Genetic selection for health beneficial long-chain omega-3 fatty acids, intramuscular fat, and fat melting point in Australian White lambs

Thesis submitted by

Shedrach Benjamin Pewan

DVM (ABU Zaria, Nigeria), MSc (ATBU Bauchi, Nigeria)

For the degree of Doctor of Philosophy

College of Public Health, Medical and Veterinary Sciences

James Cook University

Queensland, Australia

November 2022

Acknowledgements

I dedicate this work to God Almighty, who, by His grace and mercies, made me consistent in my pursuit of academic excellence, making my dreams come to fruition. Secondly, to my late father (Mr Benjamin Pewan), whose philosophies and foresight, even in death, are crystallized in my siblings and I.

I thank everyone who has played a role in this journey: My most profound gratitude goes to my Primary Supervisor and Advisor Mentor, Professor Aduli E.O. Malau-Aduli. Thank you for your unconditional, timely, professional and emotional support throughout the period of my candidature here at JCU. Your unmatched kindness, wisdom and flexibility made the PhD journey safe and trouble-free. It is a rare privilege to have studied under your guidance. My appreciation also goes to Dr Robert T. Kinobe for all your support, sacrifices and timely responses to my manuscripts. Thank you for the time you shared your wealth of knowledge with me and for your efforts in sharpening my scientific writing skills. To Dr Oyelola A. Adegboye, I appreciate your patience and unmatched statistical knowledge and guidance with all the analyses of my research data, which made the output great. Thank you all for believing in me and pushing me beyond my perceived limits! I appreciate Professor Bunmi S. Malau-Aduli who always provided moral and emotional support, especially when my family was away.

I am deeply indebted to Professor Paul Wood AO FTSE, my IMNIS (Industry Mentoring Network in STEM) mentor, who increased my understanding of the Veterinary industry in Australia and extended my professional network beyond academia and Australia. I appreciate Dr Junaidu Maina, former Chief Veterinary Officer of Nigeria, for his timely advice.

I immensely thank Dr John Otto, who made my stay in Townsville stress-free. Those molecular genetics laboratory lessons will remain memorable in my mind. I equally thank my inseparable colleague, Dr Felista Mwangi, for sharing her knowledge and experiences with my family and I. I

appreciate my colleagues, especially Dr Quang Nguyen, Dr Bénédicte Suybeng, Dr Nnamdi Mgbemena, Dr Faith Alele, Dr Andrew Adamu, Dr God'spower Okoh and Belete Mesfine, who provided the needed support to settle down easily and quickly. I equally appreciate the following individuals who assisted in data collection: Dr Benjamin W. B. Holman, Dr Richard Edmunds, Dr Michelle L. E. Henry, Dr Roger Huerlimann, Dr Sandra I. Villamil and Dr Alyssa Budd.

I would like to immensely thank the following funding agencies that provided financial support for this research: Tattykeel Australian White Pty Ltd, Australian Commonwealth Innovation Connections Grant, Science Industry Endowment Fund Ross Metcalf Research Grant and the James Cook University Postgraduate Research Scholarship. My PhD research journey would have been impossible without these grants and a living stipend scholarship. I am most grateful and indebted to these generous funding bodies and industry partners.

I want to express my deepest gratitude to my family: My wife, Mrs Larai S. Pewan; my children, Maltibweh, Benjamin, Andati and Bwekul; my mother, Mrs Damaris Pewan; my siblings, Mrs Tarfena Bati, Mrs Alice Paul, Mr Jimmy Pewan, Mrs Dorcas James and Mrs Ulo Macham, for their encouragement, guidance and sacrifices. I wish to also express my deep appreciation to my in-law, Dr Shingil Bati, who has been a pillar of support in various dimensions. He was always available and encouraged me to pursue my dreams.

Finally, I thank my employer, the National Veterinary Research Institute, Vom, Nigeria, for approving my overseas study leave that enabled me to undertake this PhD degree research programme in Australia.

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Declaration

The use of animals and all procedures performed in this thesis were approved by the James Cook University Animal Ethics Committee (Permit No. A0015657) in compliance with the Australian Code for Care and Use of Animals for Scientific Purposes (Eighth edition, 2013).

To the best of my knowledge, this thesis contains no material which has been accepted for the award of a degree by the University or any other tertiary institution and contains no material previously published or written by any other person, except where due reference is made in the text of the thesis.

Statement of the Contribution of Others

Supervision

Professor Aduli E.O. Malau-Aduli, JCU CPHMVS

Dr Robert T. Kinobe, JCU CPHMVS

Dr. Oyelola A. Adegboye, JCU CPHMVS

Experimental Design and Editing Support

Professor Aduli E.O. Malau-Aduli, JCU CPHMVS

Dr Robert T. Kinobe, JCU CPHMVS

Dr. Oyelola A. Adegboye, JCU CPHMVS

Statistical Data Analysis and Laboratory Support

Dr. Oyelola A. Adegboye, JCU CPHMVS

Dr. John R. Otto, JCU CPHMVS

Financial support

Tattykeel Australian White Pty Ltd, The Australian Commonwealth Innovation Connections Research Grant, Science Industry Endowment Fund Ross Metcalf Research Grant, and the James Cook University Postgraduate Research Scholarship, Queensland, Australia.

Meatworks Data Collection Support

Dr John R. Otto, Dr Felista W. Mwangi, Dr Benjamin W. Holman, Dr Richard C. Edmunds,

Dr Michelle L. Henry, Dr Roger Huerlimann and Dr Alyssa Budd.

Abstract

Meat quality data are mostly obtained after slaughter, and by the time an informed decision on the genetic merit for meat quality is made, the animal is already dead. This makes selection decisions about the live animal too late. Carcass estimated breeding values as the next best alternative, present major precision problems due to low accuracy. This thesis reports for the first time, a targeted next-generation sequencing (NGS) of single nucleotide polymorphisms (SNP) of lipogenic genes in Tattykeel Australian White (TAW) sheep of the MARGRA lamb brand, utilizing an innovative and minimally invasive muscle biopsy sampling technique. The primary aim was to directly quantify the genetic worth of live lambs for health-beneficial omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), intramuscular fat (IMF), and fat melting point (FMP) primarily for enhancing meat eating quality. The secondary aim was to investigate the significance of any associations between identified SNP of lipogenic genes and n-3 LC-PUFA, IMF, and FMP, together with gene expression patterns, hence underpinning potential marker-assisted selection for meat eating quality traits in TAW MARGRA lambs.

The following hypotheses were tested:

a) Variation in healthy lamb eating quality would be a function of lamb gender and not its antioxidant status or inbreeding coefficient (IC), as an index of linebreeding.
b) The inclusion of n-3 LC-PUFA in the diet would improve productive performance, carcass characteristics, wholesale cut yields and meat quality traits in TAW MARGRA lambs.
c) Fortifying feedlot pellets with omega-3 oil would enhance the human health beneficial n-3 LC-PUFA composition of edible lamb muscle tissue and organs.
d) Significant associations exist between SNP of lipid metabolism genes and n-3 LC-PUFA, IMF, and FMP, underpinning potential marker-assisted selection for meat-eating quality traits in TAW lambs.

e) Dietary fortification with omega-3 oils influences the transcriptional expression of lipogenic genes in the *Longissimus thoracis et lumborum* muscle in TAW lambs.

To achieve these, five experiments were carried out:

1) Experiment 1: Meat eating quality parameters comprising IMF content, FMP, and n-3 LC-PUFA of the *Longissimus thoracis et lumborum* muscle of 147 TAW sheep fed on antioxidant-rich ryegrass pastures were evaluated in a purely grass-fed management system.

2) Experiment 2: This study was conducted in a lot-fed management-based system. It evaluated seventy-five TAW MARGRA lambs randomly assigned to three dietary treatments of 25 lambs each, and lot-fed as a cohort for 47 days in a completely randomized experimental design that included: (a) Control grain pellets without oil plus hay; (b) Omega-3 oil fortified grain pellets plus hay; and (c) Commercial whole grain pellets plus hay. Meat eating quality indices of IMF and FMP of the *Longissimus thoracis et lumborum* muscle, feedlot performance, carcass traits and commercial wholesale cuts of lambs in response to diet fortification with n-3 LC-PUFA were evaluated.

3) Experiment 3: Evaluated the post-slaughter fatty acid composition of the *Longissimus thoracis et lumborum* muscle, liver, kidney, and heart using the gas chromatography–mass spectrophotometry technique.

4) Experiment 4: Conducted a targeted NGS of stearoyl-CoA desaturase (SCD), fatty acid binding protein-4 (FABP4), and fatty acid synthase (FASN) lipogenic genes and their associated correlations with meat quality traits; and

5) Experiment 5: Investigated gene expression patterns in the *Longissimus thoracis et lumborum* muscle of TAW lambs supplemented with whole grain and omega-3 fortified diets.

The study findings demonstrated that:

1) IC was inconsequential in influencing antioxidant status, IMF, FMP and n-3 LC-PUFA in linebred and pasture-fed TAW lambs because the observed variation in individual fatty acids was mainly driven by gender differences between ewes and rams.
2) Feedlot performance, meat eating quality traits and commercial wholesale French rack cuts were further enhanced during feedlot finishing of TAW lambs through dietary supplementation with omega-3 oils.
3) The inclusion of omega-3 oil in feedlot diets of lambs enhanced the human health beneficial n-3 LC-PUFA profiles of edible muscle tissue and organs without compromising meat quality.
4) Significant associations between SNP of lipid metabolism genes and n-3 LC-PUFA, IMF, and FMP were detected, and
5) Transcriptomic analyses revealed a differentially expressed pattern of lipogenic genes with a pronounced down-regulation of the FABP4 and FASN genes in response to dietary fortification with omega-3 oil.

Taken together, these research findings provide novel insights into the shared genetic control of the FMP, IMF content, and health-beneficial n-3 LC-PUFA composition traits that are helpful in designing breeding strategies to genetically improve meat eating quality traits in TAW lambs while they are still alive. The identified SNP of these lipid metabolism genes can also be used for breed-specific identification and marker-assisted selection of TAW lambs for the high-end meat-eating quality market.

List of Publications from Thesis

Peer-reviewed Journal Papers with 2021 Impact Factor (IF)

1. **Pewan SB**, Otto JR, Kinobe TR, Adegboye OA, Malau-Aduli AEO 2022. Fortification of diets with omega-3 long-chain polyunsaturated fatty acids enhances feedlot performance, intramuscular fat content, fat melting point and carcass characteristics of Tattykeel Australian White MARGRA lambs. *Frontiers in Veterinary Science*, *Section Animal Nutrition and Metabolism*, 9: 933038. DOI: https://doi.org/10.3389/fvets.2022.933038 **(IF 3.471).**

2. **Pewan SB**, Otto JR, Kinobe TR, Adegboye OA, Malau-Aduli AEO 2021a. Nutritional enhancement of health beneficial omega-3 long-chain polyunsaturated fatty acids in the muscle, liver, kidney and heart of Tattykeel Australian White MARGRA lambs fed pellets fortified with omega-3 oil in a feedlot system. *Biology* 10(9): 912 DOI: https://doi.org/10.3390/biology10090912 **(IF 5.168).**

3. **Pewan SB**, Otto JR, Huerlimann R, Budd AM, Mwangi FW, Edmunds RC, Holman BWB, Henry MLE, Kinobe TR, Adegboye OA, Malau-Aduli AEO 2021b. Next Generation Sequencing of single nucleotide polymorphic DNA-markers in selecting for intramuscular fat, fat melting point, omega-3 long-chain polyunsaturated fatty acids and meat eating quality in Tattykeel Australian White MARGRA lamb. *Foods* 10(10): 2288 DOI: https://doi.org/10.3390/foods10102288 **(IF 5.561).**

4. **Pewan SB**, Otto JR, Kinobe TR, Adegboye OA, Malau-Aduli AEO 2020a. MARGRA lamb eating quality and human health-promoting omega-3 long-chain polyunsaturated fatty acid profiles of Tattykeel Australian White sheep: Linebreeding and gender effects. *Antioxidants* 9(11): 1118 DOI: https://doi.org/10.3390/antiox9111118 **(IF 7.675).**

5. **Pewan SB**, Otto JR, Huerlimann R, Budd AM, Mwangi FW, Edmunds RC, Holman BWB, Henry MLE, Kinobe TR, Adegboye OA, Malau-Aduli AEO 2020b. Genetics of omega-3 long-chain polyunsaturated fatty acid metabolism and meat eating quality in Tattykeel Australian White lambs. *Genes* 11(5): 587 DOI: https://doi.org/10.3390/genes11050587 **(IF 4.141).**

Submitted peer-reviewed journal manuscript under review

6. **Pewan SB**, Otto JR, Edmunds RC, Kinobe TR, Adegboye OA, Malau-Aduli AEO 2022. Differential expressions of FASN, SCD and FABP4 genes in the *Longissimus thoracis et lumborum* muscle of Tattykeel Australia White lambs in response to supplementation with omega-3 oil. Submitted to *Scientific Reports* **(IF 4.996).**

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List of Abbreviations

ADF = acid detergent fibre

ADFI = average daily feed intake

ADG = average daily gain

A-FABP or aP2 = adipocyte fatty acid binding proteins

ALA = alpha linolenic acid

ANOVA = analysis of variance

AOAC = Association of official Analytical Chemists

ARA = arachidonic acid

BCTRC = boneless, closely trimmed retail cuts

BFT = backfat thickness

BHT = butylated hydroxytoluene

BWT body wall thickness

CART = cocaine- and amphetamine-regulated transcript

CLA = conjugated linoleic acid

CP = crude protein

CSIRO = Commonwealth Scientific and Industrial Research Organization

DE = digestible energy

DGAT1 = diacylglycerol O-acyltransferase

DHA = docosahexaenoic acid

DM = dry matter

DMD = dry matter digestibility

DOMD = digestible organic matter

DPA = docosapentaenoic acid

EBV = estimated breeding values

EE = ether extract

EPA = eicosapentaenoic acid

FA = fatty acids

FABP 4 = fatty acid binding protein 4

FAME = fatty acid methyl esters

FAO = Food and Agriculture Organisation

FADS = fatty acid desaturase

FASN = fatty acid synthase

FCTP = Folin-Ciocalteu phenolics

FMP = fat melting point

FRAP = ferric reducing antioxidant power

FSANZ = Food Standards of Australia and New Zealand

GAE = gallic acid equivalent

GC-MS = gas chromatograph–mass spectrometric

gDNA = genomic deoxy ribonucleic acid

GEBV = genomic estimated breeding values

GLM = general linear model

HSCW = hot standard carcass weight

IC = inbreeding coefficient

IMF = intramuscular fat

IQR = interquartile range

LA = linoleic acid

LCF = lipid conversion factor

LMA = eye muscle area

LWT = liveweight

MANOVA = multivariate analysis of variance

ME = metabolisable energy

MLA = Meat & Livestock Australia

MUFA = monounsaturated fatty acids

n-3 LC-PUFA = omega-3 long-chain polyunsaturated fatty acids

NADPH = nicotinamide adenine dinucleotide phosphate

NCBI = National Center for Biotechnology Information

NDF = neutral detergent fibre

NGS = next-generation sequencing

OECD = Organisation for Economic Co-operation and Development

PD = Poll Dorset

PPARγ= peroxisome proliferator-activated receptor γ

PUFA = polyunsaturated fatty acids

QTL = quantitative trait loci

REA = ribeye area

GLM = General Linear Model

SAS = Statistical Analysis System

SCD = stearoyl-CoA desaturase

SFA = saturated fatty acids

SNP =- single nucleotide polymorphisms

SOD = superoxide dismutase

SREBP1 = sterol regulatory element-binding protein 1

TAW = Tattykeel Australian White

TDN = total digestible nutrients

TLC = thin layer chromatography

t-VA = trans-vaccenic acid

TX = Texel

UFA = unsaturated fatty acids

ΣMUFA = total monounsaturated fatty acids

Σn-3 PUFA= total omega-3 polyunsaturated fatty acids

Σn-6 PUFA = total omega-6 polyunsaturated fatty acids

ΣPUFA = total polyunsaturated fatty acids

ΣSFA = total saturated fatty acids.

Chapter 1: General Introduction

The Organisation for Economic Co-operation and Development (OECD) and the Food and Agriculture Organization (FAO) of the United Nations have projected global protein availability from sheep meat to increase by 16% in 2031, and that sheep meat will overtake beef as the third most consumed meat (FAO, 2021). Meat & Livestock Australia (MLA) (2021) predicted the growth of the Australian national sheep population to 74.4 million head in 2022; the highest since 2013. The 2022-23 forecast of the value of the Australian sheep meat exports is Au$4.4 billion (ABARES, 2022). These insights into the current status of the Australian sheep meat industry are reflective of high-level management of feed resources and consistently high quality standards across the major lamb producing areas of Australia.

Ruminants are essential in man's food chain because they convert forages and plant products that humans cannot digest into readily usable nutrient sources (Chand et al., 2022). These sources include high quality proteins from meat and milk, with an excellent complement of highly digestible essential amino acids (approximately 20 g/ 100g of lean meat), fats, vitamin B-complex (especially B2, B6 and B12), and micronutrients (especially iron, zinc, copper, selenium) (Bohrer, 2017; Anzani et al., 2020). The micro-minerals play essential roles in various body metabolic pathways and processes (Cabrera & Saadoun, 2014). Fats facilitate the digestion, absorption and assimilation of fat-soluble vitamins, comprising A, D, K and E (National Health and Medical Research Council, 2017).

Meat provides essential fatty acids and calorie-dense nutrients (Ashaye et al., 2011). However, Tocher et al. (2019) and other critics of fats in the human diet have advocated for a reduction in beef, pork and lamb consumption due to high levels of saturated fatty acids (SFA) and comparatively low omega-3 long-chain polyunsaturated fatty acid (n-3 LC-PUFA) contents. In contrast, other researchers like Tobias et al. (2015), have countered this line of thought in view of the highly significant roles that red meat and animal fats play in human

nutrition (Castillo, 2019) because polyunsaturated fatty acids (PUFA) are essential constituents of cell membranes and substrates for cell signalling processes (Burdge, 2019). Cholewski et al. (2018) defined n-3 LC-PUFA as a group of fatty acids with at least one double bond between the third and fourth carbon atoms from the methyl end, and include docosapentaenoic acid (DPA, 22:5n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). They are vital for optimal foetal and neonatal growth and development (Koletzko et al., 2008), exert anti-inflammatory, therapeutic and prophylactic effects (Ding et al., 2022) against various cardiovascular diseases (Ashaye et al., 2011), cancer (Larsson & Wolk, 2006), and diabetes (Aune et al., 2009). However, n-3 LC-PUFA cannot be synthesised by mammals and must be supplied in the diet.

Health-conscious consumers increasingly demand healthier, tastier and more nutritious meat, hence there is a continuous need for Australian lamb producers to be able to meet market specifications and demand for consistently high-quality lamb. The desirable meat eating qualities include, but not limited to, moderate intramuscular fat (IMF) content, soft and low fat melting point (FMP), high n-3 LC-PUFA, tenderness, juiciness and flavour. To be able to supply these meat eating qualities demanded by consumers and meet high-end market specifications, there must be flexibility in the operational management of the Australian grass-fed and lot-fed lamb production systems.

Lamb production in Australia is largely based on an extensive production system (Bruce et al., 2021). Whereas in New Zealand, sheep farming systems are predominantly pasture-based (Clemens and Babcock, 2004), a combination of both grass-fed and grain-fed sheep production system is used in Australia (Ponnampalam et al., 2014). Pasture-based lamb production is heavily reliant on rainfall and its yearly distribution pattern, temperature, soil type and nutrients, pasture species and plant density (Hejda et al., 2022). Animals raised solely on pasture tend to experience stunted growth during droughts due to deterioration in pasture

quality and nutritive value, hence the need for supplementation to achieve set targets of meeting the nutritional requirements for meat production (Su & Chen, 2020). Grain-fed lamb production on the other hand, is an intensive management system practiced in Australian feedlots that is sheltered from fluctuations in climatic and weather variables due to reliance on grains and supplements for finishing lambs at the required market weight.

The series of experiments reported in this thesis had an overarching objective of enhancing the genetic, nutritional, and sustainable production of grass-fed and lot-fed lamb with superior eating qualities and healthy omega-3 composition without compromising animal performance and welfare. Specific aims include the direct quantification of the genetic worth of live TAW lambs for health-beneficial n-3 LC-PUFA, IMF, FMP and antioxidant status using biopsy sampling technique; nutritional fortification of feedlot diets with omega-3 oil and impact on lamb growth, feed efficiency, carcass commercial cuts and lipogenic gene transcriptional expression patterns in the muscle tissue; the use of targeted next-generation sequencing (NGS) of single nucleotide polymorphisms (SNP) of lipogenic genes to unravel the underpinning mechanism and marker-assisted selection potential for meat eating quality traits in TAW lambs; and bioinformatic computation and statistical associations between identified lipogenic gene SNP and n-3 LC-PUFA, IMF and FMP.

Therefore, this thesis is structured into the following chapters:

Chapter 1: General Introduction

Chapter 2: Literature Review: This Chapter accessed, retrieved, synthesised and critically appraised the published literature on the Australian sheep industry, genetic management, contribution of n-3 LC-PUFA consumption to human health, the synthesis, metabolism and genetics of n-3 LC-PUFA and the influence of dietary fortification with n-3 LC-PUFA on meat eating quality, lamb health, productivity and quality of edible tissues. The review also identified existing knowledge gaps, hence research opportunities, in nutrition–genetics

interactions aimed at a greater understanding of the genetics of n-3 LC-PUFA, feedlot finishing performance, carcass traits and eating quality in the TAW sheep that informed the setting up of the experimental studies reported in Chapters 3 to 7.

Chapter 3: Evaluated the *Longissimus thoracis et lumborum* muscle of 147 TAW sheep fed on antioxidant-rich ryegrass pastures for meat eating quality parameters IMF, FMP and n-3 LC-PUFA. The primary objective was to assess the impact of inbreeding coefficient (IC) as an index of linebreeding and gender on pasture-fed lamb eating quality consistency in antioxidant status, IMF, FMP, n-3 LC-PUFA. The hypothesis tested was that variation in healthy lamb eating quality will be a function of lamb gender and not its antioxidant status or IC.

Chapter 4: Investigated the effect of fortifying feedlot diets with n-3 LC-PUFA on lamb growth performance, IMF and FMP of the *Longissimus thoracis et lumborum* muscle, carcass attributes, and commercial wholesale cuts of lot-fed TAW MARGRA lambs as a result of dietary fortification of the diet with n-3 LC-PUFA. The tested hypothesis was that the inclusion of n-3 LC-PUFA in the diet will improve productive performance, carcass characteristics, wholesale cut yields and meat quality traits in TAW lambs. This 47-day feedlot trial utilised seventy-five TAW lambs at six months of age with an average liveweight of 30 ±1.2kg randomly allocated to three dietary treatments of 25 lambs each, in a completely randomised experimental design: (1) Control diet – grain pellets without omega-3 oil plus hay, (2) Commercial whole grain pellets (MSM) without omega-3 oil plus hay, and (3) grain pellets fortified with omega-3 oil plus hay.

Chapter 5: Utilised the same experimental design as in Chapter 4 to examine the nutritional enhancement of health beneficial n-3 LC-PUFA in the *Longissimus thoracis et lumborum* muscle, liver, kidney and heart of lot-fed TAW lambs in response to dietary supplementation with or without fortification with omega-3 oil. The hypothesis tested was that fortifying feedlot

pellets with omega-3 oil will enhance the human health beneficial n-3 LC-PUFA composition of edible lamb muscle tissue and organs.

Chapter 6: Conducted a targeted next-generation sequencing of stearoyl-CoA desaturase (*SCD*), fatty acid binding protein-4 (*FABP4*), and fatty acid synthase (*FASN*) lipogenic genes to detect functional SNP that offer distinctive DNA marker signatures for TAW genetics, breeding, and potential marker-assisted selection for meat-eating quality. The hypothesis tested that significant associations exist between SNP of lipogenic genes and n-3 LC-PUFA, IMF, and FMP, underpinning potential marker-assisted selection for meat-eating quality traits in TAW lambs.

Chapter 7: Was a transcriptomics experiment that investigated the differential expressions of *FASN*, *SCD* and *FABP4* genes in the *Longissimus thoracis et lumborum* muscle of TAW lambs in response to supplementation with omega-3 oil. The hypothesis tested was that dietary fortification with omega-3 oils influences the transcriptional expression of lipogenic genes in the *Longissimus thoracis et lumborum* muscle in TAW MARGRA lambs.

Chapter 8: This Chapter is a general discussion of the significant outcomes, conclusions and recommendations for future research.

Appendices: Contains all supplementary materials and copies of peer-reviewed publications from this thesis.

Chapter 2: Genetics of Omega-3 Long-Chain Polyunsaturated Fatty Acid Metabolism and Meat-Eating Quality in Tattykeel Australian White MARGRA Lambs

2.1. General overview of the Australian sheep industry and Tattykeel Australian White (TAW) MARGRA sheep

Sheep production is an important economic activity in many countries because lamb is one of the world's four major meat classes along with pork, chicken and beef (OECD, 2022). Sheep are produced mainly for their meat (lamb or mutton) and wool (Rowe, 2010) as well as milk and hides. In 2021, Australia exported 405,000 tonnes of lamb and mutton, representing a 10.5% increase over 2020 figures, and was the largest sheep exporter in the world, worth Au$4.05 billion (MLA, 2022). Australians have been among the highest consumers of lamb, estimated to be above 7kg/ capita (OECD, 2022). Thus, lamb is a very significant contributor to the Australian economy and a major part of the Australian diet.

Lamb is a very nutritious, easily digestible, and highly valued food with a healthy fatty acid composition (Milewski, 2006; Szterk et al., 2022). Lamb consumers demand meat that is safe, of consistent eating quality, healthy composition and conveniently easy to prepare (Nuernberg et al., 2008). Meat quality is the constitutional standard of lean-to-fat ratio and palatability indices that include visual appearance, aroma, drip loss, colour, texture, pH, intramuscular fat profile, tenderness, flavour, and juiciness (FAO, 2020). The entire processes of feeding, culminating in the finishing of animals, including their genetic constitution, husbandry practices and handling, all affect the overall quality of meat (MLA, 2020). There are genuine concerns about high fat consumption, especially fats of animal origin, as their profile has a significant influence on human health because excessive consumption of SFA is associated with high levels of low density-lipoproteins (LDL) and cholesterol (Scollan et al., 2014; Cardoso et al., 2016). Both LDL and hypercholesterolemia are predisposing risk factors for cardiovascular disease (De Smet et al., 2016), prostate, mammary and colorectal cancer (Calviello et al., 2009; Gu et al., 2013), dry eye disease, (Chi et al., 2019), depression (Zhang

et al., 2019), obesity, diabetes (Funaki, 2009; Sripetchwandee et al., 2018) and neuro-degenerative conditions including Schizophrenia, Alzheimer's, Parkinson's disease (Janssen and Kiliaan, 2014). Despite animal lipids being criticised as health-risk factors, it is evident that they actively support many physiological functions and provide health-beneficial omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) (Nuernberg et al., 2008). This is the basis for various animal production strategies aimed at enhancing health-beneficial fatty acids in meat and meat products (Scollan et al., 2006; Wood et al., 2008). This is because intramuscular fat, fatty acid content, water holding capacity and consistency largely influence meat organoleptic traits and retail potential namely, juiciness, tenderness, flavour, colour, shelf life and firmness (Wood et al., 2004; Webb and O'Neill, 2008).

In the quest for a meat sheep breed with good body conformation, superior eating qualities, low FMP, high IMF and healthy n-3 LC-PUFA composition, the Gilmore Family in Black Springs, Oberon, New South Wales, pioneered the development of the TAW breed over a 15-year period of rigorous breeding, culling and selection of Poll Dorset, Dorper, Texel and Van Rooy rams and ewes with an extensive utilisation of embryo transfer, artificial insemination, and natural mating. Although preliminary evidence from the data in Table 2.1 suggests that the TAW sheep breed exclusive to the MARGRA brand of lamb has an outstanding low FMP, high n-3 LC-PUFA content and IMF, comprehensive peer-reviewed publications on its eating quality attributes and n-3 LC-PUFA profile are scanty. This necessitates further research into genetic factors that may determine IMF, FMP, n-3 LC-PUFA in the TAW breed. Many genes and enzymes are responsible for fatty acid metabolism and their correlations with meat quality traits. However, the roles of stearoyl-CoA desaturase (*SCD*), fatty acid binding protein 4 (*FABP4*), and fatty acid synthase (*FASN*) genes are the most critical (Ladeira et al., 2016; Mwangi et al., 2019; 2022) and need further elucidation herein. Therefore, the primary objective of this review was to critically appraise the published

literature regarding fatty acid synthesis and metabolism, IMF, FMP, and carcass quality to identify knowledge gaps and highlight research opportunities associated with nutrition–genetics interactions influencing n-3 LC-PUFA that can inform future meat-eating quality investigations in TAW lambs.

Table 2.1. Tattykeel Australian White (TAW) carcass and meat quality characteristics (*n* = 217).

Trait	Mean ± SE	Range
Fat melting point (°C)	34.08 ± 1.4	28.0 – 39.0
Intramuscular fat (%)	4.4 ± 0.2	3.4 – 8.2
Hot standard carcass weight (kg)	24.6 ± 2.7	19.5 – 30.7
Dressing percentage	50.2 ± 2.2	47.0 – 54.4
Fat score	4.7 ± 0.6	4 – 5
GR fat depth (mm)	16.4 ± 3.5	10 – 24
Tenderness (N)	32.3 ± 5.1	20.0 – 38.5
pH	5.63 ± 0.11	5.53 – 6.83
Overall consumer liking (9-point scale)	8.2 ± 0.9	7.9 – 8.5
Omega-3 long chain PUFA (mg/100g)		
EPA (20:5n-3)	24.3 ± 5.2	17.8 – 44.8
DHA (22:6n-3)	8.3 ± 2.7	3.4 – 12.1
DPA (22:5n-3)	25.2 ± 8.0	14.0 – 80.3
EPA + DHA	32.6 ± 7.0	33.6 – 69.9
EPA + DHA + DPA	57.9 ± 13.6	49.1 – 132.5

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid.

2.2. Fatty Acids, Classifications and Functions

Lipids are preferentially utilised as the major energy source in enteral diets owing to their high caloric value (Calder et al., 2018). Fats are triglycerides comprising glycerol and fatty acids. Apart from their main biological function of energy storage, lipids are essential components of cellular membranes and signalling molecules (Kenar et al., 2017). Thus,

Patterson et al. (2012) stated that fatty acids as the "building units" of lipids, are hydrocarbon chains having a carboxyl (-COOH) group at one end and a methyl (-CH3) group at the other. When three fatty acids are attached to a glycerol molecule, energy-storing triacylglycerols are formed (Leyland et al., 2020). The amphiphilic structure of fatty acids arising from their hydrophilic carboxyl group attachment to a hydrophobic hydrocarbon chain or tail provides the ideal energy storage powerhouse that is characteristic of triacylglycerols (Webb and O'Neill, 2008). The bonds between the carbon atoms in a hydrocarbon chain differentiate between SFA and unsaturated (UFA) fatty acids. However, SFA consist of less reactive single bonds only, while UFA have one (monounsaturated, MUFA) or at least two (polyunsaturated, PUFA) reactive double bonds. Fats containing significant levels of MUFA like oleic acid (C18:1), contribute to high quality meat due to low melting point which leads to favourable meat flavour, tenderness, and juiciness (Hayakawa et al., 2015). C18:1 is the most abundant MUFA in the adipose and muscle tissues of ruminants, and it is not easily susceptible to oxidation (Melton et al., 1982). PUFA are further divided into four families: omega-3 (n-3), omega-6 (n-6), omega-7 (n-7) and omega-9 (n-9), based on the position of the initial double bond on the methyl terminal (Zhao and Wang, 2018) or the location of the last double bond relative to the terminal methyl end of the molecule (Wall et al., 2010).

Fatty acids can also be subdivided into essential and non-essential fatty acids. The latter can be synthesised de novo (mainly in the liver), without the need for dietary supplementation (Insel et al., 2018) while the former on the other hand, cannot be synthesised by mammals and need to be included in the diet (Webb et al., 2008). Essential fatty acids play significant roles in enzymatic regulation, eicosanoid synthesis, cell signalling, control of neuronal migration, neuro-modulatory and neurotransmitter activities (Yehuda et al., 2005; Khan et al., 2017). Some deficiency symptoms of essential fatty acids have been identified in several nutrition-related complications in the liver and kidneys, especially in children, to include dry and flaky

skin, diarrhoea, anaemia, stunted growth and poor wound healing as well as compromised immunity leading to secondary infections (Sampath and Ntambi, 2011). Therefore, it is important to supply this group of fatty acids in correct proportions right from conception, throughout pregnancy and infancy.

2.2.1. Omega-3 Long-Chain Polyunsaturated Fatty acids

The word "omega" (ω) in relation to fatty acids denotes the terminal carbon atom furthest from the functional carboxylic acid group (-COOH). These structural differences confer unique individual functions. Omega-3 (n-3) long-chain polyunsaturated fatty acids are a family of PUFA made up of α-linolenic acid (ALA, C18:3n-3), a precursor for the more functionally potent longer chain eicosapentaenioc acid, 20:5n-3 (EPA) and docosahexaenoic acid, 22:6n-3 (DHA) members of the family (Calder, 2014; Calder, 2016; Toa et al 2018). Omega-3 PUFA increase the stability of cell membranes, regulate immune function, block excessive inflammatory reaction (Mayer and Seeger, 2008), reduce systemic inflammatory response syndrome, various organ dysfunction syndromes, infectious complications and depress tumour growth (Tevar et al., 2002; Hayakawa et al., 2015). The most important functional n-3 LC-PUFA related to human well-being are EPA and DHA (Calder, 2009). Furthermore, the hitherto neglected roles of docosapentaenoic acid (DPA; 22:5n-3) are currently evolving (Kaur et al., 2011; Weylandt, 2016). A number of research findings have established that n-3 LC-PUFA are potent therapeutic agents for the suppression of inflammation, thus playing critical roles in a number of inflammatory conditions including diabetes, artherosclerosis, asthma and arthritis (Yehuda et al., 2005; Simopoulos, 2016).

Cardiovascular ailments and cancer are the main causes of human death globally (Nichols et al., 2014; Benjamin et al., 2017; Siegel et al., 2017). Thus, consumption of n-3 LC-PUFA decreases the danger of cardiovascular diseases by depressing systolic resting heart rate,

diastolic blood pressure (Mozaffarian et al., 2011), blood viscosity (Cartwright., 1985), plasma fibrinogen (Watanabe and Tatsuno, 2017) and platelet aggregation (Simopoulos, 2002). They also improve blood vessel function (Abeywardena and Head, 2001). In adults, increased intake of n-3 LC-PUFA has remarkable brain health benefits, reduced risk of dementia and late cognitive malfunction (Swanson et al., 2012), overall health at pregnancy (Koletzko et al., 2008), insulin resistance (Sripetchwandee et al., 2018), depression and retarding the progression of certain cancers (Astorg, 2004; Leitzmann et al., 2004). Gould et al. (2010) reported that n-3 LC-PUFA play significant roles in neural development in embryos and at infancy. High consumption of EPA and DHA has also proved useful in improving foetal brain, retinal development, and reducing the risks associated with cardiovascular and Alzheimer's diseases (Swanson et al., 2012). Welch et al. (2010) proposed DHA, EPA, n-3, ALA and LA dietary intakes of 0.16, 0.11, 1.50, 1.23 and 12.35g/d for men and 0.13, 0.09, 1.22, 0.99 and 9.42 for women, respectively. It has been recommended that patients susceptible to coronary heart disease should consume at least 1g of DPA and DHA daily; and good sources of these nutrients include seafood, particularly fatty fish (for example, mackerel, herring, sardines, salmon, trout, kippers, pilchards, eels, and tuna), whales, seals and oil supplements from fish, cod liver, krill and algae (Gould et al., 2013; Calder, 2017). However, the use of marine fish oil has some drawbacks including typical fishy smell, unpleasant taste, expensive cleansing procedure and adulteration by environmental contaminants including radioisotopes, dioxins, and heavy metals (Certik and Shimizu, 1999; Jacobs et al., 2014; Mori et al., 2014; Orsavova et al., 2015; Menzel et al., 2022). Western diets contain 1.5–10.0 g of n-6 fatty acids which are derived from plant oils rich in linoleic acid (Jeyapal et al., 2018; Dawczynski et al., 2022). ALA is also found in canola (rapeseed) oil, flaxseed (linseed) oil, rapeseed oil, soybean oil, pumpkin seeds and walnut oil (Mori, 2014; Orsavova et al., 2015; Menzel et al., 2022).

