were fed a basal diet of perennial ryegrass silage, barley and sugarbeet pulp with added linseed oil (51.4% ALA) for 90 days compared with animals fed the basal diet with the addition of Megalac (Choi et al., 2000). The increase in ALA in muscle was similar for both breeds, however, the concentration of EPA was not higher following supplementation with linseed highlighting the conversion of ALA to LCn-3PUFA is limited in some animals (Figure 16).

The concentration of EPA (15.0 vs 10.0 mg/100 g) in the *L. thoracis* of Charolais steers was higher, however, when steers were fed linseed in addition to a basal diet of silage, barley and sugarbeet pulp for 120 days compared with animals fed the basal diet with the addition of Megalac (Scollan et al., 2001). The increased response in EPA could be attributed to the longer time of feeding the supplement, or that the concentration of ALA in the diet was higher than previous studies (Choi et al., 2000).

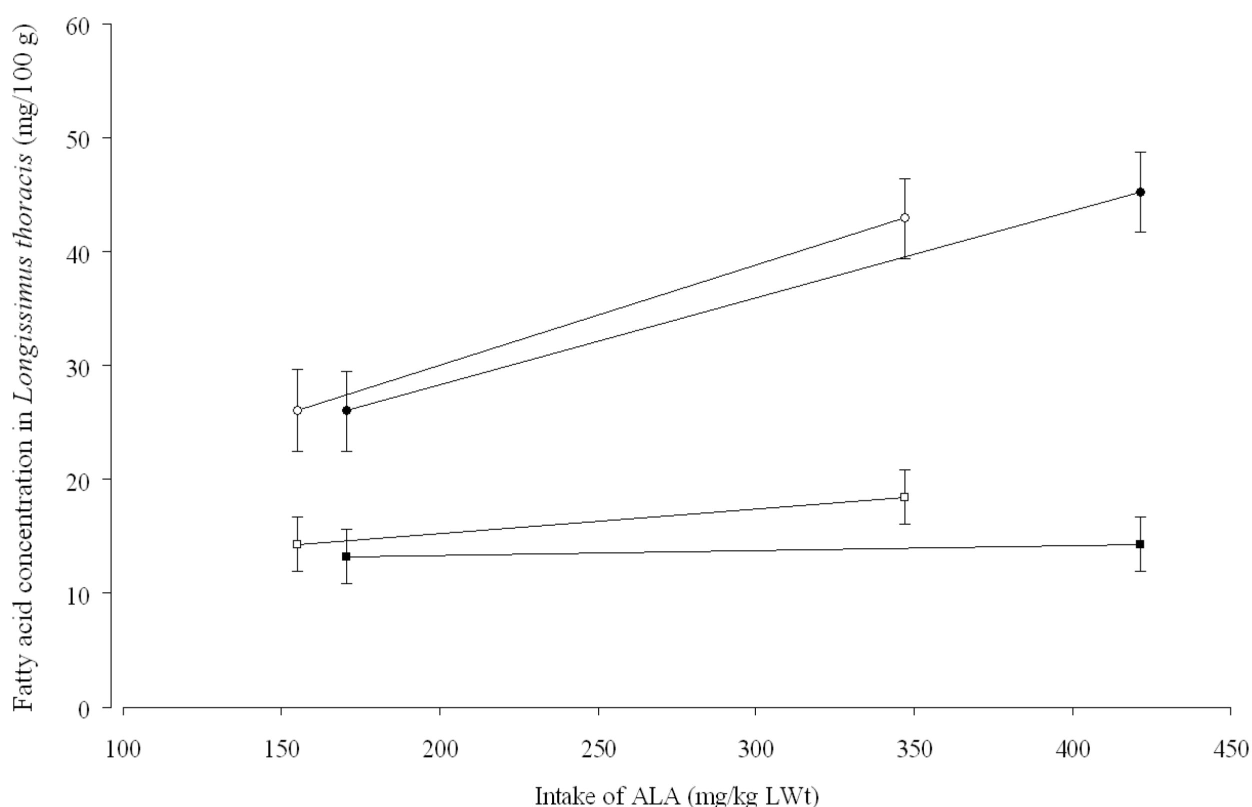


Figure 16. Change in concentrations of ALA or EPA in *Longissimus dorsi* of either Holstein-Friesian (ALA = ●, EPA = ■) or Welsh Black (ALA = ○, EPA = □) steers fed increasing doses of ALA from linseed oil. Fatty acid intake estimated from published intake of ALA per day with average body weight. Data adapted from: Choi et al. (2000).

Table 10. Omega-3 concentrations in beef following supplementation with feed sources high in α-linolenic acid (ALA, C18:3n-3).

Study	Breed	Basal Diet	Intervention	Muscle Type	Total Fat (g/100 g)	Fatty Acid Concentration (mg/100 g beef)[1] Total	Palmitic	ALA	EPA	DPA	DHA	LCn-3PUFA	n-6:n-3 Ratio
(Choi et al., 2000)	Holstein-Friesian	Perennial ryegrass silage	Megalac[2] control	*Longissimus thoracis*	-	3808	1043.9	26.03	13.21	23.06	2.65	38.92	3.43
			Linseed		-	4998	1243.9	45.29	14.23	23.32	3.23	40.78	1.98
	Welsh Blacks	Perennial ryegrass silage	Megalac control	*L. thoracis*	-	3094	837.2	26.06	14.33	22.5	2.77	39.60	2.71
			Linseed		-	2809	701.5	42.93	18.44	23.1	3.2	44.74	1.57
(Scollan et al., 2001)	Charolais	Barley + SBP[3] silage	Megalac	*L. thoracis*	-	3359	953	19.5	10	19	2.5	31.50	2.00
			Linseed		-	3618	916	38	15	20	2.7	37.70	1.19
(Kronberg et al., 2006)[4]	Hereford	Grass	Corn, field pea	*Longissimus dorsi*	-	275	35	3	7	15	2	24.0	3.59
			Peas + linseed		-	321	42	12*	10*	20*	3	33.0	2.49*
	Angus	Pasture, oats	Corn	*L. dorsi*	-	192	32.4	3	2.9	7.4	1	11.3	5.07
			Corn + linseed		-	194	29.9	13*	5.6*	8.9*	1.2*	15.7	2.66*
(Mach et al., 2006)[5]	Holstein	Maize meal	Canola 5%	*Longissimus*	2.92	1922	444	7.0	1.54	0.00	-	-	26.25
			Canola 11%		2.67	1733	380	6.7	1.90	0.00	-	-	26.46
			Linseed 5%	*Longissimus*	2.95	2030	478	15.0	2.94	0.00	-	-	15.60
			Linseed 11%		2.63	1903	421	35.5	6.30	0.00	-	-	6.31

[1]Palmitic = Palmitic acid (C16:0), ALA = α-linolenic acid (C18:3n-3), EPA = eicosapentaenoic acid (C20:5n-3), DPA = docosahexaenoic acid (C22:5n-3), DHA = docosahexaenoic acid (C22:6n-3). Fatty acids analysed in total lipid unless otherwise indicated.

[2]Megalac = Palm oil supplement high in C16:0.

[3]SBP = Sugar beet pulp.

[4]Phospholipid fatty acid fraction only.

[5]Significant differences between treatment groups not indicated. n-6:n-3 ratio calculated from % fatty acid data.

Significance for differences within study: $^{*}p < 0.05$. p-value not always indicated for total LCn-3PUFA or n-6:n3 PUFA ratio.

5.3 Manipulating omega-3 in beef by changing feed type or forage sources

Meat from animals fed grain is generally higher in total lipid concentration than animals fed forage and can be labelled as being more desirable to consumers from a flavour perspective in some markets. Individual preference for forage versus grain-fed beef is influenced by a number of factors, however, including the health attributes of the meat.

The concentrations of LCn-3PUFA in beef, lamb and pork (mg/100g edible or lean tissue) are generally higher when animals are fed forage diets compared with concentrate diets (for example, see Raes et al., 2004; McGee, 2005; Scollan et al., 2006). A number of factors contribute to these higher concentrations of LCn-3PUFA in meat, including:

- Forage-based diets are higher in ALA, which is the precursor for metabolism to LCn-3PUFA, while grain-based diets are higher in LA.
- Animals fed grain have a greater rate of *de novo* fatty acid synthesis and are fatter and, hence, have lower proportions of LCn-3PUFA to SFA.
- Ruminants fed grain have lower rumen pH. The biohydrogenation of the omega-3 fatty acid ALA may be higher than the omega-6 fatty acid LA, therefore, leaving less omega-3 available for metabolism to LCn-3PUFA in meat.

The following sections examine the influence of feed and forage types on LCn-3PUFA concentrations of meat and consider factors including feed type (grain versus forage), forage type or breed of animal. Most research in the area of feed type has been conducted with beef production. Information on the effects of feed type and forage type on LCn-3PUFA concentrations in beef has come from three main sources, including surveys of different feeding systems, experiments examining forage versus grain feeding and experiments examining feeding of different forage types. These studies have been reviewed in detail previously (for example, see Wood and Enser, 1997; Wood et al., 1999), so the main influences of feed and forage type are outlined here, with specific reference to forage type and silage. Different muscle types are also briefly reviewed, along with published data used in the National Nutrition Database (NUTTAB, FSANZ, 2006).

5.3.1 Surveys of feeding systems

The concentration of LCn-3PUFA is lower and the ratio of n-6:n-3 is higher in meat from cattle in feedlot production systems compared with pasture-based (forage) production systems (Table 11). Individual differences between studies are largely dependent on the total fat concentration in the meat examined. Few studies have surveyed LCn-3PUFA concentrations in beef from cattle in Australia.

5.3.2 Forage versus grain feeding

Many studies examining the effects of diet on LCn-3PUFA and LCn-6PUFA are confounded by differences in carcase weight and/or fatness at the time of slaughter (French et al., 2000). For example, the concentration (mg/100 g) of LCn-3PUFA in the *Semitendinosus* muscle (eye round) of Brangus x Hereford x Angus steers was not significantly different when animals were fed grain compared with forage (Table 12, Williams et al., 1983; Marmer et al., 1984), however, those animals finished on grain were fed the experimental diets for a shorter time and were heavier (482 vs 442 kg) and fatter (4.76% vs 3.08% total lipid) than animals fed fresh forage. In general, the concentration of LCn-3PUFA in meat is lower when animals are finished on grain or concentrate diets compared with forage (Table 12).

Table 11. Fatty acid concentrations (mg/100 g edible portion) in beef from surveys of different feeding systems.

Study/Country	Breed	Sex/Age	Diet/Feeding System	Muscle Type	Total Fat (g/100 g)	Fatty Acid Concentration (mg/100 g beef)[1] Total	Palmitic	ALA	EPA	DPA	DHA	LCn-3 PUFA	n6:n3 Ratio
(Enser et al., 1998)	Hereford x	Steers 12 mo	Pasture	*Longissimus*	-	2860	698	32.7	12.5	19.3	1.87	33.67	1.32
UK	Friesian	Bulls 12 mo	Barley soybean meal	*dorsi*	-	2066†	488†	10.4‡	3.79‡	8.7‡	0.92‡	13.41	9.20
	Hereford x	Steers 12 mo	Pasture	*Gluteobiceps*	-	3389	684	48.5	24	30.9	4.07	58.97	1.36
	Friesian	Bulls 12 mo	Barley soybean meal		-	2010‡	401‡	10.9‡	4.78‡	12.6‡	1.48‡	18.86	10.35
(Itoh et al., 1999)[4] New Zealand	Angus and Simmental	Steers, Old	Annual Ryegrass + red clover	*Longissimus*	9.93		293.6[b]	83.90[a]	35.01[b]	41.53[b]	BL	76.55	-
			Perennial ryegrass + white clover	*thoracis*[2]	10.55		275.7[c]	80.89[b]	40.13[a]	41.95[a]	BL	82.08	-
			Maize (70%), pasture silage (30%)		10.47		327.3[a]	13.48[c]	4.01[c]	13.76[c]	BL	17.77	-
(Rule et al.,	Beef Breed	Steer	Grass-fed	*L. dorsi*	-	1070	237.5	15.84	6.63	7.60	0.96	15.19	1.95
2002)[5,8]			Feedlot		-	2880	743.0	6.34	3.74	7.49	1.15	12.38	6.38†
USA													
	Beef Breed	Steer	Grass-fed	*Semitendinosus*	-	821	179.8	14.12	10.02	10.10	1.15	21.26	2.22
			Feedlot		-	2290	572.5	6.87	5.04	8.47	1.37	14.89	4.89†
(Raes et al., 2003a)	Limousin	Unknown	Pasture finished	*Longissimus*	-	3710	926	30.2	14.8	20.1	2.76	37.66	2.73
Belgium	Irish	Unknown	Concentrate finished	*lumborum*	-	1266‡	266‡	16.1	5.98‡	10.6‡	0.95‡	17.53	4.91‡
(Purchas et al.,	Angus cross	Steers 17 mo	Pasture - Age-matched	*L. lumborum* +	3.58	-	775.5	11.71	7.89	16.44	0.99	25.32	1.14[b]
2005)[6,8]			Pasture - Wt-matched	*Triceps brachii*	2.71	-	574.5	34.99	19.92	23.91	3.49	47.32	1.21[b]
New Zealand and USA			Corn (33%) barley (33%) potato (33%)		2.83		608.4	38.32	18.69	22.59	2.34	43.62	4.58[a]
(Alfaia et al.,	Alentejana	Bulls 20 mo	Grass-concentrate finish	*L. thoracis*	1.48	922	182.6	2.95	1.29	4.98	1.57	7.84	13.7
2006)[7,8]			Concentrate pellets		1.52	1160	237.8	3.48	0.81	4.64	0.35	5.80	20.2‡
Portugal													

Table 11. Continued.

Study/Country	Breed	Sex/Age	Diet/Feeding System	Muscle Type	Total Fat (g/100 g)	Fatty Acid Concentration (mg/100 g beef)[1] Total	Palmitic	ALA	EPA	DPA	DHA	LCn-3 PUFA	n6:n3 Ratio
(Ponnampalam et al., 2006) Australia	British	Unknown 18 mo	Pasture finished	Rump	-	2792	588^{b}	48.9^{a}	39.8^{a}	57.4^{a}	7.7	104.9^{a}	2.16^{b}
			80 days feedlot		-	2736	565^{b}	16.8^{b}	23.3^{b}	46.9^{b}	7.9	78.10^{b}	3.65^{a}
			150-200 days feedlot		-	4824	1084^{a}	21.4^{b}	20.9^{b}	47.6^{b}	6.8	75.30^{b}	4.13^{a}
	British	Unknown 18 mo	Pasture finished	Striploin	-	2120	508^{b}	32.4^{a}	24.5^{a}	36.5^{a}	4.2	65.2^{a}	1.96^{b}
			80 days feedlot		-	1538	358^{b}	10.3^{b}	11.1^{b}	23.6^{c}	3.7	38.4^{c}	3.57^{a}
			150-200 days feedlot		-	3614	899^{a}	14.9^{b}	13.1^{b}	31.6^{b}	3.7	48.4^{b}	4.01^{a}
(Razminowicz et al., 2006) Switzerland	Unknown	Heifers	Pasture	*L. dorsi*[3]	1.57	1191	-	22.9	9.6	14.3	1.7	25.6	1.7
	Unknown	Heifers	Concentrate		1.73	1355	-	16.1	7.1	11.4	1.5	20.0	3.5

[1]Palmitic = Palmitic acid (C16:0), ALA = α-linolenic acid (C18:3n-3), EPA = eicosapentaenoic acid (C20:5n-3), DPA = docosahexaenoic acid (C22:5n-3), DHA = docosahexaenoic acid (C22:6n-3). Fatty acids analysed in total lipid unless otherwise indicated. BL = below limit of detection

[2]Triglyceride + polar lipids

[3]Phospholipid + neutral lipids

[4]Fatty acid concentration (mg/100 g meat) calculated from total lipid, % lipid in each lipid class (triglyceride or polar lipid), a lipid conversion factor for each lipid class and % fatty acid.

[5]Fatty acid concentration (mg/100 g meat) calculated from total fatty acid concentration and % of each fatty acid. Therefore, no significant differences are reported for concentrations.

[6]Pasture age-matched or weight-matched to grain fed group. Concentration of fatty acid (mg/100 g edible portion) calculated from % fatty acid using total lipid content and a lipid conversion factor.

[7]Intramuscular fat extracted prior to fatty acid analysis. Pasture fed cattle were from New Zealand and concentrate fed cattle were from USA.

[8]Significant differences were reported in original publications for % fatty acids and ratio of n-6:n-3.

[9]Anatomical name of muscle not indicated in original publication.

Significance for differences between treatment groups within study: $^{*}p < 0.05$; $^{\dagger}p < 0.01$; $^{\ddagger}p < 0.001$. p-value not always indicated for total LCn-3PUFA or n-6:n3 PUFA ratio.

Table 12. Fatty acid concentrations in beef from cattle fed forage or grain-based diets under semi-controlled conditions.

Study/Country	Breed	Sex/Age	Diet	Feeding time	Muscle Type	Total Fat (g/100 g)	Fatty Acid Concentration (mg/100 g beef)[1] Total	Palmitic	ALA	EPA	DPA	DHA	LCn-3PUFA	n6:n3 Ratio
(Williams et al., 1983; Marmer et al., 1984)[5] / USA	British	Steer 348 kg	Winter Wheat Forage	202 days (older)	*Semitendinosus*[2]	3.08	1965	498.0	24.00	14.00[a]	23.00[a]	2.00[a]	39.00	2.26
			Maize	129 days (younger)	*Semitendinosus*	4.76	4105	996.0	11.00	11.00[a]	22.00[a]	3.00[a]	36.00	5.28
(Duckett et al., 1993)[6] / USA	Angus x Hereford	Steer 16 mo	Grass fed	16 months	*Longissimus*	2.52	-	574.6	21.51	3.93	5.09	24.52	33.54	4.48
			Concentrate	196 days		11.65	-	2904.7	6.42	0.00	4.28	7.49	11.76	14.77
(Enser et al., 1998) UK	Hereford x Friesian	Steers	Pasture	Lifetime	*L. dorsi*	-	2860	698.0	32.7	12.50	19.30	1.87	33.67	1.32
		Bulls	Barley SBM	Lifetime		-	2066†	488.0†	10.4‡	3.79‡	8.70‡	0.92‡	13.41	9.20
(Nuernberg et al., 2002)[7] Germany	German Simmental	Bull 6 mo	Grass + silage	12 months	*Longissimus*	1.98	-	434.4	43.62	21.08	27.26	7.27	55.62	1.3
			67% Concentrate			1.70	-	323.0	4.68	2.50	12.48	2.34	17.32	13.7*
	German Holstein	Steers 6 mo	Grass + silage	12 months	*Longissimus*	3.94	-	1012.7	32.55	14.47	21.70	7.23	43.40	1.3
			67% Concentrate			4.54	-	1142.0	8.34	2.50	10.84	1.67	15.00	13.7
(Steen et al., 2002)[8] Northern Ireland	Charolais	510 kg	Pasture	4 months		-	-	-	103.4	37.60	45.12	1.88	84.60	1.50
	Steers	575 kg	80% barley	5 months		2.43	-	-	29.3	10.67	12.80	0.53	24.00	8.00

Table 12. Continued.

Study/Country	Breed	Sex/Age	Diet	Feeding time	Muscle Type	Total Fat (g/100 g)	Fatty Acid Concentration (mg/100 g beef)[1] Total	Palmitic	ALA	EPA	DPA	DHA	LCn-3PUFA	n6:n3 Ratio
(Dannenberger et	German	Bull 6 mo	Pasture / silage	12 months	*Longissimus*[3]	2.3	2459.1	553.8	54.59	20.26	28.48	6.75	55.49	1.97
al., 2004; 2006;	Holstein		Barley			2.6	2722.5	706.0	10.76	4.38	10.42	2.45	17.25	6.18*
2007; Nuernberg et														
al., 2005)	German	Bull 6 mo	Pasture / silage	12 months	*Longissimus*[4]	-	529.03	72.6	28.98	18.37	-	2.81	-	2.11
Germany	Simmental		Barley			-	743.04	108.8	9.4	5.02	-	2.12	-	9.48*

[1]Palmitic = Palmitic acid (C16:0), ALA = α-linolenic acid (C18:3n-3), EPA = eicosapentaenoic acid (C20:5n-3), DPA = docosahexaenoic acid (C22:5n-3), DHA = docosahexaenoic acid (C22:6n-3). Fatty acids analysed in total lipid unless otherwise indicated.
[2]Neutral lipids + polar Lipids.
[3]Phospholipid + triglyceride.
[4]Phospholipid.
[5]Significant differences only available for EPA, DPA and DHA as they were found only in the phospholipid fraction and significant differences were reported in the manuscript.
[6]Concentrations of fatty acids (mg/100) calculated from concentration of neutral or polar lipid (g/100 g) with corresponding lipid conversion factor and % fatty acid in each lipid class. Ratio of n-6:n-3 fatty acids calculated from presented fatty acids.
[7]Data presented as % total fatty acids so significant differences in concentration not reported. Significant differences for n-6:n-3 ratio reported in original paper.
[8]Fatty acid concentrations estimated from total lipid, total omega-3 proportions and % of each omega-3 within total omega-3. Basal diet consisted of medium digestibility Perennial ryegrass silage. Barley diet consisted of rolled barley + soybean meal.
Fatty acids measured in total lipid fractions acids unless otherwise specified.
Significance for differences between treatment groups within study: $^{*}p < 0.05$; $^{\dagger}p < 0.01$; $^{\ddagger}p < 0.001$. p-value not always indicated for total LCn-3PUFA or n-6:n3 PUFA ratio. p-value also not given if data was reported as % fatty acid and converted to concentration (mg/100 g) in the current table.

The concentration of LCn-3PUFA decreases and the ratio of n-6:n-3 fatty acids increases with an increasing length of time of grain feeding (May et al., 1992; Duckett et al., 1993), until reaching a plateau (Figure 17A). The amount of polar lipid as a proportion of total lipid in muscle also decreases with an increased length of time of grain feeding (Figure 17B, Duckett et al., 1993). As most LCn-3PUFA is found in the polar lipid fraction of total lipid (mostly in phospholipid), as animals get fatter with an increased length of time of grain feeding, there is a larger increase in the concentration of other fatty acids, particularly, C18:0 and C18:1n-9 in total muscle lipid compared with LCn-3PUFA.