However, humans lack the enzymes required to transform n-3 from n-6 fatty acids, they also have a limited capacity to elongate and change ALA to EPA and DHA (Mori, 2014).

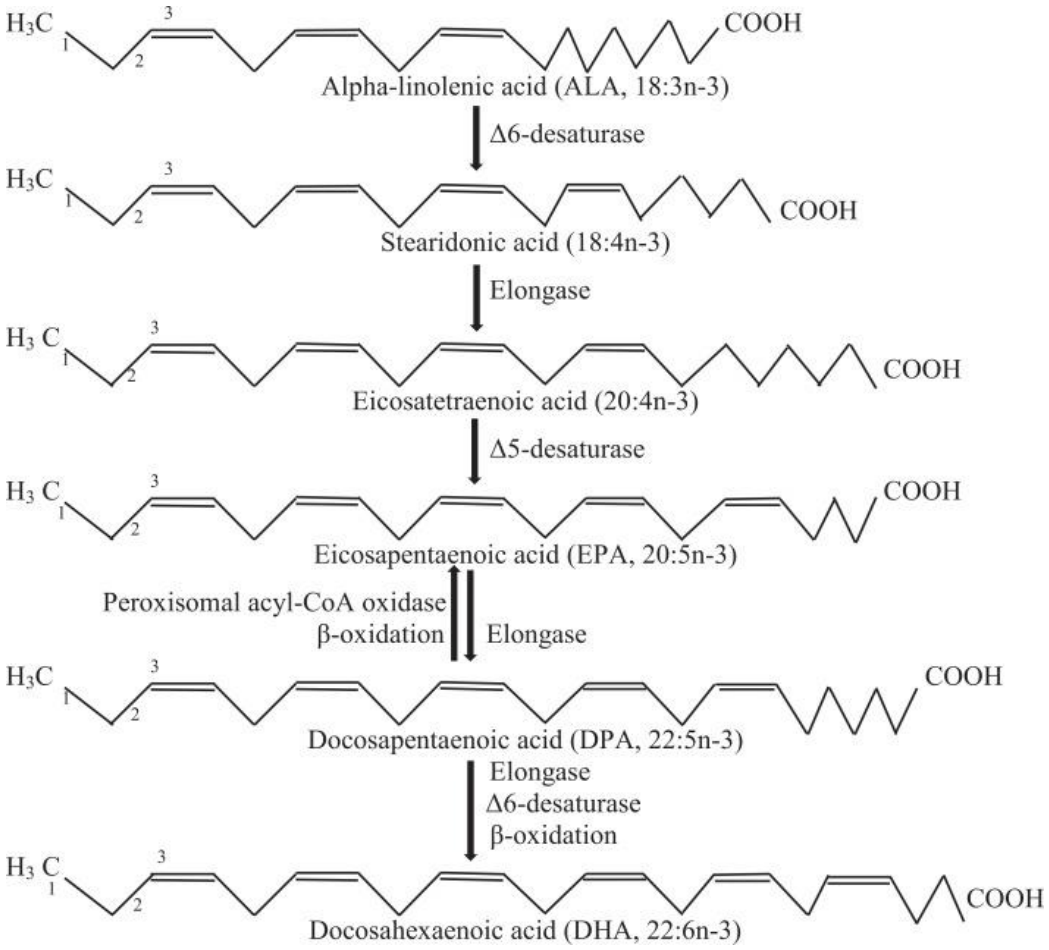

Figure 1.1. Pathway for the biosynthesis of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) from α-linolenic acid (ALA) (Calder, 2017).

Figure 1.1 depicts the pathway where EPA is produced from simpler, plant-sourced n-3 fatty acids like ALA (18:3n-3) (Calder, 2017). The enzymes involved in n-3 fatty acid interconversion are identical with the analogous n-6 fatty acid pathway for the transformation of linoleic acid (18:2n-6) to arachidonic acid (20:4n-6). The majority of these processes involve the addition of a double bond between two carbon atoms (desaturation) and addition of two carbon atoms (elongation reactions) (Brenna et al., 2009; Calder, 2017). While the enzymes

involved in elongation and desaturation pathways are well understood in monogastrics, their roles in the interconversion of n-6 to n-3 fatty acids in ruminants is less understood, especially in Tattykeel Australian White lambs, due to biohydrogenation. This represents a major knowledge gap.

Kanapanagiotidis et al. (2022) reported that even though fish oil remains an excellent source of EPA and DHA, Lum et al. (2013) recommended that attention is increasingly shifting towards cheaper but equally good substitute sources of n-3 fatty acids, including microalgae known to have high elongase and desaturase enzyme activities necessary for the biosynthesis of EPA and DHA (Gregory et al., 2013). DPA (C22:5) is similar to EPA with the same number of double bonds but has two more carbon chain units (Yazdi, 2013). Its functions were in the past, poorly understood, but currently unravelled (Kaur et al., 2011). Epidemiological trials in humans have demonstrated high levels of DPA to be favourably correlated with lesser blood triglycerides, cholesterol, inflammation and a reduced total risk of cardiovascular diseases and acute myocardial infarction (Reinders et al., 2002; Sun et al., 2008; Siegel et al., 2017). DPA is an active and potent stimulator of endothelial cell migration, an important part of the embryonic vascular system (Aase et al., 2007). It also acts as a precursor for the synthesis of resolvins which are neuroprotective in function (Kaur et al., 2011). In other studies, Phang et al. (2009) demonstrated that when applied to platelets or PC-21 human epithelial cell lines, purified DPA reduces platelet accumulation and aggregation more efficiently than EPA and DHA (Augustsson et al., 2003) and leads to endothelial cell migration (Kanayasu-Toyoda et al., 1996) and inhibition of chronic inflammation (Chen et al., 2012).

2.2.1.1. Fatty Acid Profile and Nutritional Value

The fatty acid profile of meat is related to meat quality sensory attributes, nutritional value and health benefits (Wood et al., 2008; Malau-Aduli and Holman, 2015). For instance, a direct relationship between the content of stearic acid in the fat and fat hardness exists, because as the content of stearic acid increases, so does the fat hardness. This in turn, influences marbling fat melting point and meat juiciness. The quantity and type of intramuscular fat and fatty acids in both muscle and adipose tissues influence eating quality, juiciness, tenderness, flavour, colour, shelf life and firmness of meat (Wood et al., 2004; Warren et al., 2008; Webb and O'Neill, 2008). Fat content and amount of fatty acids are quantified in mg/100g of meat (NHMR, AGDHA, 2006), whereas human nutritionists assess nutrient value of food per 100 g of serve. For food to be categorised or claimed as a source of n-3 LC-PUFA in Australia and New Zealand, its EPA and DHA contents should be greater than 30mg per serve and declared a good source if it has at least 60mg of EPA and DHA for each standard serve (NHMR, AGDHA, 2006; Ponnampalam et al., 2014; Le et al., 2019). In Europe, it is 40mg per 100g (CREU, 2014). The World Health Organization (1990) recommended that daily fat intake should be 30% of total energy, and of this, SFA should be reduced to 300mg per day. They also advocated that a reasonable balance of fatty acids in food should be established where intakes of cholesterol and SFA are decreased. The ratios between SFA and PUFA and n-6 and n-3 fatty acids determine the nutritional value of meat (Warren et al., 2008). However, Simopoulos et al. (2011) documented that in developed and industrialised countries, there is growth in the consumption of SFA, n-6 PUFA and trans fatty acids and a marked reduction in n-3 PUFA intake. The diets in these parts of the world have an n-6: n-3 PUFA ratio of about 15:1, compared to an ideal recommended ratio of 4:1 (Simopoulos, 2002; 2008). This unbalanced consumption leads to low tissue levels of DHA and EPA (Stark et al., 2016),

resulting in higher incidences of inflammatory processes, cardiovascular diseases, obesity, inflammatory bowel disease, rheumatoid arthritis, and cancer (Corsinovi et al., 2011).

2.2.2. Factors Affecting Fat Profile in Ruminant Muscle and Adipose Tissues

Fatty acid profile is influenced by biohydrogenation in the rumen, dietary concentrate supplementation versus pasture finishing and genetics (Janssen and Kiliaan, 2014; Pighin et al., 2016).

2.2.2.1. Biohydrogenation

Sheep, like all other ruminants, harbour a diverse microbial population in their rumen that enables the digestion of complex plant materials into more absorbable nutrients (Henderson et al., 2015). The rumen ecosystem is composed of anaerobic bacteria, protozoa, fungi, methanogenic archaea, and phages (Morgavi et al., 2010). Microbes play different, yet complimentary, roles in the rumen. Bacteria enzymatically convert sugars to volatile fatty acids (acetic 60–70%, propionic 15–20% and butyric acids 10–15%), which are the main energy substrates for ruminants (Demeyer et al., 2016; Doreau et al., 2016). Protozoa on the other hand, degrade complex carbohydrates and nitrogen into nutrients that are made available to the host, while anaerobic fungi engage in cellulolytic degradation activities (Jenkins et al., 2008). The type and amount of fat delivered to the rumen (Beam et al., 2000), temperature of 38–39 °C (Buccioni et al., 2012) and pH range between 6.0 and 6.7 (Van Nevel and Demeyer, 1996) dictate optimal rumen microbial function.

Ruminant diets are commonly made up of forages and concentrates with fats sometimes included, to raise the energy level in rations for lactating females or to enlarge the amount of human-health beneficial n-3 LC-PUFA, and bioactive conjugated linoleic acid in meat and milk (Dewanckele et al., 2018). Upon entry into the rumen, ingested lipids are degraded by microbial lipases via lipolysis (Jenkins et al., 2008; Buccioni et al., 2012; Edwards et al., 2017).

Lipolysis breaks down lipids and releases free fatty acids from esters, thus facilitating biohydrogenation where the number of double bonds is reduced on the carbon chain (Buccioni et al., 2012), or under ideal conditions, 85% of esterified dietary lipids in the form of galactolipids, phospholipids and triacylglycerols are hydrolysed (Palmquist et al., 2005; Buccioni et al., 2012). UFA get converted into SFA in the rumen due to microbial biohydrogenation activities involving series of consecutive conversion pathways leading to an abundance of fatty acid isomers (Dewanckele et al., 2018), and remain a major human public health issue (Li et al., 2012). The bulk of the dietary fatty acids are 18-carbon UFA (linolenic acid, 18:3n-3; linoleic acid, 18:2n-6 and oleic acid, cis-9 18:1) (Ferlay et al., 2017). However, the major biohydrogenation intermediate product in a ruminant fed forage diet is trans-vaccenic acid (trans-11 C18:1, t-VA) (Bickerstaffe ET AL., 1972). t-VA acts as a precursor required to produce SFA in the rumen to yield stearic acid (C18:0). Conversely, it is desaturated by Δ9-desaturase enzyme in the mammary gland to yield cis-9, trans-11 C18:2 and its CLA isomer that can be easily detected in milk and meat (Griinari and Bauman, 1999). Other end products of rumen metabolism are carbon dioxide, methane, and traces of hydrogen (Demeyer, 1991) used as energy sources for the reduction of carbon dioxide to methane (Moss et al., 2000). Short chain fatty acids (acetic acid (C2), propionic acid (C3), and butyric acid (C4)) produced are absorbed, transported, and metabolised by different organs in the body of the host animal while carbon dioxide and methane are expelled from the body through different cycles of eructation or belching (Ríos-Covián et al., 2016).

2.2.2.2. Influence of Concentrate or Forage Finishing on Lamb Performance and Meat Quality

Lamb finishing on pasture is cheaper than grain feeding (Fruet et al., 2019), but the viability of pasture-finishing depends on a consistent supply of good quality forage (Redfearn et al., 2002). This is achieved by growing a mixture of grasses and legumes. Legumes increase the nutritional quality through higher digestibility and protein content (Buxton et al., 1985; Sleugh et al.,

2000), healthier fatty acid composition and increased oxidative stability (Fruet et al., 2016). Meat derived from pasture-finished animals has higher CLA and PUFA content especially of the n-3 series in the *longissimus thoracis et lumborum* muscle than meat from feedlot or grain-fed ruminants and at the same time, the proportion of fat and cholesterol in meat from grass-fed ruminants is lower (Demirel et al., 2006; Scollan et al., 2006; Aidai et al., 2011). Mixed pasture finishing improves growth performance and carcass traits of grazing ruminants (Roberts et al., 2009). Apart from contributing to landscape maintenance, nature preservation, pasture feeding system is generally desired by health-conscious organic meat consumers (Nuernberg et al., 2008). However, pasture-finished meat has some limitations that include lower carcass weight (Duckett et al., 2013) and extended periods of feeding to attain market weight specifications compared to their contemporaries finished on grains (Raes et al., 2004; Realini et al., 2004; Roberts et al., 2009). Furthermore, grain finishing gives higher attainment of desired weights, better meat quality regarding tenderness, marbling, ribeye area (REA) and backfat thickness (BFT), higher stocking rate per land unit than their counterparts finished on pasture (Realini et al., 2004; Arelovich et al., 2017). In a grain finishing system, the net energy and glucose available for fat synthesis as muscles grow, reduce in older animals, and this leads to a higher fat content than obtained in a grass finishing system.

The biochemical processes outlined above are influenced by genetic differences and particularly enzymes and genes involved in fat metabolism. There are no reference materials in peer-reviewed sources on how all these biochemical processes relate to the TAW.

2.3. Lipogenic Genes and Associations with Genetic Selection for Meat Quality

Routine phenotypic data collection may be an arduous task given that live-animal proxies hardly exist for meat quality traits and the related costs of such data collection are high (Rovadoscki et al., 2018), ranging from Au$50 to 100 per animal. Genomic data therefore is

significant in the design and implementation of animal breeding and improvement programmes to rapidly increase the frequency and potency of desirable genes in the population (Goddard and Hayes, 2007; Tiezzi et al., 2015). The utilisation of genomic data can raise the accuracy level of estimated breeding values (EBV), thus increasing the rate of genetic progress (Meuwissen et al., 2001; Van Raden, 2008; Bolormaa et al., 2013). Progressive advancements in molecular genetics have resulted in an increased identification and documentation of genes or markers influencing meat quality traits (Casas et al., 2006). Casas et al. (2006) reported that DNA polymorphisms in some identified candidate genes were associated with meat tenderness. Genomic selection involves decisions that are focused on breeding values utilising genome wide markers such as SNP (Meuwissen et al., 2001). In sheep production, genomic prediction offers reliable alternatives because many traits influence fatty acid inheritance. Accurate genomic estimated breeding values (GEBV) for these traits would lead to greater genetic gains (Bolormaa et al., 2013). GEBV calculation is dependent upon the reference population that has been determined for the trait and genotyped for the markers (Bolormaa et al., 2013). Hayes et al. (2009) established the fact that the degree of accuracy of GEBV on selection candidates' rests on the proportion of this reference population and the level of the linkage disequilibrium between SNP and quantitative trait loci (QTL). Traits that are difficult or expensive to measure are quite challenging to get large reference populations for accurate GEBV prediction (Bolormaa et al., 2013).

SNP in several genes can influence the fatty acid profile of ruminants (Maharani et al., 2012), however, SNP in FASN, SCD and FABP4 would be considered in this review because of their critical roles in fatty acid metabolism. Furthermore, Bhuiyan et al. (2009) reported five SNP in the *FASN* gene in cattle, and one of the SNP was correlated with the composition of lipids and may be utilised as a marker in breeding programs. However, in sheep, there is paucity of information on these genes. From the literature, there are research attempts aimed at linking

the lipid profile of lambs with SNP (Esteves et al., 2019), but to our current knowledge, there is no published information on any identified SNP in TAW sheep. This represents a major knowledge gap.

2.3.1. Stearoyl-CoA Desaturase (*SCD*)

The *SCD* gene encodes for delta-9 desaturase enzyme and an iron-containing endoplasmic reticulum enzyme (Paton and Ntambi, 2009; Gu et al., 2019), that catalyses a rate-limiting step in the conversion of SFA into MUFA in mammalian adipose cells (Paton and Ntambi, 2009; Mannen, 2011). The principal product of the desaturase enzyme is oleic acid, which is formed by the desaturation of stearic acid (Milanesi et al., 2008). In cattle, the *SCD* gene comprises two isoforms; *SCD1* and *SCD5* (Lengi et al., 2007). The *SCD1* gene, mapped on bovine chromosome 26, codes for stearoyl-CoA desaturase (Gu et al., 2019). The fatty acid profile of stored fat reflects the earlier action of *SCD* on substrates such as palmitic or stearic acids (Kim et al., 1999). Similarly, Smith et al. (2009) reported that there are three fatty acid desaturases in animal tissues: Δ5, Δ6, and Δ9 desaturases, and that of these, only Δ9 desaturase acts upon SFA to convert them to their respective MUFA. It serves as a catalyst in the synthesis of UFA by incorporating a *cis*-bond between the 9th and 10th carbon atoms of FA with chain lengths of 10-18 carbons in adipose tissues and mammary glands (Bauman et al., 2006). Other researchers agree that the *SCD* enzyme is essential in the biosynthesis of MUFA such as oleic (C18: 1n-9) and palmitoleic (C16: 1n-9) acids, formed after the addition of a double bond in the Δ9 position of their precursors, C18: 0 and C16: 0 fatty acids (Guillou et al., 2010). It has been documented that *SCD* converts C18:1 trans-11 to C18:2 cis-9, trans-11, said to be correlated with anticarcinogenic and antiatherogenic effects (Bhattacharya et al., 2006). It also increases the ratio of MUFA to SFA (Calco et al., 2019). In sheep milk, this gene encodes the *SCD* enzyme found in a locus where a positional QTL has been identified for the CLA: VA ratio (Carta et al., 2008).

In sheep, an *SCD* SNP (SCD5, rs423661926) was found to be significantly associated with rib eye area and genotypic effects ranged from 0.035 to 0.923 (Armstrong et al., 2018). The *SCD* gene has also been reported to harbour polymorphisms that affect milk fat content, specifically, palmitoleic acid, LA, VA, SFA and MUFA and ratios of n-6: n-3 and palmitoleic acid: palmitic acid (García-Fernández et al., 2009). The expression of this gene is controlled by the diet (particularly its content of n-6 and n-3 PUFA), environmental and hormonal factors (Miyazaki and Ntambi, 2003). In sheep, as reported by Dervishi et al. (2010), grazing raises the quantities of CLA, total PUFA and n-3 PUFA in lamb, which is a favourable and desirable option in line with health-beneficial human dietary guidelines (Calco et al., 2019).

2.3.2. Fatty Acid Synthase (*FASN*)

FASN is the gene encoding for fatty acid synthase enzyme and is a versatile and valuable protein complex that controls the de novo biosynthesis of long chain fatty acids (Roy et al., 2001). According to Chirala et al. (2003), this gene plays essential roles during embryogenesis and adulthood fatty acid synthesis. Zhang et al. (2008) demonstrated associations arising from meat fatty acid profile and *FASN* candidate gene polymorphisms. In bovine species, the *FASN* gene has been mapped on BTA19 where many QTL influencing beef fatty acid profile, adipose and milk fat contents were found (Morris et al., 2007; Du et al., 2022). The four exons (39–42) in the *FASN* complex which encode for the thioesterase (TE) domain are accountable for the synthesis of fatty acids, especially C16:0, by hydrolysing the acyl-S-phosphopantetheine thioester. Consequently, Zhang et al. (2008) observed that the TE domain dictates the product chain length of *FASN* and variability in the TE domain amongst individuals is said to be a heritability candidate for variability in fatty acid profiles. Roy et al. (2006) found higher bovine *FASN* expressed in several tissues and organs especially the brain, testis and adipose tissue and less in liver and heart and *FASN* assists in catalysing the reaction steps involved in the transformation of acetyl-CoA and malonyl-CoA to palmitic acid. Similarly, *FASN* gene uses

Malonyl CoA and Acetyl CoA as substrates, while NADPH acts as a co-factor (Mohammed et al., 2013; Mozihim et al., 2022). The FASN action in mammals largely yields C16:0 with negligibly minute levels of C14:0 (Cabrits et al., 2022)).

In humans, Chakravarty et al. (2004) reported that the TE domain possesses a hydrophobic groove which contributes the fatty acyl substrate binding site with high specificity regarding C16-acyl ACP, but not C14-acyl ACP. Oh et al. (2012) demonstrated a favourable impact of *FASN* gene on fatty acid profile. *FASN* is a versatile and important protein complex which catalyses the synthesis of long-chain SFA. However, the differences in TE domain (that is exons 39–42, that account for fatty acid synthesis termination of the *FASN* gene), would be a candidate for heritable differences in fatty acid profile (Oh et al., 2012). To our current knowledge, apart from the short communication of Sanz et al. (Sanz et al., 2015) that identified novel polymorphisms in the 5′UTR of *FASN*, *PROP1, GPAM, MC4R, FADS* and *PLIN1* ovine candidate genes and their relationships with gene expression and diet in a study with Spanish sheep (Rasa Aragonesa, Roja Mallorquina and Assaf), Chinese Sunit sheep (Wang et al., 2018), and New Zealand sheep (Ekegbu et al., 2019), there is no literature published on *FASN-FADS-PROP1* genes and their correlations with growth and meat quality traits in TAW or any other sheep breed, thus presenting a significant knowledge gap.

2.3.3. Fatty Acid Binding Protein4 (*FABP4*)

To date, nine sub-types of fatty acid binding proteins (*FABP*) can be identified *(FABP1–FABP9)* and are named based on the tissues they are found in highest concentration (Kucharski and Kaczor et al., 2017). *FABP4* is also known as adipocyte fatty acid binding proteins (*A-FABP* or *aP2*). The location of *FABP4* gene varies with livestock species; for instance, in sheep and cattle, it is located on chromosomes 9 and 14, respectively (Kucharski and Kaczor et al., 2017). *FABP4* encodes for a group of fatty acid binding proteins and is abundantly expressed

in the adipose tissue where these binding proteins are important in glucose homeostasis, FA metabolism, transport, and absorption, by their association with peroxisome proliferator-activated receptors (*PPAR*) (Latruffe and Vamecq, 1997; Li et al., 2013; Szymczak-Pajor et al., 2022). Apart from differences in two regions of ovine *FABP4* in lean and fat selection lines of Coopworth (Yan et al., 2012), Romney (Yan et al., 2018), and Rasa Aragonesa (Dervishi et al., 2011), breeds, published reports on the *FABP4* gene in sheep are few and scanty.

Most of the reported studies on *FABP4* gene have been in beef cattle. Barendse et al. (2009) reported that a splice site SNP of the *FAPB4* gene appeared to relate to the deposition of IMF in the *Longissimus thoracis et lumborum* muscle. In terms of variation in the *FABP4* gene, Yan et al. (2018) found that it is linked with growth, deposition of fat and carcass traits. In Japanese black cattle, Hoashi et al. (2008) documented a relationship existing between *FABP4* and fatty acid profile, while Ardicli et al. (2017) associated SNPs in bovine FABP4 with escalation in live weight, chilled carcass weight, marbling score and back-fat thickness, but without any colour differences or carcass dimension measurements. In Aberdeen Angus and Blonde d'Aquitaine cattle, the *FABP4* SNP 7516G>C was analysed for association with IMF composition of the *Longissimus thoracis et lumborum* muscle between the 12th and 13th ribs. In Angus cattle, the CC genotype was reported to be 52% and 64% lower in myristoleic acid, and 33% and 35% lower in LA than CG and GG genotypes, respectively. On the other hand, in Blonde d'Aquitaine cattle, the CC genotype had elevated levels of arachidonic acid and EPA, and comparatively less oleic acid and total SFA than CG genotype. The GG genotype was only detected in one cow (Dujkova et al., 2015). In Wagyu × Limousine crosses, the g.7516G>C SNP were investigated for any existing relationship between marbling score and depth of subcutaneous fat. An association was established between CC genotype with lower marbling and fat depth. While GC genotype recorded the highest scores, GG genotype was intermediate

(Li et al., 2013). Furthermore, in Korean Native cattle, *FABP4* SNP had a correlation with backfat thickness (Cho et al., 2008).

In sheep, *FABP4* plays an important part in glucose and lipid metabolism in adipocytes (Backhtiarizadeh et al., 2013; Bahnamiri et al., 2018). Therefore, *FABP4* polymorphisms are believed to have a significant influence on live performance and carcass characteristics (Stoch and Corsico, 2008; Yan et al., 2018), meat tenderness, marbling score and IMF content in sheep. For instance, in Romney sheep, (Wang et al., 2018; Ekegbu et al., 2019) reported five variants (A1 − E1) in region-1 (exon 2 − intron 2) and three variants (A2 − C2) in region-2 (exon 3 − intron 3) wherein A1 was associated with a decrease in leg, loin and total meat yield, while A2 was associated with a decrease in weaning weight and pre-weaning growth rate. Haplotype A1-A2 was found to be associated with a decrease in birth weight, pre-weaning growth-rate, hot carcass weight, loin meat yield, shoulder meat yield and total meat yield, while haplotype A1-B2 was associated with increased fat depth at the 12th rib (V-GR). Taken together, their finding supports the contention that variation in *FABP4* affects growth and meat production. To our current knowledge, nothing is known about the *FABP4* gene in TAW breed and this major knowledge gap needs to be filled by researchers.

2.3.4. Other Fat Related Genes

Several other genes reported to be associated with fat are cocaine- and amphetamine-regulated transcript (*CART*) with *Longissimus thoracis et lumborum* muscle IMF content (Rempel et al., 2012). The genes encoding leptin are associated with backfat thickness and marbling score (Shin and Chung, 2006), while the gene encoding diacylglycerol O-acyltransferase (*DGAT1*) is associated with liveweight, fat thickness, rib-eye area and shoulder weight in Texel lambs (Armstrong et al., 2018) and IMF (Thaller et al., 2003). The growth hormone 1 (*GH1*) gene is weakly correlated with rump fat (Barendse et al., 2006) and sterol regulatory element-binding

protein 1 (*SREBP1*) has been reported to be correlated with FA profile (Bhuiyan et al., 2009). However, all these studies were in cattle. Similar investigations in TAW have not been published and represent major research knowledge gaps. An updated summary of candidate genes associated with meat quality in livestock (Gao et al., 2007) is shown in Table 2.2.

Table 2.2. Candidate genes associated with meat quality traits in livestock.

Animal	Candidate Genes	Traits	References
Sheep	*CAST*	Carcass	(Barebndse et al., 2006)
	MSTN	Carcass, meat quality	(Gao et al., 2007)
	FADS2, ELOVL2, SCD, CPT1α, SREBF-1	Fatty acids	(Greguła-Kania et al., 2019)
	FABP4	Carcass yield	(Scollan et al., 2001; Rainer et al., 2004)
	MYF5	Leg and loin yield	(Grochowska et al., 2019)
	Callipyge	Muscular hypertrophy	(Fan et al., 2019)
	GDF8	Muscular hypertrophy	(Wang et al., 2017)
	FAD	Omega-3 long-chain PUFA	(Freking et al., 2002)
	CAST	Tenderness	(Dervishi et al., 2011)
	FASN, FABP4, DGAT1, SCD	Fat metabolism	(Miyazaki and Ntambi et al., 2003; Malau-Aduli et al., 2011)
	FABP4, SCD, PPARG, ACACA, LPL	Fatty acid profile	(Knight et al., 2014; Mwangi et al., 2021)
	CAST	Meat tenderness	(Dervishi et al., 2011)
	Leptin/Thyroglobulin	Marbling	(Da Costa et al., 2013)
Cattle	Myostatin	Growth and profile	(Lonergan et al., 1995)
Pig	*DGAT$_1$*	IMF/marbling	(Shin et al., 2006)
	HAL	Meat quality/stress	(Mullen et al., 2006)
	MC$_4$R	Growth and fatness	(Groblet et al., 1998)
	RN, PRKAG$_3$	Meat quality	(Fujii et al., 1991)
	AFABP/FABP$_4$	IMF	(Kim et al., 2000)
	HFABP/FABP$_3$	IMF	(Milan et al., 2000)
	CAST	Tenderness	(Gerbens et al., 1998)
	IGF$_2$	Growth and fatness	(Gerbens et al., 1999)
Chicken	*EX-FABP*	Fatness	(Ciobanu et al., 2004)
	L-FABP	Fatness	(Van Laere et al., 2003)

CAST= calpastatin, *MSTN*= Myostatin, *MYF5*=myogenic factor 5, *GDF8*= growth differentiation factor *8,FADS2*= fatty acid desaturase2, *FAD*= fatty acid desaturase, *IGF2*= insulin-like growth factor 2, *IGF2*= Insulin-like growth factor 2, *CPT1α* = carnitine palmitoyltransferase 1alpha, *PPARG*= Peroxisome proliferator-activated receptor gamma, *MC$_4$R*= Melanocortin 4 receptor, *LPL*= Lipoprotein lipase, *ACACA*= Acetyl-CoA Carboxylase Alpha, *HAL*= Histidine Ammonia-Lyase, *RN*= Rendement Napole.

2.4. Meat Eating Quality

Meat eating quality is influenced mainly by marbling, juiciness, tenderness, and flavour (Pannier et al., 2018). Studies with lamb have shown that carcass intramuscular fat deposition and FA composition account for eating quality variation (Flakemore et al., 2014; Lambe et al., 2018). Consumption of lamb IMF is important to humans since it helps with the delivery and absorption of fat-soluble vitamins and exerts positive effects on immune response (Calnan et al., 2017) as exemplified by Calder's work (2017) demonstrating the relationship between fatty acid composition of immune cells and their function. Marbling score to date remains one of the most important traits and reason why carcass evaluation is carried out in the abattoir (Hocquette et al., 2005). In the United States of America for instance, it is the major index considered in assigning beef quality grades (Indurin et al., 2009) because the quantity and distribution of IMF in the longissimus muscle area have marked effects on tenderness, flavour, juiciness, and colour (Joo et al., 2013). The amount of IMF is greatly influenced by several factors. These include animal age and breed, weight at slaughter (Park et al., 2002), diet (Holman and Malau-Aduli, 2013) and growth rate (Smith et al., 2009). Adipogenesis in the animal's life commences with deposition of visceral fat, subcutaneous, intermuscular, and intramuscular fat occurs last (Hausman et al., 2009).

Deposition of IMF is a highly heritable trait and is positively correlated with overall body fatness (Joo et al., 2013). Nutritional value is an essential determinant of meat quality. Hocquette et al. (2013) reported that awareness amongst consumers has greatly increased over the years regarding the relationships that exists between diet, health and well-being which has resulted in selection of foods which are healthier and nourishing. Level of marbling, fatty acid composition, biological value of protein, minerals and vitamins are essential elements of nutritive value of any food (Wyness, 2013).

2.4.1. Influence of IMF on Lamb Eating Quality

2.4.1.1. Tenderness

Meat tenderness has been identified as the most important sensory trait consumers consider when making decisions to purchase meat (Wall et al., 2019) as it probably affects consumers' understanding of acceptability. They are prepared to pay a premium for consistently tender meat and other traits they value (Koohmaraie et al., 1990). Meat tenderness affects the profitability of the lamb meat industry. It depends on a number of factors including muscle sarcomere length, integrity, connective tissue content and composition (Koohmaraie et al., 2002). Meat tenderness differs within and between animals and the different muscles (Cohen-Zinder et al., 2017) and is influenced by age of the animal, its sex, breed, genotype, nutrition, ante-mortem stress, and post-mortem handling (Muchenje et al., 2009). The chilling of carcass soon after slaughter leads to intense contraction of the muscle fibres known as "cold shortening", which is an undesirable meat trait (Razminowicz et al., 2006). Cold shortening is the result of the rapid chilling of carcasses immediately after slaughter, before the glycogen in the muscle is converted to lactic acid. With glycogen still present as an energy source, the cold temperature induces an irreversible contraction of the muscle, thus impacting negatively on tenderness. Perlo et al. (2008) reported that meat from lambs finished on forage-based diets was less tender than meat from their counterparts' fed concentrates. In contrast, Sañudo et al. (2003) reported that meat from grazing animals was more tender than from concentrate-fed lambs. This difference could be due to variation in carcass fatness resulting in differential cooling rates during rigor development. Furthermore, the use of fatness measures as covariates during statistical analysis can provide an unbiased basis for treatment comparison to judge if the observed differences are solely due to intrinsic dietary influences (De Brito et al., 2017). Meat from young lambs is more tender, has lower fat content and preferred by most consumers compared to mutton from older sheep (Montossi et al., 2013) The metabolic processes of

lipogenesis, lipolysis and fatty acid transport culminate in IMF deposition (Yang et al., 2017). Therefore, a diet with high-energy content leads to more lipogenesis (Jurie et al., 2007). Furthermore, the level of intramuscular fatty acids is mainly regulated by either inducing or inhibiting genes encoding for specific metabolic enzymes normally linked with lipid metabolism or transcription factors (Oliveira et al., 2014).

Tenderness is a proclamation of meat texture and is regarded as a major sensory quality attribute that is related with consumer satisfaction and positively correlated with juiciness and flavour, with consumers willing to pay more for tender meat (Lusk et al., 2001; Liu et al., 2022a). It is closely related to meat structure, biochemical activity as well as time that elapses between slaughter and consumption (Elmasry et al., 2012). Ali et al. (2008) reported that meat tenderness is influenced by the rate and level of glycolysis and the onset of rigor post-slaughter. According to Starkey et al., (2016), meat tenderness is dependent on intrinsic physiological traits of the live muscle and processing elements developed after rigor, while Rhee et al. (2004) attributed it to sarcomere length. Sarcomere length governs the overall length of muscle fibres and plays a significant role in themechanical structure of muscles (Guzek et al., 2013).

2.4.1.2. Flavour

Flavour is mainly because of volatile substances that impact strongly on the sensory characteristics of red meat (Arshad et al., 2018). Meat flavour is affected by animal breed, nutrition, genotype, temperament, aging after slaughter, cooking method and their interactions (Khan et al., 2016; Arshad et al., 2018). Meat flavour is derived through cooking, as raw meat possesses slight or no aroma. During cooking, several complex reactions are observed between a number of non-volatile compounds of lean and fatty tissues making the meat flavoursome (Mottram, 1998; Calkins and Hodgen, 2007). The major reactions seen in aromatic volatile production are the Maillard reactions between amino acids and carbohydrates and heat

degradation of fats (Mottram, 1994). Gylcosylamine which is a product of condensation of amino compounds with carbonyl group of reducing sugar precipitated by heat, becomes dehydrated to yield furfural, furanone, hydroxyketones and dicarbonyl compounds (Calkins and Hodgen, 2007). However, these results of Maillard reactions arising from interaction linking carbohydrates and proteins contribute significantly to meat flavour (Jamora and Rhee, 1999). In sheep, lamb and mutton have a distinct strong species-related flavour that is influenced by various antemortem and postmortem factors such as pH, age, sex, diet, type of cooking, and curing. Post-cooking storage and modulation of lipid oxidation in mutton also has effects on flavour characteristics and various chemical compounds have been implicated as responsible for or contributing to ovine flavour (Mottram, 1998; Jamora and Rhee, 1999). Of those compounds, medium-length branched-chain fatty acids are the most important. Although processing methods that reduce or modify species flavour such as washing and extrusion with non-meat ingredients have been evaluated, definitive generalisations regarding sheep production management practices yielding meat with the most desirable flavour attributes have not yet been made (Mottram, 1998).