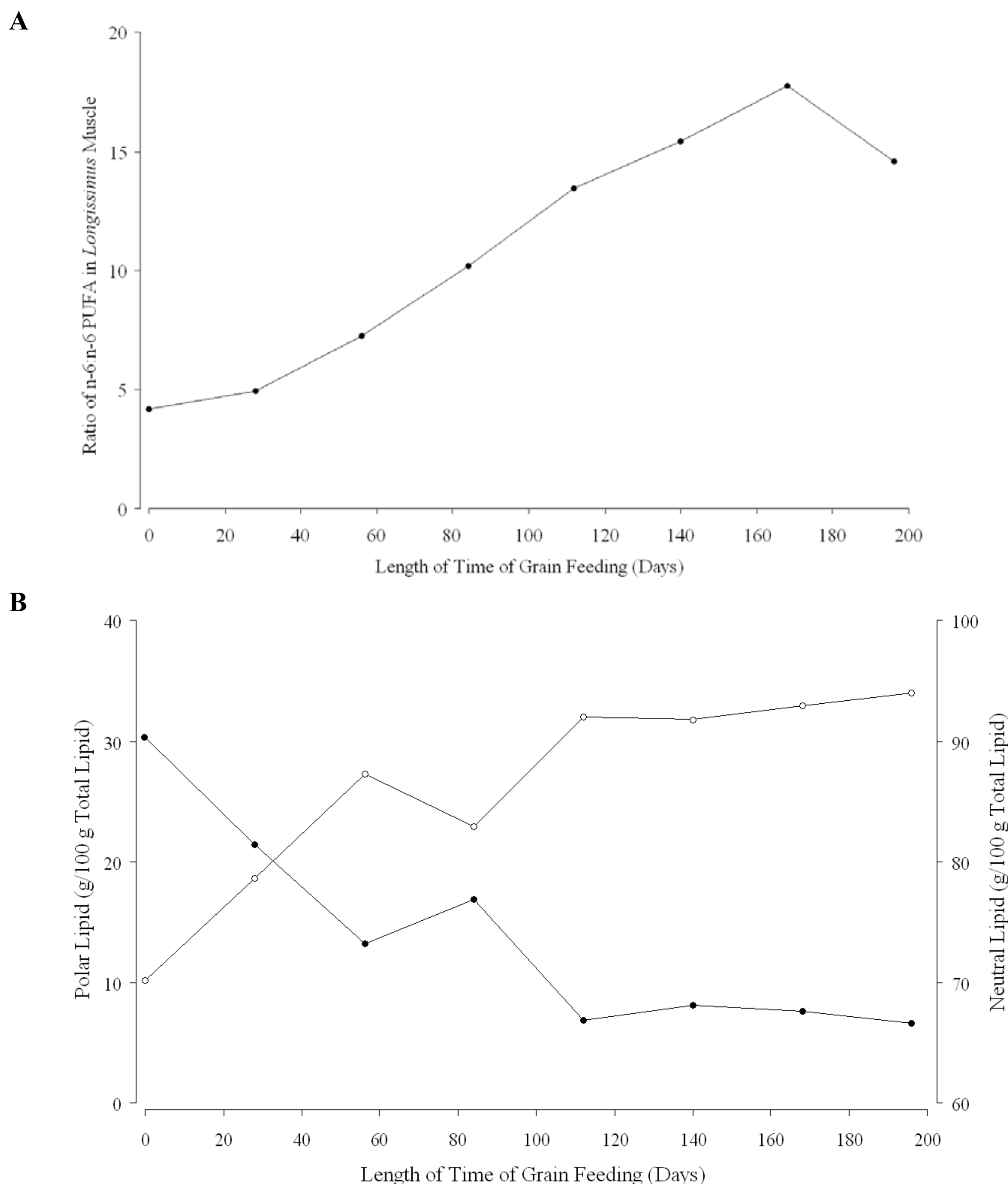


Figure 17. The ratio of n-6:n-3 PUFA (A) and the proportion of polar (●) or neutral (○) lipid in total lipid (B) in *Longissimus* muscle of Angus x Hereford steers increases with increasing length of time of grain feeding. Data adapted from: Duckett et al. (1993).

5.3.3 Feeding different types of forages

The concentration of ALA and LA in feedstuffs is influenced by the type of feed (including forage versus concentrates or grains), the type of forage (for example, perennial ryegrass versus red clover) and other factors including whether forage is consumed as fresh material compared with hay or silage. The concentration of LCn-3PUFA is lower and, the ratio of n-6:n-3 is higher, when animals consume grain, concentrates or maize compared with pasture or forage diets (Table 13). A summary of studies manipulating the concentration of fatty acids in different types of forage is summarised further in Section 7.

Similar to studies with linseed oil described in Section 5.2, the concentration of ALA, EPA and DPAn-3 in the *L. thoracis* of bulls was higher when animals were fed triticale or perennial ryegrass silage compared with maize silage when animals were slaughtered at the same carcase weight (Raes et al., 2003b). The concentration of DHA was not, however, altered by dietary manipulation, providing evidence that the conversion of ALA to LCn-3PUFA can be limited in some feeding situations. The concentration of ALA, EPA and DPA was related to the amount of ALA included in the ration of bulls (Figure 18).

5.3.4 Concentration of LCn-3PUFA in meat in the National Nutrition Database (NUTTAB)

A summary of the nutrient composition of food sources in Australia, including the concentration of LCn-3PUFA in beef and lamb, is published by Food Standards Australia and New Zealand (FSANZ) in the National Nutrition Database (NUTTAB, FSANZ, 2006). This database contains a compilation of information from a variety of surveys and research studies in Australia and values are expressed as either proportion of total fatty acids or g/100 g edible portion. The concentrations of fatty acids reported in the database are generally average values and do not take into account variation in the diet of animals prior to the collection and assessment of meat (Table 14).

Table 13. Fatty acid concentrations in beef from cattle fed different forage types under controlled conditions.

Study/Country	Breed	Sex/Age	Diet	Feeding Time	Muscle Type	Total Fat (g/100 g)	Fatty Acid Concentration (mg/100 g beef)[1] Total	Palmitic	ALA	EPA	DPA	DHA	LCn-3PUFA	n6:n3 Ratio
(French et al.,	Continental	Steer	Grass	-	*Longissimus*	4.36	-	914.2	45.23	9.21	-	-	9.21	2.33[b]
2000)[3]	x-bred	504 kg	Silage + 4kg conc	-		4.08	-	994.4	26.59	7.49	-	-	7.49	3.61[a]
Ireland			Silage + 8kg conc	-		3.41	-	857.7	22.54	3.76	-	-	3.76	4.15[a]
(Laborde et al.,	British	Steer	Lucerne silage	-	*Longissimus*	5.69	-	1405.1	24.55	10.45	17.76	3.66	31.86	3.10
2002)[3]		267 d old	Maize	-		5.89	-	1470.7	18.92	7.57	14.60	2.70	24.87	4.53‡
Canada														
(Raes et al., 2003b)	Belgian	Bulls 650	Pasture silage	-	*Longissimus*	-	670	128.0	27.9[a]	8.8[a]	12.7[a]	1.0	22.50	2.49[c]
Belgium	Blue	kg	Wheat/Maize	-		-	674	134.0	12.7[b]	6.8[b]	12.9[a]	1.29	20.99	5.08[a]
			Maize silage	-		-	651	129.0	17.1[b]	5.6[b]	10.1[b]	0.8	16.50	4.57[b]
(Varela et al., 2004)	Rubia	Steer 30	Pasture	90 days	*Longissimus*	-	2607.3	456.7	61.0	41.39	-	7.88	49.27	1.82
Spain	Gallega	mo	Maize silage			-	2824.2	581.3	40.2	38.70	-	11.35	50.04	2.85
(Noci et al., 2005)	European	Heifers	Barley + SBM	-	*L. dorsi*	2.7	-	653.4	24.79	-	-	-	-	5.19[b]
Ireland	x-bred	426 kg	Silage 38%DM	-		3.8	-	913.6	19.78	-	-	-	-	7.73[a]
			Silage 52%DM	-		2.91	-	713.8	15.44	-	-	-	-	7.90[a]
(Noci et al., 2007)	Friesian	Steer	Concentrate[4]	-	*L.dorsi*	-	6521	1519	28.16	8.43	16.3	3.16	27.89	3.89
Ireland		565 kg	Wilted silage	-		-	7078	1708	31.13	7.69	16.3	2.09	26.08	3.72

Table 13. Continued.

Study/Country	Breed	Sex/Age	Diet	Feeding Time	Muscle Type	Total Fat (g/100 g)	Fatty Acid Concentration (mg/100 g beef)[1]							n6:n3 Ratio
							Total	Palmitic	ALA	EPA	DPA	DHA	LCn-3PUFA	
(Dannenberger et	Holstein	Bull 6 mo	Pasture / silage	12 mo	*Longissimus*	2.30	-	490.9	35.26	12.25	16.89	3.17	32.30	1.94
al., 2004; 2006;			Concentrate			2.67	-	615.2	8.33	3.43	8.82	2.21	14.46	6.49
2007; Nuernberg et														
al., 2005)	Simmental	Bull 6 mo	Pasture / silage	12 mo	*Longissimus*	1.51	-	312.7	30.77	13.03	18.31	2.34	33.68	2.04
Germany			Concentrate			2.61*	-	581.3	11.02	1.92	6.95	1.20	10.06	8.34*
(Eriksson and	Hereford x	Steer 28 mo	Ryegrass pasture	-	*L. dorsi*	2.29	2004	61.52	34.87	12.22	18.04	1.60	31.86	1.26
Pickova, 2007)[2]	Limousin x	23 mo	Ryegrass silage	-		2.24	1813	558.40	20.49	9.43	13.78	1.63	24.84	1.20
Sweden	Charolais	23 mo	Silage + wheat	-		2.23	2374	733.57	21.13	8.31	13.53	1.42	23.27	1.50

[1]Palmitic = Palmitic acid (C16:0), ALA = α-linolenic acid (C18:3n-3), EPA = eicosapentaenoic acid (C20:5n-3), DPA = docosahexaenoic acid (C22:5n-3), DHA = docosahexaenoic acid (C22:6n-3).
[2]Phospholipid fatty acid fraction only. Significant differences between groups not reported as concentrations were calculated from total fatty acid concentration and fatty acid %.
[3]Fatty acid concentrations calculated from % fatty acid data using total lipid content and a lipid conversion factor of 0.918. Concentrate diet consisted of barley plus sugar beet pulp. Silage was *Lolium perenne*. Grass species was not indicated.
[4]Concentrate fed at 4.7 kg/hd. Diet also contained unwilted perennial ryegrass silage.
[5]Fatty acids calculated from fatty acid concentrations in phospholipid + triglycerides. Significant differences not reported for total fatty acid concentrations as values were reported separately for each fraction and significant differences were not indicated for total lipid.
Fatty acids measured as total fatty acids unless otherwise specified.
Significance for differences between treatment groups within study: $^{*}p < 0.05$; $^{\dagger}p < 0.01$; $^{\ddagger}p < 0.001$.

Table 14. Omega-3 concentrations (mg/100 g) published for beef in Australia and used to construct the National Nutrition Database NUTTAB.

Study	Basal Diet	Muscle Type	Total Fat (g/100 g)	Fatty Acid Concentration (mg/100 g beef)[1] Total	Palmitic	ALA	EPA	DPA	DHA	LCn-3PUFA	n-6:n-3
(Sinclair et al., 1982)	Unspecified	Rump	2.48	-	-	27.32	13.66	22.77	2.28	38.70	-
(Mann et al., 1995)	Unspecified	Rump	2.7	1904	456.0	29.00	19.00	22.00	3.00	44.00	1.8
(Mann et al., 2003)		Sirloin	2.6	1821	475.0	18.00	11.00	15.00	2.00	28.00	2.8
(Li et al., 1998)	Unspecified	T-bone	1.4	-	-	23.13	21.85	32.13	3.86	57.83	1.6
(Droulez et al., 2006)[2]	Unspecified	Rump		3200	697.6	60.8	32.0	54.4	6.4	92.80	1.90
		Scoth fillet + T-bone		4500	1039.5	49.5	31.5	49.5	9.0	90.00	2.45
		Topside		1900	431.3	9.5	13.3	28.5	3.8	45.60	3.34
(Howe et al., 2006) (Howe et al., 2007)	Unspecified			-	-	-	45	71	13	129.00	-
(FSANZ, 2006)		Median Beef		-	-	45	29.5	48.0	7.0	84.50	-
(Ponnampalam et al., 2006)[3]	Pasture finished	Rump		2792	588.0	48.9	39.8	57.4	7.7	104.90	2.16
	80 days feedlot			2736	565.0	16.8	23.3	46.9	7.9	78.10	3.65
	150-200 days feedlot			4824	1084.0	21.4	20.9	47.6	6.8	75.30	4.13
	Pasture finished	Striploin		2120	508.0	32.4	24.5	36.5	4.2	65.20	1.96
	80 days feedlot			1538	358.0	10.3	11.1	23.6	3.7	38.40	3.57
	150-200 days feedlot			3614	899.0	14.9	13.1	31.6	3.7	48.40	4.01

[1]Palmitic = Palmitic acid (C16:0), ALA = α-linolenic acid (C18:3n-3), EPA = eicosapentaenoic acid (C20:5n-3), DPA = docosahexaenoic acid (C22:5n-3), DHA = docosahexaenoic acid (C22:6n-3).
[2]Ratio of n-6:n-3 calculated from data presented. The proportion of all n-6 fatty acids were not reported.
[3]British breed cattle. All other breeds unknown.

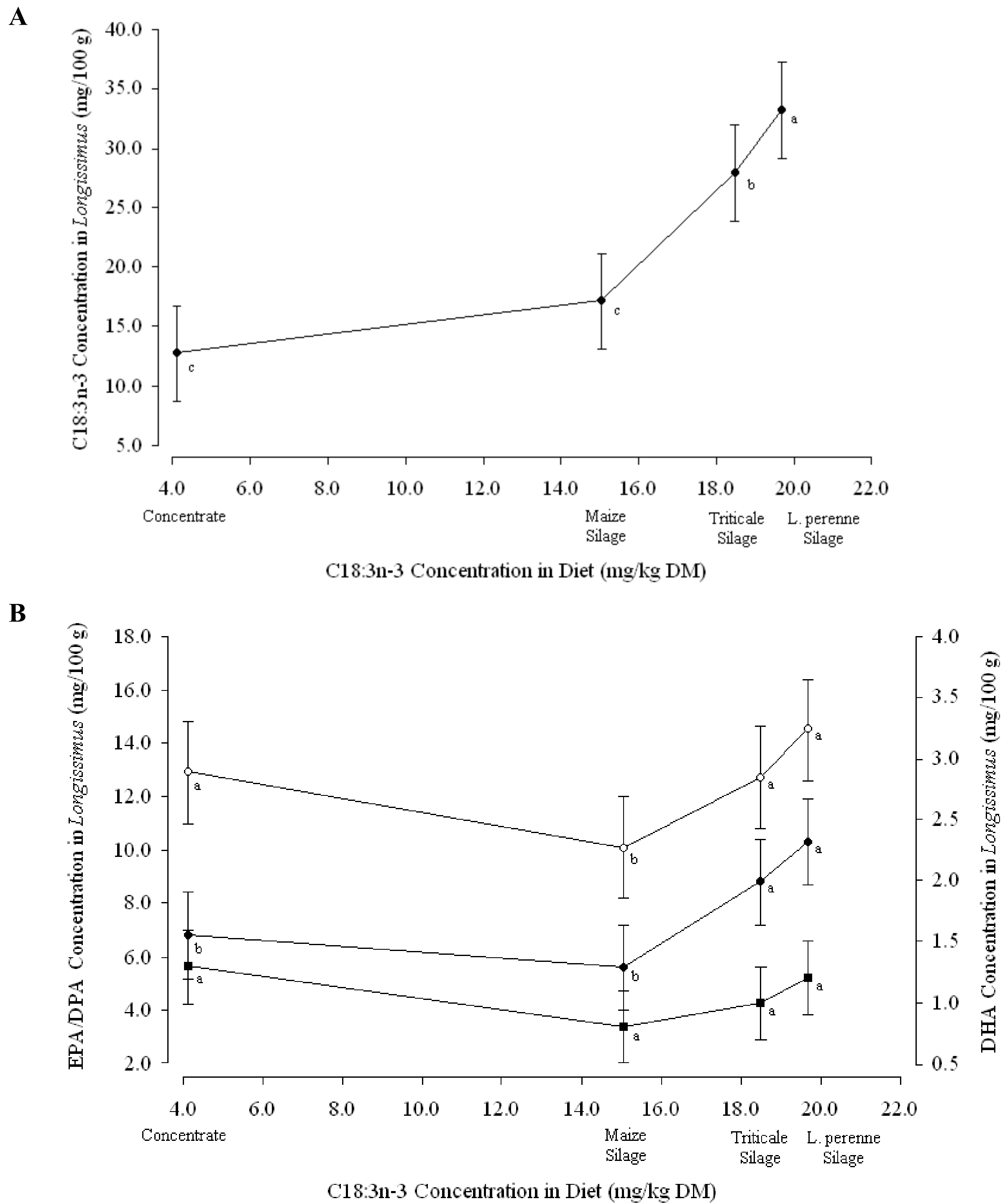


Figure 18. Concentration (mg/100 g) of (A) ALA or (B) EPA (●), DPAn-3 (○) and DHA (■) in the *Longissimus* muscle of double-muscled Belgian Blue bulls fed concentrate or silage diets varying in total concentrations of ALA (mg/kg DM). Different superscripts within the same fatty acid differ significantly ($p < 0.05$). Data adapted from: Raes et al. (2003b).

5.4 Manipulating omega-3 in lamb by changing feed type or forage sources

There is little published data available comparing LCn-3PUFA concentrations in the meat of lambs fed pasture versus concentrates in Australia. Most research examining LCn-3PUFA concentrations in lamb involves supplementation with linseed, canola, fish oil or algae (for example, see Ponnampalam et al., 2001b; Ponnampalam et al., 2002b). In a study conducted in France (Table 15) the concentration of LCn-3PUFA in the *L. thoracis* was lower when lambs were fed a diet based on barley, wheat and sugarbeet pulp compared with grass (Aurousseau et al., 2004; Aurousseau et al., 2007).

The data for concentrations of LCn-3PUFA in lamb from Australia is difficult to interpret. In one study, the authors do not present data for EPA and DHA individually (EPA + DHA are combined) and the concentration of DPAn-3 is not reported (for example, see Ponnampalam et al., 2001b). In addition, the sum of fatty acids is often greater than the total reported concentration of lipid. The authors appear to have used the sum of fatty acids to estimate total muscle lipid in some instances and, as indicated previously, lipid is not comprised entirely of fatty acids and this method of estimating total fat may lead to large errors. There is also an assumption the annual pasture fed was annual ryegrass, although this was not specified in the materials and methods (Ponnampalam et al., 2001b).

The concentration of LCn-3PUFA in the tissue of sheep is largely dependent on the concentration of ALA in feed. The concentration of ALA and EPA was significantly positively related to the concentration of ALA in silage in a series of studies conducted at the Wagga Wagga Agricultural Institute (EH Clayton, unpublished observations, Figure 19).

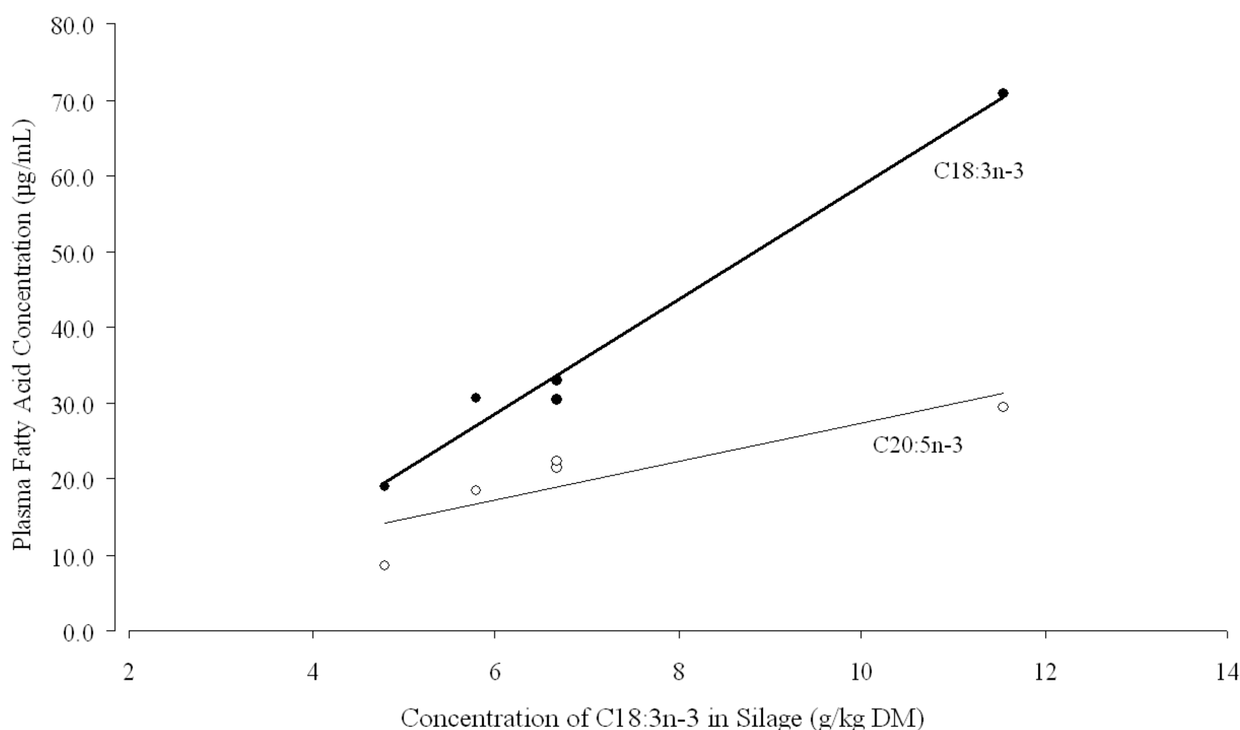


Figure 19. Correlation between the concentration of ALA in silage and the concentration of C18:3n-3 (●) or C20:5n-3 (○) in the plasma of Border Leicester x Merino ewes or Merino ewes six weeks after the commencement of feeding experimental rations. Data from EH Clayton, unpublished observations.

Table 15. Fatty acid concentrations in meat from sheep fed different diets under controlled conditions.

Study	Age	Diet	Days on Feed	Total Fat (g/100 g)	Fatty Acid Concentration (mg/100 g beef)[1]							n-6:n-3
					Total	Palmitic	ALA	EPA	DPA	DHA	LCn-3PUFA	
(Aurousseau et al., 2004)[2]	70 days	Grass-fed	93	1.58	1240	316.7	33.1	13.2	7.26	0	20.46	2.07
Longissimus thoracis		Barley wheat SBP[4]	59	2.39	1900	558.1	17.2	3.54	4.72	0	8.26	5.67
(Aurousseau et al., 2007)	100-130	Grass-fed	-	2.22	1577	314.8	40.8	27.1	37.2	9.6	73.90	1.34
Longissimus thoracis	days	Barley wheat SBP	-	2.53	1811	432.7*	22.8*	16.6*	24.8*	8.5	49.90	2.34
(Ponnampalam et al., 2012)	NI	Lucerne + Phalaris	49 days	3.03	3039	NI	61.5	NI	22.6	NI	56.1	1.84
Longissimus lumborum (LL)[3]		Annual ryegrass	49 days	2.96	2969	NI	51.8	NI	21.2	NI	51.3	2.37*

[1]Palmitic = Palmitic acid (C16:0), ALA = α-linolenic acid (C18:3n-3), EPA = eicosapentaenoic acid (C20:5n-3), DPA = docosahexaenoic acid (C22:5n-3), DHA = docosahexaenoic acid (C22:6n-3). NI = not indicated.
[2]Total fatty acid concentrations calculated from neutral lipid + phospholipid so significant differences between diets are not indicated.
[3]Concentration of DPA estimated from total LCn-3PUFA minus the concentration of EPA + DHA.
[4]SBP = Sugar beet pulp.
Significance for differences between treatment groups within study: $^{*}p < 0.05$.

6. Potential Health Effects of LCn-3PUFA in Ruminants

Most studies examining the alteration of fatty acid content of animal feeds have focussed on increasing the concentration of LCn-3PUFA in meat or milk specifically for human consumption. The effect of these diets on the health of the animal has not been studied in as much detail. Most studies examining the effects of omega-3 on aspects of animal production, other than meat or milk quality, have determined the effect of altering the fatty acid composition of feed on inflammation and reproduction. A brief summary of the reported effects of omega-3 is presented in the following section.

6.1 Inflammation

As reviewed in Section 2.2, LCn-3PUFA are precursors for the production of eicosanoids. The eicosanoids are signalling molecules associated with a number of functions in the body including inflammation (Peet and Stokes, 2005). The series-1 and series-3 PG are less inflammatory, while the series-2 PG are more inflammatory (Lands, 1992; Horrobin and Bennett, 1999). The PG, in particular, series-2 PG including $PGF_{2\alpha}$, play an important role in several aspects of reproduction, including ovulation, oestrus, embryo survival and parturition (for review, see Abayasekara and Wathes, 1999).