2.4.1.3. Juiciness

Juiciness is an organoleptic index of the quantity of moisture released from meat and the degree of salivation during the process of mastication (Muir et al., 1998). Meat juiciness is dependent on water and fat contents (McMillin and Hoffman, 2009). de Lima et al. (2019) reviewed the intrinsic factors affecting sheep meat quality and reported that the level of marbling affects different sensory attributes, especially juiciness. Cloete et al. (2012) evaluated sheep breeds and reported that the lower proportion of IMF in meat from Merino breed was responsible for lower sensory score for initial juiciness and lasting succulence compared to other sheep breeds. This therefore explains why juiciness has a positive correlation with water holding capacity as well as level of intramuscular fat in meat as demonstrated by the work of Hocquette et al.

(2010) showing that IMF has a profound effect on juiciness and flavour. Human perception of juiciness is elevated as the IMF level in meat increases (Jeremiah et al., 2003). In general terms, juiciness is more of a sensory trait for pork than flavour and tenderness (Aaslyng et al., 2007) while beef consumers rate tenderness higher (Cho et al., 2010).

2.4.2. Fat melting point

The hardness or softness of fat is determined by its melting point. Flakemore et al. (2014) stated that soft fat has a comparatively lower melting point than hard fat and this has implications for meat processors in abattoirs. MUFA are characterised by lower melting points than SFA, an attribute that favours meat flavour, tenderness, and juiciness (Hayakawa et al., 2015). Fat melting point is affected by the physical and chemical structures of fatty acids, which in turn, drive carcass evaluation, classification, and sensory characteristics of meat (Yilmaz et al., 2010). Furthermore, fat melting point is influenced by the molecular weight, the number and configuration of double or triple bonds in the fatty acid structure (Knothe and Dunn, 2009). Red meat consumers prefer fats with low melting point (Pitchford et al., 2002; Flakemore et al., 2014) because of their association with reduced risks of cardiovascular diseases (Pitchford et al., 2002). Hard fats pose meat processing and safety challenges in the boning room (Yang et al., 1999). In sheep, Holman et al. (2013) reported FMP in Merino, Dorset, Black and White Suffolk breeds ranging from 41.5 to 44.8 °C. FMP ranging from 40.6 to 48.0 °C have also been reported in Dorset, White Suffolk, and Merino breeds (Flakemore et al., 2014). However, published data on FMP in the TAW sheep breed are currently not available.

2.5. Conclusions and Future Research

The TAW sheep is a new breed derived from Texel, Van Rooy, Dorper and Poll Dorset. Mechanisms explaining the impacts and expression patterns of genes associated with intramuscular fat, fat melting points and n-3 LC-PUFA on meat sensory attributes are neither

currently published nor fully understood. Future work should attempt to unravel single nucleotide polymorphisms, expression patterns and molecular mechanisms of various fat related genes and growth responses of TAW lambs to diverse feedlot finishing diets with and without omega-3 oil inclusion.

Specific knowledge gaps include:

Early selection decision tools for meat quality traits in TAW lambs are currently non-existent. Most reported selection programmes on fatty acid profile and meat quality traits in other sheep breeds are based on carcass data after the animals have been slaughtered. Pioneering studies using biopsy sampling of the *Longissimus* muscle in rams, ewes, and lambs to directly determine n-3 LC-PUFA, IMF and FMP contents while the animals are young and alive for early selection and breeding purposes are needed.

Published data on how parents selected for their high n-3 LC-PUFA, IMF and low FMP pass these genes to their offspring are currently non-existent in the TAW breed. Pioneer studies to estimate heritability values based on actual performance data and not estimated breeding values are recommended.

SCD, FASN and *FABP4* genes have been documented to exert some influence on carcass fat traits in other bovine and ovine breeds. No such data exist for the TAW breed. There is the need to sequence the *FABP4, FASN* and *SCD* genes to provide foundational data underpinning their roles in fatty acid metabolism unique to the TAW breed.

In-depth feedlot growth studies are required for better understanding of the interactions between n-3 LC-PUFA oil diets, finishing performance, and carcass traits of TAW lambs to afford industry players the opportunity to utilise them for greater economic gains.

A cost–benefit analysis of the implication of including n-3 LC-PUFA rich oil in feedlot finishing diets will be of immense industry significance to lamb producers, feed millers and meat processors.

Chapter 3: MARGRA Lamb Eating Quality and Human Health-Promoting Omega-3 Long-Chain Polyunsaturated Fatty Acid Profiles of Tattykeel Australian White Sheep: Linebreeding and Gender effects

3.1. Introduction

The Food and Agriculture Organisation (FAO) of the United Nations defines meat quality as the constitutional standard of lean-to-fat ratio and palatability indices that include visual appearance, aroma, drip loss, colour, texture, pH, intramuscular fat content, fatty acid and fat melting point profiles, tenderness, flavour and juiciness (FAO, 2014). Meat and Livestock Australia (MLA) describes the entire processes of feeding, culminating in the finishing of animals including their genetic constitution, husbandry practices and handling, as all affect the overall quality of meat (MLA, 2020). Fat melting point (FMP), intramuscular fat (IMF) content (marbling) and fatty acid (FA) profile all influence eating quality and, ultimately, consumer preferences for consistent, safe, nutritious and tasty lamb with a healthy FA composition (Ripoll et al., 2018). Globally, meat is regarded as one of the main sources of animal protein (Elmasry et al., 2012; Guerrero et al., 2013) and lamb is known to be highly nutritious and digestible (Milewski et al., 2006), fortified with essential amino acids, iron, zinc, selenium, fatty acids, and vitamins A, B6 and B12 (Guerrero et al., 2013). Lamb also has relatively low lipid and saturated fat contents compared to meat from other ruminants (Alves et al., 2014), and its marbling, tenderness, juiciness, aroma and colour attributes have been known to influence consumer liking (Miller, 2020), carcass (Hocquette et al., 2005), meat assignment into quality grades [10] (Indurain et al., 2009), consumer food choices (Hocquette et al., 2013), and nutritional value (Valdez-Arjona and Ramírez-Mella, 2019). It is therefore very important that sheepmeat producers guarantee the consistency of their lamb products to meet consumer preferences and adapt to the dynamics of purchasing decisions based on meat eating quality.

FMP dictates fat firmness. Soft fat has a low melting point and vice versa (Flakemore et al., 2014). From a nutritional perspective, fats with low melting points consist of high levels of

unsaturated fatty acids, and, conversely, fats with high melting points have comparably higher saturated fatty acids (Webb and O'neill, 2008; Wood et al., 2008). IMF content or marbling is a main determinant of meat-eating quality in most carcass grading systems (McPhee et al., 2008). As the IMF increases, so does the eating quality (Pannier et al., 2014) because it influences meat palatability and contributes significantly to juiciness, flavour, and tenderness (Pannier et al., 2014; Thompson, 2004). Consumers therefore prefer meat with low FMP, moderate IMF, and fatty acid composition with proportionately more of the health-promoting omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). Given that humans and other vertebrates lack the capacity to synthesize n-3 LC-PUFA because they lack the enzyme Δ^{15} desaturase, they must obtain these from dietary intake sources to meet their daily requirement of 500 mg of n-3 LC-PUFA (Nichols et al., 2010). Lamb producers can tap into the omega-3 functional meat market niche by matching their sheep breeding and production system to meet this health-conscious consumer preference.

Ryegrass (*Lolium perenne*) is a popular grass species in pasture-based grazing production systems in Australia and New Zealand. Ryegrass contains many phenolic compounds such as gallic and salicylic acids (phenolic acids), tannins, coumarins, flavonoids, α-tocopherol, lignans, xanthones and anthocyanidins (Pańka et al., 2013; Stewart and Stewart, 2008). These phenolic compounds in ryegrass serve as natural antioxidants, anti-inflammatory and anti-septic agents (Choi et al., 2017) that enhance meat oxidative stability and quality attributes such as nutritive value, flavour and colour. Luciano et al. (2009a; 2009b) reported that antioxidants in dietary tannins from fresh herbage improved colour stability in Comisana lambs by halting myoglobin oxidation in the muscle and reducing meat colour deterioration. In lambs grazing ryegrass, phenolics and antioxidant enzyme activities have been demonstrated to impact oxidative stability in the *Longissimus thoracis et lumborum* muscle (Petron et al., 2007), liver and plasma (López-Andrés et al., 2014). Research investigations of perceived sheepmeat

eating quality sensory scores (O'Reilly et al., 2020) and demographic influences (O'Reilly et al., 2020) on Australian, American, and Chinese consumers demonstrated a consistent consumer response to production factors of muscle type, sire, age, and sex. Chapter 2 indicated that meat eating quality and fatty acid (FA) composition of lipids in tandem with variable fat deposition at the attainment of maturity, vary in the muscles of sheep due to differences in breed (Zhang et al., 2020; De Vargas-Junior et al., 2019;Monaco et al., 2015; Souza et al., 2013), physiological status, breeding systems (Sampath and Ntambi, 2011), grass-fed versus concentrate feeding (Hoffman et al., 2020; Nuernberg et al., 2008), and sex (Van der Merwe et al., 2020; Vnučec et al., 2016).

Linebreeding is a sheep breeding practice of mating closely related animals that can be traced back to one common ancestor with highly desirable attributes. The Tattykeel Australian White (TAW) sheep are renowned for producing the remarkably unique high-eating-quality MARGRA lamb brand and were developed from more than a decade of rigorous selection, culling and linebreeding of Texel, Van Rooy, Dorper and Poll Dorset with an extensive utilisation of natural mating, artificial insemination and embryo transfer (Chapter 2). Linebreeding increases the frequency of desirable alleles, selection intensity and homozygosity, hence a tight culling regime and close monitoring of the inbreeding coefficient are key breeding management practices that ensure uniformity and consistency in TAW lamb eating quality. A comprehensive review of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) metabolism and meat-eating quality in TAW lambs in chapter 2 identified knowledge gaps in using *Longissimus dorsi* muscle biopsy sampling of ram and ewe lambs to directly determine the impact of linebreeding and gender on n-3 LC-PUFA, IMF and FMP contents while the animals are young and alive for early selection and breeding purposes. It also recommended the need for further research to better understand the genetic and nutritional interactions between dietary n-3 LC-PUFA oil supplements versus pasture grazing, finishing

performance, carcass traits and the unique eating quality of TAW lambs to afford industry players the opportunity to consistently meet consumer preferences as well as key demand and supply determinants of profitability. This Chapter aims to fill some of these knowledge gaps by assessing the impact of linebreeding and gender on pasture-fed lamb eating quality consistency in antioxidant status, IMF, FMP, n-3 LC-PUFA and to test the hypothesis that variation in healthy lamb eating quality will be a function of lamb gender and not its antioxidant status or inbreeding coefficient (IC) as an index of linebreeding.

3.2. Materials and Methods

3.2.1. Animal Ethics

The use of animals and all procedures performed in this study were approved by the James Cook University Animal Ethics Committee (Permit No. A0015657) in compliance with the Australian Code for Care and Use of Animals for Scientific Purposes (Eighth edition, 2013).

3.2.2. Animals and Experimental Design

The animals used in this study comprised a cohort of 100 ewe and 47 ram lambs at the Tattykeel Australian White stud farm in Black Springs, Oberon, New South Wales, Australia, grazing the same ryegrass pastures in separate paddocks. They were all 10-month-old lambs, with an average liveweight of 36.8 ± 0.3 kg (range of 36– 38 kg for rams), 37.4 ± 0.4 kg (range of 37–38 kg for ewes), and an overall mean body condition score of 2.5 ± 0.01. Carcass performance and meat quality characteristics of TAW had been shown in T. An a priori power analysis was conducted using G-Power to justify an appropriate sample and effect size. As depicted in Figure 3.1, to achieve a statistical power of 95 % with a critical F-value of 2.5, a minimum total sample size of 146 lambs was sufficient for a large effect size, and a two-sided significance level of 0.05. Therefore, the cohort of 100 ewe and 47 ram lambs at the Tattykeel Australian White stud farm in Black Springs, Oberon, New South Wales, Australia grazing the same ryegrass

pastures in separate paddocks used in this study, was a sufficient and statistically robust experimental design. Total digestible nutrients (Bath and Marble, 1989) and metabolisable energy (Robinson et al., 2004) were computed from the nutritive composition of the ryegrass (Table 3.1) analysed by the Association of Official Analytical Chemists (AOAC) wet chemistry procedure.

Table 3.1. Nutrient and phenolic antioxidant compositions of ryegrass pastures grazed by Tattykeel Australian White lambs[1].

Nutrient	**Composition (% DM)**
DM	20.7
CP	19.0
ADF	26.5
NDF	30.9
EE	1.8
Ash	6.8
%TDN	62.5
DE (Mcal/kg)	2.8
ME (MJ/kg)	9.4
Phenolic Antioxidants:	
FCTP (mg GAE/g)	1.631
FRAP (mmol Fe^{2+} E/g)	6.572

[1] DM: dry matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; EE: ether extract; CP: crude protein; %TDN [40]: total digestible nutrients, calculated as (% of DM) = 82.38 − (0.7515 × ADF [% of DM]). ME [41]: metabolizable energy, calculated by converting %TDN to digestible energy (DE [Mcal/kg] = %TDN × 0.01 × 4.4) which was converted as ME = (DE (Mcal/kg) × 0.82) × 4.185; FCTP: Folin–Ciocalteu total phenolics; GAE: gallic acid equivalents; FRAP: ferric reducing antioxidant power.

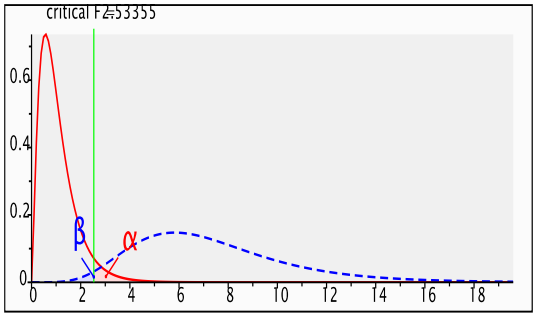

Figure 3.1. G-Power analysis for statistical power, effect and sample size.

2.3. Muscle Biopsy Sampling Procedure

Longissimus dorsi muscle biopsy samples were taken from the 12th –13th rib interface following the procedure described by Malau-Aduli et al. (1998) and are shown in Figure 3.2. Briefly, the animal was directed into a weighing chute with collapsible sides and some head restraint. The *Longissimus dorsi* muscle area on the back of the animal between the 12th and 13th ribs was shaved with a small electric clipper and cleaned with 90 % ethanol and chlorhexidine. About 15 mL of a local anaesthetic agent, lignocaine was administered intramuscularly. Five minutes following the administration of the anaesthetic, a 5–7 cm incision was made with a scalpel blade and about 5 g of the underlying fat and *Longissimus dorsi* muscle was sampled. The wound was closed via 3–4 interrupted sutures using surgilon thread. An anti-bacterial aerosol, Cetrigen, was applied to the sutured area on the skin to promote wound healing, prevent flies and the animal was released back to the paddock. No post-operative complications were reported as healing was rapid. The sutures were removed after 10–14 days. The muscle biopsy sample was immediately placed in a plastic bag on dry ice, flushed with nitrogen gas and transferred into a mobile refrigerator. Samples were transported frozen and stored at −20 °C pending further analysis in the laboratory. The muscle biopsies were analysed for IMF content, FMP and FA composition.

Figure 3.2. Muscle biopsy sampling technique in Tattykeel Australian White sheep.

3.2.4. Determination of Intramuscular Fat

The procedures of Holman et al. (2014) and Flakemore et al. (2014) were utilised for IMF determination. Briefly, the muscle sample was homogenised and 1g transferred to a labelled 50 mL plastic tube containing 20 mL of chloroform: methanol (2:1) solvent and shaken vigorously for 5 min. A filter paper was used to collect the filtrate in another labelled 50 mL tube. Approximately 5 mL of 10 % KCl was added to the filtrate to precipitate and separate the inorganic and lipid fractions into two distinct layers. The upper inorganic layer was removed and discarded, while the lower lipid layer was transferred into a clean, dry, pre-weighed and labelled ceramic crucible and evaporated in a laminar fume hood over a heating block. The crucible was cooled and further dried in a desiccator for 10–20 min before it was re-weighed. Samples were analysed in duplicates to allow for replication and reproducibility. Intramuscular fat percentage was calculated as:

[(Final crucible weight) − (Initial crucible weight) / (Initial sample weight)] × 100.

3.2.5. Determination of Fat Melting Point

The procedures of Holman et al. (2014) and Flakemore et al. (2014) were utilised for FMP determination. Briefly, the crucible containing the extracted IMF was placed in an oven at 100 °C for about 1–2 min to melt the fat. Using air suction, the melted fat was sucked into a thin capillary tube and placed in a refrigerator for about 10 min for the fat to solidify. The fat level in the capillary tube was marked with an indelible pen. The capillary tube was attached to a thermometer and vertically suspended in a beaker containing 80 mL of cold water, gradually heated over a heating block, and closely observed until the fat melted and "slipped" (rose above the mark) within the capillary tube. The temperature at which this slip occurred was recorded as the fat melting point. Samples were analysed in duplicates to allow for replication and reproducibility. TAW lamb has a very low fat melting point and can be liquid at room temperature of 25–28 °C as shown in Figure 3.3.

Figure 3.3. Tattykeel Australian White intramuscular fat (liquid at room temperature) indicating a low fat melting point (FMP).

3.2.6. Determination of Fatty Acid Composition

Fatty acid composition including n-3 LC-PUFA analysis of *Longissimus dorsi* muscle biopsy samples was analysed by means of gas chromatography–mass spectrophotometry procedure described by Malau-Aduli et al. (2016). Briefly, total lipids in 1 g of un-homogenised muscle tissue samples were extracted overnight using a modified Bligh and Dyer (1959) method. The first step was a single-phase overnight extraction using $CHCl_3$: MeOH: H2O (1:2:0.8 *v/v*). The second step involved phase separation with the addition of $CHCl_3$: saline Milli-Q H_2O (1:1 *v/v*) followed by rotary evaporation of the lower chloroform phase at 40 °C to obtain total lipids. The extracted total lipids were separated into lipid classes by thin layer chromatography (TLC) using 100 mL of the lipid extract reconstituted in hexane (Malau-Aduli et al., 1998). The extract was spotted onto silica gel G plates (200 × 200 × 0.25 mm) with a micropipette. The TLC plate was developed in an acetone/petroleum ether (1:3 vol/vol) solvent system in a tank containing a few crystals of butylated hydroxytoluene (BHT) to prevent oxidation. Triacylglycerols, cholesterol and free fatty acids migrated, while phospholipids remained at the origin of the plate. The areas corresponding to the phospholipids were scraped off the plate and each lipid class transferred to clean screw-capped test tubes for transmethylation and eventual computation of the lipid conversion factor (LCF) of 0.912 on the basis of g fatty acids/ g total lipids (0.083 phospholipids, 0.829 triacylglycerols and 0 % cholesterol because cholesterol does not contain any fatty acids). An aliquot from each total lipid extract was used for transmethylation with MeOH: $CHCl_3$: HCl (10:1:1 *v/v*) for 2 h at 80 °C. Fatty acid methyl esters (FAME) were extracted three times using hexane: $CHCl_3$ (4:1 *v/v*). A known concentration of an internal standard (19:0) was added in a 1500 µL vial containing the extracted FAME. The FAME were analysed on a 7890B gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an Equity^{TM}-1 fused 15 m silica capillary column with 0.1 mm internal diameter and 0.1 µm film thickness (Supelco, Bellefonte, PA,

USA), a flame ionisation detector, a split/splitless injector and an Agilent Technologies 7683 B Series autosampler. The gas chromatograph conditions were: splitless mode injection; carrier gas He; initial oven temperature 120 °C and then increased to 270 °C at flow rates of 10 °C/min and to 310 °C at 5 °C/min. The Agilent Technologies ChemStation software (Palo Alto, California, USA) was used to quantify fatty acid peaks. The fatty acid identities were confirmed by gas chromatograph–mass spectrometric (GC/MS) analysis using a Finnigan Thermoquest GCQ^{TM} GC/MS fitted with an on-column injector and Thermoquest Xcalibur software (Austin, Texas, USA). The gas chromatograph (GC) was equipped with an HP-5 cross-linked methyl silicone-fused silica capillary column (50 m × 0.32 mm internal diameter) which is of similar polarity to the column described above. The carrier gas was helium (head pressure 30 kPa) and GC conditions had been previously described by Miller et al. (2006). Fatty acid percentages were computed as follows:

FA% = [(individual fatty acid area) ∗ (100)]/ (sum total area of fatty acids). Fatty acid contents were calculated as follows: FA mg/100 g = (Total lipid) ∗ (LCF [0.912]) ∗ ([%FA]/100) ∗ 1000, where 0.912 was the derived lipid conversion factor like the one cited by Clayton (2014).

3.2.7. Extraction and Purification of Phenolic Compounds

Solid-phase extraction, purification, and analysis of phenolics in the ryegrass utilised the procedure described in detail by López-Andrés et al. (2014). Briefly, 2.5 g of the ryegrass was chopped to pass through a 1 mm sieve and homogenised at 4000 r.p.m. in 15 mL of acetone/water 70/30 *v/v* for 1 min and sonicated for 6 min in a water bath. Homogenates were centrifuged at 4 °C for 15 min at 3000 × g and the supernatants filtered with Whatman filter papers. About 10 mL of the filtered supernatant was acidified with 0.5 M H_2SO_4 and loaded onto reversed phase cartridges (C18 Sep-Pak Vac WAT043395, WATERS, Milan, Italy) preconditioned with methanol and distilled water to disrupt polyphenol-binding protein. The

extracted phenolics were eluted with 2 mL methanol and stored in a −30 °C freezer until ready for Folin–Ciocalteu (FCTP) and ferric reducing antioxidant power (FRAP) assays using a double-beam spectrophotometer (model UV-1601, Shimadzu Corporation, Milan, Italy) to measure the absorbance of the samples at 725 nm and 593 nm, respectively. Details of both assay procedures have been described (López-Andrés et al., 2014) and will not be repeated herein.

3.2.8. Antioxidant Enzyme Activities

Antioxidant activities of glutathione peroxidase, catalase and superoxide dismutase enzymes in the muscle were assayed as described by Petron et al. (2007). Briefly, about 5 g of the *Longissimus dorsi* muscle was homogenised in 25 mL of 0.005 M phosphate buffer (pH 7.0) and centrifuged at 4 °C for 20 min at 7000 g. The supernatant fraction was filtered through glass wool and used to determine glutathione peroxidase, catalase, and superoxide dismutase enzyme activities. By measuring the inhibition of pyrogallol autoxidation, total superoxide dismutase (SOD) activity (Cu–Zn SOD + Mn SOD) was determined where one unit was taken as the activity that inhibits the reaction by 50 %. To determine glutathione peroxidase enzyme activity, the oxidation of NADPH at 22 °C was used. The assay medium (3 mL) consisted of 1 mM reduced glutathione, 0.15 mM NADPH, 0.15 mM H_2O_2, 40 mM potassium phosphate buffer (pH 7.0), 0.5 mM EDTA, 1 mM NaN_3, 1.5 units of glutathione reductase, and 300 μL of the muscle extract. Absorbance at 340 nm was recorded over 3 min. An extinction coefficient of 6300 M^{-1} cm^{-1} was used for calculation of NADPH concentration. One unit of glutathione peroxidase enzyme activity was defined as the amount of extract required to oxidize 1 μmol of NADPH per min at 22 °C. Catalase enzyme activity was performed as described by (Petron et al., 2007). About 2 mL of the *Longissimus dorsi* muscle supernatant (2 mL) was reacted at room temperature (∼22 °C) with 1 mL of 30 mM H_2O_2 in 0.05 M phosphate buffer (pH 7.0), and the reaction (H_2O_2 decomposition) was monitored by measuring the absorbance at 240 nm

during the initial 30 s. An extinction coefficient of 0.040 cm^2 μmol^{-1} was used for calculation of H_2O_2 splitting. One unit (U) of catalase activity was defined as the amount of extract needed to decompose 1 μmol of H_2O_2 per min (Petron et al., 2007).

3.2.9. Statistical Analysis

IC as an index of linebreeding, estimates the probability that two alleles in an individual lamb will be homozygous (HH or hh) rather than heterozygous (Hh) because the parents are related and have one common ancestor. In other words, IC measures the extent to which two genes at any locus in an individual lamb are identical by descent from the common ancestor(s) of the two parents. IC was computed as:

$$F_X = \Sigma[(½)^{n+1} (1 + F_A)] \quad (1)$$

Where F_X = IC of lamb X, Σ = summation, n = number of common ancestors connecting the parents of lamb X and F_A is the IC of the common ancestor A.

Fatty acids, IMF, FMP, antioxidants, and enzyme activities were analysed as dependent variables using multivariate analysis of variance (MANOVA) after fitting the fixed effects of gender and IC in General Linear Model procedures (PROC GLM) using Statistical Analysis System software (SAS) version 9.4 (SAS Institute, Cary, NC, USA) (2013). First-order interactions between gender and IC were initially tested but later dropped from the final model due to non-significance. The initial full statistical model used for the analysis was:

$$Y = \mu + G_i + B_j + (GB)_{ij} + e_{ijk} \quad (2)$$

where Y = dependent variable (FMP, IMF, FA, antioxidants, and enzyme activities), μ = overall mean, G_i = Gender, B_j = Inbreeding Coefficient, $(GB)_{ij}$ = first-order interaction between gender and inbreeding coefficient, and e_{ijk} = residual error. Level of significance threshold was set at

$p < 0.05$ and differences between least square means were established using Tukey's pairwise comparison test.

3.3. Results

3.3.1. Nutrient Composition of the Grazed Ryegrass Pasture, Muscle Phenolics and Antioxidant Enzyme Activities

The ewe and ram lambs utilised in this study grazed high-quality ryegrass whose nutrient composition and antioxidant status is presented in Table 3.1 and fatty acid profile in Table 3.2. The low dry matter is indicative of fresh pasture with high moisture content, while the high phenolic antioxidants, crude protein, low neutral detergent and high metabolisable energy are all indicative of high palatability, digestibility and total digestible nutrients from the ryegrass pastures that are typical during spring. There were no significant differences due to gender (Table 3.3) and inbreeding coefficient (Table 3.4) in total phenolics and antioxidant enzyme activities of glutathione peroxidase, catalase and superoxide dismutase in the *Longissimus dorsi* muscle of these ryegrass pasture-fed lambs.

Table 3.2. Fatty acid composition of grazed ryegrass pasture.

Fatty Acid	% Total Fatty Acids
14:0	0.6
15:0	0.2
16:1n-9c	0.0
16:1n-7c	0.2
16:0	15.7
17:0	0.5
18:2n-6 LA	14.8
18:3n-3 ALA	57.6
18:1n-9c	1.0
18:1n-7c	0.2
18:1n-7t	0.0
18:0	0.1
20:4n-6 ARA	0.0
20:5n-3 EPA	0.0
20:3n-6	0.1
20:4n-3	0.1
20:2n-6	0.1
20:0	1.6
22:5n-6 DPA-6	0.0
22:6n-3 DHA	0.0
22:5n-3 DPA-3	0.0
22:0	1.0
23:0	0.3
24:0	0.9
∑SFA	20.9
∑MUFA	4.9
∑PUFA	73.1
∑n-3 LC-PUFA	0.1
∑n-3 PUFA	58.0
∑n-6 PUFA	15.2
∑other FA	1.0
n-6/n-3	0.3

LA, linoleic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ARA, arachidonic acid; ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; and total polyunsaturated fatty acids (ΣPUFA). ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1, 16:1n-13t, 16:1n-9, 16:1n-7, 16:1n-7t, 16:1n-5c, 17:1n-8+a17:0, 18:1n-9, 18:1n-7t, 18:1n-5, 18:1n-7, 18:1a, 18:1b, 18:1c, 19:1a, 19:1b, 20:1n-11, 20:1n-9, 20:1n-7, 20:1n-5, 22:1n-9, 22:1n-11, 24:1n-9; ∑PUFA is the sum of 18:4n-3, 18:3n-6, 18:2n-6, 18:3n-3, 20:3, 20:4n-3, 20:4n-6, 20:5n-3, 20:3n-6, 20:2n-6, 22:6n-3, 22:5n-3, 22:5n-6, 22:4n-6; ∑n-3 LC-PUFA is the sum of 20:5n-3, 20:4n-3, 22:6n-3, 22:5n-3; ∑n-3 PUFA is the sum of 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:6n-3, 22:5n-3; ∑n-6 PUFA is the sum of 18:2n-6, 18:3n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6; ∑other FA is the sum of other individual FA present at <0.1% except ARA, DHA, EPA, and DPA.

Table 3.3. Effect of gender (Means ± s.d.) on fat melting point, intramuscular fat, fatty acids, antioxidant phenolics and enzyme activities in the *Longissimus dorsi* muscle of ryegrass fed Tattykeel Australian White (TAW) lambs[1.]

Variable	Ram (*n* = 47)	Ewe (*n* = 100)	Overall (*n* = 147)	*P*-value
Fat Melting Point (°C)	35.5 ± 1.5	34.2 ± 2.4	34.6 ± 2.3	0.0001
Intramuscular fat (%)	3.4 ± 0.3	4.4 ± 1.4	4.1 ± 1.3	0.0001
FCTP (mg GAE/g)	1.142 ± 0.0036	1.171 ± 0.0042	1.156 ± 0.0039	0.4723
FRAP (mmol Fe^{2+} E/g)	5.481 ± 0.0172	5.605 ± 0.0198	5.543 ± 0.0185	0.2982
GSH-Px (U/g)	0.085 ± 0.0012	0.091 ± 0.0024	0.088 ± 0.0018	0.0921
Cat (U/g)	39.8 ± 1.3	40.1 ± 1.5	40.0 ± 1.4	0.0882
SOD (U/g)	63.8 ± 5.7	64.9 ± 6.1	64.4 ± 5.9	0.1566
Fatty Acids (mg /100 g)				
C12:0	0.1 ± 0.5	0.0 ± 0.0	0.0 ± 0.3	0.1453
C13:0	3.2 ± 5.4	0.8 ± 3.2	1.5 ± 4.2	0.0009
C14:0	438.7 ± 492.2	153.8 ± 291.9	244.9 ± 389.7	0.0001
C14:1	11.9 ± 15.7	3.0 ± 5.8	5.8 ± 10.9	0.0001
C15:0	168.6 ± 172.2	46.1 ± 98.0	85.3 ± 138.3	0.0001
C16:0	3321.2 ± 2631.5	1093.0 ± 1457.6	1805.4 ± 2170.2	0.0001
C16:1	260.7 ± 237.0	94.6 ± 134.1	147.7 ± 189.5	0.0001
C17:0	321.0 ± 307.3	109.1 ± 237.7	176.8 ± 279.1	0.0001
C17:1	233.0 ± 247.8	75.3 ± 137.5	125.7 ± 194.0	0.0001
C18.0	2692.0 ± 2283.4	1016.9 ± 1649.3	1552.5 ± 2025.2	0.0001
C18:1	4942.6 ± 4041.0	1920.3 ± 2489.0	2886.6 ± 3368.4	0.0001
C18:2 n-6 LA	423.5 ± 266.6	125.4 ± 80.0	220.7 ± 214.9	0.0001
C18:3 n-3 ALA	262.6 ± 209.0	72.5 ± 81.6	133.3 ± 161.9	0.0001
C18:3 n-6	2.1 ± 4.3	2.4 ± 6.5	2.3 ± 5.9	0.8014
C18:4 n-3	3.7 ± 8.6	1.6 ± 10.1	2.3 ± 9.7	0.2262
CLA	117.9 ± 129.7	63.7 ± 190.0	81.1 ± 174.4	0.0788
C19:1	39.1 ± 41.4	14.3 ± 26.3	22.2 ± 33.8	0.0001
C20:0	20.3 ± 18.4	7.3 ± 10.8	11.4 ± 15.0	0.0001
C20:1	26.3 ± 28.4	8.1 ± 12.2	13.9 ± 20.7	0.0001

C20:2 n-6	7.9 ± 8.9	2.1 ± 3.1	4.0 ± 6.2	0.0001
C20:3	8.4 ± 4.1	11.7 ± 12.7	10.6 ± 10.8	0.0839
C20:3 n-6	8.8 ± 4.9	6.1 ± 2.1	7.0 ± 3.5	0.0001
C20:4 n-3	4.8 ± 7.3	2.1 ± 1.5	3.0 ± 4.5	0.0005
C20:4 n-6	36.4 ± 14.2	33.7 ± 8.0	34.6 ± 10.4	0.1473
C20:5 n-3 (EPA)	26.0 ± 8.5	24.3 ± 5.2	24.9 ± 6.5	0.1402
C21:0	2.0 ± 2.5	0.6 ± 1.4	1.0 ± 1.9	0.0001
C22:0	2.8 ± 3.3	2.3 ± 1.3	2.5 ± 2.2	0.1342
C22:1	0.4 ± 1.2	0.8 ± 1.6	0.7 ± 1.5	0.1613
C22:4 n-6	0.6 ± 1.2	1.4 ± 0.5	1.2 ± 0.9	0.0001
C22:5 n-3 (DPA)	22.5 ± 11.6	25.2 ± 8.0	24.4 ± 9.4	0.097
C22:5 n-6	0.0 ± 0.1	0.2 ± 0.2	0.1 ± 0.2	0.0001
C22:6 n-3(DHA)	5.8 ± 3.7	8.3 ± 2.7	7.5 ± 3.2	0.0001
C23:0	2.5 ± 2.3	2.5 ± 0.7	2.5 ± 1.4	0.7207
C24:0	2.2 ± 2.0	2.8 ± 0.9	2.6 ± 1.4	0.008
C24:1 n-9c	1.7 ± 2.1	3.9 ± 1.8	3.2 ± 2.1	0.0001
EPA+DHA	31.9 ± 11.3	32.6 ± 7.0	32.4 ± 8.5	0.6265
EPA+DHA+DPA	54.4 ± 21.8	57.9 ± 13.6	56.7 ± 16.7	0.2388
SFA	6971.2 ± 5684.7	2434.4 ± 3700.9	3884.9 ± 4896.6	0.0001
MUFA	2120.3 ± 2772.9	5515.7 ± 4577.1	3205.9 ± 3786.7	0.0001
PUFA	380.7 ± 331.9	931.1 ± 614.7	556.7 ± 510.0	0.0001
PUFA/SFA	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.0036
∑n-3 PUFA	134.1 ± 96.5	325.5 ± 231.7	195.3 ± 176.7	0.0001
∑n-6 PUFA	479.3 ± 279.5	171.3 ± 85.2	269.8 ± 224.3	0.0001
n-6/ n-3 PUFA	1.6 ± 0.5	1.4 ± 0.3	1.5 ± 0.4	0.0001

[1]FCTP: Folin–Ciocalteu Total Phenolics; GAE: Gallic acid equivalents; FRAP: Ferric reducing antioxidant power. Antioxidant enzyme activities of GSH-Px: glutathione peroxidase, Cat: catalase (Cat) and SOD: superoxide dismutase. ∑SFA sum of saturated FAs: C12:0+C13:0+C14:0CC14:0+C15:0+C15:0+C15:0+C16:0+C17:0+C18:0+C20:0+C21:0+C22:0+ C23:0+C24:0; ∑MUFA sum of monounsaturated FAs: C14:1+ C16:1+C17:1+C18:1+C19:1+C20:1+C21:1+C22:1+ C24:1. ∑PUFA is the sum of polyunsaturated FA: C18:2 n-6+ C18:3 n-3+ C18:3 n-6+ C18:4 n-3+CLA+C20:2 n-6 +C20:3+C20:3 n-6+C20:4 n-3+ C22:4 n-6+ C20:5 n-3 +C22:5 n-3+C22:5 n-6+ C22:6 n-3. ∑n-6 PUFA is the sum of n-6 PUFA: C18:2 n-6+C18:3 n-6 +C20:2 n-6+C20:4 n-6+C20:3 n-6+C20:4 n-6+C22:5 n-6. ∑n-3 PUFA is the sum of n-3 PUFA: C18:3 n-3+C18:4 n-3+C20:4 n-3+C20:5 n-3+C22:5 n-3+C22:6 n-3.

Table 3.4. Effect of inbreeding coefficients (Means ± s.d.) on fat melting point, intramuscular fat, fatty acids, antioxidant phenolics and enzyme activities in the *Longissimus dorsi* muscle of ryegrass-fed TAW lambs[1.]

Variable	Inbreeding coefficient (%)			*P*-value
	Low (0–5) (*n* = 49)	Medium (6–10) (*n* = 49)	High (Above 10) (*n* = 49)	
Fat Melting Points (°C)	34.9 ± 2.1	34.2 ± 2.4	34.8 ± 2.8	0.225
Intramuscular fat	4.1 ± 1.4	4.0 ± 1.2	4.1 ± 1.0	0.9148
FCTP (mg GAE/g)	1.271 ± 0.0014	1.269 ± 0.0019	1.290 ± 0.0014	0.8524
FRAP (mmol Fe^{2+} E/g)	6.018 ± 0.0027	6.083 ± 0.0045	6.102 ± 0.0086	0.2352
GSH-Px (U/g)	0.091 ± 0.0041	0.086 ± 0.0036	0.089 ± 0.0062	0.0896
Cat (U/g)	40.5 ± 1.8	40.1 ± 1.6	40.7 ± 1.9	0.1843
SOD (U/g)	64.8 ± 5.7	65.0 ± 5.9	64.5 ± 5.3	0.0972
Fatty Acids (mg /100 g)				
C12:0	0.0 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.6757
C13:0	2.3 ± 4.6	0.7 ± 3.5	0.0 ± 0.0	0.0574
C14:0	340.3 ± 479.9	127.5 ± 176.7	94.6 ± 50.8	0.0033
C14:1	7.7 ± 11.9	3.6 ± 9.4	2.4 ± 1.7	0.0609
C15:0	116.6 ± 152.0	47.7 ± 112.7	27.1 ± 22.9	0.0074
C16:0	2377.1 ± 2509.5	1100.6 ± 1410.2	923.7 ± 527.9	0.0013
C16:1	194.1 ± 215.9	90.9 ± 135.4	71.8 ± 38.3	0.0033
C17:0	241.4 ± 322.4	98.9 ± 193.4	61.0 ± 32.9	0.006
C17:1	165.6 ± 202.9	78.3 ± 178.8	46.6 ± 26.2	0.0174
C18.0	2130.6 ± 2447.3	840.2 ± 938.8	655.2 ± 288.8	0.0004
C18:1	3704.7 ± 3831.0	1885.3 ± 2423.3	1552.9 ± 690.9	0.0036
C18:2 n-6 (LA)	271.1 ± 244.2	158.9 ± 156.5	139.0 ± 65.3	0.0053
C18:3 n-3 (ALA)	170.3 ± 179.0	89.0 ± 129.2	63.7 ± 33.0	0.0067
C18:3 n-6	3.1 ± 7.8	1.3 ± 0.9	1.5 ± 0.8	0.032
C18:4 n-3	3.1 ± 11.5	1.4 ± 7.0	0.4 ± 0.3	0.5311
CLA	114.4 ± 219.4	40.4 ± 75.8	24.9 ± 13.7	0.032
C19:1	29.4 ± 37.4	13.6 ± 27.5	9.1 ± 5.2	0.0139
C20:0	15.5 ± 17.7	6.5 ± 8.4	5.2 ± 2.7	0.001

C20:1	18.4 ± 22.3	8.7 ± 17.9	5.2 ± 2.2	0.0124
C20:2 n-6	5.3 ± 7.2	2.2 ± 4.3	2.0 ± 2.1	0.0092
C20:3	11.0 ± 14.3	10.2 ± 2.7	9.4 ± 2.7	0.8782
C20:3 n-6	7.6 ± 3.9	6.2 ± 2.7	5.6 ± 1.1	0.8782
C20:4 n-3	3.6 ± 5.3	2.3 ± 3.1	1.6 ± 0.6	0.171
C20:4 n-6	32.8 ± 11.6	36.6 ± 8.4	38.0 ± 8.3	0.0737
C20:5 n-3 (EPA)	24.3 ± 7.0	25.8 ± 5.9	23.0 ± 4.8	0.3091
C21:0	1.6 ± 2.3	0.4 ± 0.9	0.1 ± 0.2	0.0009
C22:0	3.0 ± 2.7	1.8 ± 1.1	1.6 ± 0.8	0.0055
C22:1	0.8 ± 1.9	0.6 ± 0.9	0.3 ± 0.3	0.5598
C22:4 n-6	1.2 ± 1.0	1.2 ± 0.7	1.3 ± 0.7	0.9005
C22:5 n-3 (DPA)	24.7 ± 11.5	23.9 ± 5.7	23.3 ± 5.0	0.8515
C22:5 n-6	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.3	0.3705
C22:6 n-3(DHA)	7.4 ± 3.8	7.6 ± 2.4	7.5 ± 3.1	0.9816
C23:0	2.7 ± 1.7	2.7 ± 1.0	2.0 ± 1.1	0.2737
C24:0	2.7 ± 1.5	2.5 ± 1.2	2.3 ± 1.3	0.674
C24:1 n-9c	2.9 ± 2.1	3.5 ± 2.1	3.7 ± 2.1	0.2498
EPA+DHA	31.8 ± 9.5	33.4 ± 7.1	30.6 ± 7.6	0.4708
EPA+DHA+DPA	56.5 ± 19.8	57.3 ± 11.9	53.8 ± 12.4	0.8738
SFA	5231.3 ± 5786.8	2228.5 ± 2775.3	1772.7 ± 913.9	0.0007
MUFA	4123.6 ± 4281.0	2084.4 ± 2782.6	1691.9 ± 761.3	0.0036
PUFA	680.1 ± 577.2	407.1 ± 373.2	341.5 ± 105.3	0.0037
PUFA/SFA	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.0781
∑n-3 PUFA	233.4 ± 196.1	149.9 ± 141.9	119.6 ± 27.5	0.0114
∑n-6 PUFA	321.2 ± 255.7	206.6 ± 162.3	187.6 ± 71.6	0.0067
n-6/ n-3 PUFA	1.5 ± 0.4	1.5 ± 0.3	1.5 ± 0.3	0.8487

[1]Abbreviations are the same as in Table 3.3.

3.3.2. Intramuscular Fat Content (IMF)

IMF ranged from 3.4 to 8.2 %, but ewe lambs had significantly higher IMF (4.4 ± 1.4 %) than ram lambs (3.4 ± 0.3 %) as shown in Figure 3.4A. Irrespective of gender, the overall IMF was 4.1 ± 1.3 % (Table 3.3). As shown in Table 3.4, IC as an index of linebreeding was classified into low (0–5 %), medium (6–10 %) and high (>10 %) and ranged from 0 to 15.6 %. As IC increased, there were no differences in IMF (Table 3.4 and Figure 3. 4B).

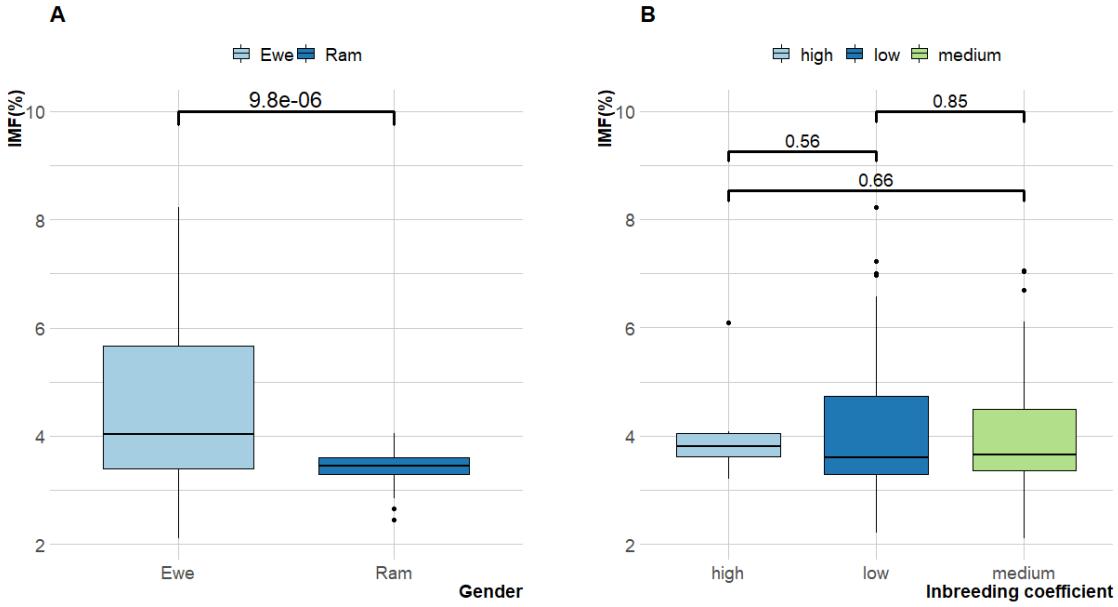

Figure 3.4. Variation in intramuscular fat (IMF) percentage of Tattykeel Australian White (TAW): (A) gender; (B) Inbreeding Coefficient (IC).3.3.3. Fat Melting Point (FMP)

FMP ranged from 28 to 39 °C, but ewe lambs had significantly lower FMP (34.26 ± 2.43 °C) than ram lambs (35.5 ± 1.5 °C) as shown in Figure 3.5. Irrespective of gender, the overall FMP was 34.6 ± 2.3 °C (Table 3.3). Similar to IMF, IC was not significantly associated with FMP (Table 3.4A and Figure 3.4B).

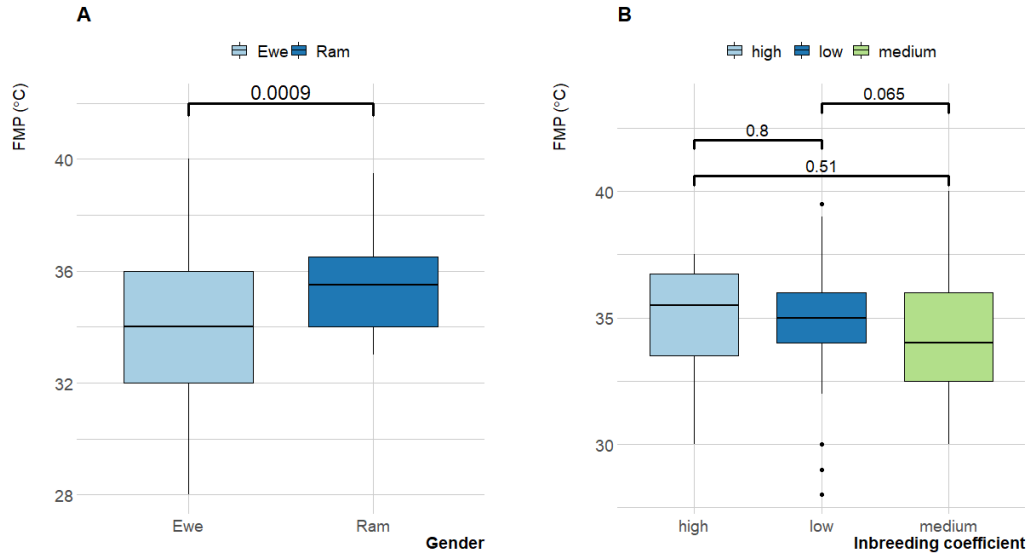

Figure 3.5. Variation in fat melting point (FMP) percentage of Tattykeel Australian White (TAW): (A) gender; (B) Inbreeding Coefficient (IC).

3.3.4. Fatty acid composition

The fatty acid composition in ewe and ram lambs in mg /100 g tissue is shown in Table 3.3. It shows that ewe lambs had significantly ($P < 0.0001$) higher C22:5n-3 (DPA), C22:6n-3 (DHA), C18:3n-6, C20:3, C22:4n-6, C22:5n-6, MUFA, PUFA and Σn-3 and lower SFA fatty acids than ram lambs. Although n-3 LC-PUFA ranged from "source" to "good source" levels of 33–69 mg /100 g in individual lambs, overall, there were no gender differences in the health-promoting EPA, DPA, EPA+DHA and EPA+DHA+DPA (Table 3.3). As IC increased, there were no differences in C20:5n-3 (EPA), DHA, DPA, EPA+DHA, EPA+DHA+DPA and ∑n-6/ ∑n-3 ratio, while increases in C18:3n-3 (ALA), MUFA, PUFA, C18:1, C18:2n-6, C18:3N-6, ∑n-3 PUFA and ∑n-6 PUFA were observed as IC decreased from high to low (Table 3.4).

3.4. Discussion

Consumer preferences, behaviours, perceptions, and satisfaction with the eating quality of meat products are intricately linked to flavour, odour, colour, aroma, taste and juiciness (de Andrade

et al., 2016; Font-i-Furnols et al., 2014). Previous studies (Watkins et al., 2013; Young et al., 2006; Sañudo et al., 2003; Young et al., 2003; Priolo et al., 2001., Young et al., 1997) identified diet-related "pastoral flavour" in lamb, also described as "milky", "barnyard", "sheepy" or "faecal" flavour, to negatively impact consumer liking. It is thought that this unpleasant "pastoral flavour" originates from skatole (3-methylindole) and indole derivatives from the degradation of tryptophan, 4-methylphenol and other branched chain fatty acids in the rumen (Watkins et al., 2014). Lamb has also been reported to have a distinct age-related "mutton flavour" and aroma associated with the three branched chain fatty acids 4-methylnonanoic, 4-methyloctanoic and 4-ethyloctanoic acids (Watkins et al., 2010). Other previous studies had demonstrated that the nutritional background of pasture-fed ruminants confers a higher muscle α-tocopherol antioxidant status compared to those on concentrate-based diets (Baldi et al., 2019; Van Elswyk et al., 2014; Bekhit et al., 2013; Zervas et al., 2011; Daley et al., 2010). Several internal and external factors influence the quantity and quality of lipids in animal products due to genetics-nutrition interactions in the expression of genes controlling fat metabolism (Malau-Aduli and Kashani, 2015) and these include the key attributes of fat melting point, intramuscular fat and fatty acid composition. The Folin–Ciocalteu total phenolics, ferric reducing antioxidant power and antioxidant enzyme activities of glutathione peroxidase, catalase and superoxide dismutase values in the present study were consistent with those reported in the *Longissimus thoracis et lumborum* muscle (Petron et al., 2007), liver and plasma (López-Andrés et al., 2014) of lambs grazing ryegrass. However, in our present study, the observation that none of the dietary phenolic compounds and antioxidant enzyme activities detected in the *Longissimus dorsi* muscle were affected by the lamb gender (Table 3) or inbreeding coefficient (Table 4) suggests that gender and linebreeding had no direct impact on the antioxidant status and deposition mechanism in the muscle tissue of MARGRA lambs. This implies that TAW sheep grazing ryegrass rich in phenolics contributed to an improved overall

meat oxidative stability with similar deposition and bioavailability in ewe and ram lambs irrespective of inbreeding coefficient.

3.4.1. FMP

The FMP range of 28–39 °C and an overall mean of 34.6 ± 2.3 °C obtained for TAW in the present study is well below the range of 40.6–48.0 °C and 41.5–44.0 °C reported by Flakemore et al. (2014) and Holman et al. (2014) in purebred and crossbred Merino, Dorset, Black and White Suffolk sheep. The presence of double or triple bonds in the FA structure leads to lower melting points because the higher the proportions of MUFA and PUFA, the more easily such bonds can be broken and the lower the fat melting points compared to the more stable SFA with high FMP. Smith et al. (1998) reported that these SFA contributed to an elevation in the hardness of fat in beef, while Flakemore et al. (2014) reported that the softness or hardness of fat has safety implications for meat processors and boning room personnel in abattoirs. From our results in the present study (Table 3), the lesser the SFA concentration and more MUFA and PUFA implied a low FMP, indicating that TAW is not only a healthier meat product for consumers, but also a safe product to meat processors in the abattoir due to ease of processing. It was also apparent that elevated proportions of SFA, especially palmitic (C16:0) and stearic (C18:0) acids in ram lambs, could have been the reason for the higher FMP than in ewe lambs. While the underpinning reasons behind the observed sex differences in FMP are not definitive from our present study, we can speculate that hormonal differences between ram and ewe lambs could have possibly had an indirect influence on FMP through IMF. This is because it has been reported that in intact bovine males, testosterone binds to receptors within the muscle and increases amino acid incorporation into protein, thus increasing muscular development, growth rate and muscle mass without simultaneous increases in IMF (Cafferky et al., 2019; Venkata Reddy et al., 2015). The lesser the IMF, the higher the FMP, and the higher the IMF as seen in TAW ewe lambs, the lower the FMP. This would seem to explain why, in the current study,

the ewe lambs had higher IMF and lower FMP than ram lambs. Further research on the likely underlying molecular mechanisms behind FMP and IMF variation through lipogenic genes controlling fat metabolism like fatty acid binding protein-4, fatty acid synthase and stearoyl-CoA desaturase in TAW lambs, would assist in shedding more light.

3.4.2. IMF

IMF influences meat palatability and contributes to its juiciness, flavour and tenderness with direct linkage between intramuscular fat deposition and gender, age, genetics and nutrition (Cafferky et al., 2019; Venkata Reddy et al., 2015; Hopkins et al., 2014). The overall mean IMF of 4.4 ± 0.2 % in the current study surpasses the suggested minimum Australian threshold of 4 % for lamb palatability by Pannier et al. (2014) who reported an overall average IMF of 4.23 ± 0.01 % in lambs from sires selected for leanness. The IMF values in TAW pasture-fed lambs in the current study are much higher than the 1.25 ± 0.22 % in lot-fed Manchega lambs reported by Gomez-Cortes et al. (2019) in contrast to the expectation that lambs sacrificed after 42 days in the feedlot should have higher IMF. This would most likely be a combination of both genetic and nutritional effects with TAW having a genetic predisposition for a comparatively higher and faster rate of IMF deposition in response to ryegrass pasture feeding than other breeds such as the Manchega lambs fed concentrate rations with high fibrous components. Published reports of gender differences in IMF are not unanimous in their findings. For instance, while Pannier et al. (2014) and McPhee et al. (2009; 2008) reported significant sex differences in IMF just as we also observed in TAW lambs, Okeudo and Moss (2007) did not find any differences in intramuscular lipid and fatty acid profiles of sheep comprising four sex-types. In beef cattle, Cafferky et al. (2019) stated that the higher IMF values in steers than intact bulls are attributed to the diminished physiological effects of androgen, which reduces plasma lipids, increases lipolysis by adipocytes and stimulates androgen receptors to directly upregulate the lipogenic gene expression of fatty acid synthase

and acetyl-CoA carboxylase (Lee et al., 2013; Xu et al., 1990) while simultaneously downregulating the lipolytic gene expression of monoglyceride lipase and adipose triglyceride lipase (Young et al., 2006). Hence, castration contributes to improved IMF deposition through increased lipogenesis and lipid uptake while decreasing lipolysis (Bong et al., 2012). Given the hormonal differences between ewe and ram lambs, similar genetic, physiological and biochemical pathways may be involved, and our lab is currently exploring the sequencing and expression of fatty acid binding protein-4, fatty acid synthase and stearoyl-CoA desaturase genes in TAW to unravel and better understand the underpinning mechanisms of fat metabolism.

It was quite interesting that IC had no impact on FMP and IMF (Table 3). This is very significant from an eating quality perspective because it indicates that TAW lambs can produce consistently high-quality MARGRA meat product with low FMP and high IMF regardless of linebreeding with IC in the 0–15.6 % range. To our current knowledge, the present study is the first of its kind to provide a significant insight into the impact of IC on meat eating quality in lamb as the only other reported research on inbreeding was in milking cows where Carrara et al. [77] reported significant ($P < 0.04$) impact of inbreeding on milk PUFA.

3.4.3. Omega-3 Long-Chain Polyunsaturated Fatty Acids

The ingestion of n-3 LC-PUFA confers several health benefits, including inhibiting cardiovascular diseases, cancer, and diabetes, obesity and neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's, and Alzheimer's (Zárate et al., 2017) as well as improve visual and brain development (Gould et al., 2013). Le et al. (2019) reported that Food Standards of Australia and New Zealand (FSANZ) guidelines stipulate that for any food or meat to be termed a 'source' of n-3 LC-PUFA, its EPA and DHA levels must be greater than 30 mg per 100 g per serve. TAW lambs had 32.4 ± 8.5 mg per 100 g of muscle, thus surpassing

the 30 mg limit set by FSANZ for 'source' claim. The main FA in pastures is ALA, a precursor of the more potent n-3 LC-PUFA (Lourenço et al., 2008), especially EPA, DHA and DPA, which have important roles to play in human health. The observations that, as IC increased, there were no differences in FMP, IMF, C20:5n-3 (EPA), DHA, DPA, EPA+DHA, EPA+DHA+DPA and Σn-6/Σn-3 ratio and that increases in C18:3n-3 (ALA), MUFA, PUFA, C18:1, C18:2n-6 and C18:3n-6 were observed as IC decreased, indicate that linebreeding in the 0–15.6 % range is not in any way detrimental to consistency in health-promoting n-3 LC-PUFA in TAW lambs. Such observations represent the first piece of experimental evidence regarding the impact of IC on omega-3 FA. Our data herein provide scientific evidence that TAW MARGRA lamb contains higher levels of health beneficial n-3 LC-PUFA than in other Australian lamb breeds previously reported by Ponnampalam et al. (2001; 2002; 2014a; 2014b; 2020), Flakemore et al. (2017), Knight et al. (2020; 2014), De Brito et al. (2017) and Fowler et al. (2019). Sex has been shown to influence heart and muscle FA composition, although the differences were restricted to only a few FA (Malau-Aduli et al., 2014). Previous studies have attributed FA variations due to sex as arising from sex-linked hormonal differences, which affect development and rumen biohydrogenation (Malau-Aduli et al., 2014).

3.5. Conclusions

The results obtained from this study provide the first detailed scientific evidence of TAW MARGRA lamb with low SFA, high IMF, MUFA, n-3 LC-PUFA and lower FMP. Therefore, the meat from TAW lambs provides anecdotal and scientific evidence for adequate meat oxidative stability and human health benefits associated with n-3 LC-PUFA to consumers. This study clearly provides a scientific confirmation of the unique meat-eating quality traits of TAW lambs. Based on nutritional value to consumers, this study reinforces the health benefits derived from consuming TAW MAGRA lamb in view of its high EPA, DHA and DPA contents. This high n-3 LC-PUFA profile of TAW MARGRA lamb has put this breed well ahead of others in

terms of healthy meat products. Our findings clearly show significant gender variation between ram and ewe lambs. The lower SFA and higher MUFA and PUFA contents make MARGRA lamb fats very soft and smooth melting in the mouth without sticking to the palate due to its low FMP and healthier composition. This study provides evidence that IC is inconsequential in affecting antioxidant status, IMF, FMP, and n-3 LC-PUFA in linebred and pasture-fed TAW sheep. This is because the observed variation in individual fatty acids was mainly driven by gender differences between ewes and rams, hence the need to accept the tested hypothesis. The practical implication is that health-conscious meat consumers are reassured by the scientific evidence herein of the consistency in the eating quality of MARGRA lamb brand from TAW sheep regardless of its linebred origin.

3.6. Summary

Health-conscious consumers increasingly demand healthier, tastier, and more nutritious meat, hence the continuous need to meet market specifications and demand for high-quality lamb. We evaluated the *longissimus dorsi* muscle of 147 Tattykeel Australian White (TAW) sheep fed on antioxidant-rich ryegrass pastures exclusive to MAGRA lamb brand for meat eating quality parameters of intramuscular fat (IMF) content, fat melting point (FMP) and omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). The aim was to assess the impact of linebreeding and gender on pasture-fed lamb eating quality and to test the hypothesis that *variation in healthy lamb eating quality is a function of lamb gender and not its antioxidant status or inbreeding coefficient* (IC). After solid-phase extraction and purification, phenolics and antioxidant enzyme activities were analysed by high-performance liquid chromatography and mass spectrometry. IMF and fatty acid composition were determined using solvent extraction and gas chromatography, respectively. IC was classified into low (0–5 %), medium (6–10 %) and high (>10 %) and ranged from 0–15.6 %. FMP and IMF ranged from 28 to 39 °C and 3.4 to 8.2 %, with overall means of 34.6 ± 2.3 °C and 4.4 ± 0.2 %, respectively, and n-

3 LC-PUFA ranged from “source” to “good source” levels of 33–69 mg/100 g. Ewes had significantly ($P < 0.0001$) higher IMF, C22:5n-3 (DPA), C22:6n-3 (DHA), C18:3n-6, C20:3, C22:4n-6, C22:5n-6, total monounsaturated (MUFA), PUFA and Σn-3 fatty acids and lower total saturated fatty acids (SFA) and FMP, than rams. As IC increased, there were no differences in FMP and IMF. Folin–Ciocalteu total phenolics, ferric reducing antioxidant power and antioxidant activities of glutathione peroxidase, catalase and superoxide dismutase enzymes did not differ by either gender or IC. This study provides evidence that IC is inconsequential in affecting antioxidant status, IMF, FMP, and n-3 LC-PUFA in linebred and pasture-fed TAW sheep because the observed variation in individual fatty acids was mainly driven by gender differences between ewes and rams, hence the need to accept the tested hypothesis. This finding reinforces the consistent healthy eating quality of MARGRA lamb brand from TAW sheep regardless of its linebred origin.

Chapter 4: Fortification of Diets with Omega-3 Long-Chain Polyunsaturated Fatty Acids Enhances Feedlot Performance, Intramuscular Fat Content, Fat Melting Point and Carcass Characteristics of Tattykeel Australian White MARGRA Lambs

4.1. Introduction

As the world's fourth most consumed meat after pork, poultry, and beef (OECD, 2022), lamb meat contributes significantly to global human nutrition since it contains nutrients of high biological value (Flakemore et al., 2017). In 2021, Australia was ranked second in global sheep production after China, and the latter still imports sheep meat as local production cannot meet its domestic needs (MLA, 2022). As the world's largest sheep exporter in 2021, Australia's overall sheep meat exports increased by 5.3% to $3.96 billion, representing a 0.5% increase in contribution to the overall worth of exports to the national economy (MLA, 2022).

Lamb consumers demand fresh, tasty, safe, and microbe-free meat with high eating quality and nutrient content, thus necessitating advanced nutrition and breeding strategies that integrate appropriate meat quality characteristics by sheep producers (de Nadai Bonin et al., 2021) to improve feedlot performance, dressing percentage, lean yield, and marbling score (Ross et al., 2021; Sood et al., 2022). To achieve accelerated lamb growth and early attainment of appropriate slaughter weights that meet market specifications, feedlotting remains a critical lamb finishing strategy for improving profitability (Arruda et al., 2021; Saldanha et al., 2022). It also facilitates the production of more uniform lamb carcasses (Brand et al., 2017) from a low mortality system that ensures more efficient use of human and technical resources to attain improved meat yield and quality (van Cleef et al., 2019). However, increased feed cost is a major limiting factor in the feedlot system (Castro et al., 2020), accounting for 65%−70% of the total cost of small ruminant production (Zhang et al., 2021). As such, nutritional strategies for increasing animal growth performance using cheap feeds without compromising carcass nutrient value and eating quality are essential elements for a profitable livestock production

enterprise to consider (Vahedi et al., 2021). Hence, there is a need for concerted research efforts in exploring diverse dietary fortification options.

The red meat industry is experiencing modernization in its production system to meet current consumer demands associated with health, quality of life, and sustainability (Fernández-López et al., 2021). The profile and quality of fatty acids in lamb can be improved by incorporating lipid sources into the diet of lot-fed lambs to boost the levels of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) known to be beneficial for human health (Fernández-López et al., 2021; Nudda et al., 2022). Meat industry producers and processors aim at producing heavy carcasses of young animals with good musculature, supplying desired meat cut yields and an attainable fat layer to protect carcasses while in cold storage (da Silva et al., 2020). Intramuscular fat (IMF), fat melting point (FMP), and pH are essential quality indicators of red meat (Dixit et al., 2021). The high marbling score represents the improved amount of IMF, water holding capacity, tenderness, flavour, juiciness, lamb palatability (Hitchman et al., 2021; Realini et al., 2021), content, and distribution of protein in the muscle fibres (Chen et al., 2019).

To the best of our current knowledge of the published literature, this is the first study to fortify the diets of TAW MARGRA lambs with n-3 LC-PUFA to enhance feedlot performance, carcass characteristics, commercial wholesale cut yields, and meat-eating quality traits.

4.2. Materials and methods

4.2.1. Animals, study location, dietary treatments, experimental design and feed intake

This lamb finishing feeding trial was conducted at the Crown Agriculture's feedlot facility at Borenore, New South Wales, Australia, from April to June 2019. Borenore is located at latitude 33°19′S and longitude 149°04′E with an elevation of 3024 feet above sea level and average

annual temperature of 11.7 °C (53.0 °F) and rainfall of 939.8 mm (37.0 inches). The feedlot was an automated facility in a well-ventilated covered building with concrete floors, density of five square meters per head with all the feeding troughs equipped with installed sensors capable of immediate data capture of each lamb's ear tag identification, entry and exit times, body weight, feed intake, and other vital parameters. These data are automatically recorded, electronically cloud-stored, and directly downloadable into Excel spreadsheets and transmitted when required. Seventy-five Tattykeel Australian White lambs exclusive to the MARGRA brand, with an average body weight of 30 kg at six months of age, were randomly assigned to the following three dietary treatments of 25 lambs each, and lot-fed as a cohort for 47 days after a 14-day adaptation period in a completely randomized experimental design: (1) Control grain pellets without oil plus hay; (2) omega-3 oil fortified grain pellets plus hay; and (3) commercial whole grain pellets plus hay. All lambs had *ad libitum* access to the basal hay diet and water. The nutrient composition of the supplementary and basal diets is presented in Table 5.1.

At the end of the feeding trial, the lambs were conveyed during the cool hours of the day to the Gundagai Meat Processing Plant, New South Wales, Australia, held in lairage and fasted overnight. The lambs were humanely sacrificed as a single mob in line with Meat Standards Australia guidelines and industry best practice standards. The carcasses were subjected to medium voltage electrical stimulation before being trimmed and dressed (Holman et al., 2021). The liver, kidney, and heart were sampled immediately following evisceration, vacuum-sealed in labelled bags, and stored at −20 °C pending fatty acid evaluation. All carcasses were held in the chiller room for 24 h at 4 °C and a sample of the *Longissimus thoracis et lumborum* muscle tissue was taken between the 12^{th} and 13^{th} ribs for fatty acid analysis.

Table 4.1. Nutrient composition of the control, n-3 LC-PUFA, MSM whole grain, and hay feeds.

Nutrient Composition (%DM) [#]	Experimental and Basal Diets			
	Control	**Omega-3**	**MSM Whole Grain**	**Hay**
Dry Matter (DM)	90.8	91.7	90.3	93.4
Moisture	9.2	8.3	9.7	6.6
Acid Detergent Fiber (ADF)	7.6	8.2	6.3	39.4
Neutral Detergent Fiber (NDF)	23.8	23.0	21.8	60.9
Crude Protein (CP)	16.9	17.0	16.4	7.5
Ash	7.8	8.2	7.2	8.1
Ether Extract (EE)	6.1	10.3	6.0	3.3
Metabolizable Energy (ME) MJ/kg	14.1	15.1	14.4	8.3
Dry Matter Digestibility (DMD)	84.9	83.8	87.5	46.8
Digestible Organic Matter (DOMD)	83.7	82.6	86.2	47.1

[#] DOMD, Digestible organic matter in the dry matter; TDN, %Total digestible nutrients (as % of DM) = 82.38−(0.7515 × ADF [% of DM]); ME, Metabolizable energy (DE [Mcal/kg] = %TDN × 0.01 × 4.4) where DE is digestible energy, which was converted as ME = (DE (Mcal/kg) × 0.82) × 4.185.

4.2.2. Feed Sample Processing and Nutrient Composition Analysis

Supplementary and basal feed samples were oven-dried for three days at 60 °C, cooled, and ground to pass through a 1 mm sieve using a laboratory mill (Thomas Model 4 Wiley® Mill; Thomas Scientific, Swedesboro, NJ, USA). Dry matter and ash percentages were determined using the AOAC standard laboratory analytical techniques (AOAC International, 1995). Neutral detergent (NDF) and acid detergent (ADF) fiber percentages were determined using an Ankom Fiber Analyzer (ANKOM2000; ANKOM Technology, Macedon, NY, USA). Nitrogen content was determined using a Thermo Finnigan EA 1112 Series Flash Elemental Analyzer (Thermo Finnigan, Poway, CA, USA) and the values were multiplied by 6.25 to provide the expected crude protein (CP) percentage. Ether extract (EE) was analyzed employing an ANKOM^{XT15} fat/oil extractor (ANKOM Technology, Macedon, NY, USA).

4.2.3. Carcass measurements

Hot standard carcass weight (HSCW) was recorded prior to chilling, and cold carcass weight was measured after removing from the chiller at 4°C after 24 h. The dressing percentage was determined as the HSCW divided by the liveweight (LWT) × 100%. In the boning room, the carcasses were cut between the 12th and 13th ribs to measure *longissimus dorsi* eye muscle area (LMA), back-fat thickness (BF), body wall thickness (BWT), loin marbling score, body and leg conformation scores, and the percentage of boneless, closely trimmed retail cuts (% BCTRC) as described previously by Jaborek et al. (2017). Wholesale primal cuts, fat trims, lean, and bone weights were recorded.

4.2.4. Determination of IMF

The technique of Flakemore et al. (2014) was used to determine the IMF content of muscle samples. This was carried out by homogenizing and extracting in $CHCl_3$: MeOH (2:1) fat-soluble solvent, phase partition in 5 ml of 10% KCl, and evaporation of the organic layer in weighed porcelain crucibles to get the fat content. The % IMF was computed as follows: [Crucible including fat weight (g) – empty crucible weight (g)]/sample weight (g) × 100.

4.2.5. Determination of FMP

The FMP was determined as described by Mwangi et al. (2021) and in Chapter3. Briefly, the muscle samples were placed in an oven at 100°C for 1–2 min to obtain fat that was used for FMP determination. Through air suction, the melted fat was sucked into thin capillary tubes and kept in a refrigerator for 10 min at 4°C to permit the fat to freeze. The fat level was marked with an indelible pen, and the capillary tube was affixed to a thermometer held in a glass beaker with ~80 ml of deionised H_2O positioned on a heating block. The heating block was slowly heated until the fat "slipped" off the mark. The temperature at which the "slip" occurred was recorded as the FMP.

4.2.6. pH and temperature measurements

pH and temperature were recorded at the 12th to 13th rib from *longissimus lumborum* muscle from the left side of each carcass as documented by Holman et al. (2021) and Hussain et al. (2021). Briefly, the initial pH measurement was performed immediately upon entry into the chiller (~30 min post-slaughter). At ~24 h *post-mortem*, four intermediate measurements were taken before the last pH measurement. A pH meter (WP-80, TPS Pty Ltd., Queensland) fitted with a polypropylene spear-type gel electrode (IJ-44, Ionode™, TPS Pty Ltd., Queensland) and calibrated using pH 4.00 and pH 7.00 standards was used for all measurements. The pH meter was initially recalibrated at each interval using standard buffers at a temperature that matched the estimated muscle temperature. This was to compensate for the influence of temperature on pH readings, as per the technical bulletin (WP-80, TPS Pty Ltd., Queensland). Muscle temperature was concurrently documented with the aid of the same pH meter, tailored with a spear-type temperature sensor (no. 121247, TPS Pty Ltd., Queensland).