The main focus of research examining the effects of LCn-3PUFA and eicosanoids concerns the inhibition of $PGF_{2\alpha}$ by EPA and DHA. Although the exact mechanism by which LCn-3PUFA supplementation inhibits $PGF_{2\alpha}$ production is not fully understood (MacLaren et al., 2006), a number of pathways may be involved. The actions of LCn-3PUFA include competitive inhibition of the action of delta-6 (Δ-6) desaturase during the synthesis of ARA, competitive exclusion of ARA incorporation into phospholipid membranes to reduce the availability for further metabolism and competitive inhibition of the action of prostaglandin H synthase (PGHS) in the metabolism of ARA to prostaglandin H_2 (for review, see Cheng et al., 2001; Cheng et al., 2005a; Caldari-Torres et al., 2006). Dietary omega-3 can also alter expression of genes involved in PG synthesis (Coyne et al., 2008). The ratio of n-6:n-3 in ruminant diets is particularly important in determining the relative availability of the precursors for eicosanoid formation. The potential secretion of $PGF_{2\alpha}$ by the uterine endometrium following an oxytocin challenge was significantly increased when ewes were fed a diet high in the omega-6 fatty acid LA compared with the omega-3 fatty acid ALA (Figure 20, Gulliver et al., 2013a).

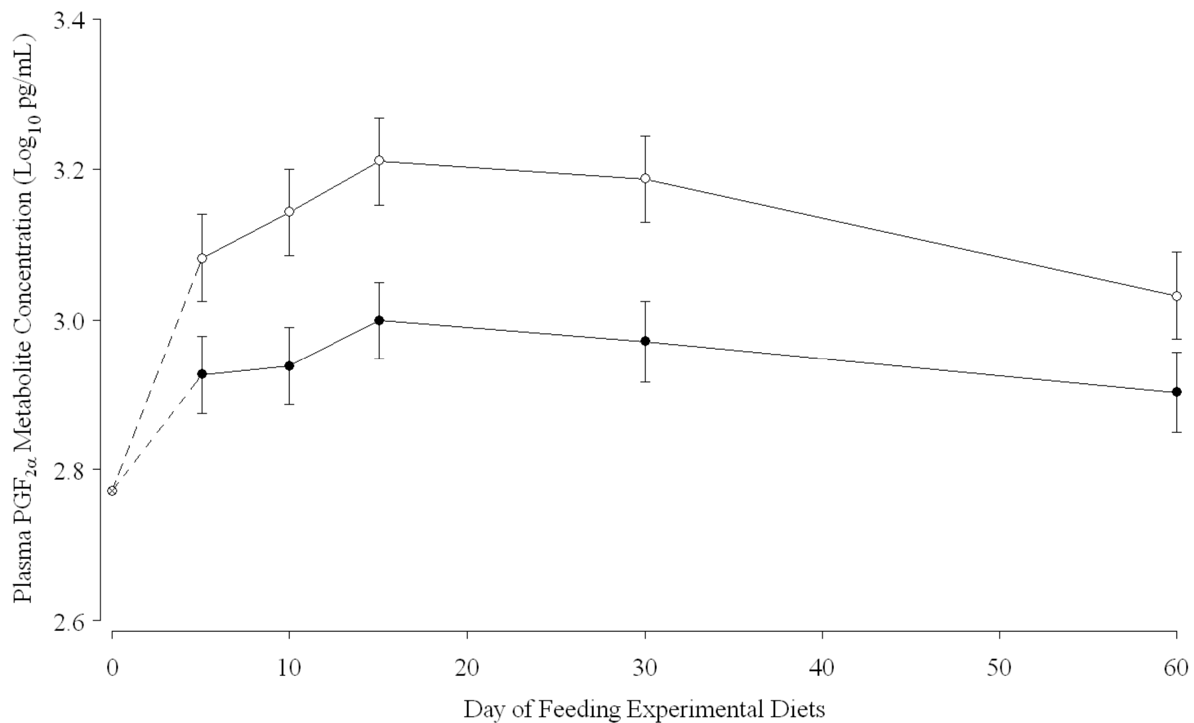


Figure 20. Change in plasma $PGF_{2\alpha}$ metabolite (PGFM) concentration over time following an oxtyocin challenge in ewes fed silage high in omega-3 (●) or oats/cottonseed meal high in omega-6 (○). Baseline PGFM concentrations for the high omega-3 and high omega-6 diets were 637.1 and 550.2 pg/mL respectively (re-transformed means) and were included in the statistical analysis as a co-variate. Significant difference between treatment diets, $p = 0.002$. Data adapted from: Gulliver et al. (2013a).

6.2 Reproduction and behaviour

A number of reviews have examined the effect of dietary fatty acids on reproduction in ruminants, however, these have primarily focused on the effects of total dietary fat and energy balance (for example, see Staples et al., 1998; Funston, 2004; Hess et al., 2008; Santos et al., 2008; Sturmey et al., 2009), rather than specific effects of omega-3. In a review examining the effects of LCn-3PUFA on reproductive success in ruminants, little evidence directly linked LCn-3PUFA intake with primary measurable reproduction outcomes compared with secondary measures such as inflammation (Gulliver et al., 2012).

The length of gestation is longer when sheep receive a diet high in omega-3 compared with omega-6 fatty acids during the last third of pregnancy (Capper et al., 2006). Prolonged gestation length following supplementation with omega-3 may increase birthweight and, therefore, improve neonatal survival, particularly in twin-bearing dams (Nowak and Poindron, 2006). Altered neonatal behaviour or improvement in milk composition following omega-3 supplementation may also improve lamb survival (Wathes et al., 2007), however, reduced colostrum production associated with n-3 supplementation during late pregnancy could negate the initial benefits of high n-3 diets (Annett et al., 2008; Annett et al., 2009).

More work is currently being undertaken by several research groups examining the enrichment of pork meat with LCn-3PUFA supplements. There may also be several health benefits to pigs following supplementing with LCn-3PUFA, including: healthy meat products, increased fertility and improved behaviour of offspring from females fed LCn-3PUFA during pregnancy and lactation (Wilkinson et al., 2014).

6.3 Sex ratio of lambs

Sheep operations would benefit from the ability to skew the sex ratio of offspring towards their preferred gender. For example, male prime lambs grow approximately 20% faster than females and have increased muscle accumulation, thereby reaching a higher market weight over a set time period. First-cross enterprises, however, prefer breeding females, which may achieve a higher sale price at weaning.

Maternal nutrition, including a number of specific nutritional factors, such as glucose (Kimura et al., 2005), total fat (Rosenfeld et al., 2003) and PUFA (Green et al., 2008) content of the diet, have been implicated in altering the sex ratio of offspring. The proportion of female offspring in mice was significantly higher when dams were fed a diet high in omega-6 fatty acids during pregnancy (Fountain et al., 2008). Similarly, the proportion of female lambs was higher when Border Leicester x Merino (Gulliver et al., 2013b) or Merino ewes (Figure 21) were fed a diet high in omega-6 compared with omega-3 fatty acids for 6 weeks prior to and, 17 days following, joining.

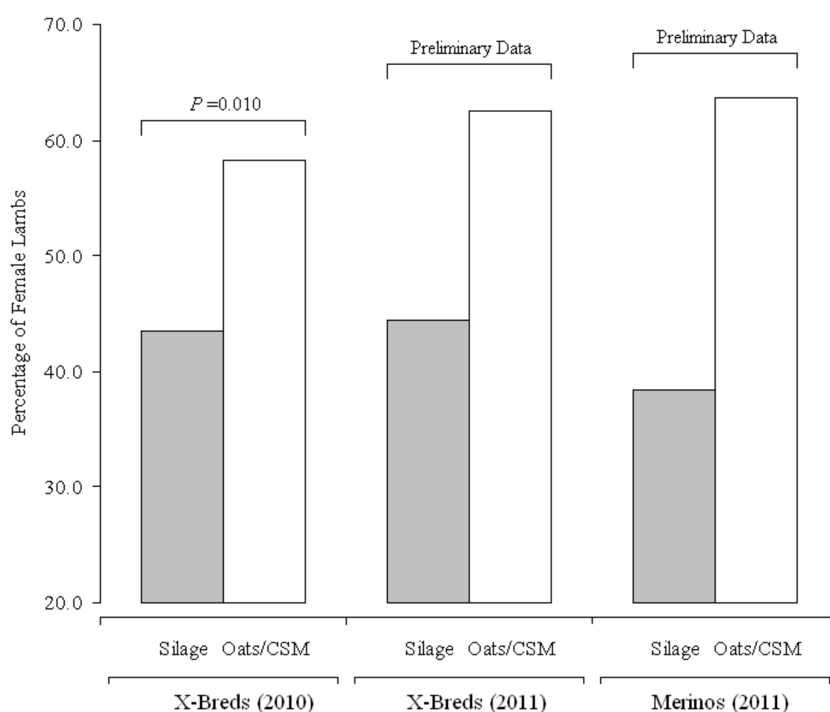


Figure 21. Proportion of female lambs in X-Bred or Merino ewes fed silage high in omega-3 (shaded bars) or oats/cottonseed meal high in omega-6 (unshaded bars) for approximately 6 weeks prior to joining and 17 days following joining. Data adapted from: Clayton et al. (2012b).

The ability to alter the concentration of omega-3 and omega-6 in animal feeds may, therefore, enable producers to feed specific diets at joining in order to target individual production systems. The effect of a diet high in n-6 fatty acids on the sex ratio of lambs when ewes are fed in an on-farm situation or in unsynchronised ewes is, however, currently unknown.

7. Manipulating the Concentration of LCn-3PUFA in Animal Feed

As reviewed previously, the type of feed, particularly grain compared with forage, can significantly alter the concentrations of LCn-3PUFA in meat. The concentration of LCn-3PUFA and LCn-6PUFA in meat are determined primarily by the amount of fatty acid precursors (ALA, C18:3n-3 and LA, C18:2n-6) available. In turn, the amount of precursors available for metabolism is dependent on a number of factors, including their concentrations in feed (Khan et al., 2012; Glasser et al., 2013) and the rate and extent of breakdown through lipolysis and biohydrogenation in the rumen prior to absorption and metabolism (Van Ranst et al., 2009; Buccioni et al., 2012). The following section outlines the primary factors affecting the concentrations of fatty acid precursors in feed and their metabolism prior to incorporation into LCn-3PUFA.

7.1 Concentrations of α-linolenic acid and linoleic acid in feedstuffs

The concentration of ALA and LA in feedstuffs is influenced by the type of feed (forage versus concentrate or grain), the type of forage (for example, perennial ryegrass versus red clover) and the nature of forage (such as fresh grass versus hay or silage). The following section reviews differences in fatty acid composition between forages and grains and examines the factors that may affect concentrations of fatty acids in different forages and relate these differences to possible alterations in LCn-3PUFA in meat.

7.1.1 Differences in fatty acid composition between forages and grains

As indicated in Section 5.3, the concentration of LCn-3PUFA in meat is lower when animals are fed grain or concentrate diets compared with fresh pasture or forage diets. There are a number of factors that influence this relationship, however, the predominant factor is the low concentration of the LCn-3PUFA precursor ALA and the high concentration of the LCn-6PUFA precursor LA found in grain (Table 16 and for review, see Dewhurst et al., 2006).

Table 16. Concentration of α-linolenic acid and the ratio of n-6:n-3 in maize silage and grain concentrates compared with fresh grass and perennial ryegrass-white clover forages.

Reference	Feed Type	Total Fat (g/100 g)	Fatty Acid Concentration (g/kg DM)[1]			n-6:n-3 Ratio
			C16:0	C18:3n-3	C18:2n-6	
(Eriksson & Pickova, 2007)[2]	Fresh grass[3]	2.58	3.02	9.71	2.66	0.27
(French et al., 2000)[2]	Fresh grass[4]	2.90	4.65	10.98	3.13	0.28
(Nuernberg et al., 2005)	Fresh grass[4]	4.44	6.65	22.00	3.85	0.17
(Nuernberg et al., 2005)	Maize silage	3.18	4.42	1.14	12.84	11.27
(Noci et al., 2007)	Barley + SBP[5]	1.31	13.2	2.02	48.93	24.22
(Eriksson & Pickova, 2007)[2]	Wheat grain	2.75	4.12	0.99	15.09	15.23
(Eriksson & Pickova, 2007)[2]	Oats grain	3.82	5.91	1.09	16.46	15.05

[1]C16:0 = palmitic acid, C18:3n-3 = α-linolenic acid, C18:2n-6 = linoleic acid.
[2]Fatty acid concentration calculated from total fat content (g/100 g) using a lipid conversion factor (LCF) of 0.770 for forage and silage and 0.860 for concentrates and grains (Weihrauch et al., 1977).
[3]Grass = mixture of *Lolium perenne*, *Trifoloium repens*.
[4]No details of grass species given.
[5]SBP = sugarbeet pulp (high in starch) - mixed ration containing 34.5% barley, 36% SBP and 14% soyabean meal.

Fatty acid concentrations also vary between forage types and within forage type between processing methods, however, these changes are usually small compared with differences between forages and concentrates. Relatively minor alterations in fatty acid concentrations in feed can significantly affect levels in milk, however, there is less evidence of the impact of these alterations in meat.

7.1.2 Effect of stage of growth on fatty acid composition

One of the major factors influencing fatty acid composition is the stage of growth of the plant. The concentration of total lipid and most major fatty acids including ALA and LA is lower when forages are cut at a later stage of development (for review, see Khan et al., 2012; Glasser et al., 2013). For example, the concentration of ALA and LA was lower and the ratio of LA(n-6):ALA(n-3) was higher when Timothy grass (*Phleum pratense*) was cut at later stages of development (Table 17, Boufaied et al., 2003a). The lower fatty acid concentration with later stages of development is the result of a lower leaf to stem ratio with increasing plant maturity, as leaf contains higher concentrations of fatty acids, in particular, ALA, compared with stem (Dewhurst et al., 2006). When comparing differences in fatty acid composition between forages, it is important, therefore, to compare cultivars or species at similar stages of development.

Table 17. Concentration of fatty acids in *Phleum pratense* cut at different stages of development.

Stage of	Fatty Acid Concentration (g/kg DM)[1]				
Growth	Total	C16:0	C18:3n-3	C18:2n-6	n-6:n-3
Stem elongation	18.01	3.27	8.71	3.97	0.46
Early head	15.09	2.96	6.86	3.51	0.51
Late head	14.42	2.82	6.37	3.39	0.53
Early flowering	13.72	2.70	5.96	3.19	0.54
Sem[2]	0.447	0.076	0.255	0.093	NI

[1]C16:0 = palmitic acid, C18:3n-3 = α-linolenic acid and C18:2n-6 = linoleic acid.
[2]Sem = pooled standard error of the mean across stage of development. NI = not indicated.
Data adapted from: Boufaied et al. (2003a).

7.1.3 Differences in fatty acid composition between forage species

The concentration of ALA was lower in annual ryegrass (*Lolium rigidum*) compared with other annual grasses (Table 18, Boufaied et al., 2003a), however, annual ryegrass was cut earlier (vegetative stage) than other perennial grasses (early head stage), so a direct comparison cannot easily be made. The concentration of ALA was higher and the ratio of n-6:n-3 was lower in white clover (*Trifolium repens*) compared with red clover (*T. pratense*) and lucerne (*Medicago sativa*) when fresh forage samples were collected from plants at the 10% bloom stage (Table 18).

The concentration of ALA and LA was higher in silage produced from red and white clover compared with ryegrass (Dewhurst et al., 2003b; Lee et al., 2003), however, the ratio of n-6:n-3 was lower in a mixed ryegrass pasture compared with clover (Table 19). The concentration of ALA in forage is also higher following the application of nitrogen fertiliser (Boufaied et al., 2003a; Witkowska et al., 2008), primarily due to a higher leaf to stem ratio following fertilisation (Dewhurst et al., 2006).

Table 18. Concentration of fatty acids in different forages grown to similar stages of development.

Species (cultivar)	Stage of Growth	Fatty Acid Concentration (g/kg DM)[1] Total	C16:0	C18:3n-3	C18:2n-6	n-6:n-3 Ratio
Annual ryegrass (Maris Ledger)	Vegetative	31.77	4.79	20.56	3.97	0.19
Tall fescue (Kokanee)	Early head	20.77	4.03	11.82	2.77	0.23
Timothy (Champ)	Early head	17.01	3.45	7.87	3.72	0.47
White clover (California)	10% bloom	29.76	5.22	16.52	4.62	0.28
Red clover (AC Charlie)	10% bloom	22.00	4.16	9.54	5.11	0.54
Lucerne (Arrow)	10% bloom	17.59	4.10	6.90	4.05	0.59
LSD[2]		2.00	0.40	1.20	0.31	NI

[1]C16:0 = palmitic acid, C18:3n-3 = α-linolenic acid and C18:2n-6 = linoleic acid.
[2]LSD = Estimated least significant difference ($p < 0.05$) reported within species to allow a general comparison between species. NI = Not indicated.
Data adapted from: Boufaied et al. (2003a).

Table 19. Concentration of fatty acids in silages produced from ryegrass or clover.

Source	Species	Total Fat (g/100 g)	Fatty Acid Concentration (g/kg DM)[1] Total	C16:0	C18:3n-3	C18:2n-6	n-6:n-3 Ratio[4]
(Lee et al., 2003)	Perennial ryegrass	3.25	18.20[b]	4.61[b]	8.59[b]	2.56[c]	0.30
	Red clover	2.42	22.00[a]	5.28[a]	9.65[ab]	3.96[b]	0.41
	White clover	2.89	24.10[a]	5.39[a]	10.80[a]	4.48[a]	0.41
(Dewhurst et al., 2003b)[2]	Ryegrass[3]	-	14.40	3.16	7.75	2.40	0.31
	Red clover	-	21.00	3.99	10.92	4.20	0.38
	White clover	-	25.34	4.57	13.92	4.68	0.34

[1]C16:0 = palmitic acid, C18:3n-3 = α-linolenic acid and C18:2n-6 = linoleic acid.
[2]Fatty acid concentration converted to g/kg oven dry matter from g/kg freeze-dried dry matter.
[3]Mixture of perennial ryegrass (*Lolium perenne*) and Italian ryegrass (*L. multiflorum*).
[4]Ratio of n-6:n-3 calculated from concentrations of C18:2n-6:C18:3n-3. Significant differences between silages not indicated in original papers.
Values in the same column with different superscripts for the study by Lee et al. (2003) differ significantly ($p < 0.05$). Significant differences between fatty acid concentrations not indicated for the study by Dewhurst et al. (2003b).

7.1.4 Change in fatty acid composition by conserving forage as silage

There is little agreement between studies examining the change in fatty acid concentration in forages prior to and following ensiling. Several authors report decreased concentrations of ALA and LA following silage production while others report either no change or increased fatty acid concentrations. The following sections summarise results of studies where concentrations decreased, increased or were unaltered following ensiling.

Decreased concentration of ALA following ensiling
The concentration of ALA was lower in silage and hay compared with fresh forage when *Lolium perenne* was wilted for 32 hr prior to forage conservation (Moloney, 2007). This lower concentration of ALA was associated with a lower concentration of LCn-3PUFA in meat when cattle consumed silage compared with fresh perennial ryegrass (Moloney, 2007).

The concentration of ALA was higher when ryegrass forage mixtures were ensiled using an *in vitro* system compared with fresh forage, however, the ratio of n-6:n-3 fatty acids was not altered (Table 20, Eriksson and Pickova, 2007). The concentration of EPA and DPAn-3 was not significantly lower in meat produced from animals fed diets supplemented with this silage compared with fresh forage (data not shown). Cattle fed the silage-based diets were younger and heavier at slaughter as all animals were slaughtered at the same time, which makes interpretation of differences in fatty acid composition difficult. The loss of fatty acids during lipolysis and ensiling may be reduced by the action of polyphenol oxidase (PPO, van Ranst, 2009). Some species of 'stay green' red clover have altered concentrations of PPO.

Table 20. Change in fatty acid composition of perennial ryegrass cultivars prior to and, following, ensiling using an *in vitro* system.

Forage Type	Total Lipid (g/100 g DM)	Fatty Acid Concentration (g/kg DM)[1] C16:0	C18:3n-3	C18:2n-6	n-6:n-3 Ratio
Grass[2]	2.58	3.02	9.71	2.66	0.274
Silage[3]	2.08	2.58	7.96	2.18	0.274

[1]C16:0 = palmitic acid, C18:3n-3 = α-linolenic acid, C18:2n-6 = linoleic acid. Fatty acid concentration calculated from total fat content (g/100 g) using a lipid conversion factor (LCF) of 0.770 (Weihrauch et al., 1977). Significant differences between forage types were not reported.
[2]Grass = *Lolium perenne*, *Trifoloium repens*
[3]Silage = *Lolium perenne*, *Phleum pretense*, *T. repens*, *T. pretense*
Data adapted from: Eriksson and Pickova (2007).

The concentration of ALA and LA was significantly lower following the *in vitro* ensiling of several cultivars of *Lolium perenne* compared with fresh forage prior to ensiling (Table 21, Elgersma et al., 2003). The ratio of n-6:n-3, however, was not altered in several species and the difference in the n-6:n-3 ratio between all species was small.

Table 21. Concentration of α-linolenic acid and the ratio of n-6:n-3 ratio in perennial ryegrass cultivars prior to and, following, ensiling.

	Lipid (g/100 g DM)		Fatty Acids (mg/kg DM) Total		C18:3n-2		C18:2n-6		Ratio n-6:n-3	
Cultivar	Fresh	Silage	Fresh	Silage	Fresh	Silage	Fresh	Silage	Fresh	Silage
Agri	4.41	5.32	29.75	19.64	20.29	12.83	3.13	2.31	0.15	0.18
AberGold	4.30	5.08	28.62	18.08	19.96	11.58	2.85	2.09	0.14	0.18
Respect	4.69	5.55	27.58	20.98	19.11	13.66	2.84	2.41	0.15	0.18
Herbie	3.78	5.22	27.22	17.57	18.56	11.33	3.19	2.08	0.17	0.18
Barezane	4.17	5.25	31.54	20.93	21.35	13.61	3.45	2.50	0.16	0.18
Barnhem	4.21	5.29	30.07	19.76	20.16	12.67	3.07	2.45	0.15	0.19
p-value[1]	NI		< 0.001		< 0.001		< 0.001		NI	

[1]*p*-value for the main effect of silage versus fresh. NI = not indicated.
Data adapted from: Elgersma et al. (2003).

The concentration of ALA was significantly lower and the concentration of LA significantly higher in ensiled lucerne compared with fresh lucerne (Table 22, Whiting et al., 2004). Lucerne was wilted for three days prior to ensiling, which may have resulted in a significant loss of fatty acids, particularly ALA, through lipolysis. The reported intake of ALA (94.42 versus 81.71 g/day; $p < 0$ 001) and concentration of ALA in milk (45.99 versus 34.20 mg/100 g milk; *p*-value not indicated) was higher when dairy cattle were offered fresh lucerne compared with lucerne silage (Whiting et al., 2004), which was in agreement with the higher reported concentrations of ALA in fresh forage.

Table 22. Change in fatty acid composition of fresh compared with ensiled lucerne.

Analysis	Forage Type		
	Fresh	Silage[3]	*p*-value
	(% DM)		
Total Fat	1.80	1.70	0.29
Fatty acid[1]	**(g/kg DM)**		
Total	13.99	12.99	0.004
C16:0	2.97	2.88	0.180
C18:3n-3	5.38	4.53	0.001
C18:2n-6	2.46	2.78	0.004
C20:5n-3	0.030	0.026	0.240
C22:5n-3	0.023	0.018	0.130
C22:6n-3	0.012	0.020	0.200
n-6:n-3 Ratio[2]	0.46	0.61	NI

[1]C16:0 = palmitic acid, C18:3n-3 = α-linolenic acid, C18:2n-6 = linoleic acid, C20:5n-3 = eicosapentaenoic acid (EPA), C22:5n-3 = docosapentaenoic acid (DPA) and C22:6n-3 = docosahexaenoic acid (DHA).
[2]Significant differences between forage types for the ratio of n-6:n-3 not indicated in the paper.
[3]Silage was wilted for 3 days prior to ensiling and was ensiled by storing in an upright silo for 3 weeks.
Data adapted from: Whiting et al. (2004).