4.2.7. Statistical analysis

The data were analyzed utilizing nonparametric analytical methods in R. Each animal was regarded as an experimental unit. Meat quality, carcass characteristics, and wholesale cut yields were presented as medians and interquartile range (IQR) and visualized in boxplots after adjustment for treatment effect. Spearman's correlation procedure was used to quantify the relationship between variables. To analyse the effect of treatment on FMP, IMF, and carcass traits, the treatment was defined as the fixed effect. The Kruskal–Wallis test was also used to decide whether or not a statistically significant difference existed between the different feed treatments as previously described (R Core Team, 2021).Tukey's adjusted multiple comparisons were also used for the pairwise comparison test at $p < 0.05$.

4.3. Results

4.3.1. Liveweight, Average daily feed intake, average daily gain, and feed cost

Figure 4.1 shows the liveweights, average daily feed intake (ADFI), average daily gain (ADG), and feed cost per unit gain as influenced by treatment. In all treatment groups, the liveweights at commencement and the 3-week adaptation period were similar and not significantly different from each other. Similarly, at the end of the 47-day feeding trial, the final liveweights did not differ significantly between treatments. However, lambs fed the omega-3 diet had a significantly ($p < 0.0140$) lower ADFI (1.01 kg/day) compared to those fed the control (1.57 kg/day) and MSM whole grain (1.69 kg/day) diets. Lambs fed omega-3 diet gained the most weight with an ADG of 230 g/head/day, followed by MSM whole grain (224 g/head/day) and control (194 g/head/day) ($p < 0.0390$). The result also revealed that n-3 LC-PUFA-fortified dietary treatment had the lowest cost in terms of ADFI, followed by control, while MSM whole grain was the most expensive feed. The cost of producing a ton of the control diet was Au$381.55, Au$426.44 for MSM whole grain, and Au$528.30 for the omega-3 diets.

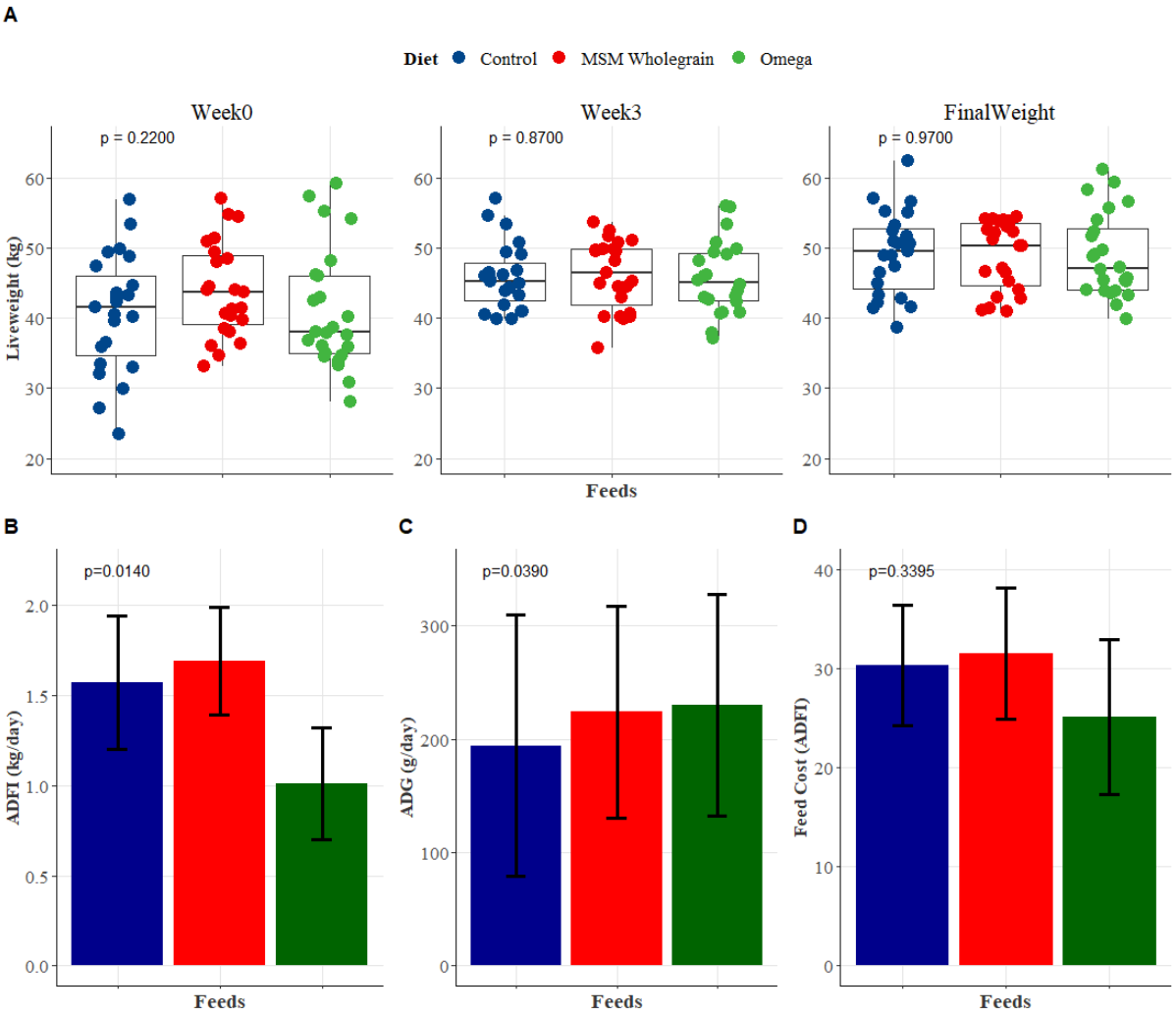

Figure 4.1. (A–D) Liveweights, average daily feed intake, daily gain, and feed cost per unit gain in lot-fed Tattykeel Australian White lambs in different treatment groups. Hays without omega-3 (control); whole grain pellets (MSM); hay plus pellets fortified with omega-3 (Omega). [abc]Superscript letters indicate a significant difference among the means in each variable (Tukey's adjusted).

4.3.2. Carcass characteristics, IMF and FMP

Table 4.1 shows that IMF and FMP differed significantly among treatments ($p < 0.0001$). The IMF from lambs fed omega-3 pellets was markedly higher than those fed control and MSM whole grains. The FMP of meat from lambs fed omega-3 diet was the lowest at 30.15°C (29.62–31.85°C) than the control at 34.75°C (34.3–35.3°C) and MSM whole grain at 36.8°C (36.8–37.22°C). The diet fortified with omega-3 led to a marked decrease in FMP ($p \leq 0.0001$). Overall, the median (IQR) pH level of TAW MARGRA lambs was 6.13 (6.06–6.19) and

significantly differed between treatments (p = 0.0380; Table 4.1). On the contrary, there was no significant difference in temperature. Using the Kruskal–Wallis test, fortification with omega-3 had no influence on final LWT, GR fat depth, HSCW, and dressing percentage. Regarding cut yields of lot-fed Tattykeel Australian White MARGRA lambs, only wholesale French rack (p = 0.0300) and bones (p = 0.0190) showed significant differences. Tukey's adjusted tests for pairwise comparisons between treatment groups indicate a significant difference in pH between control and MSM whole grain **(**−0.33, 95% CI: −0.60 to −0.06, p = 0.0198). The omega-3 diet had the lowest FMP and highest IMF.

Table 4.1. Median (inter-quartile range) of meat quality and carcass characteristics in lot-fed Tattykeel Australian White MARGRA lambs in different treatment groups

Variables	Overall	Control	MSM	Omega	P-value[a]
Meat quality					
pH	6.13 (6.06– 6.19)	6.19 (6.16– 6.22)	6.08 (6.06–6.13)	6.12 (6.02–6.17)	0.0380
Temperature (°C)	23.23 (22.42 – 23.86)	22.37 (21.86 – 23.36)	23.60 (23.2–23.97)	23.29 (22.79–23.98)	0.1000
FMP (°C)	34.75 (31.70 – 36.80)	34.75 (34.3 – 35.30)	36.80 (36.8–37.22)	30.15 (29.62–31.85)	0.0001
IMF (%)	3.50 (2.73– 4.07)	3.50 (3.15– 3.58)	2.40 (2.02–2.68)	4.15 (4.03–4.42)	0.0001
Carcass characteristi					
Final LWT (kg)	48.20 (45.03–52.38)	46.95 (43.67– 51.67)	51.23(47.94–53.42)	46.52 (45.27 –51.47)	0.6400
GR Fat Depth (cm)	16.00 (14.00– 18.00)	16.00 (13.25– 17.50)	15.50 (14.00 –18.25)	15.50 (14.00 – 18.75)	0.8300
Fat Score	5.00 (5.00–5.00)	5.00 (5.00– 5.00)	5.00 (5.00 –5.00)	5.00 (5.00 – 5.00)	0.7900
HSCW (kg)	23.75 (22.72–26.45)	23.35 (22.00– 25.52)	25.95(24.15–26.67)	23.25 (22.72 – 25.45)	0.2000
Dressing (%)	50.18 (49.13–52.23)	49.72 (48.77– 50.89)	51.17(50.37–53.69)	49.78 (49.00 – 51.08)	0.0980
Cut yields (kg)					
Eye of loin	0.41 (0.36 – 0.46)	0.46 (0.37 –0.50)	0.43 (0.36 –0.44)	0.37 (0.34 –0.43)	0.1900
French Rack	1.89 (1.63 – 2.07)	1.90 (1.76 –2.04)	2.08 (1.96 –2.32)	1.71 (1.59 –1.80)	0.0300
Tenderloin	0.22 (0.20 – 0.24)	0.22 (0.22 –0.24)	0.23 (0.2 –0.26)	0.22 (0.18 –0.24)	0.6900
Banjo Shoulder	2.53 (2.28 – 2.67)	2.55 (2.40 –2.79)	2.44 (2.28 –2.57)	2.41 (2.26 –2.64)	0.3800
Neck	0.58 (0.48 – 0.68)	0.51 (0.47 –0.66)	0.62 (0.52 –0.72)	0.49 (0.48 –0.58)	0.3100
Leg shank	5.47 (5.21 – 5.73)	5.60 (5.47 –5.77)	5.37 (5.22 –5.52)	5.18 (4.95 –5.73)	0.1900
Rump	1.05 (0.94 – 1.15)	1.07 (1.04 –1.19)	0.98 (0.94 –1.04)	1.01 (0.94 –1.04)	0.1500
Rib set	0.77 (0.66 – 0.88)	0.75 (0.70 –0.84)	0.80 (0.64 –0.94)	0.71 (0.66 –0.85)	0.7700
Breast and Flank	0.88 (0.80 – 0.96)	0.90 (0.80 –1.03)	0.88 (0.79 –0.95)	0.88 (0.84 –0.94)	0.8600
Lean Trim	2.96 (2.56 – 3.40)	3.19 (3.11 –3.71)	2.74 (2.58 –3.01)	2.65 (2.42 –3.17)	0.1300
Fat Trim	2.27 (1.77 – 2.77)	2.05 (1.50 –2.62)	2.46 (1.82 –3.29)	2.10 (1.78 –2.62)	0.4200
Bones	4.00 (3.46 – 4.34)	4.30 (4.01 –4.64)	4.04 (3.48 –4.41)	3.55 (3.29 –3.91)	0.0190
EMAW	64.13 (60.00 – 69.50)	62.00 (60.25 –67.50)	65.00 (60.5 –72.25)	61.00 (60.00– 67.00)	0.5100
Total Retail Meat Yield	9.23 (7.94 – 10.20)	10.13 (9.06 –10.21)	9.26 (7.88 –10.43)	8.34 (7.91 –9.53)	0.2200
Trims & Bones	7.53 (6.90 – 8.09)	7.63 (7.32 –8.37)	7.50 (6.62 –7.95)	7.28 (6.85 –8.20)	0.4100
Saleable Meat Yield	16.77 (15.74– 18.05)	17.57 (16.04– 18.18)	16.12(15.83– 17.38)	15.58 (14.79– 16.86)	0.2300

[a]Based on Kruskal-Wallis test; FMP fat melting point; IMF intramuscular fat; HSCW hot standard carcass weight; LWT liveweight; EMAW eye muscle width;

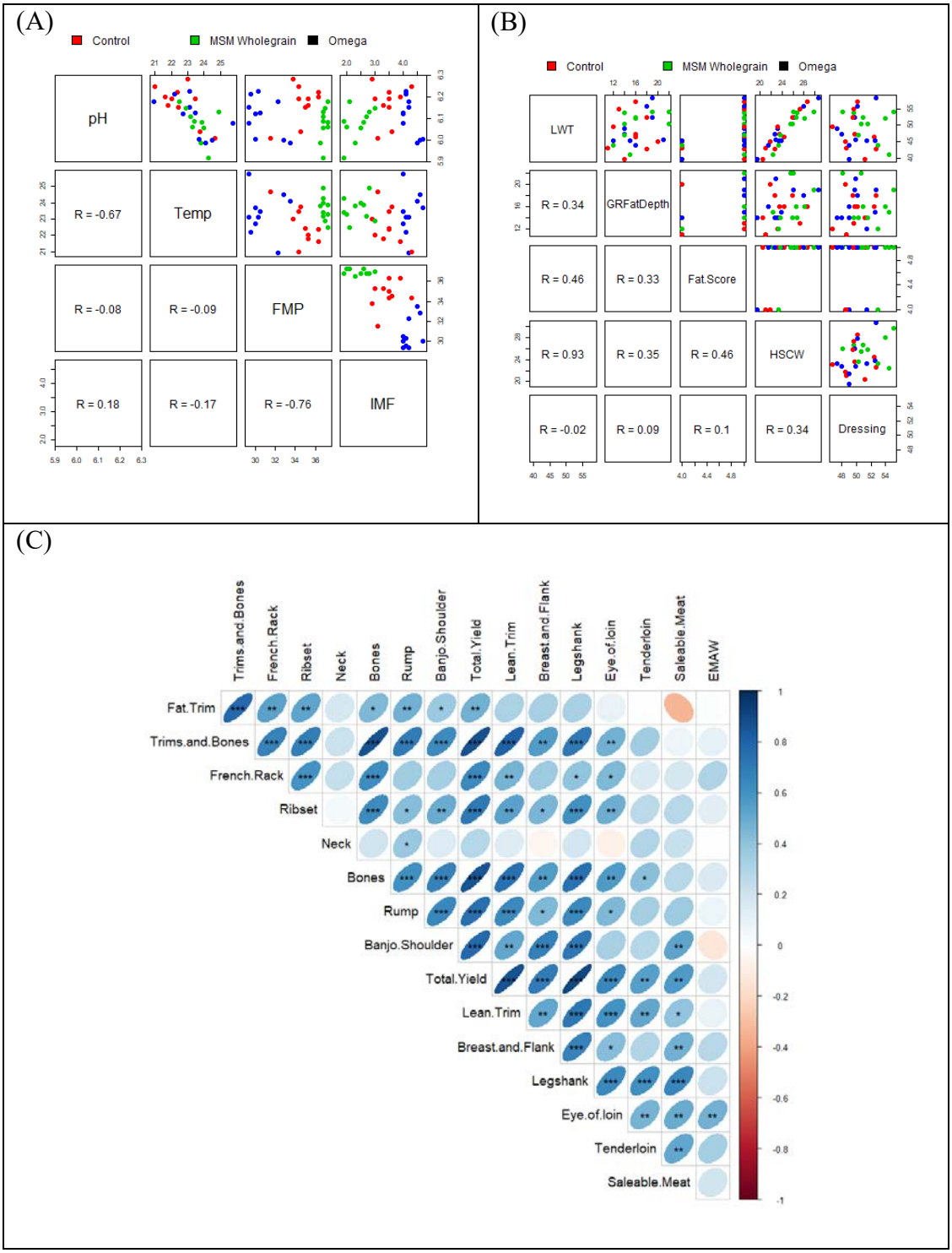

Figure 4.2 A-C. There was a significantly strong negative correlation between temperature and pH ($r = -0.67$, $p < 0.05$) and between FMP and IMF ($r = -0.76$, $p < 0.05$; Figure 4.2A). As shown in Figure 4.2B, there was a very strong positive and significant relationship between LWT and HSCW ($r = 0.93$), while a moderate and positive relationship ($r = 0.46$) was observed between LWT and fat score and between fat score and HSCW ($r = 0.46$). Moderately positive and significant correlations ($p < 0.001$) were observed between fat trim and total yield, bones, and eye of loin ($p < 0.001$), lean trim and tenderloin ($p < 0.001$) in Figure 4.2C. There was a weak and negative correlation between saleable meat and fat trim ($p < 0.001$).

4.3.3. Correlations

There was a significantly strong negative correlation between temperature and pH ($r = -0.67$, $p < 0.05$) and between FMP and IMF ($r = -0.76$, $p < 0.05$; Figure 4.2A). As shown in Figure 4.2B, there was a very strong positive and significant relationship between LWT and HSCW ($r = 0.93$), while a moderate and positive relationship ($r = 0.46$) was observed between LWT and fat score and between fat score and HSCW ($r = 0.46$). Moderately positive and significant correlations ($p < 0.001$) were observed between fat trim and total yield, bones, and eye of loin ($p < 0.001$), lean trim and tenderloin ($p < 0.001$) in Figure 4.2C. There was a weak and negative correlation between saleable meat and fat trim ($p < 0.001$).

4.4. Discussion

The n-3 LC-PUFA, especially DHA (docosahexaenoic acid; C22:6n-3) and EPA (eicosapentaenoic acid; C20:5n-3), are mainly found in large quantities in oily cold-water fish and seafood, but in insufficient amounts in ruminant meat and milk (Widmann et al., 2011).The n-3 LC-PUFA are known to perform vital physiological roles in maintaining and growing fetuses, neonates, and infant brains (Abdel-Tawwab et al., 2021).The low concentration of n-3 LC-PUFA in ruminant muscle tissue is mainly due to extensive lipolysis and biohydrogenation of unsaturated fatty acids (UFA) by ruminal microbes (Amills et al., 2020; Chiofalo et al., 2020). The content of a lamb's diet influences the composition and value of its tissues (Holman et al., 2021). Furthermore, several trials have indicated that numerous feeding approaches can facilitate the deposition of n-3 LC-PUFA in muscle tissues in lambs, resulting in healthier meat (Flakemore et al., 2017; Le et al., 2018; Nguyen et al., 2018; Le et al., 2019; da Silva et al., 2020; Gravador et al., 2020; Guerreiro et al., 2020; Forwood et al., 2021). The use of vegetable seed oils in ruminant diets was found to increase energy and the level of UFA deposited in meat (Diogénes et al., 2020). Thus, the resultant meat product had reduced levels of FA that were termed undesirable and enhanced levels that were characterized as beneficial to human

health (Shan et al., 2017). Meat quality indices can be improved by the inclusion of lipid supplements in diets fed to animals raised under feedlot conditions to increase the levels of oleic, linoleic, and linolenic acids and other n-3 LC-PUFA (Fusaro et al., 2021; Torres et al., 2022). To the best of our knowledge, this is the first study that evaluated the supplementation of diets in TAW MARGRA with promising results.

4.4.1. Liveweight, Average daily feed intake, average daily gain, and feed cost

The inclusion of omega-3 oils decreased dry matter intake but led to significantly higher ADG, an indication of better feed conversion efficiency. The higher average daily gain with less feed consumption implies a better utilization of absorbed nutrients from the abomasum either due to a modification of the rumen environment and ecology that favours less biohydrogenation and more by-pass proteins from the rumen or a higher turnover rate of volatile fatty acid absorption. In economic terms, the relatively higher feed cost of the omega-3 fortified diet was offset by the highest feed efficiency of consuming less but growing the fastest in terms of average daily gains, thus leading to improved profitability (Nascimento et al., 2021). Therefore, fortification of diets with omega-3 resulted in better feed conversion efficiency, and this may assist lot-fed lambs to attain finishing weight early, thus saving costs and improving profitability for sheep farmers.

4.4.2. Intramuscular fat

Fats are essential nutrients associated with vital physiological functions and are widely distributed within animal tissues and organs such as subcutaneous, intermuscular, and intramuscular fat. Malgwi et al. (2022) defined IMF as the quantity of fat resident in meat also referred to as marbling fat. In lambs, Pannier et al. (2014) reported IMF values of 1.5–9.5%. In this current study, lambs fed omega-3 diet had IMF values between 1.5 and 9.5% (Pannier et al., 2014) recommended for consumers. Australian lamb consumers prefer a 4–5% IMF

threshold for palatability and tenderness (Hopkins et al., 2006). Lambs fed control diet had IMF values between 3 and 4%, while lambs fed MSM whole grain diets had IMF values between 2 and 3%. IMF has a significant influence on tenderness, flavor, and juiciness, overall liking (Holman and Hopkins, 2021), meat processing (Alvarenga et al., 2021), and water holding capacity (Tao et al., 2021). Tenderness, according to Zhao et al. (2015), is an essential factor for the valuation of meat quality, and it affects consumer purchasing and market acceptability decisions. IMF is influenced by both genetic and environmental factors, depending on species, breed, genotype, muscle type, age, gender, and nutritional status (Bao et al., 2021; Xiao et al., 2021). Consequently, increasing the level of IMF is fundamental to improving meat quality (Scollan et al., 2017; Xiao et al., 2021). The n-3 LC-PUFA profile of muscle tissue and organs of TAW MARGRA lambs in this experiment had been presented in Chapter 5. Herein, lambs fed the omega-3 fortified diet had better growth performance indicators than the lambs fed the control and MSM whole grain diets.

Generally, consumers prefer lean lamb with low SFA and high n-3 LC-PUFA. The association between total fat content and relative proportions of fatty acids has long been established owing to the minor impact of membrane phospholipids (Bessa et al., 2015; Manni et al., 2018). Furthermore, Scollan et al. (2017) reported that the potential for IMF accumulation rests on the equilibrium between uptake, synthesis, and degradation of triacylglycerols. This enhances the accessibility of net energy for fat production during finishing and results in higher IMF content.

4.4.3. Fat melting points

The proportions of single and double bonds influence FMP constituents of fatty acids. For instance, the SFA stearic acid (18:0) and UFA α-linolenic acid (18:3) have melting temperatures of 69.7 and −11°C, respectively (Toral et al., 2018). The UFA are softer with little heat or energy required to melt them compared to SFA, which are harder requiring more

energy to melt. Therefore, the omega-3 fortified diet increased the level of UFA in the muscle tissue, thus accounting for the low FMP recorded in this study.

4.4.4. Muscle pH

The mean pH values (>6.00) obtained were slightly outside the range of 5.3–5.8 after 24 h of slaughter reported by Yagoubi et al. (2018), but there were no incidences of dark, firm, and dry (DFD) muscles. This study's results were in accordance with the report of Inserra et al. (2014), who fed lambs on diets containing 0% citrus, 24% citrus, and 35% citrus, but in contrast to those of Chiofalo et al. (2020) and Ozdogan et al. (2017) in cattle and lambs supplemented with olive oil cake. A series of physiological processes, especially glycolysis, occurs before muscles are converted to meat. Under anaerobic conditions after slaughter, the glycogen stored in muscle tissues is converted to lactic acid, leading to a drop in pH (Stenberg et al., 2020) and the onset of rigor mortis. Stenberg et al. (2020) reported that lambs fed high-energy diets tend to have higher pH values than their counterparts fed low-energy diets. These lambs could have lost a lot of glycogen during transport from the feedlot facility to the abattoir. Generally, muscle pH is a significant indicator of *post-mortem* animal muscle glycolysis, which is related to water-holding capacity and meat colour (Hughes et al., 2020; Abhijith et al., 2021). Lower pH values make the muscle more acidic, bacteriostatic, and fungistatic, thereby hindering bacterial and fungal growth. Lambs fed energy-dense diets have a better capacity to store, replenish glycogen in the muscle tissue, and are more capable of coping with pre-slaughter processing, including transport (Fusaro et al., 2021). According to Holman et al. (2021), the degree of pH decline is significant as meat tenderness is dependent on it. When muscle temperatures decline rapidly, the meat assumes a more rigid state and cold shortening ensues.

4.4.5. Muscle temperature

Dietary treatment did not influence ($p > 0.05$) temperature, probably due to identical protein, carbohydrate contents, and dry matter intake (Vodolazska et al., 2020; Fusaro et al., 2021). It is highly unlikely that other physiological factors could have affected the observations in muscle pH since all the animals were wethers of the same breed and age. Stress and excessive exercise before slaughter and electrical inputs during dressing should be minimized as much as possible to reduce muscle temperature.

4.4.6. Wholesale commercial meat cut yields

Lamb cuts have been designed and marketed based on their nutritional quality, offering processors and retailers the ability to use heavy lambs more efficiently (Fowler et al., 2019). The percentage of bone has been reported to be higher and edible tissues lower in younger than older animals (del Mar Campo et al., 2021). This implies that other tissues develop with advancing age, where the proportion of edible tissues rises as the animal ages. This, unfortunately, has its drawbacks, as the meat becomes less tender, but has a more intense odour and flavour (Schönfeldt et al., 1993). Carcasses of lambs on the omega-3 fortified diet were of better conformation, hence the superior French rack yield, more saleable meat, and lean-to-bone ratio than their counterparts fed both MSM and control diets. Body measurements are vital in estimating liveweights for farm animals (Canul-Solis et al., 2020), and this valuable information contributes to decisions in terms of selection and husbandry system aimed at raising the edible lean meat yield and reduction in fat content in carcasses (Sood et al., 2022). In sheep and goats, cut yields are predictors of overall carcass tissue composition (Barcelos et al., 2021). In this study, the measures of the primal cut weights were identical in the three groups, except for bones and French racks. The proportions of bone, muscle, and fat shift during the growth of an animal concurrently with its carcass water, protein, fat, and mineral contents. The animal's age, weight, breed, sex, nutritional status (Owens et al., 1993; de

Albuquerque Borges et al., 2022), and production system influence these. The weight and yield of carcasses are significant determinants of commercial value with better returns (Fernandes et al., 2022). The mean tissue content of the leg showed that the muscles had the greatest contribution (66.70%), followed by bone (18.89%) and fat (10.09%) (da Trindade Silva et al., 2021). Bautista-Díaz et al. Bautista-Díaz et al. (2020) reported that bones made up 1.46 ± 0.27 kg of suckling lambs. On account of the weights of primal cuts recorded in this study, the carcasses provided reasonable cut yields when the leg, loin, and shoulder were considered (de Oliveira et al., 2018). Da Trindade Silva et al. (2021) reported that these are responsible for an estimated 60% of the entire yield of cuts. Results generated from this study with other carcass traits could therefore be useful when making decisions regarding the selection and the most appropriate husbandry system to employ.

4.6. Conclusion

The results of this study showed that dietary fortification with n-3 LC-PUFA enhanced feedlot performance in TAW lambs with significant improvement in health-beneficial intramuscular fat content, low-fat melting point, and French rack primal cut yield. The results align with the tested hypothesis that the inclusion of n-3 LC-PUFA in feedlot diets will improve productive performance, carcass characteristics, wholesale commercial French rack primal cut yields, and meat quality traits in TAW lambs. The inclusion of omega-3 oils in feedlot diets decreased dry matter intake, increased feed efficiency resulting in faster growth, and healthier meat from supplemented lambs.

4.7 Summary

Meat eating quality indices such as intramuscular fat content (IMF) and fat melting point (FMP) of the *Longissimus thoracis et lumborum* muscle and the feedlot performance, carcass traits, and commercial wholesale cuts of lot-fed Tattykeel Australian White (TAW)

MARGRA lambs as a result of dietary fortification of the diet with omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) were evaluated. A total of 75 TAW MARGRA lambs at 6 months of age with an average liveweight of 30 ± 1.2 kg were used. The lambs were randomly allocated to the following three dietary treatments of 25 lambs each in a 47-day feeding trial using a completely randomized experimental design: (1) control diet of hay plus pellets without omega-3 oil, (2) hay plus commercial whole grain pellets (MSM) without omega-3 oil, and (3) hay plus pellets fortified with omega-3 oil. It was hypothesized that dietary supplementation with omega-3 fortified pellets will improve feedlot performance, meat-eating quality indices of IMF, FMP, and carcass characteristics. Lot-fed lambs on the MSM whole grain had the highest feed intake of 1.69 kg/day, followed by the control at 1.57 kg/day and the lowest in the omega-3 diet at 1.01 kg/day ($p = 0.0001$). However, the omega-3 diet had the highest average daily gain of 230 g/head/day ($p = 0.0001$), indicating the greatest feed efficiency since it had the best growth response with minimal feed intake. Post-slaughter evaluation of the *Longissimus thoracis et lumborum* muscle revealed significant treatment variations in IMF ($p = 0.0001$), FMP ($p = 0.0001$), pH ($p = 0.0380$), and wholesale French rack primal cut ($p = 0.0001$). Strong correlations ($p < 0.05$) between liveweight, temperature, pH, FMP, and IMF were observed. Similarly, significant correlations between carcass characteristics of total saleable meat yield, lean trim, fat trims, bones, and leg shank were evident ($p < 0.05$). However, there were no treatment differences in the final liveweight, GR fat depth, hot standard carcass weight, or dressing percentage. The findings indicate that feedlot performance, meat-eating quality traits such as IMF and FMP, and commercial wholesale French rack cuts can be further improved during feedlot finishing of TAW lambs through dietary supplementation with omega-3 oils, and hence the tested hypothesis of improved meat quality attributes is partially confirmed.

Chapter 5: Nutritional Enhancement of Health Beneficial Omega-3 Long-Chain Polyunsaturated Fatty Acids in the Muscle, Liver, Kidney, and Heart of Tattykeel Australian White MARGRA Lambs Fed Pellets Fortified with Omega-3 Oil in a Feedlot System

5.1. Introduction

Functional foods are among the fastest-growing markets in developed countries, where the average consumer prefers omega-3 enrichment with information about the food's production process (Boncinelli et al., 2021). Functional foods influence satiety and a healthier lifestyle (Munekata et al., 2021). The main strategies for creating healthier and functional foods with increased satiety include modifying of dietary fat, fibre, and sugar compositions (Munekata et al., 2021). Ansorena and Astiasarán (2013) provided insights into modifying the formulations of fresh, cooked, and fermented meat products to increase omega-3 fatty acid content without modifying animal diets. The fortification of functional beef burgers with microencapsulated cod liver oil (Morsy and Elsabagh, 2021), algal and wheat germ oil emulsions (Barros et al., 2021) are examples of methods for enriching foods with omega-3 fatty acids.

The demand for high-quality meat is on the increase as consumer preferences for edible animal-based protein sources shift toward eating quality with increased human health benefits. Meat, an essential component of the human diet, is rich in nutrients including protein, fatty acids, iron, zinc, copper, selenium, and B-complex vitamins (Juárez et al., 2021); Omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) are essential fatty acids that play diverse roles in human health and disease prevention. They include the following longer chain derivatives of alpha-linolenic acid (ALA, C18:3n-3): Eicosapentaenoic (EPA, C20:5n-3), docosapentaenoic (DPA, C22:5n-3), and docosahexaenoic (DHA, C22:6n-3) acids. EPA + DPA + DHA are known to promote intellectual development in infancy, relieve inflammation, boost the immune system, reduce incidences of cardiovascular diseases, some cancers, diabetes, allergies, behavioural disorders, and sustain retinal functions (Chappus-McCendie et

al., 2019; Fu et al., 2021; Ponnampalam et al., 2021; Rizos et al., 2021). However, humans, like all mammals, cannot synthesize n-3 LC-PUFA because they are unable to produce Δ12 and Δ15-desaturase enzymes (Suito et al., 2020), hence, they rely on dietary sources like leafy vegetables, oilseeds, nuts, eggs, and seafood, especially fish and crustaceans (Mazzocchi et al., 2021; Ponnampalam et al., 2021), edible marine algae, bacteria, fungi, diatoms, fruits, and herbs (Núñez-Sánchez et al., 2021), to meet their daily n-3 LC-PUFA requirements. Oilseeds commonly used in human diets include rapeseed (Sharafi et al., 2015) and soybean (Joki´c et al., 2013), while seed oils from waste food by-products such as tomato (Giuffrè, A.M.; Capocasale, 2016) and citrus (Angelo et al., 2020) are cheap animal feed sources that can enhance the healthy fatty acid composition.

Fatty acid composition influences meat's nutritive value and organoleptic traits including tenderness, flavour, and juiciness (Gonzales-Barron et al., 2021). The fatty acid content of meat can be affected by the animal production system (Malau-Aduli et al., 2019; Hoffman et al., 2020), breed or genotype, gender (Sari et al., 2019), age at slaughter (Belaunzaran et al., 2018), liveweight (Miguel et al., 2021), level of fatness (Gonzales-Barron et al., 2021), type of muscle and feed. In lamb (Matar et al., 2020), cattle, swine, and poultry (Wood and Enser, 2017), it has been suggested that dietary manipulation can be utilised to improve the fatty acid content and nutritional value of meat that more closely meets nutritional guidelines. However, due to extensive rumen microbial biohydrogenation in ruminants, dietary polyunsaturated fatty acids (PUFA) are converted to saturated fatty acids (SFA), absorbed in the small intestine, and deposited in edible tissues (muscles), products (milk), and organs (liver, kidney, and heart), thereby causing more health challenges to consumers (Garcia-Galicia et al., 2020; Vahmani et al., 2020). Ruminant meat research aims to reduce saturated fatty acids and increase the proportion of health-beneficial n-3 LC-PUFA (Garcia-Galicia et al., 2020). Therefore, dietary supplementation with rumen-protected plant and fish-based n-3 LC-PUFA oil, forages, and

concentrates containing bioactive enriched microalgae (Dewanckele et al., 2018) are some of the steps taken by livestock farmers to improve the nutritional and health values of meat.

To date, there is presently no published literature on n-3 LC-PUFA metabolism in the *Longissimus thoracis et lumborum* muscle, heart, kidney, and liver of lot-fed Tattykeel Australian White (TAW) MARGRA lambs in response to dietary supplementation with omega-3 oil. The research reported in this present study intends to fill this knowledge gap. It was hypothesised that *fortifying feedlot pellets with omega-3 oil will enhance the human health beneficial n-3 LC-PUFA composition of edible lamb muscle tissue and organs*. Therefore, the primary objective of this study was to evaluate and compare the fatty acid profiles in the tissues and organs of TAW lambs raised in a feedlot production system in response to dietary supplementation with or without fortification with omega-3 oil.

5.2. Materials and Methods

5.2.1. Animals, Dietary Treatments, and Experimental Design

The animals, study location, dietary treatments, and experimental design have already been described in detail in Chapter 4 of this Thesis. Briefly, TAW MARGRA lamb breed was developed from the rigorous selection, culling, and linebreeding of Texel, Van Rooy, Dorper, and Poll Dorset with an extensive utilization of natural mating, artificial insemination, and embryo transfer as described in Chapter 3. TAW MARGRA lamb is a special breed of lamb with a low FMP (28–39ºC) compared to the ranges of 40.6–48.0°C and 41.5–44.0°C reported in the study by Flakemore et al. (2015) and Holman et al. (2014) for purebred and crossbred Merino, Dorset, Black, and White Suffolk sheep. Similarly, TAW lambs contained EPA+DHA content of 32.4 ± 8.5 mg per 100 g of muscle, surpassing the 30 mg limit set by Food Standards of Australia and New Zealand (FSANZ) for the “source” claim (Van Le et al., 2019).

The feedlot finishing feeding trial was performed at Crown Agriculture's lamb feedlot facility at Borenore, New South Wales, Australia, from April to June 2019. The study utilized 75 TAW MARGRA wethers at 6 months of age with an average liveweight of 30 ±1.2 kg. The lambs were dewormed and allowed a 14-day adjustment period with *ad libitum* access to water and the gradual introduction of three experimental diets to minimize any gastrointestinal disorders. The lambs were randomly allocated to the following three dietary treatments of 25 lambs each in a 47-day feeding trial using a completely randomized experimental design: (1) control diet of hay plus pellets without omega-3 oil, (2) hay plus commercial whole grain pellets (MSM) without omega-3 oil, and (3) hay plus pellets fortified with omega-3 oil. All lambs were fed in groups (control, MSM, and omega-3). All these three diets were formulated to be isonitrogenous (CP = 14%) and isocaloric (ME = 10.258 Mj/kg DM). Details of the nutrient compositions are presented in Chapter 5. The feeding troughs had electronic sensors, where each animal's identification, liveweight, feed intake, average daily gains, and other vital parameters were automatically recorded, cloud-stored, and downloaded at the required time. At the end of the feeding trial, the lambs were transported to the Gundagai Meat Processing plant, held in lairage overnight, and humanely sacrificed as a single mob in line with Meat Standards Australia specifications. The carcasses were subjected to medium-voltage electrical stimulation before being trimmed and dressed (Holman et al., 2021). Carcasses were kept in the chiller room for 24 h during which *in situ* pH and temperature were recorded.

5.2.2. Fatty Acid Analysis

Fatty acid analysis of feed, muscle tissue, liver, kidney, and heart samples were carried out at the Commonwealth Scientific and Industrial Research Organization (CSIRO), Food Nutrition and Bio-based Products, Oceans and Atmosphere Laboratory, Hobart, Tasmania, Australia. The gas chromatography–mass spectrophotometry total lipids and muscle phospholipids extraction procedures of Malau-Aduli et al. (2016; 1998) based on an amended Bligh and Dyer

technique (1959), were utilized for fatty acid composition analysis where total lipids in 1 g of un-homogenized muscle tissue samples were extracted overnight. The original phase was a single-phase overnight extraction utilizing $CHCl_3$: MeOH: H2O (1:2:0.8 *v*/*v*). The second segment involved phase separation with the addition of $CHCl_3$: saline Milli-Q H_2O (1:1 *v*/*v*) followed by rotary evaporation of the lower chloroform phase at 40 °C to acquire total lipids. The extracted cumulative lipids were separated into lipid classes by thin-layer chromatography (TLC) using 100 mL of the lipid extract reconstituted in n-hexane. The extract was marked onto silica gel G plates (200 × 200 × 0.25 mm^3) using a micropipette. The TLC plate was developed in an acetone/petroleum ether (1:3 vol/vol) solvent system in a tank comprising a few crystals of butylated hydroxytoluene (BHT) to hinder oxidation. Triacylglycerols, cholesterol, and free fatty acids migrated, while phospholipids remained at the origin of the plate. The phospholipids were scraped off the plate into clean screw-capped test tubes for transmethylation and eventual computation of the lipid conversion factor (LCF) of 0.912 based on g fatty acids/g total lipids (0.083 for phospholipids, 0.829 for triacylglycerols, and 0% for cholesterol since cholesterol does not have any fatty acids). An aliquot from each total lipid extract was utilized for transmethylation with MeOH: $CHCl_3$: HCl (10:1:1 *v*/*v*) for two hours at 80 °C. Fatty acid methyl esters (FAME) were extracted thrice using n-hexane: $CHCl_3$ (4:1 *v*/*v*). A known concentration of an internal standard (C19:0) was added in a 1500 µL vial encompassing the extracted FAME. The FAME was analyzed on a 7890B gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) furnished with an Equity™ -1 fused 15 m silica capillary column with 0.1 mm internal diameter and 0.1 µm film thickness (Supelco, Bellefonte, PA, USA), a flame ionization sensor, a split/splitless injector, and an Agilent Technologies 7683 B Series autosampler. The gas chromatograph settings were splitless mode injection; carrier gas He; original oven temperature 120 °C and then increased to 270 °C at flow rates of 10 °C/min and to 310 °C at 5 °C/min. The Agilent Technologies ChemStation

software (Palo Alto, CA, USA) was used to measure fatty acid peaks. The fatty acid identities were established using a Finnigan Thermoquest GCQTM GC/MS fitted with an on-column injector and Thermoquest Xcalibur software (Austin, TX, USA) as described in detail by Miller et al. (2006). Fatty acid percentages were calculated as follows: FA% = [(individual fatty acid area) × (100)]/ (sum total area of fatty acids). Fatty acid contents were calculated as follows: FA mg/100 g = (Total lipid) × (LCF [0.912]) × ([% FA]/100) × 1000, where 0.912 was the resultant lipid conversion factor (Clayton, 2014). FA contents were presented in mg/100 g tissue as per Food Standards of Australia and New Zealand recommendations.

5.2.3. Statistical Analyses

Data analysis was performed as a completely randomized design using R statistical software version 3.6.3 (2021). Statistical inference was based on a 5% level of significance. Summary statistics of fatty acids composition were presented as means and standard deviations. The effect of dietary treatment was statistically analyzed separately using one-way analysis of variance (ANOVA) in the general linear model (GLM) procedure to investigate the fatty acid profile differences in the muscle, liver, kidney, and heart of the TAW lambs.

The model utilized was:

$$FA_{ij} = \mu + Feed_i + \epsilon_{ij}$$

where *FA* is the fatty acid composition; μ is the overall mean response; $Feed_i$ is the effect due to the i^{th} treatment (i = 1 to 3; control, omega-3, MSM whole grain); and ϵ_{ij} is the random error. Dunn's post-hoc test (Pohlert, 2014; Dunn, 1964) for multiple comparisons of groups with Hochberg's adjustment (Benjamin and Hochberg, 1995; Kendall, 1938) was used to further examine which treatment was responsible for the differences among means of fatty acids that were statistically significant in the one-way ANOVA.

5.3. Results

5.3.1. Fatty Acid Composition of Basal and Supplementary Feeds

The fatty acid profiles of the basal (hay), control (without oil), omega-3 oil-fortified (omega-3), and whole grain (MSM) pelleted diets are shown in Table 5.2. All the supplementary diets were formulated to be isocaloric (metabolizable energy of 14–15 MJ/kg) and isonitrogenous (crude protein of 16.4–17.0%) with a dry matter digestibility of 83.8–87.5%. As depicted in Table 5.1, the omega-3 oil-infused diet had higher EPA + DHA + DPA, n-3 LC-PUFA and ALA (2.74, 19.18, and 15.39 mg/100 g, respectively), and lower total n-6 PUFA and ratios of n-6/n-3 and PUFA/SFA (28.16, 1.47, and 0.75 mg/100 g, respectively) than the control and MSM whole grain diets. The MSM whole grain diet had the highest proportions of C18:2n-6 (linoleic acid) and oleic acid (C18:1) (92.38 and 75.06 mg/100 g, respectively). The control diet had a higher n-6/n-3 ratio value of 9.08, while the basal hay diet had the highest proportion of C20:2n-6 and C20:4n-6 (arachidonic acid).

5.3.2. Fatty Acid Profile of the Longissimus thoracis et lumborum Muscle

The fatty acid composition of the *longissimus thoracis et lumborum* muscle tissue is presented in Table 4.3. The omega-3 oil diet produced lamb muscles with the highest contents of n-3 LC-PUFA, DHA, EPA, DPA, C18:3n-3, C18:1, C18:0 (stearic acid), total SFA, MUFA, and PUFA/SFA ratio. The MSM whole grain diet produced muscles with the highest n-6/n-3 PUFA ratio. A boxplot of Hochberg's adjusted multiple comparisons of significant differences between the treatment groups in muscle fatty acid profiles are depicted in Figure 5.1, where the omega-3 diet consistently shows a significantly higher fatty acid concentration than the control and MSM whole grain diets.

Table 5.1. Fatty acid composition (mg/100 g) of basal and supplementary diets [#].

Fatty Acid	Omega-3	Control	MSM Whole Grain	Basal Hay
13:0	0.00	0.00	0.01	0.00
14:1	0.00	0.00	0.00	0.00
14:0	2.23	0.45	0.25	0.33
15:0	2.62	0.36	0.30	0.21
16:1	1.02	0.79	1.05	1.88
16:0	35.96	27.19	30.52	12.53
17:1n8c + a17:0	0.64	0.27	0.25	0.18
17:0	1.09	0.33	0.30	0.13
18:3n6	0.32	0.00	0.00	0.24
18:4n3	0.32	0.00	0.00	0.20
18:2n6 (LA)	25.25	81.75	92.38	30.02
18:3n3 (ALA)	15.39	8.84	11.21	6.13
CLA	0.65	0.10	0.29	0.00
18:0	8.25	4.59	4.13	2.74
18:1	31.92	57.34	75.06	51.23
19:1	0.00	0.04	0.06	0.04
20:4n6 (ARA)	0.00	0.00	0.00	0.53
20:5n3 (EPA)	0.22	0.18	0.23	0.00
20:3	0.00	0.00	0.01	0.00
20:3n6	0.80	0.15	0.16	0.18
20:4n3	0.30	0.00	0.00	0.02
20:2n6	0.32	0.13	0.21	0.33
20:1	1.51	1.71	2.02	1.79
20:0	2.80	0.60	0.81	0.42
21:5n3	0.43	0.00	0.00	0.00
21:0	1.17	0.16	0.08	0.00
22:5n6	0.87	0.01	0.08	0.00
22:6n3 (DHA)	1.53	0.03	0.05	0.93
22:4n6	0.60	0.08	0.05	0.00
22:5n3 (DPA)	0.99	0.00	0.17	0.22
22:1	2.39	0.47	0.34	0.25
22:0	4.63	0.44	0.60	0.23
23:1	0.64	0.06	0.00	0.00
23:0	1.72	0.01	0.14	0.02
24:1	0.94	0.25	0.28	0.14
24:0	3.54	0.40	0.54	0.15
Total FA	147.11	185.91	220.96	110.69
EPA + DHA	1.74	0.21	0.28	0.93
EPA + DHA + DPA	2.74	0.21	0.45	1.16
SFA	64.02	34.52	37.66	16.77
MUFA	35.09	60.13	78.46	55.12
PUFA	48.00	91.26	104.84	38.81
PUFA/SFA	0.75	2.64	2.78	2.31
∑n3PUFA	19.18	9.05	11.66	7.51
∑n6PUFA	28.16	82.12	92.88	31.30

n6/n3PUFA	1.47	9.08	7.97	4.17

[#] LA, linoleic acid; ALA, α-linolenic acid; CLA, conjugated linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ΣSFA, total saturated fatty acids; FA, fatty acid; ΣMUFA, total monounsaturated fatty acids; and total polyunsaturated fatty acids (ΣPUFA); ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1, 16:1, 17:1n-8 + a17:0, 18:1, 19:1, 20:1, 22:1, 23:1, 24:1; ∑PUFA is the sum of 18:4n-3, 18:3n-6, 18:2n-6, 18:3n-3, 20:3, 20:4n-3, 20:4n-6, 20:5n-3, 20:3n-6, 20:2n-6, 22:6n-3, 22:5n-3, 22:5n-6, 22:4n-6; ∑n-3 LC-PUFA is the sum of 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:6n-3, 22:5n-3; ∑n-6 PUFA is the sum of 18:2n-6, 18:3n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6.

Table 5.2. Fatty acid profile (mg/100 g) of *Longissimus thoracis et lumborum* muscle tissue[#].

Fatty Acid	Omega-3	Control	MSM Whole Grain	*p*-Value
13:0	0.05 ± 0.11	0.03 ± 0.04	0.00 ± 0.00	0.1108
14:1	0.63 ± 0.65	0.31 ± 0.33	0.19 ± 0.34	0.0417
14:0	24.25 ± 18.62	16.80 ± 7.84	14.22 ± 8.06	0.0809
15:1	4.44 ± 2.36	2.26 ± 1.22	1.58 ± 0.85	0.0005
16:0	320.50 ± 182.90	209.83 ± 83.05	169.06 ± 79.43	0.0106
16:1	25.86 ± 17.40	16.93 ± 7.29	14.43 ± 8.57	0.0398
17:1n8c + a17:0	13.83 ± 7.99	9.40 ± 3.68	7.85 ± 3.55	0.0201
17:0	16.85 ± 9.12	10.80 ± 4.08	8.31 ± 3.10	0.0035
18:3n6	1.09 ± 0.38	0.93 ± 0.28	0.71 ± 0.31	0.0116
18:4n-3	0.34 ± 0.28	0.01 ± 0.04	0.03 ± 0.05	0.0006
18:2n-6 (LA)	99.35 ± 25.12	57.06 ± 14.44	46.89 ± 10.66	0.0000
18:3n-3 (ALA)	15.70 ± 5.01	8.76 ± 2.46	6.87 ± 2.64	0.0000
CLA	3.83 ± 2.05	2.65 ± 1.03	1.79 ± 0.93	0.0030
18:0	208.83 ± 119.80	139.11 ± 54.40	99.63 ± 40.82	0.0042
18:1	582.31 ± 364.95	395.67 ± 173.74	305.06 ± 147.93	0.0174
19:1	1.31 ± 0.68	0.89 ± 0.37	0.93 ± 0.51	0.1222
20:4n-6 (ARA)	21.87 ± 8.20	11.26 ± 8.18	4.78 ± 3.64	0.0000
20:5n-3 (EPA)	9.68 ± 3.68	4.25 ± 2.15	2.36 ± 1.01	0.0000
22:3	0.19 ± 0.31	0.21 ± 0.22	0.18 ± 0.23	0.9168
20:3n-6	4.61 ± 1.17	2.10 ± 0.68	1.74 ± 0.58	0.0000
20:4n-3	0.02 ± 0.05	0.10 ± 0.18	0.15 ± 0.24	0.0945
20:2n-6	1.66 ± 0.41	0.52 ± 0.20	0.45 ± 0.29	0.0000
20:0	1.71 ± 1.01	1.15 ± 0.40	0.82 ± 0.37	0.0051
20:1	4.45 ± 2.34	1.71 ± 0.63	1.30 ± 0.68	0.0001
21:5n-3	0.15 ± 0.13	0.14 ± 0.12	0.11 ± 0.12	0.4905
21:0	0.13 ± 0.22	0.04 ± 0.09	0.10 ± 0.14	0.5992
22:5n-6	0.27 ± 0.22	0.26 ± 0.25	0.24 ± 0.11	0.6978
22:6n-3 (DHA)	5.59 ± 1.63	1.85 ± 0.81	1.28 ± 0.67	0.0000
22:4n-6	1.10 ± 0.39	0.93 ± 0.33	0.91 ± 0.25	0.2010
22:5n-3 (DPA)	9.69 ± 3.36	5.48 ± 1.75	4.42 ± 1.25	0.0000
22:0	0.48 ± 0.27	0.34 ± 0.17	0.36 ± 0.43	0.3908
22:1	0.68 ± 0.31	0.52 ± 0.14	0.43 ± 0.21	0.0218
23:1	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.05	0.2268
23:0	0.78 ± 0.18	0.52 ± 0.16	0.41 ± 0.09	0.0000
24:1	1.37 ± 0.33	0.66 ± 0.16	0.66 ± 0.24	0.0000
24:0	0.98 ± 0.29	0.61 ± 0.17	0.53 ± 0.25	0.0004
Total FA	1384.60± 766.56	904.11 ± 342.20	698.78 ± 307.88	0.0056
EPA + DHA	15.28 ± 5.12	6.11 ± 2.88	3.64 ± 1.49	0.0000
EPA + DHA + DPA	24.97 ± 8.27	11.58 ± 4.54	8.05 ± 2.56	0.0000
∑SFA	579.40 ± 333.52	381.87 ± 149.39	295.27 ± 131.95	0.0079
∑MUFA	630.23 ± 393.61	425.92 ± 185.28	330.79 ± 161.51	0.0172
∑PUFA	175.14 ± 47.59	96.53 ± 28.59	72.90 ± 19.03	0.0000
PUFA/SFA	0.36 ± 0.14	0.29 ± 0.12	0.27 ± 0.10	0.1210
∑n-3PUFA	41.36 ± 12.83	20.81 ± 6.43	15.38 ± 5.24	0.0000
∑n-6PUFA	133.78 ± 35.37	75.71 ± 22.91	57.51 ± 14.31	0.0000
n-6/n-3PUFA	3.33 ± 0.52	3.72 ± 0.63	3.87 ± 0.65	0.0499

[#] Abbreviations as in Table 2.

5.3.3. Fatty Acid Content of Liver

In the liver (Table 5.4), it was evident that the sheer volume of total fatty acid metabolism output was greater than in the muscle, kidney, and heart. The omega-3 oil diet had significantly higher CLA ($p < 0.0346$), EPA + DHA ($p < 0.0000$), EPA + DHA + DPA ($p < 0.0002$), PUFA/SFA ($p < 0.0365$), n-3 LC-PUFA ($p < 0.0004$), n-6 PUFA ($p < 0.0008$), and n-6/n-3 PUFA ratio ($p < 0.0000$) than the other treatment groups. A boxplot of Hochberg's adjusted multiple comparisons of significant differences between the treatment groups in liver fatty acid profiles are depicted in Figure 2, where the omega-3 oil diet maintained a significantly higher fatty acid content than the control and MSM whole grain diets. However, the compositions of C14:0, C17:1n8c + a17:0, C18:3n-6, C19:1, C20:4n-6, C20:3, CLA, C22:5n-6, C22:4n-6, C23:0, n-6 PUFA, and n-6/n-3 PUFA ratio in the MSM whole grain diet were higher than in the control and omega-3 oil diets.

Table 5.3. Fatty acid profile (mg/100 g) of the liver in TAW lambs [#].

Fatty Acid	Omega-3	Control	MSM Whole Grain	*p*-Value
13:0	0.24 ± 0.26	0.16 ± 0.26	0.36 ± 0.50	0.3890
14:1	0.84 ± 0.92	1.45 ± 1.21	0.55 ± 0.86	0.6411
14:0	26.95 ± 8.36	31.22 ± 8.74	40.09 ± 13.89	0.0190
15:1	19.20 ± 6.55	15.49 ± 5.47	18.81 ± 7.39	0.7370
16:0	755.15 ± 151.51	765.95 ± 134.32	906.84 ± 205.56	0.0938
16:1	66.47 ± 18.67	83.97 ± 26.54	89.50 ± 28.94	0.0783
17:1n8c + a17:0	46.43 ± 11.84	62.51 ± 16.78	78.31 ± 25.78	0.0017
17:0	73.54 ± 16.08	76.10 ± 16.51	101.28 ± 26.67	0.0143
18:3n6	9.67 ± 3.17	13.31 ± 4.89	15.45 ± 5.60	0.0160
18:4n-3	6.10 ± 3.34	2.34 ± 2.22	2.15 ± 3.18	0.0098
18:2n-6 (LA)	508.28 ± 68.59	438.04 ± 84.82	570.01 ± 99.23	0.2330
18:3n-3 (ALA)	72.78 ± 18.68	50.46 ± 9.52	58.65 ± 12.48	0.0655
CLA	12.45 ± 2.57	15.13 ± 3.13	18.05 ± 8.47	0.0346
18:0	1050.30 ± 82.46	879.11 ± 139.95	1058.24 ± 190.38	0.9770
18:1	1414.42 ± 210.44	1414.52 ± 268.81	1521.50 ± 345.97	0.4875
19:1	7.25 ± 1.88	8.93 ± 2.98	12.35 ± 3.47	0.0010
20:4n-6 (ARA)	213.71 ± 41.72	310.53 ± 69.00	368.35 ± 71.25	0.0000
20:5n-3 (EPA)	122.60 ± 36.37	40.03 ± 9.65	43.99 ± 10.29	0.0000
22:3	2.08 ± 2.23	6.35 ± 2.14	7.16 ± 1.72	0.0001

20:3n-6	58.65 ± 9.41	33.88 ± 5.74	49.26 ± 16.35	0.1700
20:4n-3	6.07 ± 0.84	5.00 ± 2.59	6.03 ± 1.75	0.9550
20:2n-6	12.96 ± 3.02	6.72 ± 1.79	8.59 ± 1.24	0.0033
20:0	33.42 ± 7.26	19.28 ± 5.06	21.45 ± 3.60	0.0652
20:1	5.02 ± 0.28	6.25 ± 1.61	6.38 ± 1.97	0.0007
21:5n-3	1.58 ± 0.71	3.27 ± 2.79	2.76 ± 2.20	0.2770
21:0	0.37 ± 0.21	0.28 ± 0.29	0.51 ± 0.26	0.2643
22:5n-6	5.23 ± 3.89	17.08 ± 3.63	21.53 ± 5.93	0.0000
22:6n-3 (DHA)	286.77 ± 95.79	116.09 ± 30.92	124.56 ± 30.70	0.0001
22:4n-6	16.44 ± 9.99	39.84 ± 11.41	54.47 ± 11.01	0.0000
22:5n-3 (DPA)	185.12 ± 30.84	133.09 ± 29.99	154.08 ± 24.89	0.0515
22:0	6.78 ± 1.87	2.05 ± 0.87	2.12 ± 0.74	0.0000
22:1	9.76 ± 0.84	8.20 ± 1.11	9.31 ± 1.81	0.5057
23:1	0.56 ± 0.57	0.14 ± 0.22	0.17 ± 0.27	0.0504
23:0	15.48 ± 0.82	16.69 ± 2.77	20.36 ± 4.07	0.0011
24:1	17.44 ± 2.66	11.81 ± 2.62	11.14 ± 1.71	0.0000
24:0	16.10 ± 1.00	15.49 ± 2.52	17.48 ± 2.82	0.2397
Total FA	5085.02 ± 632.20	4650.60 ± 806.74	5421.65 ± 1044.59	0.5016
EPA + DHA	409.37 ± 127.95	156.12 ± 37.43	168.54 ± 35.69	0.0000
EPA + DHA + DPA	594.49 ± 153.14	289.22 ± 63.49	322.62 ± 56.93	0.0002
∑SFA	1972.10 ± 229.59	1814.93 ± 295.51	2179.67 ± 431.38	0.2769
∑MUFA	1593.04 ± 242.26	1604.51 ± 317.62	1736.91 ± 404.01	0.4181
∑PUFA	1519.88 ± 191.39	1231.16 ± 228.83	1505.07 ± 261.64	0.8519
PUFA/SFA	0.77 ± 0.06	0.68 ± 0.06	0.70 ± 0.07	0.0365
∑n-3PUFA	682.48 ± 170.17	356.63 ± 72.64	399.37 ± 68.16	0.0004
∑n-6PUFA	1045.88 ± 117.45	1167.97 ± 233.56	1452.52 ± 275.64	0.0008
n-6/n-3PUFA	1.69 ± 0.83	3.30 ± 0.43	3.66 ± 0.49	0.0000

[#] Abbreviations as in Table 2.

5.3.4. Fatty Acid Profile of the Kidney

The fatty acid profile of the kidney is presented in Table 4.5. Within the n-3 LC-PUFA, the contents of ALA, EPA, DHA, DPA, EPA + DHA, and EPA + DHA + DPA were greater in the omega-3 oil diet than in the control and MSM whole grain diets. The overall increase in the contents of 18:3 n-3 and its long chain metabolites remained statistically significant. A boxplot of Hochberg's adjusted multiple comparisons of significant differences between the treatment groups in kidney fatty acid profiles are depicted in Figure 5.3, where the omega-3 oil diet maintained a significantly higher fatty acid content than the control and MSM whole grain diets. However, the control diet had the highest contents of C22:0, C20:2n-6, C22:5n-6,

C22:4n-6, and n-6/n-3 PUFA ratio, while the MSM whole grain diet led in C20:0 and C21:5n-3 contents.

Table 5.4. Fatty acid profile (mg/100 g) of the kidney in TAW lambs [#].

Fatty Acid	Omega-3	Control	MSM Whole Grain	*p*-Value
13:0	0.22 ± 0.21	0.16 ± 0.22	0.28 ± 0.22	0.5721
14:1	0.1 ± 0.14	0.05 ± 0.15	0.04 ± 0.07	0.2296
14:0	7 ± 2.29	6.96 ± 2.3	5.42 ± 1.27	0.0886
15:1	5.33 ± 1.39	5.73 ± 1.45	3.72 ± 1.33	0.0217
16:0	317.14 ± 52.86	339.32 ± 63.53	283.75 ± 48.67	0.2055
16:1	10.57 ± 2.87	11.21 ± 2.56	11.39 ± 2.94	0.5105
17:1n8c + a17:0	12.44 ± 1.91	14.29 ± 3.33	12.04 ± 2.77	0.7594
17:0	25.4 ± 3.66	28.7 ± 7.39	23.32 ± 5.09	0.4353
18:3n6	1.75 ± 0.54	1.28 ± 0.28	0.98 ± 0.25	0.0001
18:4n-3	0.00 ± 0.00	0.04 ± 0.12	0.01 ± 0.03	0.7995
18:2n-6 (LA)	281.42 ± 66.94	280.63 ± 73.32	249.57 ± 56.92	0.2849
18:3n-3 (ALA)	12.43 ± 3.02	8.4 ± 1.62	7.63 ± 1.61	0.0001
CLA	3.5 ± 0.82	3.78 ± 0.85	3.11 ± 1.14	0.3653
18:0	351.69 ± 51.59	327.73 ± 62.89	279.14 ± 47.83	0.0054
18:1	320.27 ± 53.92	314.51 ± 56.7	293.23 ± 58.49	0.2854
19:1	2.17 ± 0.44	3.84 ± 1.28	2.9 ± 1.01	0.1645
20:4n-6 (ARA)	169.81 ± 27.05	246.86 ± 57.97	209.05 ± 37.93	0.0940
20:5n-3 (EPA)	69.1 ± 17.67	16.87 ± 4.66	16.57 ± 2.71	0.0000
20:3	1.1 ± 0.62	2.98 ± 0.75	3.06 ± 1.12	0.0001
20:3n-6	19.6 ± 3.26	17.41 ± 5.3	12.88 ± 3.59	0.0010
20:4n-3	1.95 ± 0.45	1.91 ± 0.92	2.54 ± 1.71	0.2627
20:2n-6	7.9 ± 1.65	8.77 ± 2.68	6.62 ± 2.3	0.2320
20:0	5.35 ± 1.12	5.84 ± 1.13	4.91 ± 0.85	0.3655
20:1	11.88 ± 2.14	9.14 ± 2.2	8.52 ± 2.19	0.0020
21:5n-3	0.48 ± 0.13	0.99 ± 0.38	1.04 ± 0.35	0.0006
21:0	0.69 ± 0.13	0.82 ± 0.16	0.65 ± 0.13	0.5464
22:5n-6	0.44 ± 0.49	3.03 ± 0.73	2.33 ± 0.62	0.0003
22:6n-3 (DHA)	58.76 ± 10.76	25.17 ± 5.59	25.3 ± 5.21	0.0000
22:4n-6	3.52 ± 0.76	15.04 ± 5.17	9.88 ± 3.32	0.0133
22:5n-3 (DPA)	43.39 ± 6.14	33.76 ± 8.04	29.14 ± 4.96	0.0000
22:0	35.02 ± 6.6	36.52 ± 8.02	29.06 ± 5.3	0.0000
22:1	9.01 ± 1.71	5.12 ± 2.16	3.62 ± 1.08	0.0659
23:1	0.56 ± 0.23	0.96 ± 0.34	0.8 ± 0.28	0.1012
23:0	8.84 ± 1.35	9.96 ± 1.97	7.94 ± 1.71	0.2826
24:1	30.55 ± 5.54	33.12 ± 7.47	32.73 ± 7.26	0.4763
24:0	34.42 ± 5.47	37.53 ± 7.62	31.21 ± 6.79	0.3120
Total FA	1499.2 ± 208.97	1515.42 ± 308.98	1322.14 ± 193.73	0.1155
EPA + DHA	127.86 ± 26.2	42.04 ± 9.14	41.87 ± 7.28	0.0000
EPA + DHA + DPA	171.26 ± 29.76	75.8 ± 15.82	71.01 ± 10.98	0.0000
∑SFA	439.41 ± 67.09	471.55 ± 89.38	390.25 ± 66.92	0.1743
∑MUFA	385.11 ± 61.94	377.95 ± 69.57	353.22 ± 71.1	0.2933
∑PUFA	674.69 ± 91.83	665.92 ± 156.17	578.66 ± 79.86	0.0698

PUFA/SFA	1.54 ± 0.13	1.4 ± 0.12	1.51 ± 0.29	0.7367
∑n-3PUFA	187.22 ± 32.15	90.12 ± 18.16	85.28 ± 13.7	0.0000
∑n-6PUFA	318.13 ± 70.45	329.97 ± 86.61	285.38 ± 60.57	0.3264
n-6/n-3PUFA	1.74 ± 0.42	3.65 ± 0.64	3.44 ± 1.14	0.0003

[#] Abbreviations as in Table 5.2.

5.3.5. Fatty Acid Profile of the Heart

Table 5.6 shows the fatty acid contents of the heart. The hearts from lambs on the omega-3 oil diet had the highest ALA, EPA, C20:2n-6, EPA + DHA, EPA + DHA + DPA, n-3 LC-PUFA, DHA, and DPA contents and lowest n-6/n-3 PUFA ratio than in the control and MSM whole grain diets. However, the hearts of lambs fed the control diet had the highest contents of C23:0, C22:0m and C22:4n-6, while those on the MSM whole grain diet had the highest C20:3 and C21:5 n-3 contents. As shown in Figure 5.4, a boxplot of Hochberg's adjusted multiple comparisons of significant differences between the treatment groups in the heart fatty acid profiles shows that the omega-3 oil diet maintained a significantly higher EPA, DHA, EPA + DHA, EPA + DHA + DPA, and ∑n-3PUFA content than the control and MSM whole grain diets, while the control diet had higher C22:4n-6 content and n-6/n-3 PUFA ratio than both omega-3 oil and MSM whole grain diets.

Table 5.5. Fatty acid profile (mg/100 g) of the heart in TAW lambs [#].

Fatty Acid	Omega-3	Control	MSM Whole Grain	*p*-Value
13:0	0.14 ± 0.35	0.02 ± 0.07	0.03 ± 0.1	0.2533
14:1	0.51 ± 0.35	0.61 ± 0.3	0.36 ± 0.47	0.3935
14:0	45.97 ± 95.07	13.63 ± 9.65	23.69 ± 32.81	0.3994
15:1	11.42 ± 20.53	4.75 ± 2.84	6.68 ± 5.79	0.3391
16:0	539.22 ± 573.68	402.04 ± 132.98	389.7 ± 233.75	0.3616
16:1	59.06 ± 91.19	34.61 ± 18.23	41.34 ± 35.23	0.4919
17:1n8c + a17:0	33.28 ± 40.65	21.73 ± 10.42	26.43 ± 18.68	0.5655
17:0	57.87 ± 82.96	37.03 ± 17.28	41.53 ± 29.75	0.4822
18:3n6	2.81 ± 1.01	2.55 ± 0.55	2.24 ± 0.57	0.0919
18:4n-3	0.31 ± 0.46	0.15 ± 0.29	0.06 ± 0.12	0.0823
18:2n-6 (LA)	543.33 ± 141.44	591.85 ± 100.17	466.72 ± 122.48	0.1901
18:3n-3 (ALA)	31.32 ± 26.24	18.55 ± 7.28	22.03 ± 15.47	0.2636

CLA	709.39 ± 866.87	492.75 ± 233.75	459.86 ± 332.73	0.3144
18:0	862.44 ± 1163.49	557.84 ± 334.03	601.47 ± 462.38	0.4367
18:1	9.67 ± 10.06	6.03 ± 2.24	5.68 ± 3	0.1578
19:1	3.32 ± 3.49	2.59 ± 1.28	3.41 ± 2.54	0.9383
20:4n-6 (ARA)	128.74 ± 35.44	166.3 ± 33.44	143.74 ± 48.1	0.4257
20:5n-3 (EPA)	38.59 ± 11.44	18.01 ± 3.9	14.87 ± 4.96	0.0000
22:3	1.6 ± 0.83	3.05 ± 0.79	2.23 ± 0.62	0.1398
20:3n-6	18.01 ± 4.67	12.78 ± 3.01	9.71 ± 2.57	0.0000
20:4n-3	0.22 ± 0.28	0.48 ± 0.72	0.11 ± 0.24	0.6342
20:2n-6	5.12 ± 2.19	2.45 ± 0.87	1.82 ± 0.6	0.0000
20:0	6.11 ± 8.08	4.76 ± 2.14	4.27 ± 2.66	0.4158
20:1	11.82 ± 10.49	6.45 ± 2.36	5.24 ± 3.19	0.0301
21:5n-3	0.29 ± 0.55	0.62 ± 0.32	0.39 ± 0.26	0.6112
21:0	0.62 ± 0.74	0.48 ± 0.13	0.61 ± 0.46	0.9544
22:5n-6	1.26 ± 0.58	2.49 ± 0.57	2.21 ± 0.64	0.0045
22:6n-3 (DHA)	36.74 ± 9.42	15.37 ± 4.1	11.99 ± 3.73	0.0000
22:4n-6	2.91 ± 0.89	6.06 ± 1.29	5.5 ± 1.16	0.0003
22:5n-3 (DPA)	34.82 ± 9.38	19.03 ± 14.44	23 ± 6.92	0.0301
22:0	2.95 ± 1.25	0.97 ± 0.33	0.69 ± 0.38	0.0000
22:1	4.47 ± 2.07	5.03 ± 1.97	3.72 ± 1.83	0.4011
23:1	0.43 ± 0.28	0.17 ± 0.19	0.02 ± 0.05	0.0000
23:0	4.58 ± 1.46	6.38 ± 1.24	4.43 ± 1.42	0.8409
24:1	10.03 ± 3.11	8.43 ± 1.31	5.59 ± 1.55	0.0000
24:0	4.19 ± 1.24	4.65 ± 1.82	3.72 ± 1.03	0.4712
Total FA	3223.54 ± 3074.77	2470.68 ± 880	2335.08 ± 1176.42	0.3140
EPA + DHA	75.32 ± 20.32	33.38 ± 7.73	26.86 ± 8.05	0.0000
EPA + DHA + DPA	110.14 ± 28.19	52.41 ± 18.48	49.86 ± 14.91	0.0000
∑SFA	1384 ± 1645.79	971.52 ± 396.97	938.25 ± 632.49	0.3409
∑MUFA	983.83 ± 1309.31	633.39 ± 366.3	684.53 ± 519.99	0.4274
∑PUFA	855.71 ± 225.88	865.77 ± 149	712.3 ± 183.06	0.1016
PUFA/SFA	1 ± 0.42	0.98 ± 0.25	0.99 ± 0.48	0.9636
∑n-3PUFA	143.88 ± 41.03	75.26 ± 23.68	74.68 ± 23.06	0.0000
∑n-6PUFA	711.84 ± 189.55	790.5 ± 130.94	637.62 ± 168.09	0.3408
n-6/n-3PUFA	5.01 ± 0.68	11.12 ± 2.43	8.82 ± 1.92	0.0041

[#] Abbreviations as in Table 2.

Figure 5.1. Boxplots showing the distribution of selected fatty acid composition in the muscle tissue. Each plot tested the mean fatty acid in omega-3 versus control, omega-3 versus MSM whole grain, and control versus MSM whole grain with Hochberg's adjusted multiple comparisons. *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant ($p > 0.05$).