Unaltered or increased concentration of ALA following ensiling

The concentration of ALA and LA in *Phleum pratense* (Timothy grass) was significantly higher following ensiling using an *in vitro* system compared with fresh forage (Table 23 Boufaied et al., 2003a), however, the ratio of n-6:n-3 was not altered by ensiling. The concentration of ALA and LA in a mixture of *Phelum pratense* and *Festuca pratensis* (meadow fescue) was lower following ensiling compared with fresh forage when the forage was wilted for 6 hr with no inoculant prior to ensiling (Table 23, Shingfield et al., 2005). The concentration of ALA and LA was higher, however, when silage was produced following the addition of formic acid during the wilting process (Table 23). The treatment of forage with formic acid may have stabilised leaf tissue and inhibited some lipolysis during the storage process, however, this effect has not been investigated in details and the authors did not report significant differences between treatment groups (Shingfield et al., 2005).

Table 23. Effect of wilting prior to conserving forage as hay or silage *in vitro* on the concentration of fatty acids in *Phleum pratense* L. cv. 'Champ'.

Reference	Forage type	Fatty acid concentration (g/kg DM)[1]				n-6:n-3
		Total	C16:0	C18:3n-3	C18:2n-6	Ratio
(Boufaied et al., 2003a)	Fresh	19.29[b]	3.42[b]	9.26[b]	4.54[b]	0.49
	Grass hay[2]	16.91[c]	3.14[c]	8.25[c]	3.77[c]	0.46
	Silage[3]	20.70[a]	3.64[a]	10.31[a]	4.98[a]	0.48
	p-value[4]	< 0.05	< 0.05	< 0.01	< 0.01	NI
(Shingfield et al., 2005)	Fresh	1894	3.91	10.36	3.14	0.303
	Silage (no inoculant)	1727	3.30	9.17	2.92	0.318
	Silage (enzyme)	1795	3.34	9.52	3.06	0.321
	Silage (formic acid)	2039	3.33	11.70	3.45	0.295

[1]C16:0 = palmitic acid, C18:3n-3 = α-linolenic acid, C18:2n-6 = linoleic acid.
[2]Grass hay was wilted for 3 days to obtain 850 g/kg dry matter
[3]Silage produced from fresh forage and forage wilted for "a few hours" to reach 400 g/kg dry matter.
[4]*p*-value = fresh versus silage. NI = not indicated.

Effect of wilting prior to ensiling

Wilting forage prior to ensiling is associated with significant losses of lipid through lipoloysis (see Section 7.2 below). The concentration of ALA was lower in silage produced from perennial ryegrass when forage was wilted prior to ensiling compared with forage that was unwilted (Table 24, Noci et al., 2007). The concentration of ALA and EPA in meat was not significantly altered, however, when animals consumed silage from wilted compared with unwilted silage (see Tables 9 and 24). Although the concentration of ALA and LA is commonly lower in perennial ryegrass following wilting and ensiling compared with fresh forage, the ratio of n-6:n-3 fatty acids may not be altered and the change in concentrations of fatty acids in meat may also be affected by differences in rumen lipoloysis and biohydrogenation of fatty acids (Dewhurst and King, 1998; Dewhurst et al., 2002). These factors need to be taken into account when assessing the effect of manipulating the concentration of ALA in feed.

Table 24. Fatty acid concentration in silage produced from perennial ryegrass ensiled without wilting compared with wilting for 32 hr and subsequent fatty acid concentrations in meat following consumption of silages by Friesian steers.

	Fatty Acids in Silage (g/kg DM)[1]			Fatty Acids in Meat (mg/100 g)			
	C18:3n-3	C18:2n-6	n-6:n-3 Ratio	C18:3n-3	C20:5n-3	C22:5n-3	n-6:n-3 Ratio
Non-wilted	13.86	3.49	0.25	28.16	8.43	16.3	3.89
Wilted	11.39	3.42	0.30	31.13	7.69	16.3	3.72
Sed[2]	0.394	0.131	NI	3.387	2.131	2.637	0.206

[1]C18:3n-3 = α-linolenic acid, C18:2n-6 = linoleic acid, C20:5n-3 = EPA, C22:5n-3 = DPAn-3.
[2]Sed = pooled standard error of difference calculated from standard error of means for wilted versus non-wilted silage.
Data adapted from: Noci et al. (2007).

The ability of plants to withstand the wilting process has been manipulated through breeding in order to maximise the amount of lipid and fatty acid remaining during the ensiling process. The concentration of ALA in perennial ryegrass remained significantly higher following ensiling when silage was produced from a 'stay-green' variety of forage compared with conventional forage (Table 25, Dewhurst et al., 2002; Dewhurst et al., 2003c). Although there were significant decreases in C18:3n-3 concentrations in both wilted silages, the ratio of n-6:n-3 did not change following wilting.

Table 25. Concentration of fatty acids in fresh conventional or 'stay-green' perennial ryegrass and silage produced from these forages.

Perennial Ryegrass Variety	Wilting	Fatty Acid Concentration (g/kg DM)[1]				n-6:n-3 Ratio[2]
		Total	C16:0	C18:3n-3	C18:2n-6	
Normal	Fresh forage	29.4[a]	5.37[a]	17.91[a]	3.92[a]	0.219
	Wilted 48 hr	21.1[b]	4.02[b]	12.72[c]	2.75[b]	0.216
Stay-green	Fresh forage	28.7[a]	4.98[a]	17.92[a]	3.94[a]	0.220
	Wilted 48 hr	22.2[b]	3.97[b]	13.92[b]	2.76[b]	0.198

[1]C16:0 = palmitic acid, C18:3n-3 = α-linolenic acid and C18:2n-6 = linoleic acid.
[2]Ratio of n-6:n-3 calculated as ratio of C18:2n-6:C18:3n-3. Significant differences not indicated in original paper.
Where indicated, values in the same column with different superscripts differ significantly ($p < 0.05$). Data adapted from: Dewhurst et al. (2002).

7.1.5 Potential future research

The fatty acid profiles in forage species grown in Australia have not been reported in detail. Several factors determine the changes in fatty acid concentrations following ensiling of forages, including the time of wilting prior to ensiling and the method of silage production (including whether additives are used). The effect of ensiling under Australian conditions on the concentration of fatty acids in forage, the availability of these fatty acids for ruminants and subsequent accumulation in meat has not been examined.

There is also a need to further examine the relationship between concentrations of ALA in forage compared with those found in milk. The concentration of ALA is lower when forage has been dried for hay production compared with undried forage (Shingfield et al., 2005), however, the concentration of ALA in milk is often similar when animals consume hay compared with fresh forage. The effect of ensiling on the biohydrogenation of fatty acids from ensiled forage, in particular, has not been examined in Australia.

7.2 Factors affecting loss of fatty acids in the rumen

As reviewed in Section 2.4, fatty acids in feedstuffs are lost through the combined processes of lipolysis and biohydrogenation. A number of factors affect these processes and the subsequent loss of fatty acids in the rumen prior to absorption and metabolism to meat or milk. The following section provides details about several factors affecting lipoloysis or biohydrogenation of lipids and fatty acids in forage.

7.2.1 Lipolysis and release of free fatty acids

The concentration of fatty acid in phospholipid and triglyceride lipid is important for metabolism and conservation for meat or milk. If fatty acids are released from phospholipid and triglyceride during lipolysis to the free fatty acid form (NEFA), these fatty acids may be more easily hydrogenated to C18:0 in the rumen and, therefore, not available for conversion to LCn-3PUFA in meat.

There is considerable hydrolysis and loss of structural lipids, including phospholipid and diglyceride and an increase in the proportion of lipid as NEFA following ensiling (Dewhurst et al., 2006). The change in the class of lipid in silages produced in Australia has not been investigated in detail, however, in an unpublished experiment conducted at the Wagga Wagga Agricultural Institute, the proportion of lipid as phospholipid was lower and the proportion as NEFA was higher in oat/pea silage compared with fresh forage (Table 26, EH Clayton, unpublished observations).

Table 26. Lipid classes (proportion of total lipid) in fresh oat/pea forage compared with silage.

Proportion of Total Lipid (%)	Fresh Forage	Silage	*p*-value
Neutral lipid	61.7 (± 1.69)	59.9 (± 1.76)	0.412
Phospholipid	26.2 (± 1.21)	21.2 (± 0.99)	0.008
NEFA	11.5 (± 0.86)	16.9 (± 0.75)	< 0.001

Data from EH Clayton, unpublished observations.

The proportion of ALA in the complex lipid fraction (likely to be predominantly phospholipid) of total lipid from a mix of grass species (predominantly *Lolium perenne*) was lower following ensiling compared with fresh forage (Table 27, Lough and Anderson, 1973). In one silage sample, there was no ALA in the PL fraction of lipid (Table 27). This decrease in ALA in phospholipid may lead to a subsequent increase in hydrogenation of non-esterified ALA compared with LA, thereby resulting in proportionately more LA being available for absorption and metabolism.

Table 27. Proportion of fatty acids in the phospholipid, free fatty acid (NEFA) and total fatty acid classes of a mixture of *Lolium perenne*, *Phleum pratense* and *Festuca pratensis* prior to and following ensiling.

	Fatty Acid Proportion[1] (% in each lipid class)								
	C18:3n-3			C18:2n-6			n-6:n-3 Ratio		
Forage	NEFA	PL	Total	NEFA	PL	Total	NEFA	PL	Total
Fresh grass	0.68	53.28	66.72	0.24	7.40	9.84	0.35	0.14	0.15
Silage 1	0.68	5.18	11.14	2.20	10.36	17.32	1.82	2.00	1.55
Silage 2	0.12	0.00	7.82	7.40	7.40	12.66	5.33	n-6[2]	1.62

[1]C18:3n-3 = α-linolenic acid, C18:2n-6 = linoleic acid, PL = phospholipid, NEFA = non-esterified fatty acid (free fatty acid). Data are proportion of fatty acid in each lipid class.
[2]Phospholipid class of Silage 2 contained 100% n-6 fatty acids and 0% n-3 fatty acids.
Data adapted from: Lough and Anderson (1973).

7.2.2 Biohydrogenation of fatty acids from different forages

The concentration of ALA was higher in red clover and white clover silage than perennial ryegrass silage (Lee et al., 2003). The conservation of ALA in cattle from mouth to duodenum was less than 100% for red clover (93%) and white clover (95%), however, the apparent conservation of ALA from perennial ryegrass silage was 119%. The biohydrogenation of ALA from perennial ryegrass silage may have been lower than ALA from clover (Lee et al., 2003), thereby allowing more ALA to be available for incorporation into meat or milk. The effect of forage species on the biohydrogenation of fatty acids in Australian forages is currently unknown.

7.2.3 Relationship between rumen pH and biohydrogenation of fatty acids

The activity of the enzymes responsible for the initiation of the biohydrogenation of ALA and LA (see Section 2.4.2) is highest at a rumen pH of 7.0 and approximately half at a rumen pH of 6.0 (Kepler and Tove, 1967). The biohydrogenation of LA, but not ALA, is reduced when rumen pH is below 7.0 following grain feeding (Lee et al., 2006). The loss of ALA compared with LA is higher, therefore, when rumen pH decreases towards 6.0, resulting in a relative increase in the amount of LA being available for absorption and incorporation into meat or milk (Figure 22, van Nevel and Demeyer, 1996). The effect of lower rumen pH on the dose of ALA and LA available for meat, however, remains unclear under practical feeding situations.

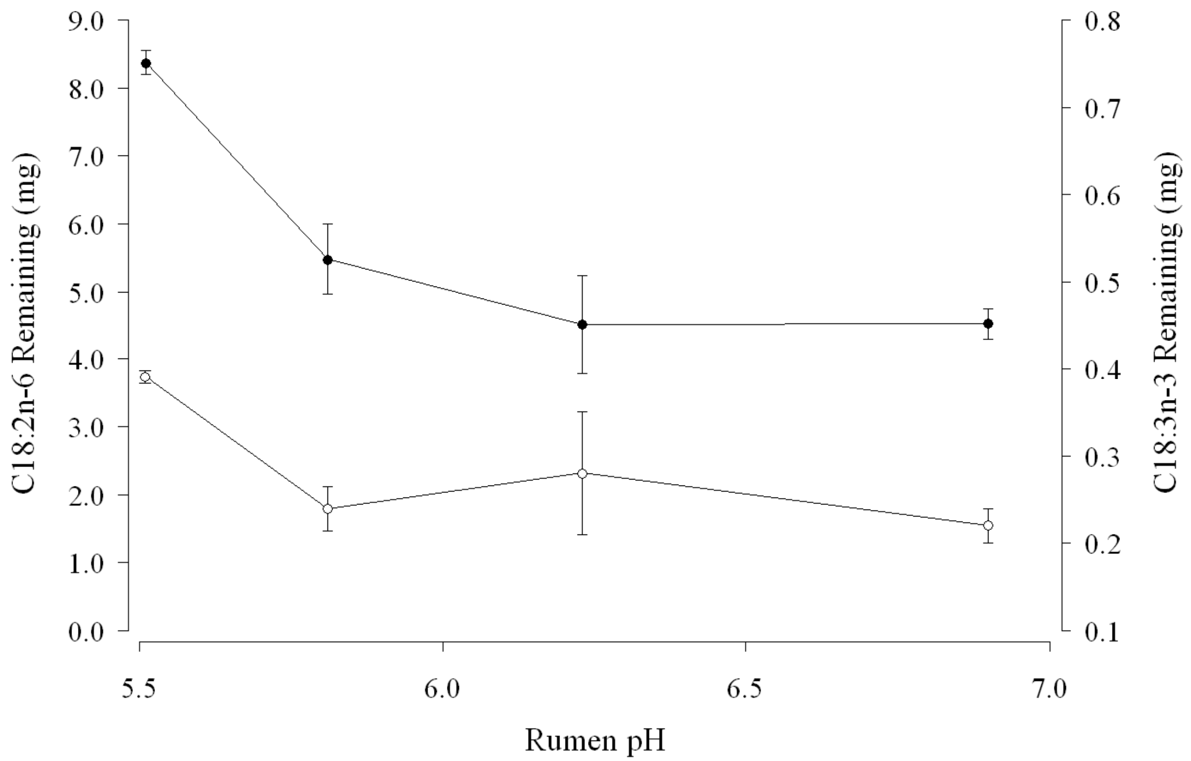


Figure 22. Amount (mg) of linoleic acid (C18:2n-6, ●) and α-linolenic acid (C18:3n-3, ○) remaining in the rumen after biohydrogenation to C18:0 at different rumen pH. Data adapted from: van Nevel and Demeyer (1996).

8. Meat Production Systems in Australia

As reviewed previously, the concentration of fatty acids in meat is largely determined by the diet consumed by sheep or cattle. Beef is produced by a number of different production systems throughout Australia and the diet available to cattle varies depending on the system employed. Different production and feeding systems are more common either in northern or southern Australia (Thomason, 2007), for example;

Northern Australia	- Predominantly *Bos indicus* (eg. Brahman)
	- Mainly pasture-based systems (grass-fed)
Southern and central Australia	- Predominantly European and British (eg. Angus, Hereford)
	- Mixture of pasture and grain feeding

In southern and central Australia, there is a variety of production systems, including pasture/grass-fed, which results in low levels of intramuscular fat (marbling); short-fed grain (grain-finished for 40–70 days), which does not affect marbling to a great extent; and long-fed grain (grain-fed for 100–300 days), which increases marbling specifically.

Approximately 30% (about 3 million head) of Australian cattle turned off eachr year (out of a total of 8–9 million hd/year) are finished in feedlots, having been either short or long-term grain fed. The total capacity of Australian feedlots is approximately 1.15 million head and the number of cattle on-feed at any one time varied considerably from 1991 to 2012. The percentage of cattle in feedlots fed grain long-term for export markets was between 60–70% from 2001 and 2007 (Figure 23).

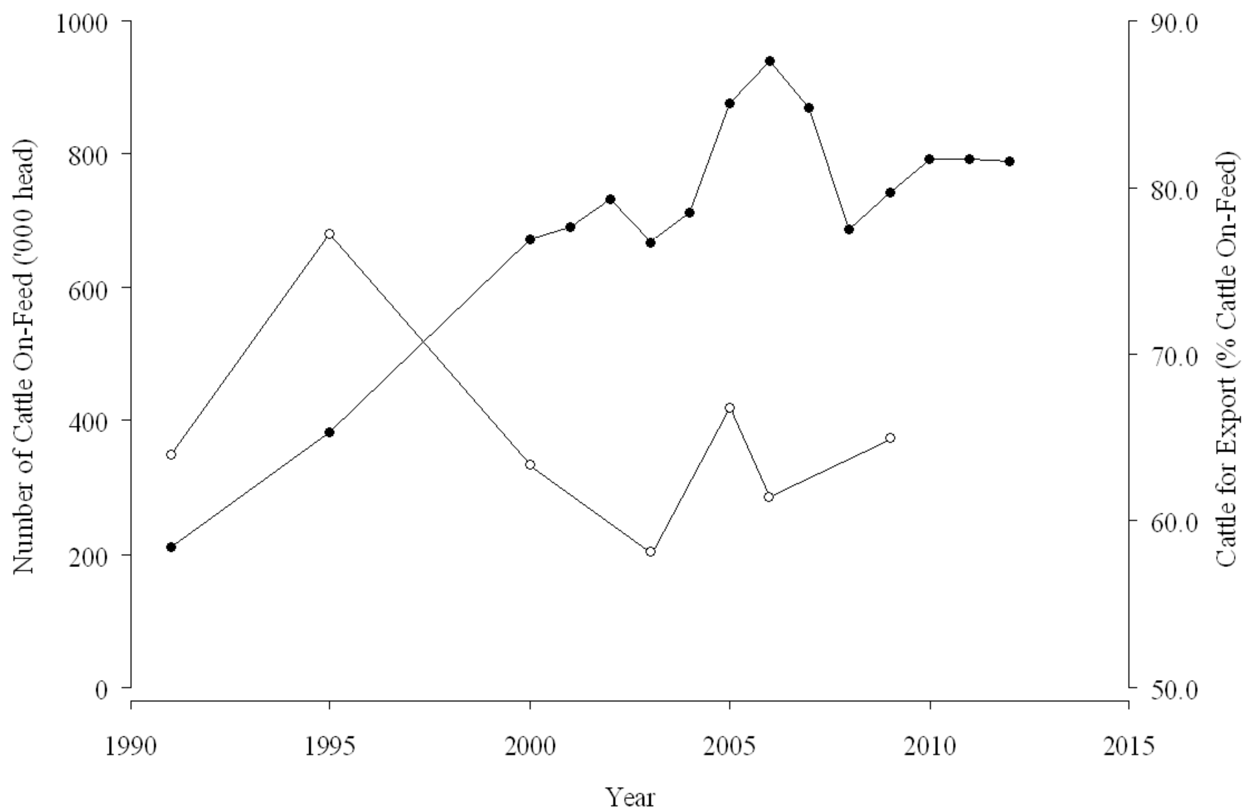


Figure 23. Number of feedlot cattle on-feed (●) and percentage of feedlot cattle grown for the export market (○) in Australia per year. Data adapted from: ABARE and MAF (2006); McRae and Thomas (2013).

The majority of cattle in feedlots in Australia are fed grain (on-feed) for between 50–100 days (Thomason, 2007) and the percentage of cattle on-feed decreases for longer periods of feeding (Figure 24). The concentration of total LCn-3PUFA in beef decreases substantially from approximately 70 mg/100 g to 50 mg/100 g when cattle are fed grain for between 50–100 days compared with being fed grain for less than 50 days (Duckett et al., 1993).

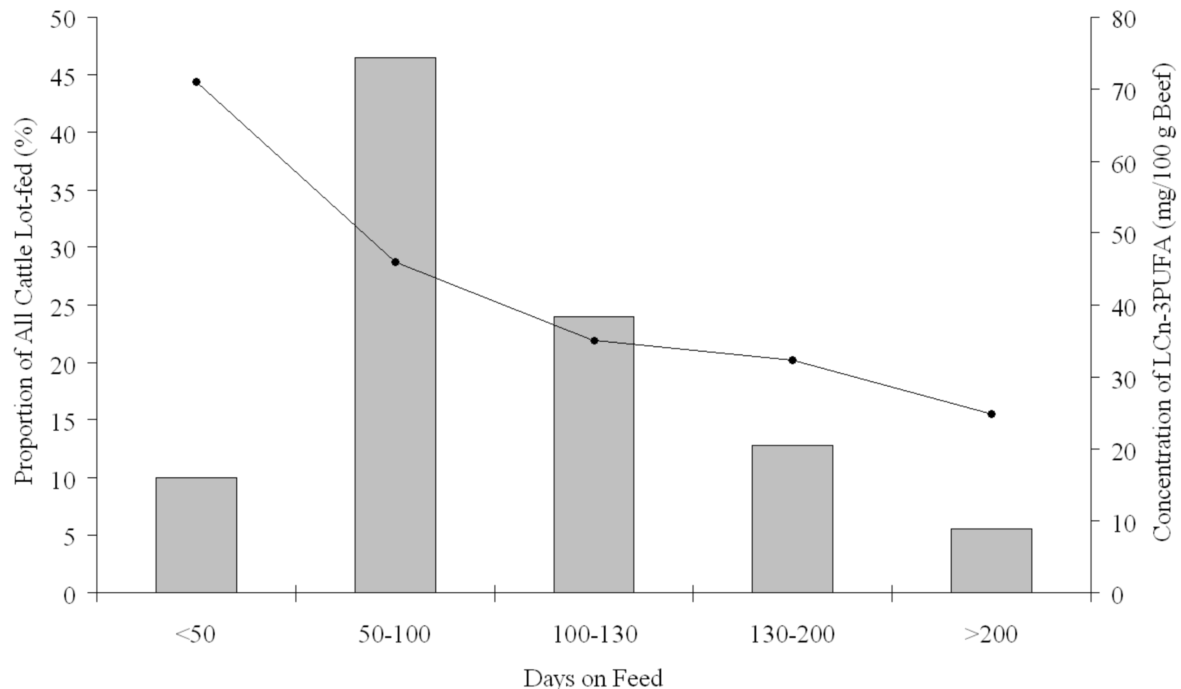


Figure 24. Percentage of Australian lot-fed cattle for increasing days on feed (bars) with the estimated concentration of LCn-3PUFA (mg/100 g) in beef produced from cattle fed grain for those lengths of time. Data for percentage of cattle lot-fed adapted from (Thomason, 2007) and data for concentrations of LCn-3PUFA derived from Clayton et al. (2009b) together with data from Duckett et al. (1993).

Most beef and lamb meat in Australia is considered relatively lean (Williams, 2007), due primarily to animals being pasture fed for the majority of their lives (Thomason, 2007). As presented in Section 9, many cuts of beef and lamb from Australian production systems are considered a ‘source’ or a ‘good source’ of omega-3 fatty acids and are considered healthy products for consumers.

The key to improving the health attributes of meat from beef or lamb produced in Australia is to optimise the inclusion of forage into current feeding systems. There are many opportunities to include fresh forage or silage into beef production systems, for example, even when animals are grain-fed for varying times in the feedlot industry. The production of fresh forage or silage of high quality that can meet market specifications for the efficient production of high-quality beef is a high priority.

9. Recommended and Reported Intakes of LCn-3PUFA in Humans

The primary interest in manipulating the fatty acid profile of meat, in particular, increasing the concentration of LCn-3PUFA, is related to the possible health benefits to humans from consuming these meat products. There are several consensus guidelines worldwide for recommended intakes of LCn-3PUFA and biological markers indicating LCn-3PUFA status. In addition, there are several studies reporting LCn-3PUFA intake both in Australia and overseas by which recommended intakes can be compared. In order to understand the contribution that altering the LCn-3PUFA status of meat through dietary manipulation may have on total LCn-3PUFA intake on a population basis, it is necessary to review the guidelines for recommended intakes of LCn-3PUFA and the contribution of meat to this intake.

9.1 Recommended intakes

There are several different recommendations for intakes of LCn-3PUFA from different organisations in Australia, as well as, internationally. The recommended intake (Adequate Intake, AI) of LCn-3PUFA for the Australian population is 160 mg/day for men and 90 mg/day for women and lower for adolescents (Table 28). Recommended intakes for LCn-3PUFA in Australia include EPA + DPA + DHA, whereas most international guidelines only include EPA + DHA. A higher Suggested Dietary Targets (SDT) to prevent chronic disease is recommended for adults, but not adolescents.