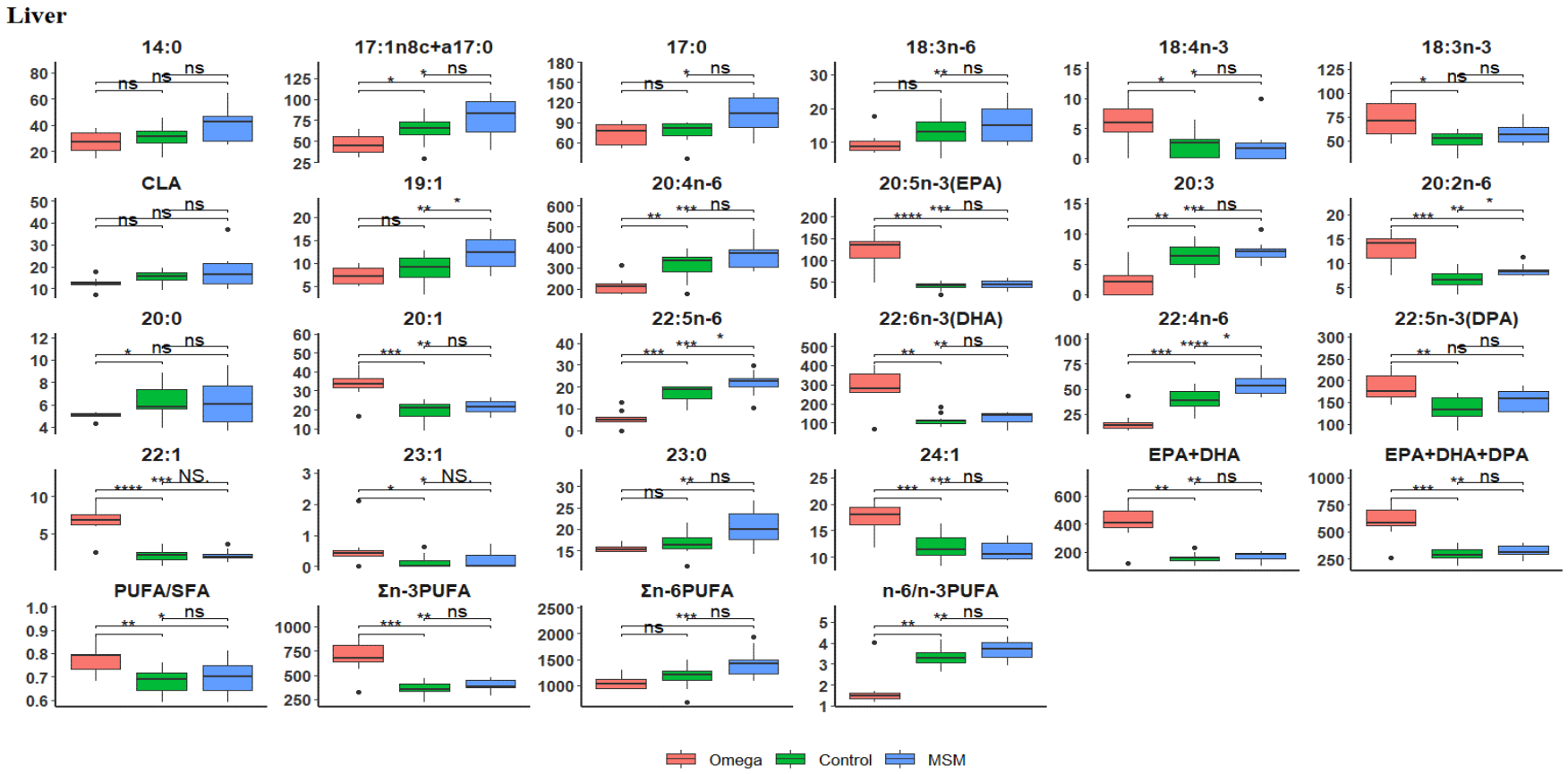

Figure 5.2. Boxplots showing the distribution of selected fatty acids composition in the liver. Each plot tested the mean fatty acid in omega-3 versus control, omega-3 versus MSM whole grain, and control versus MSM whole grain with Hochberg's adjusted multiple comparisons. *p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001; ns, not significant (p > 0.05).

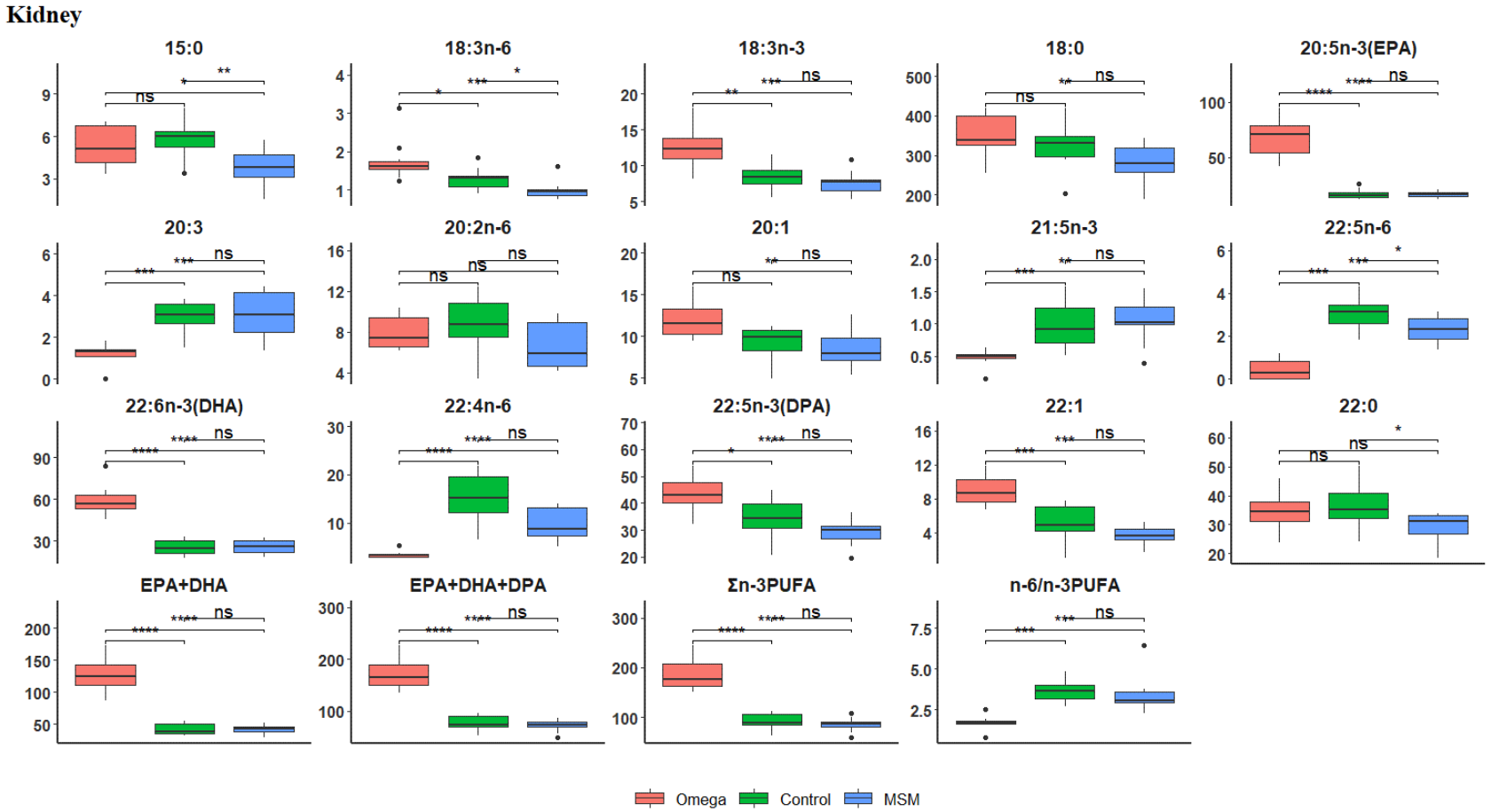

Figure 5.3. Boxplots showing the distribution of selected fatty acids composition in the kidney. Each plot tested the mean fatty acid in omega-3 versus control, omega-3 versus MSM whole grain and control versus MSM whole grain with Hochberg's adjusted multiple comparisons. *$p <$ 0.05; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant ($p > 0.05$).

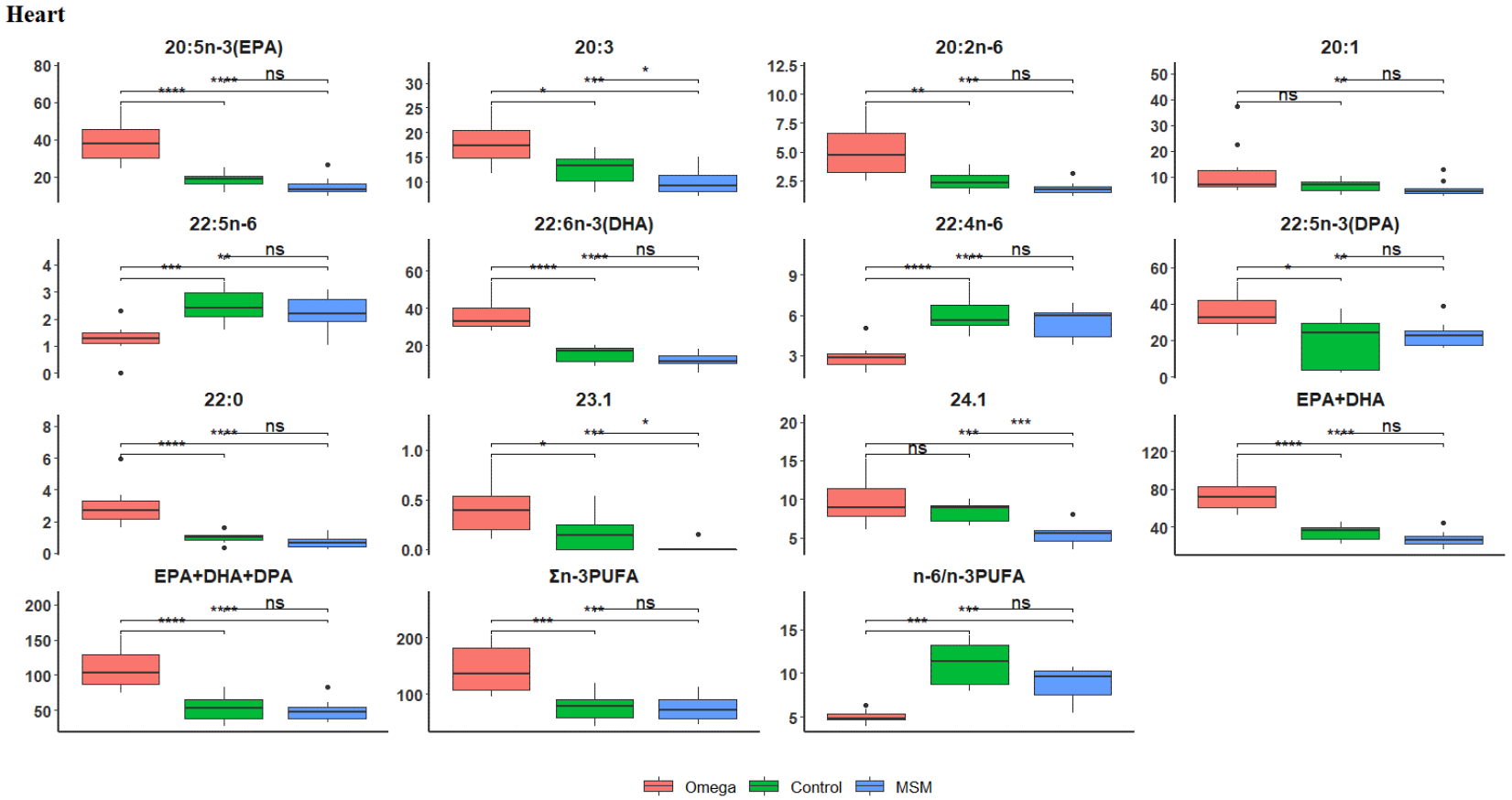

Figure 5.4. Boxplots showing the distribution of selected fatty acids composition in the heart. Each plot tested the mean fatty acid in omega-3 versus control, omega-3 versus MSM whole grain, and control versus MSM whole grain with Hochberg's adjusted multiple comparisons. *$p <$ 0.05; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant ($p > 0.05$)**.**

5.4. Discussion

A prospective cohort study of men in the USA (Al-Shaar et al., 2020) and a cross-sectional survey of Korean adults (Jo et al., 2020) both reinforced the need to enhance a healthier composition of red meat among consumers to minimize the dietary risks of coronary heart disease, cardiometabolic, and cancer mortality burdens. Previous studies (Nguyen et al., 2017; Le ta al., 2019; Malau-Aduli et al., 2019; Nguyen et al., 2019; Urrutia et al., 2020; Alba et al., 2021) established that the fatty acid profiles of muscles and organs could be modified by dietary supplementation with n-3 LC-PUFA, leading to higher human health beneficial EPA + DHA + DPA and lower n-3/n-6 ratio (Celada and Sánchez-Múniz, 2016). It is important to strike a balance between attaining higher total PUFA deposition in the muscle and oxidative stability (Gruffat et al., 2020) because meat colour, flavour, nutritional value, shelf-life, and overall consumer acceptance can be compromised by lipid oxidation (De Lima Júnior et al., 2013; Renna et al., 2019). Therefore, in this study, the time-tested and oxidatively stable omega-3 oil-infused pellets previously reported in Le et al. (2019) were utilized in the comparative analysis with non-oil and whole grain pellets.

The level of incorporation and abundance of both monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the muscle is consistent with previously reported intramuscular fatty acid compositions (Facciolongo et al., 2018). A review of pre-clinical and human trials with conjugated linoleic acid CLA (C18:2n-6) revealed positive effects on cancer, obesity, and atherosclerosis (Simopoulos, 2010; Den Hartigh, 2019), and the muscle in the present study had significantly incorporated levels of CLA and ALA (C18:3n-3). ALA is the precursor for the synthesis of n-3 LC-PUFA through desaturation and/or chain-elongation by desaturase and elongase enzymes (Widmann et al., 2011; Urrutia et al., 2016). In the present study, it was evident that supplementation of lambs with omega-3 oil-fortified pellets increased the muscle contents of ALA by two to three-folds, which translated into higher contents of

EPA + DHA + DPA than in muscles from the control and whole grain diets. Furthermore, the high and significant levels of C20:4n-6 in the muscle could be a result of LA being subjected to elongation and desaturation by delta-5 and delta-6 desaturases and elongase enzymes (Lee et al., 2016), since all dietary treatments favored *de novo* fatty acid synthesis due to high levels of C18:1 originating from elongation and desaturation of C16:0 into palmitoleic and C18:0 into oleic acids (Mueller-Harvey et al., 2019; Vasta et al., 2019; Freitas et al., 2020). In terms of dietary n-6/n-3 PUFA ratio, the human nutrition guidelines recommend an ideal value of not more than 4 (Wood et al., 2008), because n-3 PUFA plays an anti-inflammatory role (Lee et al., 2018), while n-6 PUFA exerts pro-inflammatory effects (Calder, 2006) in asthma and rheumatoid arthritis (Yates et al., 2014) and increased risk of cancer (Yang et al., 2014). This study clearly demonstrated that supplementing lambs with omega-3 oil is an excellent nutritional strategy for lowering the n-6/n-3 ratio in the muscle for a healthier meat.

The liver is responsible for the metabolism, uptake, and dissemination of lipids through free fatty acids, lipoproteins, and de novo lipogenesis (Lee et al., 2017). It plays a role in mitochondrial fatty acid β-oxidation and facilitates key catabolic pathways in hepatocytes (Lee et al., 2017) and ruminant meat production (Castillo, 2019). In the liver of lambs supplemented with omega-3 oil, the significantly higher proportions of EPA + DHA + DPA compared to the control diet equivalent to three-folds of what is obtainable in the kidney and heart (Tables 5.5 and 5.6) in the present study, align with earlier reports (Malau-Aduli et al., 2016; Nguyen et al., 2017; Le ta al., 2019). These figures are well above the Food Standards of Australia and New Zealand recommendations of 30 mg/100 g as 'source' and 60 mg/100 g as 'good source' levels (Le ta al., 2019), and are in agreement with previous studies showing that the lamb liver is one of the richest sources of EPA + DHA + DPA, comprising approximately 185 mg/100 g of hepatocytes from omega-3 oil supplemented diet (Byelashov et al., 2015). The liver levels of CLA were also high in lambs supplemented with omega-3 oil, and given its reported

immuno-modulatory, anti-obesity, and anti-carcinogenic properties (Hennessy et al., 2011; Kuhnt et al., 2016), the liver could be considered a healthy product. The major synthesis pathway of the CLA isomer, rumenic acid, in the tissues of ruminants is primarily from the biohydrogenation of ALA into vaccenic acid (C18:1n-7) catalyzed by reductase enzymes (Gómez et al., 2015; Guerrero et al., 2018). The variation patterns and ranges in liver fatty acid profiles in the present study were like previously reported findings (Moibi and Christopherson, 2001; Borowiec et al., 2004; Demirel et al., 2004; Kim et al., 2007; Bernacka et al., 2013; Coleman et al., 2019). The liver has also been reported to have a high nutrient content of essential amino acids, fatty acids, iron, zinc, magnesium, selenium, calcium, vitamins B1, B6, B12, and folic acid (Florek et al., 2012; Biel et al., 2019) and may explain why duck liver attracts a price premium and is consumed in France (Hicks et al., 2018).

Mashek and Coleman (2006) showed that the kidney plays a role in cellular fatty acid uptake and contributes to metabolism, with strong suggestions that the metabolic demand for fatty acids is a major driving force governing fatty acid uptake in the kidney. Although the mechanism is unknown, it appears that converting fatty acids to acyl-CoAs and downstream metabolic intermediates increases cellular fatty acid uptake, probably by limiting efflux (Mashek and Coleman, 2006). Hagve et al. (1998) demonstrated that increasing levels of n-3 fatty acids in membranes affect the uptake and intracellular metabolism of fatty acids as well as membrane fluidity in the kidney. Evidence from both gain and loss-of-function experiments indicates that fatty acid uptake can be modulated by activation at both the plasma membrane and internal organ sites by intracellular fatty acid binding proteins, and by enzymes in synthetic or degradative metabolic pathways (Mashek and Coleman, 2006). In a study of long-chain polyunsaturated fatty acids metabolism in kidney cells, Liabo et al. (2003) argued that only little is known about the metabolism of fatty acids in the kidney, because it is controversial whether the kidney possesses the ability to desaturate long-chain fatty acids or kidney cells are

dependent on pre-formed polyunsaturated fatty acids transported from the liver. However, they concluded that the kidney, at least in part, must obtain its C-20 and C-22 fatty acids from circulation, while the active delta5-desaturase suggests that pre-formed C-20 fatty acids can be converted to more unsaturated homologues in the kidney. This could probably explain the significant increases in the contents of C18:3n-3 and its long chain ALA, EPA, DHA, and DPA metabolites being more significant in the kidney of lambs supplemented with omega-3 oil than in control and MSM whole grain diets observed in the present study (Table 5.4 and Figure 5.3).

Schaap et al. (1998) reported that long-chain fatty acids are essential to fuel molecules in the heart, because their oxidation in the mitochondria provides the bulk of the energy required for cardiac functioning. However, the cellular transport of fatty acids in aqueous solutions is impaired due to low solubility. To circumvent this hurdle, cardiac tissues contain several fatty acid-binding proteins (FABP) capable of non-covalently binding to fatty acids, thus facilitating both cellular uptake and intracellular transport of fatty acids. The majority of fatty acids taken up by the heart seem to pass the sarcolemma through a carrier-mediated translocation mechanism consisting of one or more membrane-associated FABP (Bernacka et al., 2013). Perhaps the observed significant differences between the treatment groups in the heart fatty acid profiles in the present study where the omega-3 oil diet maintained a significantly higher EPA, DHA, EPA + DHA, EPA + DHA + DPA, and ∑n-3PUFA content than the control and MSM whole grain diets, could probably be indicative of higher activities of the FABP in intracellular transport and cellular uptake of long chain fatty acids.

Edible parts also known as offal, of some internal organs such as liver, kidney, heart, spleen, brain, tongue etc derived from slaughter are an important part of the diet in some countries across the globe. This however varies with culture and region may appear more nutritious than in muscular tissues, for instance, according to Florek et al. (2012) and Biel et al. (2019) the liver has high content of nutrients such as vitamins (B1, B2, B6, and folic acid), iron, zinc,

magnesium, selenium and calcium. Furthermore, in some countries, certain ani-mal organs are consumed as delicacies, including duck liver in France and beef tongue in Latin America. In Australia, offal are supplemented with pet diets.

5.5. Conclusions

This is the first study that evaluated and compared the fatty acid profiles in the tissues and organs of TAW MARGRA lambs raised in a feedlot production system in response to dietary supplementation with or without fortification with omega-3 oil. It was primarily to shed some light on n-3 LC-PUFA metabolism in the *Longissimus thoracis et lumborum* muscle, heart, kidney, and liver of lot-fed TAW MARGRA lambs in response to dietary supplementation with omega-3 oil. The findings suggest that dietary manipulation can be utilised to improve the fatty acid content and nutritional value of muscle and organs of TAW MARGRA lambs to meat that more closely meets nutritional guidelines of higher levels of health-beneficial n-3 LC-PUFA. The data clearly portray the liver, kidney, and heart of TAW MARGRA lambs with the highest contents of the healthiest omega-3 fatty acids well beyond the FSANZ 'good source' levels. Therefore, the hypothesis that *fortifying feedlot pellets with omega-3 oil will enhance the human health beneficial n-3 LC-PUFA composition of edible lamb muscle tissue and organs* holds and is worthy of acceptance.

5.6. Summary

This research aimed to evaluate the nutritional enhancement of omega-3 long-chain polyunsaturated fatty acid (n-3 LC-PUFA) composition of edible lamb *Longissimus thoracis et lumborum* muscle, heart, kidney, and liver in response to dietary supplementation of lot-fed lambs with or without omega-3 oil fortified pellets. The hypothesis tested was that fortifying feedlot pellets with omega-3 oil will enhance the human health beneficial n-3 LC-PUFA composition of edible lamb muscle tissue and organs. Seventy-five Tattykeel

Australian White lambs exclusive to the MARGRA brand, with an average body weight of 30 kg at six months of age, were randomly assigned to the following three dietary treatments of 25 lambs each, and lot-fed as a cohort for 47 days in a completely randomized experimental design: (1) Control grain pellets without oil plus hay; (2) Omega-3 oil fortified grain pellets plus hay; and (3) Commercial whole grain pellets plus hay. All lambs had *ad libitum* access to the basal hay diet and water. The gas chromatography–mass spectrophotometry technique determined the post-slaughter fatty acid composition of the *Longissimus thoracis et lumborum* muscle, liver, kidney, and heart. Results indicated significant variations ($p < 0.05$) in fatty acid profiles between tissues and organs. Omega-3 oil fortified pellets significantly ($p < 0.05$) increased ≥C20 n-3 LC-PUFA (C20:5n-3 eicosapentaenoate, EPA + C22:5n3 docosapentaenoate, DPA + C22:6n3 docosahexanoate DHA); C18:3n-3 alpha-linolenate, ALA; C18:2 conjugated linoleic acid, CLA; total monounsaturated fatty acids, MUFA; polyunsaturated fatty acids, PUFA contents; and reduced the ratio of omega-6 to omega-3 fatty acids in all lamb organs and tissues without impacting shelf-life. The findings demonstrate that the inclusion of omega-3 oil in feedlot diets of lambs enhances the human health beneficial omega-3 long-chain polyunsaturated fatty acid profiles of edible muscle tissue and organs without compromising meat quality.

Chapter 6: Next Generation Sequencing of Single Nucleotide Polymorphic DNA-Markers in Selecting for Intramuscular Fat, Fat Melting Point, Omega-3 Long-Chain Polyunsaturated Fatty Acids and Meat Eating Quality in Tattykeel Australian White MARGRA Lamb

6.1. Introduction

Eating quality is the most significant determinant of consumer acceptability and satisfaction with meat products. The eating and nutritional quality of lamb is influenced by intramuscular fat (IMF) content (Thomas et al., 2021), fat melting point (FMP), tenderness, juiciness, flavour and health-promoting omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) that optimise retinal, maternal and childhood brain functions while minimising the risks associated with cardiovascular and chronic diseases (Heck et al., 2021).

In a recent review of the development, calibration and validation of objective measurement technologies for carcass composition, lean, fat and meat-eating quality traits in the Australian and New Zealand livestock industries, Gardner et al. (2021) highlighted the inherent difficulties associated with the poor measurement of meat eating quality and lean meat yield. Attempts to predict IMF (Alvarenga et al., 2021; Fowler et al., 2021; Lambe et al., 2021), intramuscular connective tissue (Andueza et al., 2021), composition and quality characteristics (Patel et al., 2021), tenderness, ultimate pH and IMF content (Dixit et al., 2021; 2020; Knight et al., 2019) from near infra-red based regression equations were characterised by low accuracy, inconsistency and divergence between calibration and validation data. Such inaccuracies lead to lamb inefficiencies and an estimated annual value-chain wastage cost of $130 million to the Australian beef industry (Gardner et al., 2021).

However, meat quality data can only be obtained after slaughter when selection decisions about the live animal are already too late. Carcass estimated breeding values (Knight et al., 2020; Anderson et al., 2016), visual marbling score and meat imaging camera marbling systems (Stewart et al., 2021) and dual X-ray absorptiometry scanner-based computed tomography

determined fat, lean muscle and bone compositions of lamb carcasses (Connaughton et al., 2021) are all useful technological advancements, but still present precision problems due to low accuracy, and by the time an informed decision on the genetic merit for meat quality is made, the animal is already dead. In a study of associations of sire estimated breeding values and objective meat quality measurements with sensory scores in Australian lamb, Pannier et al. (2014) confirmed the growing concerns that selecting for lean meat yield would reduce consumer eating quality and concluded that careful monitoring of selection programmes is needed to maintain lamb eating quality. In an experimental trial to understand the impact of sire lean meat yield breeding value on carcass composition, meat quality, nutrient and mineral content of Australian lamb, Knight et al. (2020) concluded that to avoid deterioration in meat quality, the nutritional content of lamb and fresh meat colour, Australian sheep producers will need to incorporate other aspects of meat quality when selecting sires with increased lean meat yield. To date, conventional laboratory-based fat extraction, 'slip point' and gas chromatography methods remain the most accurate techniques for measuring IMF, FMP, and n-3 LC-PUFA, and predicting consumer acceptance of beef and sheep meat (Holman and Hopkins, 2021). Herein, we report for the first time, a combination of an innovative and minimally invasive *longissimus dorsi thoracis et lumborum* muscle biopsy sampling of Tattykeel Australian White (TAW) sheep exclusive to MARGRA lamb brand, laboratory-based IMF, FMP and fatty acid analyses and advanced genomics technique of next generation sequencing (NGS) of single nucleotide polymorphisms (SNP) of lipid metabolism genes for directly quantifying the genetic worth of live lambs for health-beneficial n-3 LC-PUFA, IMF and FMP. The primary objective was to conduct a NGS of stearoyl-CoA desaturase (*SCD*), fatty acid binding protein-4 (*FABP4*) and fatty acid synthase (*FASN*) lipogenic genes to identify functional SNP that provide unique DNA marker signatures for TAW genetics, breeding and selection programmes for meat eating quality. The hypothesis tested was that *significant*

associations exist between SNP of lipid metabolism genes and n-3 LC-PUFA, FMP and FMP underpinning potential marker-assisted selection for meat eating quality traits in TAW lambs.

6.2. Materials and Methods

6.2.1 Animals and Experimental Design

The experimental design for the selection, breeding and evaluation of n-3 LC-PUFA, IMF and FMP in Tattykeel Australian White (TAW) sheep is shown in Figure 6.1.

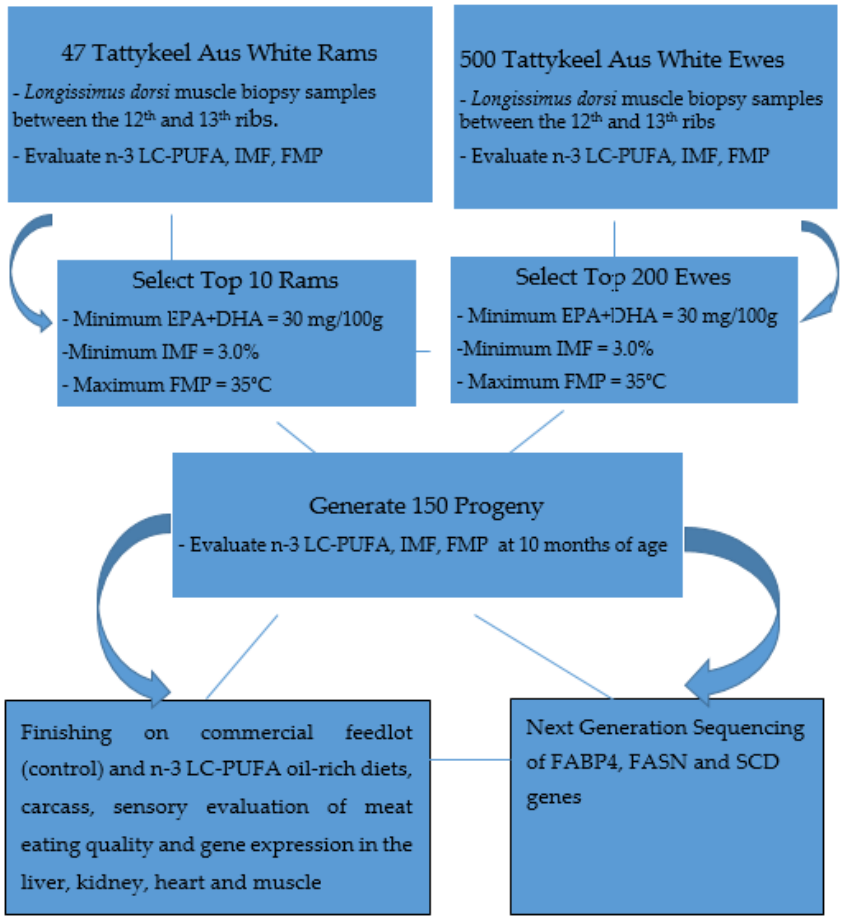

Figure 6.1. Experimental design for the selection, breeding and evaluation of n-3 LC-PUFA, IMF and FMP in Tattykeel Australian White sheep

Three composite generations - parental, first (F_1) and second (F_2) composite generations of lambs were bred, raised and maintained under the same management at the Tattykeel Australian White Stud in Black Springs, Oberon, New South Wales, Australia. The parental composite generation comprised 47 rams mated to 500 ewes after evaluating their *longissimus dorsi thoracis et lumborum* muscle biopsy samples for health-beneficial n-3 LC-PUFA, IMF and FMP with minimum thresholds set at 30 mg/ 100 g, 3.0 % and 35 °C, respectively. The top 10 rams and 200 ewes were selected and mated to generate 150 progeny whose muscle biopsy samples were laboratory tested for n-3 LC-PUFA, IMF, FMP and genomic DNA sequenced at 10 months of age prior to being finished at a commercial feedlot. The Poll Dorset and Texel were used as positive control and the Rambouillet as the negative control in assessing extracted genomic DNA, polymerase chain reaction products and next generation sequencing procedures in the laboratory. Details of the muscle biopsy procedure and laboratory analyses of IMF, FMP and fatty acid composition had been presented in Chapter 3 and are summarised below for reproducibility.

6.2.2. Muscle biopsy sampling procedure

The biopsy procedure for sampling the *Longissimus dorsi* muscle from the 12^{th} -13^{th} rib was first described in cattle (Malau-Aduli et al., 1998) and modified in sheep. Details of the biopsy procedure in sheep have been presented in Chapter 3 and these will not be repeated here.

6.2.3. Determination of intramuscular fat

Details of the procedures for laboratory analysis of intramuscular fat have been published by Holman et al. (2014) and Flakemore et al. (2014) and will not be repeated here.

6.2.4. Determination of fat melting point

Details of the laboratory analysis of fat melting point have been published by Holman et al. (2014) and Flakemore et al. (2014) and needless to repeat herein.

6.2.5. Determination of fatty acid composition

Fatty acid composition including n-3 LC-PUFA analysis of *Longissimus dorsi* muscle biopsy samples was analysed by means of gas chromatography – mass spectrophotometry procedure described in detail by Malau-Aduli et al. (2016) based on modified Bligh and Dyer (1959), Miller et al. (2006) and Clayton (2014) methods. Details have been also been presented in Chapter 3.

6.2.6 Blood collection and genomic DNA extraction

About 10 ml of blood was collected from Tattykeel Australian White, Poll Dorset and Texel (positive control) lambs of the same age and under the same management conditions by jugular venipuncture into vacutainers containing EDTA. Blood samples were stored at -80°C until ready for genomic DNA (gDNA) extraction. gDNA was extracted from 2 ml of blood using NucleoSpin Blood Kits (Macherey-Nagel GmbH & Co. KG, Neumann-Neander-Str. 6-8. 52355 Duren, Germany) according to the manufacturer's protocol. gDNA yield was quantified with a NanoDrop ND-1000 spectrophotometer (NanoDrop, Analytical Technologies, Biolab).

6.2.7 Primer design

6.2.7.1 FASN, FABP4 and SCD primers

All primers were designed using Geneious Prime Software Program 2020 v.2.2 (http://www.geneious.com). A targeted candidate gene approach of lipid metabolism genes (*FASN*, *FABP4* and *SCD*) was utilized. Single coding sequences of each gene deposited in the National Center for Biotechnology Information (NCBI) database (Genbank) of *FASN*, *FABP4* and *SCD* of *Ovis aries* breed were used as reference points. To amplify the 18 kb of the *FASN* gene (Accession Number: NC_040262.1), a long-range PCR approach was used to split the gene sequence into three overlapping fragments of 8.5 kb each (*FASN*1, *FASN*2 & *FASN*3), comprising approximately 91 % of the total gene sequence. For the 4 kb *FABP4*

(NC_040260.1) and 12 kb *SCD* (NC_040273.1) gene fragments, a single primer set was designed as shown in Table 1. All primers were synthesised at Integrated DNA Technologies Pte. Ltd, Melbourne, Australia (itddna.com).

6.2.7.2 Long-range PCR

Due to the different fragment lengths and DNA composition, it was necessary to use three different long-range PCR approaches to amplify the *FASN*, *FABP4* and *SCD* genes. During optimization, all three approaches were tested for all three genes, but only the best performing combinations were utilised.

6.2.7.3 FASN gene

FASN PCR amplification assay was performed using the TakaRa PrimeSTAR GXL Master Mix (TaKaRa Bio Inc.). PCR reaction assay was set up in a total volume of 50 µL containing 10 µL of 5x TakaRa PrimeSTAR GXL Buffer, 200 µM of TaKaRa dNTP Mixture, 1.25 units of TaKaRa PrimeSTAR GXL DNA Polymerase, 0.2 µM of each primer (IDT, Melbourne, Australia), and 100 ng of DNA template. PCR was performed in a SimpliAmp™ Thermal Cycler (Thermofisher Scientific, Melbourne, Australia), in a 2-step protocol using the following conditions: 98 °C initial denaturation for 1 minute (1 cycle); 98 °C denaturation for 10 seconds; 68 °C annealing/extension for 10 minutes for 30 cycles. PCR success was checked in 0.8 % agarose gel electrophoresis as depicted in Figures 6.2-4.