Table 28. Australian guidelines for recommended daily intakes of LCn-3PUFA for different populations (Nutrient Reference Values, NRVs).

	Recommended Daily Intake of LCn-3PUFA (mg/day)[1]			
	Adolescents (14-18 yrs)		Adults (19+ yrs)	
	Boys	Girls	Men	Women
Adequate Intake (AI)	125	85	160	90
Suggested Dietary Target (SDT)	NI	NI	610	430

[1]LCn-3PUFA = eicosapentaenoic acid (EPA) + docosapentaenoic acid (DPA) + docosahexaenoic acid (DHA).
NI = not indicated in NRVs.
Data adapted from: Australian Nutrient Reference Values (NHMRC and Health, 2006).

The AI for Australia is based on the 50th percentile of estimated intake in the Australian population (median intake) based on a 24 hr dietary recall from the 1995 National Nutrition Survey (NNS, McLennan and Podger, 1999a; McLennan and Podger, 1999b), whereas the SDT for the prevention of chronic disease (NHMRC and Health, 2006) is based on the 90th percentile of Australian intake (Meyer et al., 2003; Howe et al., 2006). The National Health and Medical Research Council (NHMRC) states that the AIs do not necessarily reflect optimal intakes, but are normal values found in a population where there is no obvious sign of deficiency. These guidelines do not take into account a change in the risk of disease that may have been examined in any other research studies with LCn-3PUFA in Australia or overseas.

International guidelines recommend intakes of EPA+DHA between 200–650 mg/day for the general population to 1000 mg/day for people at risk of CVD (Table 29). The American Heart Association recommends 2 fatty fish meals per week or 400–500 mg/day EPA+DHA for the general population (Murphy et al., 2007).

Table 29. International guidelines for recommended daily intakes of LCn-3PUFA for different populations.

Organisation[1]	Recommended Intake of EPA + DHA (mg/day)[2]	Population
British Nutrition Foundation Task Force	500–1000	People at risk of CVD
UK Department of Health	200	General population
European Academy of Nutritional Sciences	200	General population
ISSFAL	650	General population
AHA	1000	People at risk of CVD
	Oily fish (twice/week)	General population
	>3 g/day	To reduce triglyceride levels
NIH	300	Pregnant and lactating females

[1]ISSFAL = International Society for the Study of Fatty Acids and Lipids, AHA = American Heart Association, NIH = National Institute of Health.
[2]EPA = Eicosapentaenoic acid (C20:5n-3); DHA = Docosahexaenoic acid (C22:6n-3).
Note: International guidelines for recommended daily intakes of LCn-3PUFA only include EPA + DHA whereas Australian guidelines (NHMRC and Health, 2006) include EPA, DPA and DHA as sources of LCn-3PUFA.
Data adapted from: Garg et al. (2006); Gebauer et al. (2006); Murphy et al. (2007).

There are currently no recommendations in Australia for an optimal balance of n-6:n-3 fatty acids in the whole diet. However, there are several recommendations internationally suggesting the optimal human diet should contain a ratio of n-6:n-3 of approximately 5:1 (for example, see Simopoulos, 1991, 1999).

9.1.1 Food sources of LCn-3PUFA

Nutrition claims have been introduced by FSANZ where foods can be labelled as a 'source' of omega-3 if they contain at least 30 mg of EPA and/or DHA per serving and a 'good source' of omega-3 if they contain at least 60 mg of EPA and/or DHA per serving together with less than 5 g saturated/trans fat (FSANZ, 2000, 2002). There appears to be little agreement, however, about what constitutes a 'standard serve' of meat. A standard serve of beef, veal and lamb is reported to be 135 g (Williams, 2007). According to information available on the FSANZ website on standard measures, a standard serve of T-bone steak (separable lean, grilled) is 135 g, but there are no other references to 135 g for standard serves of meat.

The average serve of cooked meat (beef, lamb, pork or chicken) according to the Australian Guide to Healthy Eating (Smith et al., 1998) is 85 g (65–100 g) and an average serve of fish is 100 g. Considering the cooking loss reported for *L. dorsi* is approximately 33% (for example, see Wythes et al., 1988), these values for cooked meat translate to a serving size of approximately 123 g (97–149 g) for raw meat. Other databases report average serving sizes of steak, roast and mince of 205, 88 and 100 g respectively. Researchers promoting the health benefits of meat may try to increase the serving size in order to increase the likelihood of reaching the target of 30 or 60 mg/serve EPA + DHA to be considered a 'source' or 'good source' of omega-3 (see Table 30)

Currently the NRVs for LCn-3PUFA intake in Australia include EPA+DPA+DHA whereas the nutrition claims by FSANZ for 'sources' of omega-3 only include EPA and DHA. As such, many cuts of meat do not currently meet the criteria to be considered a 'good source' of omega-3 even though they are contributing significantly to current AIs and SDTs. Currently there is a push to include DPAn-3 in the FSANZ nutrition claims, which would make total LCn-3PUFA in most meat produced in Australia in the high range.

Table 30. Mean concentration (mg/100 g) of LCn-3PUFA in different meat sources in Australia and the consideration of these as a 'sources' of omega-3 depending on criteria used to calculate LCn-3PUFA.

Fatty Acid[1]	Beef	Veal	Lamb	Mutton	Chicken	Pork	White Fish	Oily Fish
	Concentration per serve of 100 g							
EPA	31	28	28	44	5	0	48	913
DPA	51	33	44	53	9	6	21	194
DHA	6	3	13	20	9	4	111	1118
EPA+DHA	37†	31†	41†	**64**‡	14	4	**159**‡	**2031**‡
LCn-3PUFA	**88**‡	**64**‡	**85**‡	**117**‡	23	10	**180**‡	**2225**‡
	Concentration per serve of 123 g							
EPA+DHA	45.5†	38.1†	50.4†	**78.7**‡	17.2	4.9	**196**‡	**2498**‡
LCn-3PUFA	**108.2***	**78.7***	**104.6***	**143.9**‡	28.3	12.3	**221**‡	**2737**‡
	Concentration per serve of 135 g							
EPA+DHA	50.0†	41.9†	55.4†	**86.4**‡	18.9	5.4	**215**‡	**2742**‡
LCn-3PUFA	**118.8***	**86.4***	**114.8***	**158.0**‡	31.1†	13.5	**243**‡	**3004**‡

†Considered a "source of omega-3" according to (FSANZ, 2002).
‡Considered a "good source of omega-3" according to (FSANZ, 2002).
*Considered a "good source of omega-3" if DPA is included in the calculation of LCn-3PUFA content.
[1]EPA = eicosapentaenoic acid (C20:5n-3), DHA = docosahexaenoic acid (C22:6n-3), LCn-3PUFA = EPA+DPA+DHA.
Data adapted from: Williams (2007).

9.2 Reported intakes of LCn-3PUFA in Australia

The average reported intake of total LCn-3PUFA in Australia for adults 19–64 years old is 298 mg/day for males and 195 mg/day for females (Table 31). The reported median intake of total LCn-3PUFA is 160 and 90 mg/day for adult males and females, respectively, over 19 years of age and 125 and 85 mg/day for boys and girls, respectively, aged 14–18 years (Howe et al., 2006). The previous reported median intakes of EPA, DPAn-3, DHA and total LCn-3PUFA were 8, 6, 15 and 29 mg/day, respectively, for adults over 19 years of age (Meyer et al., 2003). These differences between mean and median values indicate there is a substantial positive skew in intake data in the Australian population. The median intakes reported in 2006 correspond to the 50th percentile AIs recommended in the Australian NRVs (NHMRC and Health, 2006).

Table 31. Mean reported daily intakes of ALA and LCn-3PUFA for different age groups in Australia.

	Reported Intake of Fatty Acids (mg/day)						
	Reported in 2003			Reported in 2006			
	Males and Females			Males and Females		Male	Female
Fatty Acid	12–15	16–18	19–64	12–18	19–24	19–64	
C18:3n-3	1220	1290	1210	1180 ± 20	1070 ± 10	1280 ± 10	870 ± 10
C20:5n-3	32	41	55	57 ± 4	77 ± 4	91 ± 3	60 ± 2
C22:5n-3	22	20	27	63 ± 2	81 ± 2	90 ± 1	52 ± 1
C22:6n-3	63	77	106	75 ± 7	94 ± 6	117 ± 5	83 ± 3
LCn-3PUFA	117	138	188	195 ± 12	253 ± 11	298 ± 8	195 ± 5

NB. Data from the National Nutrition Survey (NNS) of Australia in 1995 (McLennan and Podger, 1999a).
[1]Data for 2003 from: Meyer et al. (2003), data for 2006 from: Howe et al. (2006). The intake of LCn-3PUFA was calculated using two different databases for the LCn-3PUFA content of foods.

The database (Mann et al., 1995; Mann et al., 2003) used to estimate the intake of LCn-3PUFA (Meyer et al., 2003) from dietary information recorded in the National Nutrition Survey (NNS, Australian Bureau of Statistics, ABS, 1998) was also marketed in the FoodWorks program (Xyris Software, Brisbane, version 3.01 2002). Reported intakes of LCn-3PUFA were subsequently revised using the same intake data from the NNS, but with a revised database for the LCn-3PUFA content in food, particularly meat (Howe et al., 2006). The significant increase in reported intakes was due largely to significant increases in reported concentrations of DPAn-3 in red meat (Tables 32 and 33).

Table 32. Reported concentrations (mg/100 g) of LCn-3PUFA in beef used in databases to estimate the daily intake of LCn-3PUFA in the Australian population.

	Reported Concentration of Omega-3 (mg/100 g)				
Reference	ALA	EPA	DPA	DHA	LCn-3PUFA
(Mann et al., 1995; Meyer et al., 2003)	29.0	19.0	22.0	3.0	44.0
(FSANZ, 2006)[1]	45.0	29.5	48.0	7.0	84.5
(Howe et al., 2006)	-	45.0	71.0	13.0	129.0

[1]Median value of fatty acids from all cuts of meat.

Table 33. Reported concentrations (mg/100 g) of DPAn-3 in different meat types used in databases to estimate the daily intake of LCn-3PUFA in the Australian population.

	Reported Concentration of Omega-3 (mg/100 g)			
Reference	Beef	Lamb	Poultry	Pork
(Mann et al., 1995; Meyer et al., 2003)	22.0	36.9	14.0	11.0
(FSANZ, 2006)[1]	48.0	58.0	7.0	29.0
(Howe et al., 2006)	71.0	83.0	18.0	28.0

[1]Median value of fatty acids from all cuts of meat.

The reported mean intake of EPA (83.0 mg/day), DPAn-3 (43.8 mg/day), DHA (186.6 mg/day) and total LCn-3PUFA (313.4 mg/day) was higher in a population of men and women aged between 20–64 years in the Wollongong region of NSW compared with the general population (Patch et al., 2005). These figures are likely to be higher than the general population due to the participant sample (n = 94) being significantly overweight and their total intakes of total fat and energy would have been relatively high.

The average reported intake of total LCn-3PUFA in children and adolescents in Australia is 137.5 mg/day (Table 34, Clayton et al., 2008). These values are comparable to those reported previously in a similar population (Meyer et al., 2003). The median reported intake for the same population is 86.8 mg/day (Clayton et al., 2008). The mean reported intake of LCn-3PUFA was not significantly different between children and adolescents diagnosed with bipolar disorder compared to age and sex-matched controls after adjusting for socio-economic status (SES) as a co-variate in the analysis (Table 34).

Table 34. Mean reported daily intakes of LCn-3PUFA in a population of children and adolescents (mean age = 14.8 years) in Australia using a food frequency questionnaire (FFQ).

Fatty Acid intake (mg/day)[1]	Control	Bipolar	*p*-value[2]
EPA	33.6 (± 4.22)	22.4 (± 3.90)	0.087
DPA	22.2 (± 3.71)	22.2 (± 3.71)	0.150
DHA	58.0 (± 7.25)	38.0 (± 6.69)	0.079
LCn-3PUFA	121.5 (± 13.09)	83.4 (± 12.09)	0.068

[1]Values are least squares means ± standard error of the least squares mean adjusted for SES as a co-variate for 15 age and sex-matched pairs; n = 5 males (mean age = 12.6 yrs) and n=10 females (mean age = 15.4 yrs).
[2]Differences between groups adjusted for SES as a co-variate in the analysis.
Adapted from: Clayton et al. (2008).

The reported mean intake of EPA + DHA (Table 34) is higher than those values previously reported in children 8 years old using a food frequency questionnaire (FFQ, Oddy et al., 2004). The reported intake of EPA + DHA was 30 mg/day when children reported their consumption of fish as ‘never’ or ‘rarely’, whereas, the reported intake was 46 mg/day when children reported eating fish more than twice per week (Oddy et al., 2004). The intake of LCn-3PUFA has previously been reported to increase with increasing age of children.

The data for reported intakes of LCn-3PUFA for different populations in Australia can be compared with the intakes recommended in the Australian NRV (NHMRC and Health, 2006). The reported intake of LCn-3PUFA for Australian males and females is above the AI recommended from the Australian NRV, but not as high as the SDT (Figure 25). The reported intake of LCn-3PUFA for children and adolescents is above the reported AI from the NRVsand higher than a SDT estimated from the 90th percentile of reported intake from an Australian study (Clayton et al., 2008).

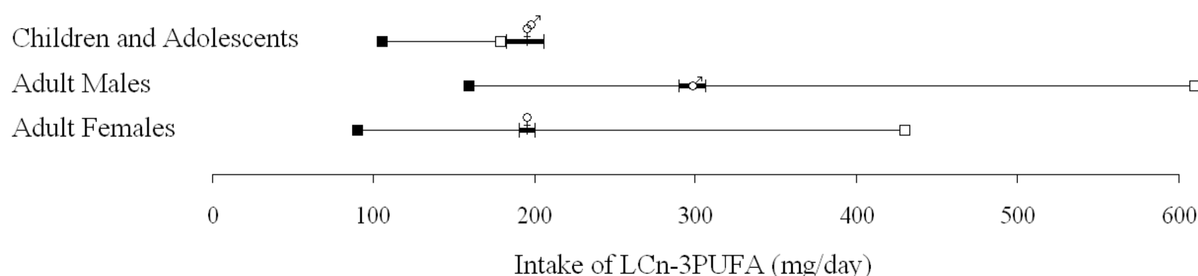


Figure 25. Mean (± se) estimated intake of LCn-3PUFA by female (♀) and male (♂) children and adolescents or adults in Australia (McLennan and Podger, 1999a; Howe et al., 2006) compared with recommended Adequate Intakes (AI ■) and Suggested Dietary Targets (SDT, □) from the Australian Nutrient Reference Values (NRV, NHMRC and Health, 2006) or a SDT for children and adolescents estimated from the 90th percentile of reported intake from an Australian study (Clayton et al., 2008).

9.3 Reported intake of meat and fish and contribution to LCn-3PUFA intake

9.3.1 Reported intake of meat and fish in Australia

The intake of meat and fish is estimated using a variety of methods, including 24-hour dietary recall, 24-hour dietary record, FFQ and Apparent Consumption. The Apparent Consumption per capita is also calculated from the sum of local production plus imports minus exports (ABS, 2000) and is then corrected for the dressing percentage of different cuts of meat (Cashel and Greenfield, 1994). The reported mean intake of beef and lamb in adults in Australia is approximately 45 and 25 g/day, respectively, (Table 35) and this intake has remained relatively constant since 2000. The mean reported intake of meat other than fish in Australian children and adolescents varies according to the method of data collection and the population studied (Table 36). The reported intake of meat is higher in Australia than values reported in the UK (Enser et al., 1996; MAFF, 2001).

Table 35. Reported intakes of meat and fish (g/day per head) for adults in the Australian population.

Study	Age	Year	Method[5]	Mean Intake (g/day)					
				Beef	Lamb	Pork	Poultry	Total Meat other than Fish	Fish
(Cashel and Greenfield, 1994)[1]	Population	1983	Apparent	47.4	25.1	20.2	23.0	115.7	18.7
(Cashel and Greenfield, 1994)[2]	Population	1983	24 hr recall	67.0	27.6	20.5	26.0	141.1	18.4
(ABS, 2000)[3]	Population	1989	Apparent	45.2	25.4	26.1	29.0	125.7	18.6
(ABS, 2000)[3]	Population	1998	Apparent	41.1	18.7	29.7	37.1	126.6	24.5
(Cook et al., 2001)	Adults	1995	24 hr recall	-	-	-	-	161.0	28.0
(Howe et al., 2006)[4]	Adults	1995	24 hr recall	43.9	11.2	18.3	54.7	128.1	19.5
(Ollis et al., 1999)	29-66	1996	3-day	-	-	-	-	164.0	28.0

[1]Intake data calculated from Apparent Consumption from the National Dietary Survey of Adults (1983) and is presented as weight of boneless, cooked edible weight equivalent after adjusting for carcass yield (Cashel et al., 1986). Beef = beef + veal.
[2]Intake data calculated from 24 hour diet recall in adults (English et al., 1988). Beef = beef + veal. Lamb = Lamb + mutton.
[3]Intake calculated from Apparent Consumption adjusting for carcass yield (Cashel et al., 1986). Beef = beef + veal. Lamb = Lamb + mutton.
[4]Mean intake calculated for all age groups from reported values for LCn-3PUFA intake and percentage contribution of each meat source to intake together with reported weighted concentrations of LCn-3PUFA in meat (Howe et al., 2007).
[5]FFQ = Food Frequency Questionnaire, Apparent = Apparent Consumption, calculated from local production + imports - exports, 24 hr recall = 24 hour dietary recall, 3-day = 3-day weighed food record, 4-day = 4-day weighed food record.

Table 36. Reported intakes of meat and fish (g/day per head) for children and adolescents in the Australian population.

Study	Age	Year	Method[4]	Mean Intake (g/day)					
				Beef	Lamb	Pork	Poultry	Total Meat other than Fish	Fish
(Laing et al., 1999)[1]	10-12 M	1994	4-day	54.7	28.3	20.4	42.2	145.5	-
	10-12 F	1994	4-day	37.0	34.9	24.5	41.4	137.8	-
	14-16 M	1994	4-day	88.4	44.4	47.9	83.0	263.7	-
	14-16 F	1994	4-day	44.8	33.6	31.3	50.0	159.7	-
(Baghurst, 1999)[2]	12-15 M	1995	24 hr recall	69		-	-	-	-
	12-15 F	1995	24 hr recall	52		-	-	-	-
	16-18 M	1995	24 hr recall	82		-	-	-	-
	16-18 F	1995	24 hr recall	52		-	-	-	-
(Cook et al., 2001)	10-15 M + F	1995	24 hr recall	-	-	-	-	123.0	16.0
(Clayton et al., 2008)[3]	12.6 M	2007	FFQ	26.9	13.6	17.4	46.0	103.9	12.8
	15.4 F	2007	FFQ	24.7	14.3	12.8	44.0	95.8	11.0

[1]Intake estimated using a 4-day weighed food record. Data extracted from Figure 2 from the consumption of individual meat sources over a 4 day period and estimating the proportion of participants eating each type of meat type, Beef = 92.1%, Lamb = 84.2%, Poultry = 97.4% and Pork = 81.6%.
[2]Data for "red meat" = beef + veal + lamb.
[3]Mean intake in child and adolescents aged between 8-18 years. F = Females, M = Males.
[4]FFQ = Food Frequency Questionnaire, Apparent = Apparent Consumption, calculated from local production + imports - exports, 24 hr recall = 24 hour dietary recall, 3-day = 3-day weighed food record; 4-day = 4-day weighed food record.

The Apparent Consumption of poultry increased while the consumption of red meat decreased in Australia over the 20 years from 1980 to 2000. The reported intake of fish in Australia has also increased steadily since 1980 (Figure 26). Apparent consumption figures have not been published by the ABS since 2000, which covered the period from 1998–1999, however, recent data can be estimated from production, import and export data for meat in Australia, but are not collated here.

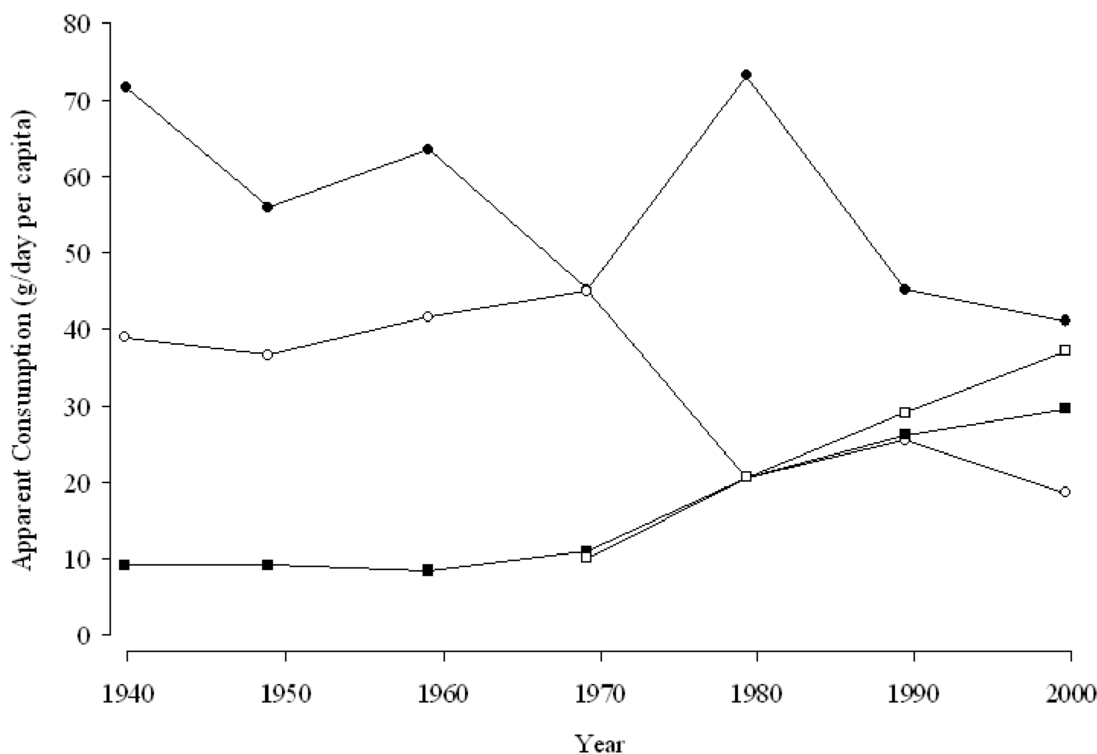


Figure 26. Apparent Consumption (g/day per capita) of beef (●) lamb (○), poultry (■) and fish (□) in Australia from 1939 to 2000. Data adapted from: ABS (2000) using dressing percentage from Cashel and Greenfield (1994).

9.3.2 Contribution of meat to LCn-3PUFA intake

Meat (other than fish) is an important contributor to LCn-3PUFA intake in the Australian population (Howe et al., 2006). Meat contributes approximately 42% of the total intake LCn-3PUFA in Australian adults (Howe et al., 2006) and 54.4% in children and adolescents (Table 37). The values reported for the Australian population are higher than those reported in the Illawarra region of NSW, where meat contributed approximately 30% (90 mg/day) of the total intake of LCn-3PUFA per day (Ollis et al., 1999). It is clear that red meat (including beef and lamb) contributes significantly to the total intake of LCn-3PUFA in the Australian diet, particularly, when the intake of DPA is considered.

Table 37. Contribution of meat (%) to total reported intake of LCn-3PUFA in adults or children and adolescents in the Australian population.

	Proportion of Total LCn-3PUFA Intake from Meat (%)				
Study	Beef	Lamb	Poultry	Pork	Total
(Ollis et al., 1999)[1]	-	-	-		30.0
(Howe et al., 2006)[2]	22.3	5.9	10.0	3.9	42.1
(Clayton et al., 2008)[3]	20.2	11.4	17.4	5.5	54.4

[1]Adults 29–66 years of age.
[2]Adults 19–64 years of age.
[3]Children and adolescents 8-18 years of age. Intake percentage adjusted for socio-economic status (SES) as a co-variate in the analysis.

Meat (particularly red meat) is high in DPAn-3 and the importance of DPAn-3 in relation to human health is becoming increasingly studied. Concentrations of DPAn-3 are also high in seal meat and oil. The Greenland Inuits, who formed the basis of epidemiological research into fish and omega-3 intake being associated with a reduced incidence of CVD (Bang et al., 1980), actually ate a diet high in seal and, therefore, high in DPAn-3 rather than EPA or DHA alone (Howe et al., 2007).

9.4 Biological markers of LCn-3PUFA intake

The success of clinical studies examining the ability of different food sources to alter LCn-3PUFA status is determined, to a large extent, by the correct selection of biological indicators of LCn-3PUFA intake. These intake markers are compared prior to and following dietary intervention, or are correlated with disease incidence. There are several markers for LCn-3PUFA intake including blood and tissue measures.

The concentration of fatty acids in plasma is related to dietary fatty acid intake over a short time period (approximately 24–48 hr, Katan et al., 1991; Katan et al., 1997), while plasma phospholipid concentration represents fatty acid intake during the past 1–2 weeks (Sztern and Harris, 1991). Red blood cell membrane (RBC) concentration reflects intake in the previous 14–120 days (Brown et al., 1991; Pala et al., 2001) and adipose tissue concentration is related to intake over the previous 1–2 years (Marckmann et al., 1995; Katan et al., 1997).

Concentrations of EPA and DHA in RBC are strongly correlated with long-term fatty acid intake. For example, reported intake of fish (Mina et al., 2007) and LCn-3PUFA (Sullivan et al., 2005) is positively correlated with RBC percentage EPA (spearman rho = 0.47 and 0.33, respectively) and DHA (spearman rho = 0.33 and 0.40, respectively) in Australian adults. Reported intakes of EPA and DHA were also significantly related to RBC concentrations of EPA ($r = 0.59$, $p = 0.026$) and DHA ($r = 0.64$, $p = 0.013$) in Australian children and adolescents (Clayton et al., 2008). Similar relationships have been reported for adults in Japan (Kuriki et al., 2006; Kuriki et al., 2007) and Sweden (Wirfalt et al., 2004).

The omega-3 index refers to RBC membrane EPA% + DHA% and may be an indicator of the risk of morbidity and mortality from CVD (Harris, 2007). At present, an omega-3 index of 0–4% is considered a 'high risk' for CVD, an index of 4–8% is considered a 'medium risk', while an index >8% is considered a 'low risk' for CVD. Presently, DPAn-3 is not included in this index as a risk factor for CVD. As indicated previously, several recommendations for intake of LCn-3PUFA currently include EPA, DPA and DHA, although research linking DPAn-3 to a reduced risk of CVD is presently lacking. There is a significant push from some groups to include DPAn-3 in dietary guidelines, as this will mean many cuts of meat would be considered a 'good source' of omega-3 in Australia.

10. Altering Consumer Intake of LCn-3PUFA by Enhancing Animal Products

Few studies have examined the effect the consumption of animal products with differing LCn-3PUFA concentrations has on disease incidence. The intake of LCn-3PUFA and systemic fatty acid concentrations have been increased by manipulating the concentration of LCn-3PUFA in food, however, these changes have not always been associated with a reduced risk of disease.

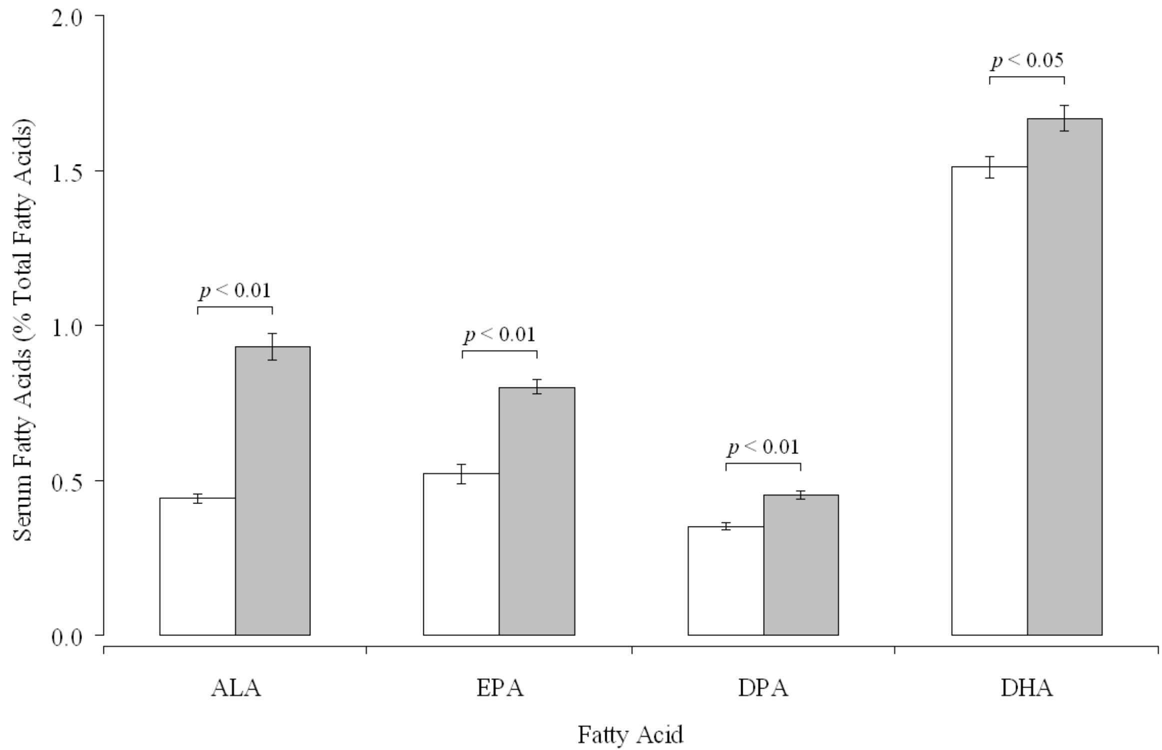
The concentration of ALA, but not EPA and DHA, was increased in milk, pork, chicken meat and eggs when animals were fed a basal diet of maize, wheat and barley with the addition of linseed (dairy cows = 1000 g/day, pigs = 62 g/day, layer hens = 13 g/day and broiler chickens = 4 g/day) compared with the basal diet alone (Weill et al., 2002). The concentration of ALA and LCn-3PUFA was increased in serum and the concentration of ALA, EPA and DPA was increased in RBC when participants consumed food from animals fed the omega-3 enriched diet compared with the un-enriched diet (Figure 27). The concentration of cholesterol and serum triglycerides was not significantly affected by dietary intake, however, indicating some risk factors of CVD were not modified by enriching food with omega-3 from ALA.

In an Australian study, participants consumed a range of foods enriched with LCn-3PUFA during the manufacturing process, including biscuits, bread, cheese, chocolate and other items manufactured by Goodman Fielder, Maritex, Denmark and Pace Farm Pty Ltd (Murphy et al., 2007). Foods were intended to contain 125 mg EPA + DHA per serve, but analyses indicated the content was extremely variable, between 13–150 mg/serve. Dietary intake was assessed using a combination of a diet history and a 3-day weighed food record (Patch et al., 2005).

The reported intake of EPA + DHA increased significantly when participants consumed the LCn-3PUFA enriched compared with un-enriched foods (Table 38). The concentration of EPA, DPAn-3 and DHA in RBC also increased significantly following the consumption of foods enriched with LCn-3PUFA (Table 39). RBC membrane fatty acids were not correlated to intake pre-supplementation, but were related to intake following supplementation, most likely due to the larger range in values observed. No risk factors associated with CVD measured during the study were altered following the intake of omega-3 enriched foods (Murphy et al., 2007).

The proportion of DHA, but not EPA, in RBC membranes was increased when healthy men and women consumed 1000 g/week of pork enriched with omega-3. The omega-3 content of pork was increased by incorporating PorcOmega^{TM} into a basal diet of barley grain (Coates et al., 2009). The concentration of triacylglycerols (TAG) in serum decreased following the consumption of omega-3 enriched pork for 12 weeks compared with un-enriched pork (Coates et al., 2009) indicating one of the possible risk factors for CVD may have been reduced. The study is very difficult to interpret, however, as the concentration of TAG was higher (1.1 ± 0.1 versus 0.79 ± 0.08 mmol/L) at baseline prior to the consumption of omega-3 enriched pork, even though the authors state the difference between groups was not statistically significant (Coates et al., 2009). The analysis only reported the change in serum TAG from baseline (baseline = 0), whereas, a more powerful analysis would have determined the change in actual concentrations of TAG over time using baseline values as a co-variate in the analysis. More importantly, the authors did not present results for the total estimated intake of fatty acids by study participants either prior to, or following, dietary intervention. It is impossible to determine, therefore, whether the results observed were due to the dietary treatments imposed or some other difference in dietary intake between groups.

A



B

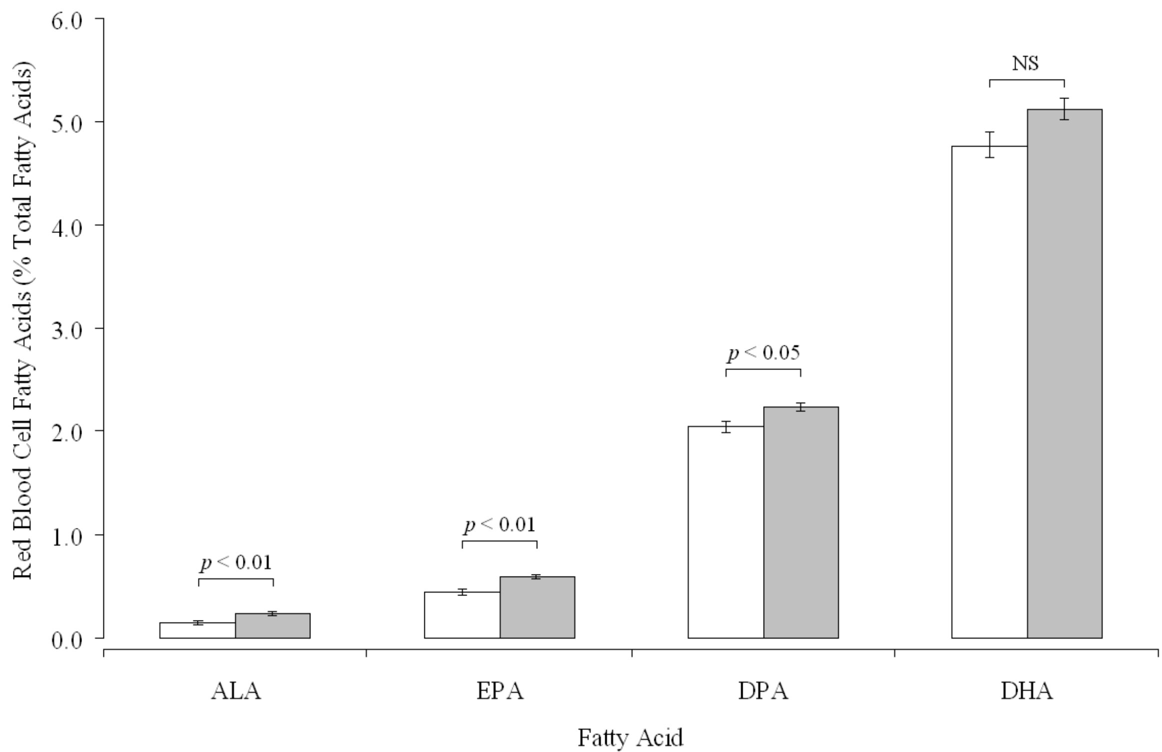


Figure 27. Serum (A) or RBC (B) fatty acids (% total fatty acids) for participants prior to (unshaded bars) and following (shaded bars) the consumption of milk, pork, chicken and eggs enriched with LCn-3PUFA containing a total of 110 or 250 mg/day LCn-3PUFA. Data are means ± standard error of the means adapted from: Weill et al. (2002).

Table 38. Reported mean daily intakes of LCn-3PUFA (mg/day) prior to and following the consumption of a range of food products enriched with LCn-3PUFA.

Group	Reported Intake of LCn-3PUFA (mg/day)		
	Baseline	3 months	6 months
Control	150 ± 40	190 ± 40	110 ± 30
Enriched	200 ± 40	1060 ± 40	960 ± 110

Data adapted from: Murphy et al. (2007).

Table 39. Red blood cell (RBC) membrane LCn-3PUFA (% total fatty acids) prior to and following the consumption of a range of food products enriched with LCn-3PUFA.

Fatty Acid	Group	Fatty Acid Proportion (% total fatty acids)		
		Baseline	3 months	6 months
EPA	Control	0.61 ± 0.04	0.64 ± 0.03	0.63 ± 0.03
	Enriched	0.55 ± 0.04	1.00† ± 0.05	1.20† ± 0.06
DPA	Control	2.0 ± 0.11	2.2 ± 0.07	2.2 ± 0.10
	Enriched	2.0 ± 0.12	2.2 ± 0.08	2.3 ± 0.08
DHA	Control	3.8 ± 0.23	4.1 ± 0.13	3.9 ± 0.20
	Enriched	3.4 ± 0.04	5.3† ± 0.05	5.9† ± 0.06

†Denotes significantly different from baseline using repeated measures ANOVA analysis.
Data adapted from: Murphy et al. (2007).

10.1 Increasing LCn-3PUFA intake by the consumption of meat other than fish

As seen in previous sections, it is possible to increase the intake of LCn-3PUFA by consuming foods enriched (or fortified) with EPA and DHA. There is, however, significant interest in being able to increase intakes of LCn-3PUFA from whole, unfortified foods, such as meat. Meat from animals fed forage is significantly higher in LCn-3PUFA than animals fed grain (see Section 4.3). The intake of LCn-3PUFA from meat and the contribution to recommended intakes can, therefore, be estimated for meat from different production systems (De Henauw et al., 2007). The estimated intake of LCn-3PUFA from meat may increase from 26 to 54 mg/day if the concentration of LCn-3PUFA in meat is maximised by the incorporation of forages into production feeding diets (Table 40).

Table 40. Reported intake of meat in Australia and estimated intake of LCn-3PUFA from meat if animals are fed predominantly grain or forage diets.

Meat[1]	Intake[2] (g/day)	LCn-3PUFA content (mg/100 g)[3]		LCn-3PUFA intake (mg/day)		% Daily NRV[4]	
		Grain	Forage	Grain	Forage	Grain	Forage
Beef	41.1	14.0	37.8	5.75	15.54	3.60	9.71
Lamb	18.7	35.5	73.6	6.64	13.76	4.15	8.60
Pork	29.7	40.1	69.0	11.91	20.49	7.44	12.81
Poultry	37.1	4.5	10.90	1.67	4.04	1.04	2.53
Total	126.6	-	-	25.97	53.84	16.23	33.65

[1]Beef = beef + veal, lamb = lamb + mutton. Includes mixed dishes
[2]Intake calculated from Apparent Consumption (ABS, 2000) adjusted for carcase yield (Cashel and Greenfield, 1994).
[3]LCn-3PUFA content of meat from data reviewed previously (Table 14).
[4]Percentage of recommended daily intake (AI) of LCn-3PUFA for adult males according to the Australian and New Zealand Nutrient Reference Values (NRV, NHMRC and Health, 2006).

11. Summary and Recommendations

The analysis and interpretation of the lipid and fatty acid composition of plants and animal tissue is complicated. A sound understanding of the principles of analysis and the interpretation of data is required in order to determine the effects of different feeding systems on fatty acid concentrations in ruminants. A significant amount of research has examined the effects of animal diets on LCn-3PUFA concentrations in meat and, in general, animals fed forage diets have higher concentrations of LCn-3PUFA in meat than animals fed concentrates.

There are a wide range of concentrations of lipid and omega-3 in different forages and the concentration is dependent on a range of factors, in particular, stage of growth. Little information is available, however, on the omega-3 concentration of forages grown in Australia and the factors that alter these concentrations. A survey of pasture and feeding systems in Australia would allow producers to select forages or production systems that may maximise the amount of LCn-3PUFA available in meat.

In Australia, the effects of different forage conservation practices on both the concentrations of LCn-3PUFA precursors in forage and the availability and metabolism of these precursors in ruminants for the production of LCn-3PUFA in meat is largely unknown. An understanding of these factors may allow meat to be produced with a higher concentration of LCn-3PUFA. Differences in the concentration and availability of LCn-3PUFA in the phospholipid and free fatty acid lipid fractions of plant material between fresh forage and silage should be examined in order to determine the possible role of silage in production feeding rations. Differences in biohydrogenation and loss of fatty acids in the rumen between different forage types and, between forages conserved using different methods, should also be examined.

The intake of meat other than fish is an important contributor to LCn-3PUFA intake in the Australian population. If the concentration of LCn-3PUFA in meat can be maximised through alterations in the production systems employed, then Australian beef and lamb may be considered an excellent source of LCn-3PUFA and may have the potential to contribute significantly to a healthy diet. Meat from animals fed either fresh forage or silage should be compared with meat from grain feeding systems in order to determine more accurately the potential health benefits in humans from the consumption of these meat products. Until researchers can provide evidence there is a significant measurable decrease in the risk of disease following the consumption of meat with higher LCn-3PUFA, a premium price will not be offered for this meat. Lowest-cost production systems will, therefore, be likely to remain in place. Consumers may, therefore, select meat based on factors such as taste, juiciness or tenderness, or on cost alone, which may lead to the selection of meat from grain-fed rather than pasture or forage-based systems.

12. References

ABARE and MAF (2006). "Agricultural Economies of Australia and New Zealand: Past, Present and Future" Australian Bureau of Agricultural and Resource Economics, Canberra.

Abayasekara DR and Wathes DC (1999). Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility. *Prostaglandins Leukotrienes and Essential Fatty Acids* **61 (5):** 275-287.

ABS (1998). "National Nutrition Survey nutrient intakes and physical measurements Australia" Australian Bureau of Statistics, Australian Government Publishing Services, Canberra.

ABS (2000). "Apparent Consumption of Foodstuffs" Australian Bureau of Statistics, Canberra.

AFIA (2006). Determination of crude fat (ether extract). *AFIA Laboratory Methods Manual* **Method 3.1D (Version 1):** 87-90.

Alfaia CMM, Ribeiro VSS, Lourenco MRA, Quaresma MAG, Martins SIV, Portugal A, Fontes C, Bessa RJB, Castro MLF and Prates JAM (2006). Fatty acid composition, conjugated linoleic acid isomers and cholesterol in beef from crossbred bullocks intensively produced and from Alentejana purebred bullocks reared according to Carnalentejana-PDO specifications. *Meat Science* **72 (3):** 425-436.

Al-Mabruk RM, Beck NF and Dewhurst RJ (2004). Effects of silage species and supplemental vitamin E on the oxidative stability of milk. *Journal of Dairy Science* **87 (2):** 406-412.

Anderson BA (1976). Comprehensive evaluation of fatty acids in foods. VII. Pork products. *Journal of the American Dietetic Association* **69 (1):** 44-49.

Anderson BA, Kinsella JA and Watt BK (1975). Comprehensive evaluation of fatty acids in foods. II. Beef products. *Journal of the American Dietetic Association* **67 (1):** 35-41.

Annett RW, Carson AF and Dawson LER (2008). Effects of digestible undegradable protein (DUP) supply and fish oil supplementation of ewes during late pregnancy on colostrum production and lamb output. *Animal Feed Science and Technology* **146 (3-4):** 270-288.

Annett RW, Dawson LER, Edgar H and Carson AF (2009). Effects of source and level of fish oil supplementation in late pregnancy on feed intake, colostrum production and lamb output of ewes. *Animal Feed Science and Technology* **154 (3-4):** 169-182.

AOAC (1990). Method 920.39 - Crude Fat. *In* "Official Methods of Analysis of AOAC International". Association of Official Analytical Chemists, Arlington, VA.

AOAC (2003). Method 954.02 - Fat (Crude) or Ether Extract in Pet Food. *In* "Official Methods of Analysis of AOAC International" (W Horwitz, ed.), pp. 33. Association of Official Analytical Chemists, Arlington, VA.

AOAC (2006). "Official Methods of Analysis of AOAC International" 18th/Ed. Association of Official Analytical Chemists, Gaithersburg, Maryland.

AOCS (1998). "Official Methods and Recommended Practices of the American Oil Chemists Society" Fifth/Ed. American Oil Chemists' Society, Washington DC.

Aurousseau B, Bauchart D, Calichon E, Micol D and Priolo A (2004). Effect of grass or concentrate feeding systems and rate of growth on triglyceride and phospholipid and their fatty acids in the *M. Longissimus thoracis* of lambs. *Meat Science* **66 (3):** 531-541.

Aurousseau B, Bauchart D, Faure X, Galot AL, Prache S, Micol D and Priolo A (2007). Indoor fattening of lambs raised on pasture: (1) Influence of stall finishing duration of lipid classes and fatty acids in the *Longissimus thoracis* muscle. *Meat Science* **76 (2):** 241-252.

Baghurst K (1999). Red meat consumption in Australia: intakes, contributions to nutrient intake and associated dietary patterns. *European Journal of Cancer Prevention* **8 (3):** 185-191.

Bang HO, Dyerberg J and Sinclair HM (1980). The composition of the Eskimo food in north western Greenland. *American Journal of Clinical Nutrition* **33 (12):** 2657-2661.

Bauchart D, Verite R and Remond B (1984). Long-chain fatty acid digestion in lactating cows fed fresh grass from spring to autumn. *Canadian Journal of Animal Science* **64 (Suppl 1):** 330-331.

Bligh EG and Dyer WJ (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37 (8):** 911-917.

Boufaied H, Chouinard PY, Tremblay GF, Petit HV, Michaud R and Belanger G (2003a). Fatty acids in forages. I. Factors affecting concentrations. *Canadian Journal of Animal Science* **83 (3):** 501-511.

Boufaied H, Chouinard PY, Tremblay GF, Petit HV, Michaud R and Belanger G (2003b). Fatty acids in forages. II. *In vitro* ruminal biohydrogenation of linolenic and linoleic acids from timothy. *Canadian Journal of Animal Science* **83 (3):** 513-522.

Brown AJ, Pang E and Roberts DC (1991). Persistent changes in the fatty acid composition of erythrocyte membranes after moderate intake of n-3 polyunsaturated fatty acids: study design implications. *American Journal of Clinical Nutrition* **54 (4):** 668-673.

Buccioni A, Decandia M, Minieri S, Molle G and Cabiddu A (2012). Lipid metabolism in the rumen: New insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. *Animal Feed Science and Technology* **174 (1-2):** 1-25.

Buckley DJ, Gray JI, Asghar A, Price JF, Crackel RL, Booren AM, Pearson AM and Miller ER (1989). Effects of dietary antioxidants and oxidized oil on membranal lipid stability and pork product quality. *Journal of Food Science* **54 (5):** 1193-1197.

Caldari-Torres C, Rodriguez-Sallaberry C, Greene ES and Badinga L (2006). Differential effects of n-3 and n-6 fatty acids on prostaglandin $F_{2\alpha}$ production by bovine endometrial cells. *Journal of Dairy Science* **89 (3):** 971-977.

Capper JL, Wilkinson RG, Mackenzie AM and Sinclair LA (2006). Polyunsaturated fatty acid supplementation during pregnancy alters neonatal behavior in sheep. *Journal of Nutrition* **136 (2):** 397-403.

Cashel KM, English R, Bennett S, Berzins J, Brown GH and Magnus P (1986). "National Dietary Survey of Adults. Report No. 1. Foods Consumed" Australian Government Publishing Service, Canberra.

Cashel KM and Greenfield H (1994). Principal sources of dietary fat in Australia: evidence from apparent consumption data and the national dietary survey of adults. *British Journal of Nutrition* **71 (5):** 753-773.

Cheng Z, Robinson RS, Pushpakumara PG, Mansbridge RJ and Wathes DC (2001). Effect of dietary polyunsaturated fatty acids on uterine prostaglandin synthesis in the cow. *Journal of Endocrinology* **171 (3):** 463-473.

Cheng Z, Elmes M, Kirkup SE, Chin EC, Abayasekara DR and Wathes DC (2005a). The effect of a diet supplemented with the n-6 polyunsaturated fatty acid linoleic acid on prostaglandin production in early- and late-pregnant ewes. *Journal of Endocrinology* **184 (1):** 165-178.

Cheng ZG, Abayasekara DRE and Wathes DC (2005b). The effect of supplementation with n-6 polyunsaturated fatty acids on 1-2-and 3-series prostaglandin F production by ovine uterine epithelial cells. *Biochimica Et Biophysica Acta - Molecular and Cell Biology of Lipids* **1736 (2):** 128-135.

Choi NJ, Enser M, Wood JD and Scollan ND (2000). Effect of breed on the deposition in beef muscle and adipose tissue of dietary n-3 polyunsaturated fatty acids. *Animal Science* **71:** 509-519.

Chow TT, Fievez V, Moloney AP, Raes K, Demeyer D and De Smet S (2004). Effect of fish oil on *in vitro* rumen lipolysis, apparent biohydrogenation of linoleic and linolenic acid and accumulation of biohydrogenation intermediates. *Animal Feed Science and Technology* **117:** 1-12.

Christie WW (1982). "Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids." 2nd Ed. Pergamon Press, Oxford.

Christie WW (2003). "Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids." 3rd Ed. The Oily Press, Bridgwater, England.

Clayton EH, Hanstock TL, Garg ML and Hazell PL (2007). Long-chain omega-3 polyunsaturated fatty acids in the treatment of psychiatric illnesses in children and adolescents. *Acta Neuropsychiatrica* **19 (2):** 92-103.

Clayton EH, Hanstock TL, Kable CJ, Hirneth SJ, Garg ML and Hazell PL (2008). Long-chain omega-3 polyunsaturated fatty acids in the blood of children and adolescents with juvenile bipolar disorder. *Lipids* **43 (11):** 1031-1038.

Clayton EH, Hanstock TL, Kable CJ, Hirneth SJ, Garg ML and Hazell PL (2009a). Reduced mania and depression in juvenile bipolar disorder associated with long-chain omega-3 polyunsaturated fatty acid supplementation. *European Journal of Clinical Nutrition* **63 (8):** 1037-1040.

Clayton EH, Hanstock TL and Watson JF (2009b). Estimated intakes of meat and fish by children and adolescents in Australia and comparison with recommendations. *British Journal of Nutrition* **101 (12):** 1731-1735.

Clayton EH, Gulliver CE, Piltz JW, Taylor RD, Blake RJ and Meyer RJ (2012a). Improved extraction of saturated fatty acids but not omega-3 fatty acids from sheep red blood cells using a one-step extraction procedure. *Lipids* **47 (7):** 719-727.

Clayton EH, Gulliver CE, Wilkins JF, King BJ, Meyer RJ and Friend MA (2012b). Increasing the proportion of female lambs by supplementary feeding oats high in omega-6 fatty acids at joining. *In* "Proceedings of the 27th Annual Conference of the Grassland Society of NSW Inc." Wagga Wagga, NSW, pp. 107-113.

Clayton EH, Lamb TA, Refshauge G, Kerr MJ, Bailes KL, Friend MA and Hopkins DL (2014). Differential response to an algae supplement high in DHA mediated by maternal peri-conceptional diet - Intergenerational effects of omega-6 fatty acids. *Lipids* **49 (8):** 767-775.

Coates AM, Sioutis S, Buckley JD and Howe PRC (2009). Regular consumption of n-3 fatty acid-enriched pork modifies cardiovascular risk factors. *British Journal of Nutrition* **101 (4):** 592-597.

Cook T, Rutishauser IHE and Allsopp R (2001). "The Bridging Study - Comparing results from the 1983, 1985 and 1995 Australian National Nutrition Surveys" Commonwealth of Australia, Canberra.

Cordain L, Eaton SB, Miller JB, Mann N and Hill K (2002a). The paradoxical nature of hunter-gatherer diets: meat-based, yet non-atherogenic. *European Journal of Clinical Nutrition* **56 (Suppl 1):** S42-S52.

Cordain L, Watkins BA, Florant GL, Kelher M, Rogers L and Li Y (2002b). Fatty acid analysis of wild ruminant tissues: evolutionary implications for reducing diet-related chronic disease. *European Journal of Clinical Nutrition* **56 (3):** 181-191.

Coyne GS, Kenny DA, Childs S, Sreenan JM and Waters SM (2008). Dietary n-3 polyunsaturated fatty acids alter the expression of genes involved in prostaglandin biosynthesis in the bovine uterus. *Theriogenology* **70 (5):** 772-782.

Dannenberger D, Nuernberg G, Scollan N, Schabbel W, Steinhart H, Ender K and Nuernberg K (2004). Effect of diet on the deposition of n-3 fatty acids, conjugated linoleic and C18:1trans fatty acid isomers in muscle lipids of German Holstein bulls. *Journal of Agricultural and Food Chemistry* **52 (21):** 6607-6615.

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. *Journal of Agricultural and Food Chemistry* **54 (1):** 182-188.

Dannenberger D, Nuernberg G, Scollan N, Ender K and Nuernberg K (2007). Diet alters the fatty acid composition of individual phospholipid classes in beef muscle. *Journal of Agricultural and Food Chemistry* **55 (2):** 452-460.

De Henauw S, Van Camp J, Sturtewagen G, Matthys C, Bilau M, Warnants N, Raes K, Van Oeckel M and De Srnet S (2007). Simulated changes in fatty acid intake in humans through n-3 fatty acid enrichment of foods from animal origin. *Journal of the Science of Food and Agriculture* **87 (2):** 200-211.

De Smet S, Raes K and Demeyer D (2004). Meat fatty acid composition as affected by fatness and genetic factors: a review. *Animal Research* **53:** 81-98.

Demeyer D and Doreau M (1999). Targets and procedures for altering ruminant meat and milk lipids. *Proceedings of the Nutrition Society* **58 (3):** 593-607.

Dewhurst RJ and King PJ (1998). Effects of extended wilting, shading and chemical additives on the fatty acids in laboratory grass silages. *Grass and Forage Science* **53 (3):** 219-224.

Dewhurst RJ, Moorby JM, Dhanoa MS, Evans RT and Fisher WJ (2000). Effects of altering energy and protein supply to dairy cows during the dry period. 1. Intake, body condition, and milk production. *Journal of Dairy Science* **83 (8):** 1782-1794.

Dewhurst RJ, Moorby JM, Scollan N, Tweed JK and Humphreys MO (2002). Effects of a stay-green trait on the concentrations and stability of fatty acids in perennial ryegrass. *Grass and Forage Science* **57 (4):** 360-366.

Dewhurst RJ, Evans RT, Scollan ND, Moorby JM, Merry RJ and Wilkins RJ (2003a). Comparison of grass and legume silages for milk production. 2. *In vivo* and *in sacco* evaluations of rumen function. *Journal of Dairy Science* **86 (8):** 2612-2621.

Dewhurst RJ, Fisher WJ, Tweed JK and Wilkins RJ (2003b). Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. *Journal of Dairy Science* **86 (8):** 2598-2611.

Dewhurst RJ, Scollan ND, Lee MR, Ougham HJ and Humphreys MO (2003c). Forage breeding and management to increase the beneficial fatty acid content of ruminant products. *Proceedings of the Nutrition Society* **62 (2):** 329-336.

Dewhurst RJ, Shingfield KJ, Lee MRF and Scollan ND (2006). Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology* **131 (3-4):** 168-206.

Doreau M and Chilliard Y (1997). Digestion and metabolism of dietary fat in farm animals. *British Journal of Nutrition* **78 (Suppl 1):** S15-S35.

Dozier BL, Watanabe K and Duffy DM (2008). Two pathways for prostaglandin $F_{2\alpha}$ synthesis by the primate periovulatory follicle. *Reproduction* **136 (1):** 53-63.

Droulez V, Williams P, Levy G, Stobaus T and Sinclair AJ (2006). Composition of Australian red meat 2002 2. Fatty acid profile. *Food Australia* **58 (7):** 335-341.

Duckett SK, Wagner DG, Yates LD, Dolezal HG and May SG (1993). Effects of time on feed on beef nutrient composition. *Journal of Animal Science* **71 (8):** 2079-2088.

Elgersma A, Ellen G, van der Horst H, Muuse BG, Boer H and Tamminga S (2003). Comparison of the fatty acid composition of fresh and ensiled perennial ryegrass (*Lolium perenne* L.), affected by cultivar and regrowth interval. *Animal Feed Science and Technology* **108 (1-4):** 191-205.

English R, Cashel KM, Lewis JL, Bennett S, Berzins J, Waters A and Magnus P (1988). "National Dietary Survey of Schoolchildren Aged 10-15 Years. Report No. 1. Foods Consumed" Australian Government Publishing Service, Canberra.

Enser M, Hallett K, Hewitt B, Fursey GAJ and Wood JD (1996). Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science* **42 (4):** 443-456.

Enser M, Hallett KG, Hewett B, Fursey GAJ, Wood JD 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 Science* **49 (3):** 329-341.

Eriksson SF and Pickova J (2007). Fatty acids and tocopherol levels in M. *Longissimus dorsi* of beef cattle in Sweden - A comparison between seasonal diets. *Meat Science* **76 (4):** 746-754.

Farquharson J, Cockburn F, Patrick WA, Jamieson EC and Logan RW (1992). Infant cerebral cortex phospholipid fatty-acid composition and diet. *Lancet* **340 (8823):** 810-813.

Folch J, Lees M and Sloane Stanley GH (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226 (1):** 497-509.

Fortier MA, Krishnaswamy K, Danyod G, Boucher-Kovalik S and Chapdelaine P (2008). A postgenomic integratred view of prostaglandins in reproduction: implications for, other body systems. *Journal of Physiology and Pharmacology* **59 (Suppl 1):** 65-89.

Fountain ED, Mao J, Whyte JJ, Mueller KE, Ellersieck MR, Will MJ, Roberts RM, Macdonald R and Rosenfeld CS (2008). Effects of diets enriched in omega-3 and omega-6 polyunsaturated fatty acids on offspring sex-ratio and maternal behavior in mice. *Biology of Reproduction* **78 (2):** 211-217.

French P, Stanton C, Lawless F, O'Riordan EG, Monahan FJ, Caffrey PJ and Moloney AP (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* **78 (11):** 2849-2855.

FSANZ (2000). Amendment 53 to the Food Standards Code: Part 1.2.8 Div 3: Conditions for Making Certain Nutrition Claims, 13-Claims in Relation to Omega- Fatty Acid Content. Vol. P 30, pp. 68. Commonwealth of Australia Gazette, Food Standards Australia New Zealand, Canberra.

FSANZ (2002). Standard 1.2.8 Nutrition Information Requirements. *In* "Australia New Zealand Food Standards Code", Vol. 2, pp. 68. Food Standards Australia New Zealand, Information Australia, Canberra.

FSANZ (2006). "NUTTAB 2006. Online Database of the Nutritional Composition of Australian Foods" Food Standards Australia New Zealand, Canberra.

Funston RN (2004). Fat supplementation and reproduction in beef females. *Journal of Animal Science* **82 (E-Suppl):** E154-E161.

Garg ML, Wood LG, Singh H and Moughan PJ (2006). Means of delivering recommended levels of long chain n-3 polyunsaturated fatty acids in human diets. *Journal of Food Science* **71 (5):** R66-R71.

Gebauer SK, Psota TL, Harris WS and Kris-Etherton PM (2006). n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *American Journal of Clinical Nutrition* **83 (6 Suppl):** 1526S-1535S.

Gerson T, John A and Sinclair BR (1983). The effect of dietary N on *in vitro* lipolysis and fatty-acid hydrogenation in rumen digesta from sheep fed diets high in starch. *Journal of Agricultural Science* **101 (AUG):** 97-101.

Gerson T, John A and King ASD (1985). The effects of dietary starch and fiber on the *in vitro* rates of lipolysis and hydrogenation by sheep rumen digesta. *Journal of Agricultural Science* **105 (AUG):** 27-30.

Glasser F, Doreau M, Maxin G and Baumont R (2013). Fat and fatty acid content and composition of forages: A meta-analysis. *Animal Feed Science and Technology* **185 (1-2):** 19-34.

Green MP, Spate LD, Parks TE, Kimura K, Murphy CN, Williams JE, Kerley MS, Green JA, Keisler DH and Roberts RM (2008). Nutritional skewing of conceptus sex in sheep: effects of a maternal diet enriched in rumen-protected polyunsaturated fatty acids (PUFA). *Reproduction Biology and Endocrinology* **6 (1):** 21.

Gulliver CE, Friend MA, King BJ and Clayton EH (2012). The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. *Animal Reproduction Science* **131 (1-2):** 9-22.

Gulliver CE, Friend MA, King BJ, Robertson SM, Wilkins JF and Clayton EH (2013a). Increased prostaglandin response to oxytocin in ewes fed a diet high in omega-6 polyunsaturated fatty acids. *Lipids* **48 (2):** 177-183.

Gulliver CE, Friend MA, King BJ, Wilkins JF and Clayton EH (2013b). A higher proportion of female lambs when ewes were fed oats and cottonseed meal prior to and following conception. *Animal Production Science* **53 (5):** 464-471.

Harfoot CG, Noble RC and Moore JH (1973). Factors influencing the extent of biohydrogenation of linoleic acid by Rumen micro-organisms *in vitro*. *Journal of the Science of Food and Agriculture* **24 (8):** 961-970.

Harfoot CG and Hazlewood GP (1997). Lipid metabolism in the rumen. *In* "The Rumen Microbial Ecosystem" (PN Hobson and CS Stewart, eds.), pp. 382-426. Blackie, London.

Harris WS (2007). Omega-3 fatty acids and cardiovascular disease: a case for omega-3 index as a new risk factor. *Pharmacology Research* **55 (3):** 217-223.

Hertzman C, Göransson L and Rudérus H (1988). Influence of fishmeal, rape-seed, and rape-seed meal in feed on the fatty acid composition and storage stability of porcine body fat. *Meat Science* **23 (1):** 37-53.

Hess BW, Moss GE and Rule DC (2008). A decade of developments in the area of fat supplementation research with beef cattle and sheep. *Journal of Animal Science* **86 (14 Suppl):** E188-E204.

Hirashima F, Parow AM, Stoll AL, Demopulos CM, Damico KE, Rohan ML, Eskesen JG, Zuo CS, Cohen BM and Renshaw PF (2004). Omega-3 fatty acid treatment and t2 whole brain relaxation times in bipolar disorder. *American Journal of Psychiatry* **161 (10):** 1922-1924.

Hopkins DL, Clayton EH, Lamb TA, van de Ven RJ, Refshauge G, Kerr MJ, Bailes KL, Lewandowski P and Ponnampalam EN (2014). The impact of supplementing lambs with algae on growth, meat traits and oxidative status. *Meat Science* **98 (2):** 135-141.

Horrobin DF, Manku MS, Hillman H, Iain A and Glen M (1991). Fatty acid levels in the brains of schizophrenics and normal controls. *Biological Psychiatry* **30 (8):** 795-805.

Horrobin DF and Bennett CN (1999). Depression and bipolar disorder: relationships to impaired fatty acid and phospholipid metabolism and to diabetes, cardiovascular disease, immunological abnormalities, cancer, ageing and osteoporosis. Possible candidate genes. *Prostaglandins Leukotrienes and Essential Fatty Acids* **60 (4):** 217-234.

Howe P, Buckley DJ and Meyer B (2007). Long-chain omega-3 fatty acids in red meat. *Nutrition and Dietetics* **64 (Suppl 4):** S135-S139.

Howe P, Meyer B, Record S and Baghurst K (2006). Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. *Nutrition* **22 (1):** 47-53.

Howe PR, Downing JA, Grenyer BF, Grigonis-Deane EM and Bryden WL (2002). Tuna fishmeal as a source of DHA for n-3 PUFA enrichment of pork, chicken, and eggs. *Lipids* **37 (11):** 1067-1076.

Innis SM (2003). Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. *Journal of Pediatrics* **143 (4 Suppl):** S1-S8.

Ip C, Banni S, Angioni E, Carta G, McGinley J, Thompson HJ, Barbano D and Bauman D (1999). Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *Journal of Nutrition* **129 (12):** 2135-2142.

ISO (1999). "Animal Feeding Stuffs - Determination of Fat Content" International Organisation for Standardization, Geneve, Switzerland.

Itoh M, Johnson CB, Cosgrove GP, Muir PD and Purchas RW (1999). Intramuscular fatty acid composition of neutral and polar lipids for heavy-weight Angus and Simmental steers finished on pasture or grain. *Journal of the Science of Food and Agriculture* **79 (6):** 821-827.

Kaluzny MA, Duncan LA, Merritt MV and Epps DE (1985). Rapid separation of lipid classes in high yield and purity using bonded phase columns. *Journal of Lipid Research* **26 (1):** 135-140.

Katan MB, van Birgelen AP, Deslypere JP, Penders M and van Staveren WA (1991). Biological markers of dietary intake, with emphasis on fatty acids. *Annals of Nutrition and Metabolism* **35 (5):** 249-252.

Katan MB, Deslypere JP, van Birgelen AP, Penders M and Zegwaard M (1997). Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *Journal of Lipid Research* **38 (10):** 2012 - 2022.

Kepler CR and Tove SB (1967). Biohydrogenation of unsaturated fatty acids. 3. Purification and properties of a linoleate delta-12-cis, delta-11-trans-isomerase from *Butyrivibrio fibrisolvens*. *Journal of Biological Chemistry* **242 (24):** 5686-5692.

Khan NA, Cone JW, Fievez V and Hendriks WH (2012). Causes of variation in fatty acid content and composition in grass and maize silages. *Animal Feed Science and Technology* **174 (1-2):** 36-45.

Kim HY and Salem N, Jr. (1990). Separation of lipid classes by solid phase extraction. *Journal of Lipid Research* **31 (12):** 2285-2289.

Kimura K, Spate LD, Green MP and Roberts RM (2005). Effects of D-glucose concentration, D-fructose, and inhibitors of enzymes of the pentose phosphate pathway on the development and sex ratio of bovine blastocysts. *Molecular Reproduction and Development* **72 (2):** 201-207.

Kitessa SM and Young P (2009). Echium oil is better than rapeseed oil in enriching poultry meat with n-3 polyunsaturated fatty acids, including eicosapentaenoic acid and docosapentaenoic acid. *British Journal of Nutrition* **101 (5):** 709-715.

Knight TW, Knowles S and Death AF (2003). Factors affecting the variation in fatty acid concentrations in lean beef from grass-fed cattle in New Zealand and the implications for human health. *New Zealand Journal of Agricultural Research* **46 (2):** 83-95.

Kris-Etherton PM, Harris WS and Appel LJ (2003). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology* **23 (2):** e20-30.

Kronberg SL, Barcelo-Coblijn G, Shin J, Lee K and Murphy EJ (2006). Bovine muscle n-3 fatty acid content is increased with flaxseed feeding. *Lipids* **41 (11):** 1059-1068.

Kuriki K, Wakai K, Hirose K, Matsuo K, Ito H, Suzuki T, Saito T, Kanemitsu Y, Hirai T, Kato T, Tatematsu M and Tajima K (2006). Risk of colorectal cancer is linked to erythrocyte compositions of fatty acids as biomarkers for dietary intakes of fish, fat, and fatty acids. *Cancer Epidemiology Biomarkers and Prevention* **15 (10):** 1791-1798.

Kuriki K, Hirose K, Wakai K, Matsuo K, Ito H, Suzuki T, Hiraki A, Saito T, Iwata H, Tatematsu M and Tajima K (2007). Breast cancer risk and erythrocyte compositions of n-3 highly unsaturated fatty acids in Japanese. *International Journal of Cancer* **121 (2):** 377-385.

Laborde FL, Mandell IB, Tosh JJ, Buchanan-Smith JG and Wilton JW (2002). Effect of management strategy on growth performance, carcass characteristics, fatty acid composition, and palatability attributes in crossbred steers. *Canadian Journal of Animal Science* **82 (1):** 49-57.

Laing DG, Oram N, Burgess J, Ram PR, Moore G, Rose G, Hutchinson I and Skurray GR (1999). The development of meat-eating habits during childhood in Australia. *International Journal of Food Sciences and Nutrition* **50 (1):** 29-37.

Lands WE (1992). Biochemistry and physiology of n-3 fatty acids. *FASEB Journal* **6 (8):** 2530-2536.

Leaf A and Weber PC (1987). A new era for science in nutrition. *American Journal of Clinical Nutrition* **45 (5 Suppl):** 1048-1053.

Lee MR, Harris LJ, Dewhurst RJ, Merry RJ and Scollan N (2003). The effect of clover silages on long chain fatty acid rumen transformations and digestion in beef steers. *Animal Science* **76 (3):** 491-501.

Lee MR, Winters AL, Scollan ND, Dewhurst RJ, Theodorou MK and Minchin FR (2004). Plant-mediated lipolysis and proteolysis in red clover with different polyphenol oxidase activities. *Journal of the Science of Food and Agriculture* **84 (13):** 1693-1645.

Lee MR, Tweed JK, Dewhurst RJ and Scollan ND (2006). Effect of forage:concentrate ratio on ruminal metabolism and duodenal flow of fatty acids in beef steers. *Animal Science* **82 (1):** 31-40.

Lepage G and Roy CC (1986). Direct transesterification of all classes of lipids in a one-step reaction. *Journal of Lipid Research* **27 (1):** 114-120.

Li D, Ng A, Mann NJ and Sinclair AJ (1998). Contribution of meat fat to dietary arachidonic acid. *Lipids* **33 (4):** 437-440.

Lough AK and Anderson LJ (1973). Effect of ensilage on the lipids of pasture grasses. *Proceedings of the Nutrition Society* **32 (2):** 61A-62A.

Mach N, Devant M, Diaz I, Font-Furnols M, Oliver MA, Garcia JA and Bach A (2006). Increasing the amount of n-3 fatty acid in meat from young Holstein bulls through nutrition. *Journal of Animal Science* **84 (11):** 3039-3048.

MacLaren LA, Guzeloglu A, Michel F and Thatcher WW (2006). Peroxisome proliferator-activated receptor (PPAR) expression in cultured bovine endometrial cells and response to omega-3 fatty acid, growth hormone and agonist stimulation in relation to series 2 prostaglandin production. *Domestic Animal Endocrinology* **30 (3):** 155-169.

MAFF (2001). "The National Food Survey" Ministry of Agriculture, Fisheries and Food, The Stationery Office, London.

Mann N (2007). Meat in the human diet: An anthropological perspective. *Nutrition and Dietetics* **64 (Suppl 4):** S102-S107.

Mann NJ, Johnson LG, Warrick GE and Sinclair AJ (1995). The arachidonic acid content of the Australian diet is lower than previously estimated. *Journal of Nutrition* **125 (10):** 2528-2535.

Mann NJ, Sinclair AJ, Percival P, Lewis JL, Meyer BJ and Howe PRC (2003). Development of a database of fatty acids in Australian foods. *Nutrition and Dietetics* **60 (1):** 42-45.

Marckmann P, Lassen A, Haraldsdottir J and Sandstrom B (1995). Biomarkers of habitual fish intake in adipose tissue. *American Journal of Clinical Nutrition* **62 (5):** 956-969.

Marmer WN, Maxwell RJ and Williams JE (1984). Effects of dietary regimen and tissue site on bovine fatty-acid profiles. *Journal of Animal Science* **59 (1):** 109-121.

May SG, Dolezal HG, Gill DR, Ray FK and Buchanan DS (1992). Effect of days fed, carcass grade traits, and subcutaneous fat removal on postmortem muscle characteristics and beef palatability. *Journal of Animal Science* **70 (2):** 444-453.

McGee M (2005). Recent developments in feeding beef cattle on grass silage-based diets. *In* "Silage Production and Utilisation. Proceedings of the XIVth International Silage Conference, a satellite workshop of the XXth International Grassland Congress" (RS Park and MD Stronge, eds.), Wageningen Academic Publishers, Belfast, Northern Ireland, pp. 51-64.

McLennan W and Podger A (1999a). "National Nutrition Survey - Foods Eaten, Australia" Australian Bureau of Statistics, Commonwealth Department of Health and Aged Care, Canberra.

McLennan W and Podger A (1999b). "National Nutrition Survey - Users' Guide" Australian Bureau of Statistics, Commonwealth Department of Health and Aged Care, Canberra.

McNamara RK and Carlson SE (2006). Role of omega-3 fatty acids in brain development and function: Potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins Leukotrienes and Essential Fatty Acids* **75 (4-5):** 329-349.

McRae T and Thomas B (2013). "Australian Cattle - Industry Projections" Meat and Livestock Australia, North Sydney.

Meyer BJ, Tsivis E, Howe PRC, Tapsell LC and Calvert GD (1999). Polyunsaturated fatty acid content of foods: differentiating between long and short chain omega-3 fatty acids. *Nutrition Reviews* **51 (3):** 82-95.

Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair AJ and Howe PRC (2003). Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. *Lipids* **38 (4):** 391-398.

Mina K, Fritschi L and Knuiman M (2007). A valid semiquantitative food frequency questionnaire to measure fish consumption. *European Journal of Clinical Nutrition* **61 (8):** 1023-1031.

Mirnikjoo B, Brown SW, Seung Kim HF, Marangell LB, Sweatt JD and Weeber EJ (2001). Protein kinase inhibition by w-3 fatty acids. *Journal of Biological Chemistry* **276 (14):** 10888 - 10896.

Moloney AP (2007). Enrichment of omega-3 fatty acids and CLA in beef by diet modification. *Irish Veterinary Journal* **60 (3):** 180-185.

Moore SA, Yoder E, Murphy S, Dutton GR and Spector AA (1991). Astrocytes, not neurons, produce docosahexaenoic acid (22:6 omega-3) and arachidonic acid (20:4 omega-6). *Journal of Neurochemistry* **56 (2):** 518-524.

Morrison WR, Mann DL, Soon W and Coventry AM (1975). Selective extraction and quantitative analysis of non-starch and starch lipids from wheat flour. *Journal of the Science of Food and Agriculture* **26 (4):** 507-521.

Muir PD, Deaker JM and Bown MD (1998). Effects of forage- and grain-based feeding systems on beef quality: A review. *New Zealand Journal of Agricultural Research* **41 (4):** 623-635.

Murphy RC and Gijon MA (2007). Biosynthesis and metabolism of leukotrienes. *Biochemical Journal* **405:** 379-395.

Murphy KJ, Meyer BJ, Mori TA, Burke V, Mansour J, Patch CS, Tapsell LC, Noakes M, Clifton PA, Barden A, Puddey IB, Beilin LJ and Howe PR (2007). Impact of foods enriched with n-3 long-chain polyunsaturated fatty acids on erythrocyte n-3 levels and cardiovascular risk factors. *British Journal of Nutrition* **97 (4):** 749-757.

NHMRC and Health NZMo (2006). "Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes" National Health and Medical Research Council, Commonwealth of Australia, Canberra.

Nilzen V, Babol J, Dutta PC, Lundeheim N, Enfalt AC and Lundstrom K (2001). Free range rearing of pigs with access to pasture grazing - effect on fatty acid composition and lipid oxidation products. *Meat Science* **58 (3):** 267-275.

Noble RC, Moore JH and Harfoot CG (1974). Observations on the pattern on biohydrogenation of esterified and unesterified linoleic acid in the rumen. *British Journal of Nutrition* **31 (1):** 99-108.

Noci F, O'Kiely P, Monahan FJ, Stanton C and Moloney AP (2005). Conjugated linoleic acid concentration in m. longissimus dorsi from heifers offered sunflower oil-based concentrates and conserved forages. *Meat Science* **69 (3):** 509-518.

Noci F, Monahan FJ, Scollan ND and Moloney AP (2007). The fatty acid composition of muscle and adipose tissue of steers offered unwilted or wilted grass silage supplemented with sunflower oil and fishoil. *British Journal of Nutrition* **97 (3):** 502-513.

Nowak R and Poindron P (2006). From birth to colostrum: early steps leading to lamb survival. *Reproduction Nutrition Development* **46 (4):** 431-446.

Nuernberg K, Nuernberg G, Ender K, Lorenz S, Winkler K, Rickert R and Steinhart H (2002). N-3 fatty acids and conjugated linoleic acids of longissimus muscle in beef cattle. *European Journal of Lipid Science and Technology* **104 (8):** 463-471.

Nuernberg K, Dannenberger D, Nuernberg G, Ender K, Voigt J, Scollan ND, Wood JD, Nute GR and Richardson RI (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 Production Science* **94 (1-2):** 137-147.

Oddy WH, Sherriff JL, Kendall GE, de Klerk NH, Mori TA, Blake KV and Beilin LJ (2004). Patterns of fish consumption and levels of serum phospholipid very-long-chain omega-3 fatty acids in children with and without asthma, living in Perth, Western Australia. *Nutrition and Dietetics* **61 (1):** 30-37.

Ollis TE, Meyer BJ and Howe PR (1999). Australian food sources and intakes of omega-6 and omega-3 polyunsaturated fatty acids. *Annals of Nutrition and Metabolism* **43 (6):** 346-355.

Pala V, Krogh V, Muti P, Chajes V, Riboli E, Micheli A, Saadatian M, Sieri S and Berrino F (2001). Erythrocyte membrane fatty acids and subsequent breast cancer: a prospective Italian study. *Journal of the National Cancer Institute* **93 (14):** 1088-1095.

Parker G, Gibson NA, Brotchie H, Heruc G, Rees AM and Hadzi-Pavlovic D (2006). Omega-3 fatty acids and mood disorders. *American Journal of Psychiatry* **163 (6):** 969-978.

Patch CS, Tapsell LC, Mori TA, Meyer BJ, Murphy KJ, Mansour J, Noakes M, Clifton PM, Puddey IB, Beilin LJ, Annison G and Howe PR (2005). The use of novel foods enriched with long-chain n-3 fatty acids to increase dietary intake: a comparison of methodologies assessing nutrient intake. *Journal of the American Dietetic Association* **105 (12):** 1918-1926.

Peet M and Stokes C (2005). Omega-3 fatty acids in the treatment of psychiatric disorders. *Drugs* **65 (8):** 1051-1059.

Ponnampalam EN, Sinclair AJ, Egan AR, Blakeley SJ, Li D and Leury BJ (2001a). Effect of dietary modification of muscle long-chain n-3 fatty acid on plasma insulin and lipid metabolites, carcass traits, and fat deposition in lambs. *Journal of Animal Science* **79 (4):** 895-903.

Ponnampalam EN, Sinclairt AJ, Egan AR, Blakeley SJ and Leury BJ (2001b). Effect of diets containing n-3 fatty acids on muscle long-chain n-3 fatty acid content in lambs fed low- and medium-quality roughage diets. *Journal of Animal Science* **79 (3):** 698-706.

Ponnampalam EN, Sinclair AJ, Egan AR, Ferrier GR and Leury BJ (2002a). Dietary manipulation of muscle long-chain omega-3 and omega-6 fatty acids and sensory properties of lamb meat. *Meat Science* **60 (2):** 125-132.

Ponnampalam EN, Sinclair AJ, Hosking BJ and Egan AR (2002b). Effects of dietary lipid type on muscle fatty acid composition, carcass leanness, and meat toughness in lambs. *Journal of Animal Science* **80 (3):** 628-636.

Ponnampalam EN, Mann NJ and Sinclair AJ (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 Pacific Journal of Clinical Nutrition* **15 (1):** 21-29.

Ponnampalam EN, Warner RD, Kitessa S, McDonagh MB, Pethick DW, Allen D and Hopkins DL (2010). Influence of finishing systems and sampling site on fatty acid composition and retail shelf-life of lamb. *Animal Production Science* **50 (8):** 775-781.

Ponnampalam EN, Burnett VF, Norng S, Warner RD and Jacobs JL (2012). Vitamin E and fatty acid content of lamb meat from perennial pasture or annual pasture systems with supplements. *Animal Production Science* **52 (4):** 255-262.

Purchas RW, Knight TW and Busboom JR (2005). The effect of production system and age on concentrations of fatty acids in intramuscular fat of the *Longissumus* and *Triceps brachii* muscles on Angus-cross heifers. *Meat Science* **70 (4):** 597-603.

Raes K, Balcaen A, Dirinck P, De Winne A, Claeys E, Demeyer D and De Smet S (2003a). Meat quality, fatty acid composition and flavour analysis in Belgian retail beef. *Meat Science* **65 (4):** 1237-1246.

Raes K, De Smet S, Balcaen A, Claeys E and Demeyer D (2003b). Effect of diets rich in n-3 polyunsatured fatty acids on muscle lipids and fatty acids in Belgian Blue double-muscled young bulls. *Reproduction Nutrition Development* **43 (4):** 331-345.

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. *Animal Feed Science and Technology* **113:** 199-221.

Randall EL (1974). Improved method for fat and oil analysis by a new process of extraction. *Journal of the Association of Official Analytical Chemists* **57 (5):** 1165-1168.

Razminowicz RH, Kreuzer M and Scheeder MRL (2006). Quality of retail beef from two grass-based production systems in comparison with conventional beef. *Meat Science* **73 (2):** 351-361.

Rosenfeld CS, Grimm KM, Livingston KA, Brokman AM, Lamberson WE and Roberts RM (2003). Striking variation in the sex ratio of pups born to mice according to whether maternal diet is high in fat or carbohydrate. *Proceedings of the National Academy of Sciences of the USA* **100 (8):** 4628-4632.

Rule DC, Broughton KS, Shellito SM and Maiorano G (2002). Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. *Journal of Animal Science* **80 (5):** 1202-1211.

Santos JEP, Bilby TR, Thatcher WW, Staples CR and Silvestre FT (2008). Long chain fatty acids of diet as factors influencing reproduction in cattle. *Reproduction in Domestic Animals* **43 (1):** 23-30.

Sastry PS (1985). Lipids of nervous tissue: composition and metabolism. *Progress in Lipid Research* **24 (2):** 69-176.

Scollan ND, Choi NJ, Kurt E, Fisher AV, Enser M and Wood JD (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *British Journal of Nutrition* **85 (1):** 115-124.

Scollan ND, Enser M, Gulati SK, Richardson I and Wood JD (2003). Effects of including a ruminally protected lipid supplement in the diet on the fatty acid composition of beef muscle. *British Journal of Nutrition* **90 (3):** 709-716.

Scollan N, Hocquette J-F, Nuernberg K, Dannenberger D, Richardson I and Moloney AP (2006). Innovations in beef production systems that enhance the nutritional health value of beef lipids and their relationship with meat quality. *Meat Science* **74 (1):** 17-33.

Scollan ND, Dannenberger D, Nuernberg K, Richardson I, MacKintosh S, Hocquette JF and Moloney AP (2014). Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science* **97 (3):** 384-394.

Seung Kim HF, Weeber EJ, Sweatt JD, Stoll AL and Marangell LB (2001). Inhibitory effects of omega-3 fatty acids on protein kinase C activity *in vitro*. *Molecular Psychiatry* **6 (2):** 246-248.

Shingfield KJ, Salo-Vaananen P, Pahkala E, Toivonen V, Jaakkola S, Piironen V and Huhtanen P (2005). Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk. *Journal of Dairy Research* **72 (3):** 349-361.

Shrapnel B and Baghurst K (2007). Lack of nutritional equivalence in the 'meats and alternatives' group of the Australian guide to healthy eating. *Nutrition and Dietetics* **64 (4):** 254-260.

Simopoulos AP (1991). Omega-3 fatty acids in health and disease and in growth and development. *American Journal of Clinical Nutrition* **54 (3):** 438-463.

Simopoulos AP (1999). Essential fatty acids in health and chronic disease. *American Journal of Clinical Nutrition* **70 (3 Suppl):** S560-S569.

Sinclair AJ, Slattery WJ and O'Dea K (1982). The analysis of polyunsaturated fatty acids in meat by capillary gas-liquid chromatography. *Journal of the Science of Food and Agriculture* **33:** 771-776.

Sinclair AJ and O'Dea K (1993). The significance of arachidonic acid in hunter-gatherer diets: Implications for the contemporary Western diet. *Journal of Food Lipids* **1:** 143-157.

Sinclair AJ, Johnson L, O'Dea K and Holman RT (1994). Diets rich in lean beef increase arachidonic acid and long-chain omega 3 polyunsaturated fatty acid levels in plasma phospholipids. *Lipids* **29 (5):** 337-343.

Smith WL, Marnett LJ and Dewitt DL (1991). Prostaglandin and thromboxane biosynthesis. *Pharmacology & Therapeutics* **49 (3):** 153-179.

Smith A, Kellett E and Schmerlaib Y (1998). The Australian Guide to Healthy Eating: Background Information for Nutrition Educators. *In* "Publication No. 2361". Commonwealth Department of Health and Family Services, Canberra.

Staples CR, Burke JM and Thatcher WW (1998). Influence of supplemental fats on reproductive tissues and performance of lactating cows. *Journal of Dairy Science* **81 (3):** 856-871.

Steen RWJ, Kilpatrick DJ and Porter MG (2002). Effects of the proportions of high or medium digestibility grass silage and concentrates in the diet of beef cattle on leveweight gain, carcass composition and fatty acid composition of muscle. *Grass and Forage Science* **57 (3):** 279-291.

Stoll AL, Locke CA, Marangell LB and Severus WE (1999). Omega-3 fatty acids in bipolar disorder: a review. *Prostaglandins Leukotrienes and Essential Fatty Acids* **60 (5&6):** 329-337.

Sturmey RG, Reis A, Leese HJ and McEvoy TG (2009). Role of fatty acids in energy provision during oocyte maturation and early embryo development. *Reproduction in Domestic Animals* **44:** 50-58.

Sukhija PS and Palmquist DL (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *Journal of Agricultural and Food Chemistry* **36 (6):** 1202-1206.

Sullivan B, Brown J, Williams PG and Meyer BJ (2005). A valid and reproducible food frequency questionnaire to estimate long chain omega-3 polyunsaturated fatty acid intakes. *Asia Pacific Journal of Clinical Nutrition* **14 (Suppl 1):** S56.

Sztern MI and Harris WS (1991). Short-term effects of fish oil on human plasma lipid levels. *Journal of Nutritional Biochemistry* **2 (5):** 255-259.

Tappia PS, Ladha S, Clark DC and Grimble RF (1997). The influence of membrane fluidity, TNF receptor binding, cAMP production and GTPase activity on macrophage cytokine production in rats fed a variety of fat diets. *Molecular and Cellular Biochemistry* **166 (1-2):** 135-143.

Thiex NJ, Anderson S and Gildemeister B (2003). Crude fat, hexanes extraction, in feed, cereal grain, and forage (Randall/Soxtec/submersion method): Collaborative study. *Journal of AOAC International* **86 (5):** 899-908.

Thomas PC, Robertson S, Chamberlain DG, Livinstone RM, Garthwaite PH, Dewey PJS, Smart R and Whyte C (1988). Prediction of the metabolisable energy (ME) content of compounded feeds for ruminants. *In* "Recent Advances in Animal Nutrition" (W Haresign and DJA Cole, eds.), pp. 127-146. Butterworths, London, UK.

Thomason D (2007). Production practices for red meat in Australia. *Nutrition and Dietetics* **64 (Suppl 4):** S192-S195.

Ulijaszek S, Mann N and Elton S (2012). "Evolving Human Nutrition" Cambridge University Press, New York.

van Nevel CJ and Demeyer DI (1996). Effect of pH on biohydrogenation of polyunsaturated fatty acids and their Ca-salts by rumen microorganisms *in vitro*. *Archives of Animal Nutrition* **49:** 151-157.

van Nevel CJ and Demeyer DI (1996). Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro. *Reproduction Nutrition Development* **36 (1):** 53-63.

Van Ranst G, Fievez V, De Riek J and van Bockstaele E (2009). Influence of ensiling forages at different dry matters and silage additives on lipid metabolism and fatty acid composition. *Animal Feed Science and Technology* **150 (1-2):** 62-74.

Vaneslow B (2007). The loaves and the fishes. The modern day human dietary imbalance of omega-3 and omega-6 fatty acids and the input from animal production methods. *In* "NSW DPI Beef Conference", NSW Department of Primary Industries, Hawks Nest.

Varela A, Oliete B, Moreno T, Portela C, Monserrrat L, Carballo JA and Sanchez L (2004). Effect of pasture finishing on the meat characteristics and intramuscular fatty acid profile of steers of the Rubia Gallega breed. *Meat Science* **67 (3):** 515-522.

Wang N and Anderson RE (1993). Synthesis of docosahexaenoic acid by retina and retinal pigment epithelium. *Biochemistry* **32 (49):** 13703-13709.

Watanabe K (2002). Prostaglandin F synthase. *Prostaglandins & Other Lipid Mediators* **68 (9):** 401-407.

Wathes DC, Abayasekara DRE and Aitken RJ (2007). Polyunsaturated fatty acids in male and female reproduction. *Biology of Reproduction* **77 (2):** 190-201.

Weihrauch JL, Posati LP, Anderson BA and Exler J (1977). Lipid conversion factors for calculating fatty acid contents of foods. *Journal of the American Oil Chemists Society* **54 (1):** 36-40.

Weill P, Schmitt B, Chesneau G, Daniel N, Safraou F and Legrand P (2002). Effects of introducing linseed in livestock diet on blood fatty acid composition of consumers of animal products. *Annals of Nutrition and Metabolism* **46 (5):** 182-191.

Whiting CM, Mutsvangwa T, Walton JP, Cant JP and McBride BW (2004). Effects of feeding either fresh alfalfa or alfalfa silage on milk fatty acid content in Holstein dairy cows. *Animal Feed Science and Technology* **113 (1-4):** 27-37.

Wilkinson SJ, Downing JA, Thomson PC and Newman RE (2014). Dietary fatty acids affect the growth, body composition and performance of post-weaning gilt progeny. *Animal Production Science* **In Press:** Accepted for Publication 14 May 2013.

Willatts P and Forsyth JS (2000). The role of long-chain polyunsaturated fatty acids in infant cognitive development. *Prostaglandins Leukotrienes and Essential Fatty Acids* **63 (1-2):** 95-100.

Williams JE, Wagner DG, Walters LE, Horn GW, Waller GR, Sims PL and Guenther JJ (1983). Effect of production systems on performance, body-composition and lipid and mineral profiles of soft-tissue in cattle. *Journal of Animal Science* **57 (4):** 1020-1028.

Williams P (2007). Nutritional composition of red meat. *Nutrition and Dietetics* **64 (Suppl 4):** S113-S119.

Wirfalt E, Vessby B, Mattisson I, Gullberg B, Olsson H and Berglund G (2004). No relations between breast cancer risk and fatty acids of erythrocyte membranes in postmenopausal women of the Malmo Diet Cancer cohort (Sweden). *European Journal of Clinical Nutrition* **58 (5):** 761-770.

Witkowska IM, Wever C, Gort G and Elgersma A (2008). Effects of nitrogen rate and regrowth interval on perennial ryegrass fatty acid content during the growing season. *Agronomy Journal* **100 (5):** 1371-1379.

Wood JD and Enser M (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition* **78 (Suppl 1):** S49-S60.

Wood JD, Enser M, Fisher AV, Nute GR, Richardson RI and Sheard PR (1999). Manipulating meat quality and composition. *Proceedings of the Nutrition Society* **58 (2):** 363-370.

Woods VB, Forbes EGA, Easson DL and Fearon AM (2005). "Dietary sources of unsaturated fatty acids for animals and their subsequent availability in milk, meat and eggs." Occasional Publication No. 4, May 2005. Agri-Food and Biosciences Institute: Global Research Unit, AFBI Hillsborough, Hillsborough, Northern Ireland, pp. 94.

Wythes J, Arthur R, Dodt R and Shorthose W (1988). Cattle handling at abattoirs. II. The effects of rest in transit and duration of the resting period before slaughter on carcass weight, bruising and muscle properties. *Australian Journal of Agricultural Research* **39 (1):** 97-107.

APPENDIX I - Nomenclature and common names for fatty acids of interest in animal and human nutrition

Fatty Acid	Scientific Name	Common Name
SFA		
C8:0	octanoic acid	Caprylic
C9:0	nonanoic acid	Pelargonic
C10:0	decanoic acid	Capric
C11:0	undecanoic acid	Undecanic
C12:0	dodecanoic acid	Lauric acid
C14:0	tetradecanoic acid	Myristic acid
iC15:0	*iso*-pentadecanoic acid	-
aiC15:0	*anteiso*-pentadecanoic acid	-
C15:0	pentadecanoic acid	Pentadecylic acid
C16:0	hexadecanoic acid	Palmitic acid
iC17:0	*iso*-heptadecanoic acid	-
aiC17:0	*anteiso*-heptadecanoic acid	-
C17:0	heptadecanoic acid	Margaric (daturic)
C18:0	octadecanoic acid	Stearic acid
C20:0	eicosanoic acid	Arachidic
C21:0	heneicosanoic acid	Heneicosylic acid
C22:0	docosanoic acd	Behenic
C23:0	tricosanoic acid	Tricosylic acid
C24:0	tetracosanoic acid	Lignoceric acid
MUFA		
C11:1n-1	10-undecanoic	Undecylenic
C12:1n-7	5-lauroleic acid	Lauroleic acid
C13:1n-1	12-tridecenoic acid	-
C14:1n-5	9-tetradecaenoic acid	Myristoleic acid
C15:1n-5	cis-10-pentadecenoic acid	Pentadecanoic
C16:1n-7t	9-hexadecaenoic acid (*trans*)	Palmitelaidic acid
C16:1n-7	9-hexadecaenoic acid (*cis*)	Palmitoleic acid
C17:1n-7	cis-10-heptadecenoic acid	-
C18:1n-9t	9-octadecanoic acid (*trans*)	Trans Elaidic acid
C18:1n-7t	11-octadecanoic acid (*trans*)	Trans Vaccenic acid
C18:1n-12	cis-6-octadecenoic acid	Petroselenic acid
C18:1n-9	9-octadecanoic acid (*cis*)	Oleic acid
C18:1n-7	11-octadecanoic acid (*cis*)	Vaccenic acid
C19:1n-12	7-nonadecanoic acid	-
C20:1n-15	5-eicosenoic acid	Eicosenoic acid
C20:1n-12	8-eicosenoic acid	Eicosenoic acid
C20:1n-9	11-eicosenoic acid	Gondoic acid
C22:1n-9	13-docosaenoic acid	Erucic acid
C24:1n-9	15-tetracosaenoic acid	Nervonic acid

Fatty Acid	Scientific Name	Common Name
n-3 PUFA		
C18:3n-3	9,12,15-octadecatrienoic acid	α-linolenic **(ALA)**
C18:4n-3	6,9,12,15-octadecatetraenoic acid	Stearidonic acid **(SDA)**
C20:3n-3	11,14,17-eicosatrienoic acid	Eicosatrienoic acid **(ETA)**
C20:5n-3	5,8,11,14,17-eicosapentaenoic acid	Timnodonic acid **(EPA)**
C22:5n-3	7,10,13,16,19-docosapentaenoic acid	Clupanodonic acid **(DPA n-3)**
C22:6n-3	4,7,10,13,16,19-docosahexaenoic acid	**DHA**
n-6 PUFA		
C18:2n-6t	9,12-octadecadienoic acid (*trans*)	Trans Linolelaidic acid
C18:2n-6	9,12-octadecadienoic acid (*cis*)	Linoleic acid **(LA)**
C18:3n-6	6,9,12-octadecatrienoic acid	γ-Linolenic **(GLA)**
C20:2n-6	11,14-eicosadienoic acid	Eicosadienoic acid
C20:3n-6	8,11,14-eicosatrienoic acid	Dihomo-γ-linolenic acid **(DGLA)**
C20:4n-6	5,8,11,14-eicosatetraenoic acid	Arachidonic acid **(ARA)**
C22:4n-6	7,10,13,16-docosatetraenoic acid	Adrenic acid
C22:5n-6	4,7,10,13,16-docosapentaenoic acid	**DPA n-6**

All bonds are in the *cis* configuration unless otherwise stated. Sources: Wirfalt et al. (2004), http://www.lipomics.com/resources/fatty_acids/index.htm.