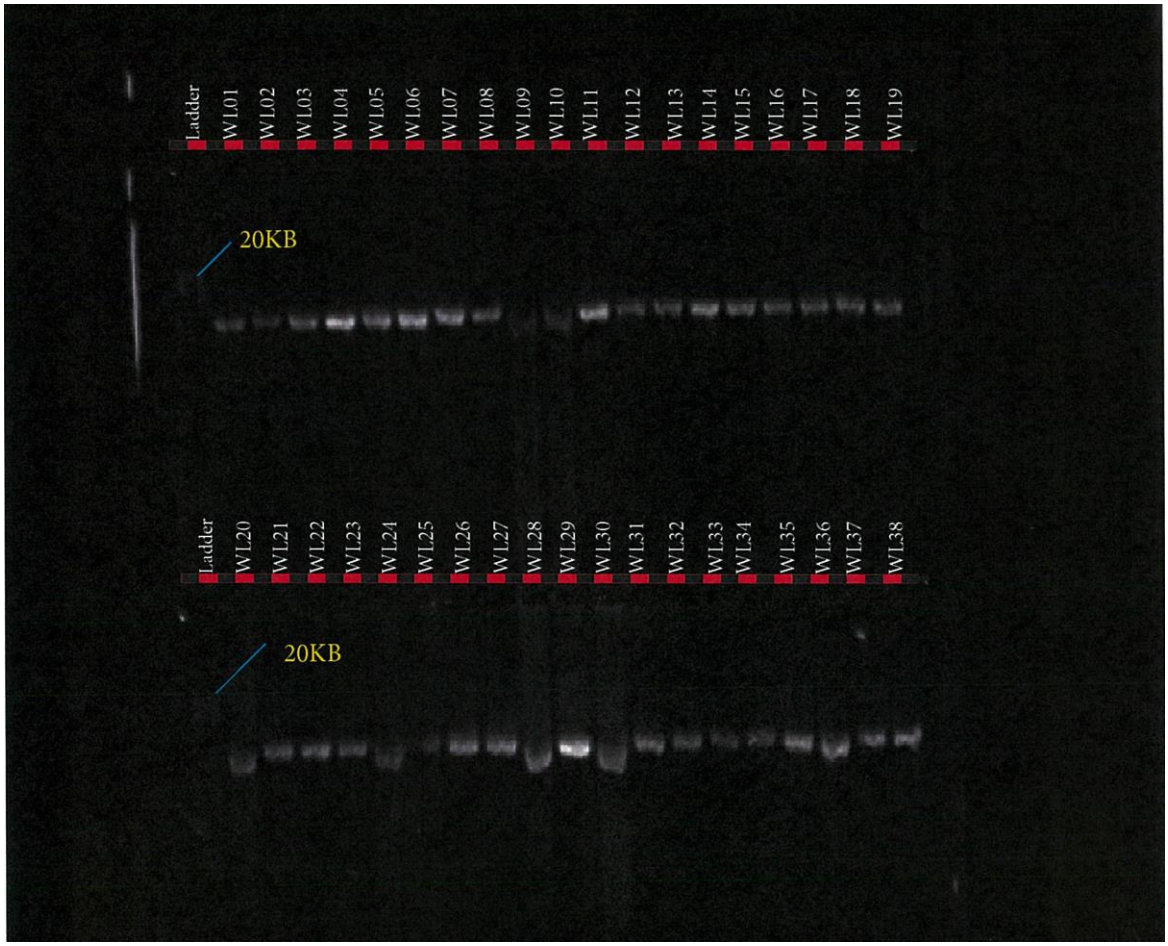

Figure 6.2. *FASN* fragment 1 PCR product in Tattykeel Australian White (WL), Poll Dorset (PD) and Texel (TX) lambs.

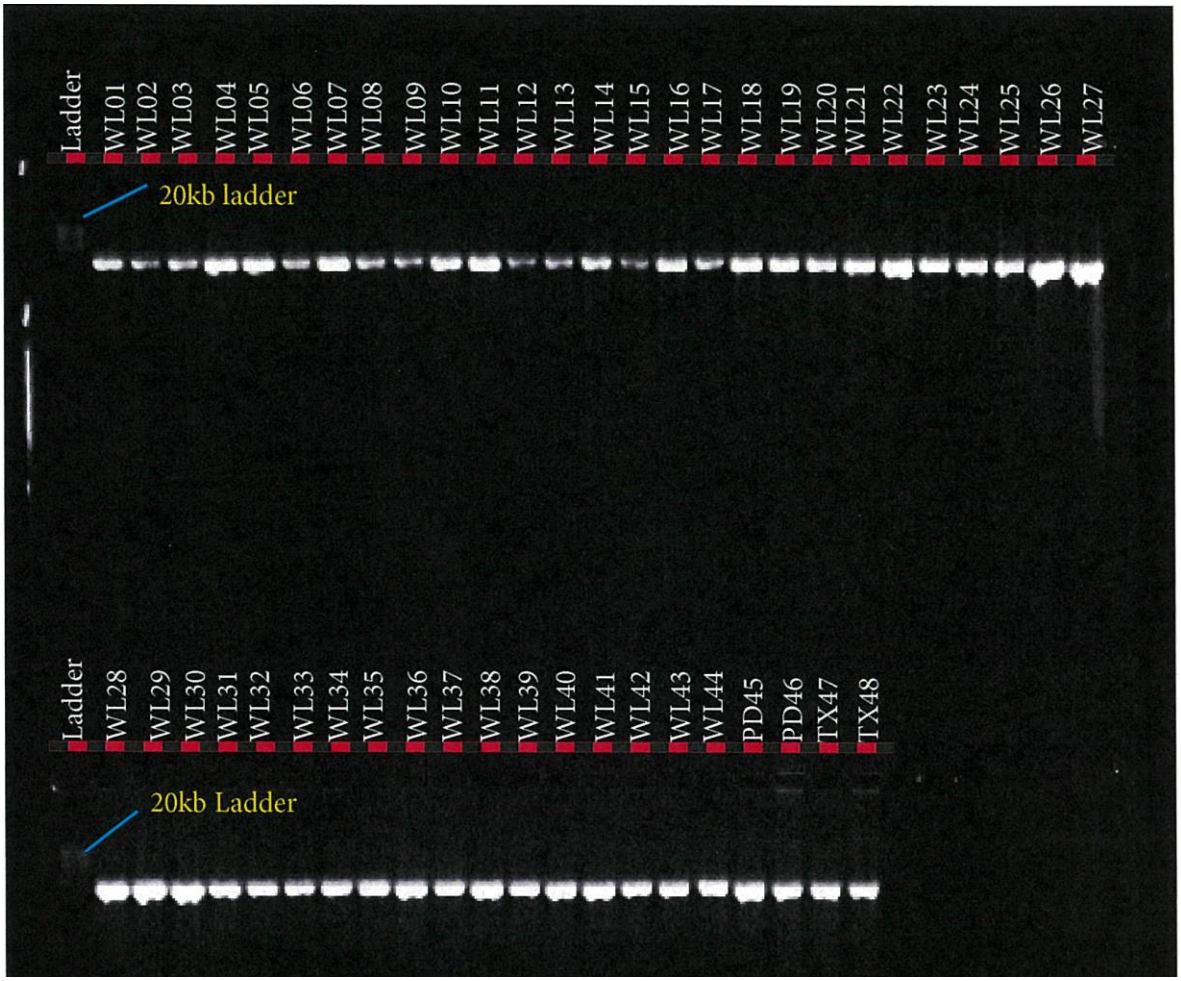

Figure 6.3. *FASN* fragment 2 PCR product in Tattykeel Australian White (WL), Poll Dorset (PD) and Texel (TX) lambs.

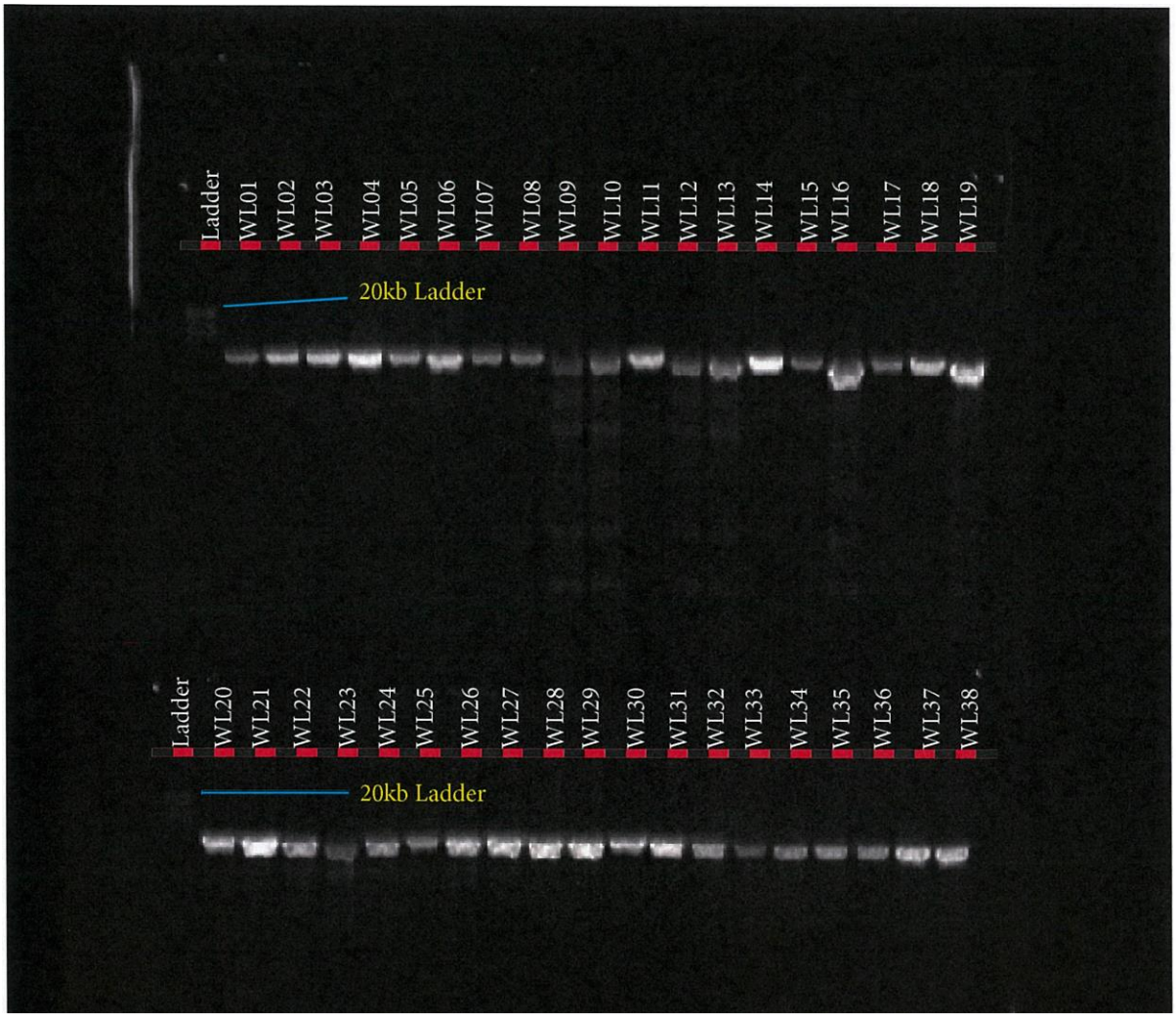

Figure 6.4. *FASN* fragment 3 PCR product in Tattykeel Australian White (WL), Poll Dorset (PD) and Texel (TX) lambs.

6.2.7.4 FABP4 and SCD

For the FAPB4 gene, Platinum™ SuperFi™ II PCR Master Mix (Thermofisher Scientific, Australia) was used, while for *SCD* gene, Hot Start II High-Fidelity PCR Master Mix (Thermofisher Scientific, Australia) was used under the same PCR conditions. The amplification reactions were performed in a total volume of 50 µL containing 25 µL of 2X Platinum™ SuperFi™ II PCR Master Mix or Phusion Hot Start II High-Fidelity PCR Master Mix (Thermofisher Scientific, Australia), 0.5 µM of each primer (IDT, Australia), and 100 ng of DNA template. PCR was performed in a SimpliAmp™ Thermal Cycler (Thermofisher

Scientific, Australia), in a 3-step protocol, using the following conditions: 98 °C initial denaturation in 1 minute (1 cycle); 98 °C for denaturation 15 seconds; 60 °C (*FABP4*)/and 65 °C (*SCD*) annealing for 15 seconds; 72 °C extension for 9 minutes; 72 °C final extension for 9 minutes; 4 °C hold for 35 cycles. PCR success was checked in 0.8% agarose gel electrophoresis as depicted in Figures 6.5 and 6.6.

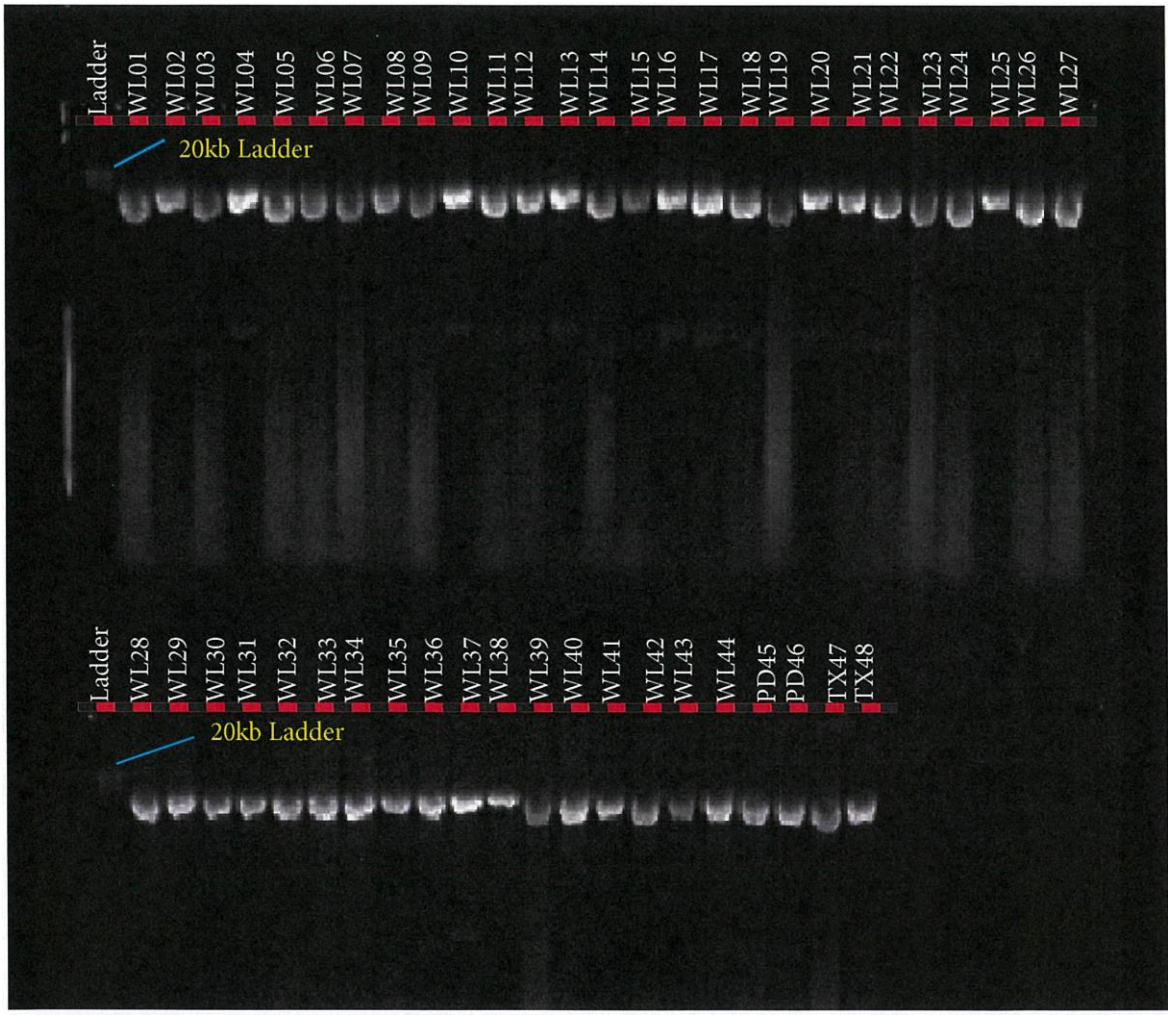

Figure 6.5. *SCD* PCR product in Tattykeel Australian White (WL), Poll Dorset (PD) and Texel (TX) lambs.

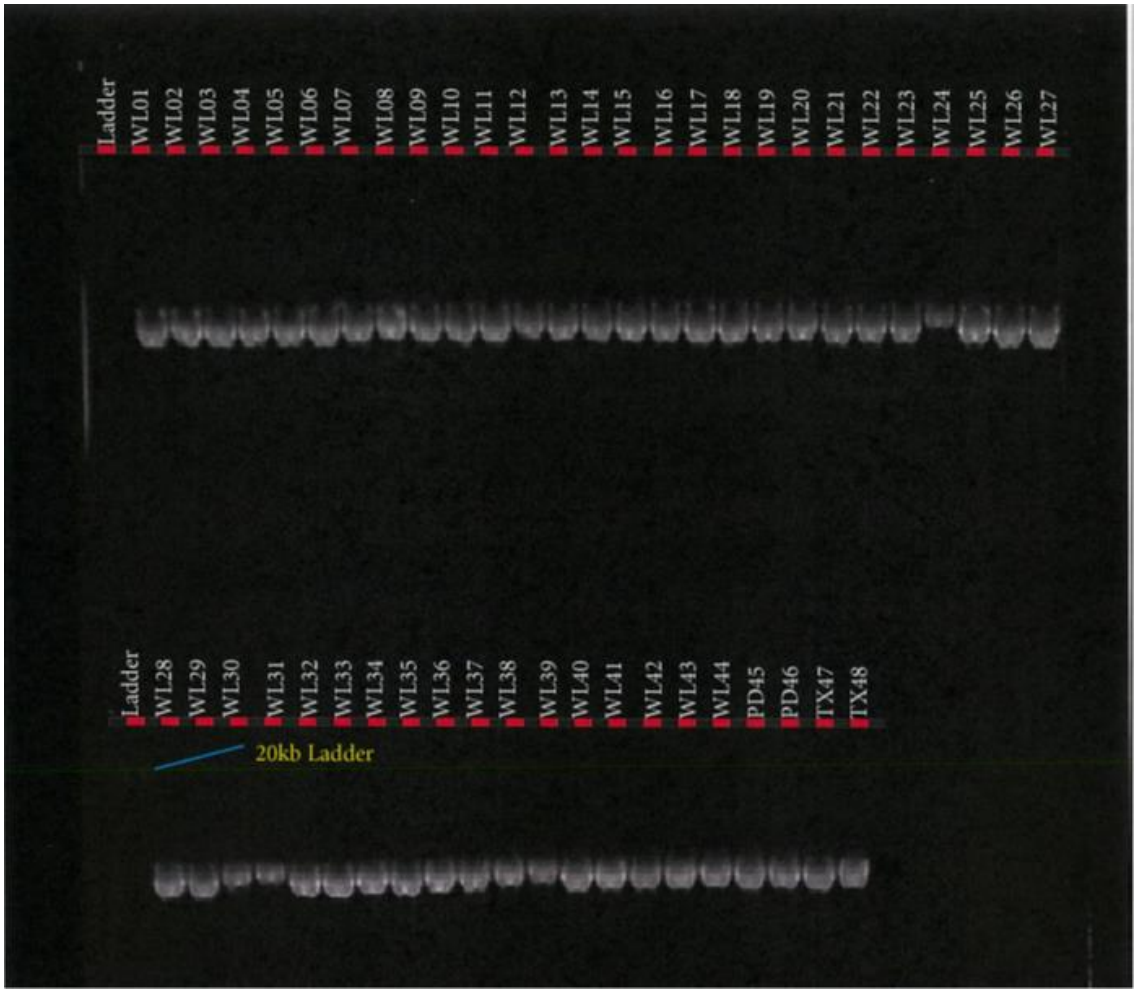

Figure 6.6. *FABP4* PCR product of Tattykeel Australian White (WL), Poll Dorset (PD), and Texel (TX) lambs

6.2.8. PCR Clean-up

Sera-Mag™ SpeedBeads was prepared according to Faircloth et al. (2014) and used to clean the PCR products using a Zephyr NGS Workstation (Caliper Lifesciences, Perkin-Elmer) and quantified using a Promega dsDNA Quantifluor System Kit (Ref: E2670, 00002484139) on an Enspire Workstation (Perkin-Elmer). The five different PCR products were pooled at approximately 0.4 nM to ensure even coverage during sequencing using Quantifluor dsDNA System (Promega, USA). The products were normalized to 2 ng/ µL using 10 mM Tris-HCl (pH 8.0). Final dilution to 0.2 ng/ µL with 10 mM Tris-HCl (pH 8.0) was conducted in

preparation for library preparation and final accuracy checks using the Illumina Nextera^{XT} DNA.

6.2.9 Library preparation, quantification, normalization, and sequencing

Libraries were prepared using Nextera XT DNA Library Prep kit (Illumina, Ca, USA) in accordance with the manufacturer's protocols using the recommended input of 5 µL of 0.2 ng/ µL gDNA per sample. This was followed by Sera-Mag™ SpeedBeads purification using 0.6 x beads and two washes using 80 % ethanol to select fragments > 250 bp and remove unincorporated adapters. Each DNA library fragment size and concentration was determined using Agilent High Sensitivity D5000 reagents and ScreenTape on the Tape Station 4200 Instrument (Agilent Technologies, USA) according to the Agilent assay quick guide. Additionally, all individual libraries were quantified using QuantiFluor® dsDNA System (Promega, USA) to give an additional concentration estimate. The resultant size and concentration data from Tape Station and Quantifluor system were used to normalize each library to 4 nM by diluting with 10 mM Tris-HCl (pH 8.5) prior to pooling. An equal volume of 5 µL was pooled and sequenced on an Illumina MiSeq benchtop sequencer, using a 500-cycle MiSeq Reagent Nano Kit v2 with a 10 pM input and 10 % PhiX spike-in.

6.2.10. Bioinformatics and Next Generation Sequencing Data Analysis

Genomic data analysis was performed using commercial bioinformatics program Geneious Prime software program 2020 v.2.24 (http://www.geneious.com) to analyze the sequences. The following reference sequences deposited in the NCBI database were used for comparative analysis: NC_040262.1, NC_040260.1 and NC_040273.1 for *FASN*, *FABP4* and *SCD* genes, respectively. Next Generation Sequenced data were retrieved from Illumina Dashboard-BaseSpace Sequence Hub (https://basespace.illumina.com/dashboard) as paired read data in two separate forward and reverse read lists in FASTQ format. The retrieved raw reads were

subjected to quality control measures. Reads were trimmed and adapters removed using BBDuk trimmer in Geneious Prime 2020 v.2.2 with the default setting for paired-end reads. The Quality (Q) value of Phred score was set at 20 to improve sequenced data and increase the likelihood of calling true SNPs to 99 %. Short reads with a minimum length of 20 bp were discarded, resulting in clean reads. Regions of low coverage were excluded when calling SNPs using the Annotate and Predict → Find Low/High Coverage. The reads were mapped to reference in Geneious. The reference sequences were retrieved from NCBI database (Genbank) of *FASN*, *FABP4* and *SCD* of Ovis aries breed. The Sensitivity was set on Medium Sensitivity/ Fast and Fine-Tuning (iterate up to 5 times) option selected to improve the results by aligning reads to each other in addition to the reference sequence. Major allele frequencies from the next generation sequence data based on observed and expected genotypes were computed using the Hardy-Weinberg equilibrium principle as described by Graffelman et al. (2017).

6.2.11. Statistical Analyses

All statistical analyses of the associations between detected SNP of the three genes and meat-eating quality traits were performed using R statistical software version 3.6.3 (2021). Linkage disequilibrium as an index of non-random association between alleles of different loci, was estimated as the difference between the frequency of gametes carrying the pair of alleles A and B at two loci (pAB) and the product of the frequencies of those alleles (pA and pB), D_{AB} = pAB – pApB, where the allele pair AB is a haplotype and pAB is the haplotype frequency (Slatkin, 2008). Major and minor allele frequencies were computed, and the Hardy-Weinberg Equilibrium tested using chi-square test. Pearson's residual correlation analysis was carried out to examine the relationships between genomic variants and meat quality traits (FA, FMP and IMF). Linear mixed models procedure was used to investigate differences in FMP, IMF and fatty acid profiles of the TAW lambs due to *FABP4*, *SCD* and *FASN* variants fitting the fixed effect of allele substitution for individual SNP and random effect of animal (for pedigree)

accounting for generation effects. Functional allele mutations at the coding regions of identified *FABP4*, *SCD* and *FASN* loci were statistically analysed for genome association with FMP, IMF and fatty acids. Least-square means were compared using the Tukey-adjusted multiple comparisons test. The full statistical model was:

$$Y_{ij} = \mu + \alpha i + \gamma_1 FA_{ij} + \gamma_2 SC_{ij} + \gamma_3 SK_{ij} + e_{ij}$$

Where Y_{ij} = dependent variable (FMP, IMF, FA) of j^{th} TAW of i^{th} composite generation, μ= overall mean, αi = effect of the i^{th} composite generation, FA = the genotype *FASN* (AA, GA, and GG), SC = the genotype *SCD* (CC, CT and TT), SK = the genotype *FABP4* (GG, GA and AA), γ=effect of the genotype, and e_{ij} = residual error.

6.3. Results

This study of *SCD*, *FASN* and *FABP4* lipogenic genes SNP in TAW lamb muscle biopsy samples bred, selected and evaluated as per the experimental design shown in Figure 6.1, was based on the Geneious-designed primers whose sequences are presented in Table 6.1 and successful polymerase chain reactions (PCR) products are presented in Figures 6.2-6.

Table 6.1. Primer sequences for *FABP4*, *FASN* and *SCD* polymerase chain reaction assays[#]

Gene		Sequence	Length (bp)	T_a (°C)	Fragment length (bp)
FASN 1	Forward	CCTACTTTCCCATGCTCAGAGAA	23	68	7890
	Reverse	CTACGTTGCTGAGGAAGAACTCTA	24	68	
FASN 2	Forward	ACCGTCTCTCCTTCTTCTTTGAC	23	68	8798
	Reverse	GAAGTTGAGGGAGGCGTAATAGAT	24	68	
FASN 3	Forward	CTAGAGTTCTTCCTCAGCAACGTA	24	68	9288
	Reverse	GCCAGGGAGCTGTGAATAATACTA	24	68	
FABP4	Forward	TTGTTGAATGGCTGGGCTTATAAC	24	60	4107
	Reverse	TAAGAAAATACTTCCTGGGGCACA	24	60	
SCD	Forward	CAAACTTAGGTCTGCAACTTTCGT	24	65	11545
	Reverse	TTTCCCACTTCAACTCACCCTATT	24	65	

[#]*FASN*, Fatty Acid Synthase; *FABP4*, Fatty Acid Binding Protein 4; *SCD*, Stearoyl-CoA Desaturase; T_a, annealing temperature.

6.3.1. SCD, FASN and FABP4 gene SNP variants and genotypes

Using the Poll Dorset and Texel as positive controls, and Rambouillet as negative controls, eight *SCD* gene SNP loci (g.23880613A>G; g.23881050T>C; g.23883280G>A; g.23885910C>A; g.23887165A>G; g.23888763C>T; g.23889346T>G; g.23890209T>C) with major allele frequencies ranging from 0.53 to 0.93 were identified as depicted in Table 6.2. It was evident from Table 6.2 that TAW lambs were all heterozygous at three loci (g.23881050T>C, g.23883280G>A g.23885910C>A) in the parental, first and second

generations, thereby presenting a genetic divergence from the homozygous variants seen in the Poll Dorset, Texel and Rambouillet controls.

Table 6.2. *SCD* gene SNP (major allele frequency) in TAW[1], Poll Dorset (+ control) and Rambouillet (- control) lambs.

Lamb breed, generation, type of control and genotypes (major allele frequencies in brackets) Parental composites **1**st (F_1) and 2nd (F_2) composites Positive (+) and negative (-) controls						
SNP locus	TAW Parents (n=147)	TAW F_1 (n=75)	TAW F_2 (n=75)	Poll Dorset (+ n=2)	Texel (+ n=2)	Rambouillet (- n=2)
g.23880613A>G	GG (0.82)	GG (0.93)	GG (0.73)	GG	GG	AA
g.23881050T>C	CT (0.58)	CT (0.54)	CT (0.90)	CC	CC	TT
g.23883280G>A	AG (0.53)	AG (0.71)	AG (0.60)	AA	AA	GG
g.23885910C>A	AC (0.57)	AC (0.71)	AC (0.53)	CC	CC	CC
g.23887165A>G	GA (0.69)	GG (0.82)	GG (0.70)	GG	GG	AA
g.23888763C>T	TC (0.58)	TC (0.54)	CC (0.93)	CC	CC	CC
g.23889346T>G	GT (0.68)	GG (0.82)	GG (0.70)	GG	GG	TT
g.23890209T>C	CT (0.67)	CC (0.82)	CC (0.70)	CC	CC	TT

[1] TAW, Tattykeel Australian White.

As depicted in Table 6.3, nine functional SNP covering 91 % of the *FASN* gene sequence were identified. The genotypes at the nine loci were all the same in TAW, indicating a consistent heredity pattern from the TAW parents to the first and second generations which were all distinguishable from the Rambouillet negative control breed. For the *FABP4* gene, three SNP loci were genotyped with major allele frequencies ranging from 0.50 to 0.97 (Table 6.4).

Table 6.3. *FASN* gene SNP (major allele frequency) in TAW[1], Poll Dorset (+ control) and Rambouillet (- control) lambs.

Lamb breed, generation, type of control and genotypes (major allele frequencies in brackets) Parental composite **1**st (F_1) and 2nd (F_2) composites Positive (+) and negative (-) controls						
SNP locus	TAW Parents (n=147)	TAW F_1 (n=75)	TAW F_2 (n=75)	Poll Dorset (+ n=2)	Texel (+ n=2)	Rambouillet (- n=2)
g.12316077T>G	GG (0.89)	GG (0.86)	GG (0.95)	GG	GG	TT
g.12318491A>G	GG (0.89)	GG (0.86)	GG (0.95)	GG	GG	AA
g.12320583T>C	CC (0.89)	CC (0.86)	CC (0.97)	CC	CC	TT
g.12321671T>C	CC (0.89)	CC (0.86)	CC (0.97)	CC	CC	TT
g.12323864A>G	GA (0.70)	GA (0.69)	GA (0.70)	GG	GG	AA
g.12324288G>A	AG (0.69)	AG (0.68)	AG (0.69)	AA	AA	GG
g.12326992T>C	CC (0.88)	CC (0.79)	CC (0.90)	CC	CC	TT
g.12327084->CT	CT (0.50)	CT (0.50)	CT (0.50)	CT	CT	TT
g.12328120T>C	CC (0.89)	CC (0.86)	CC (0.97)	CC	CC	TT

[#]TAW, Tattykeel Australian White

Table 6.4. *FABP4* gene SNP (major allele frequency) in TAW[1], Poll Dorset (+ control) and Rambouillet (- control) lambs[#]

Lamb breed, generation, type of control and genotypes (major allele frequencies in brackets) Parental composites 1st (F_1) and 2nd (F_2) composites Positive (+) and negative (-) controls						
SNP locus	TAW Parents (n=147)	TAW F_1 (n=75)	TAW F_2 (n=75)	Poll Dorset (+ n=2)	Texel (+ n=2)	Rambouillet (- n=2)
g.62826961T>C	CT (0.61)	TT (0.64)	CT (0.60)	TT	TT	TT
g.62826965C>G	GC (0.61)	GC (0.57)	GC (0.60)	GG	GG	CC
g.62829478A>T	AT (0.55)	AT (0.61)	AT (0.53)	AA	AA	AA

[#]TAW, Tattykeel Australian White

6.3.2. Correlations between SCD, FASN and FABP4 gene SNP, FMP, IMF and fatty acids

Figure 6.7 shows significant correlations between detected *SCD* SNP loci, several fatty acids and other meat-eating quality traits. Among *SCD* SNP loci, the highest correlations of 0.98 were observed between g.23888763C>T and g.23881050T>C; g.23889346T>G and g.23887165A>G. Moderate correlations between health promoting n-3 LC-PUFA (EPA, DHA and DPA), and g.23888763C>T and g.23881050T>C loci ranging from 0.37 to 0.47 were observed. IMF was moderately to highly correlated with n-3 LC-PUFA (0.38-0.66), while FMP was negatively correlated with IMF (-0.66) and DHA (-0.42). Among the different fatty acids and their summations, very high correlations of up to 0.99 were evident (Figure 6.7).

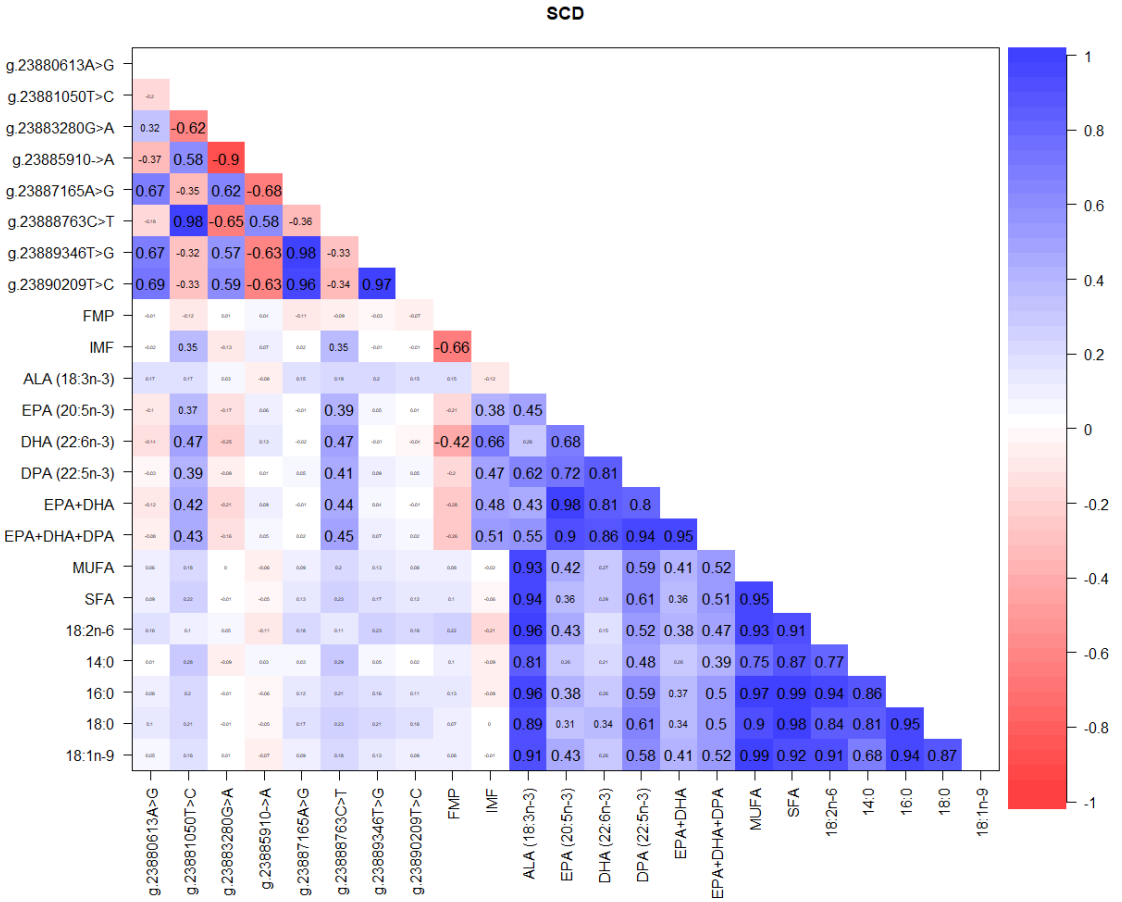

Figure 6.7. Correlations between *SCD* gene SNP loci, IMF, FMP and fatty acids in TAW lambs.

Figure 6.8 shows that among *FASN* gene SNP, there were highly significant correlations between the loci, while correlations between the g.12323864A>G locus and most fatty acids were negative ranging from -0.3 to -0.34. Negative correlations between IMF and FMP (-0.66) and DHA (-0.42) were also observed, while the highest positive correlations were between the various fatty acids (Figure 6.8).

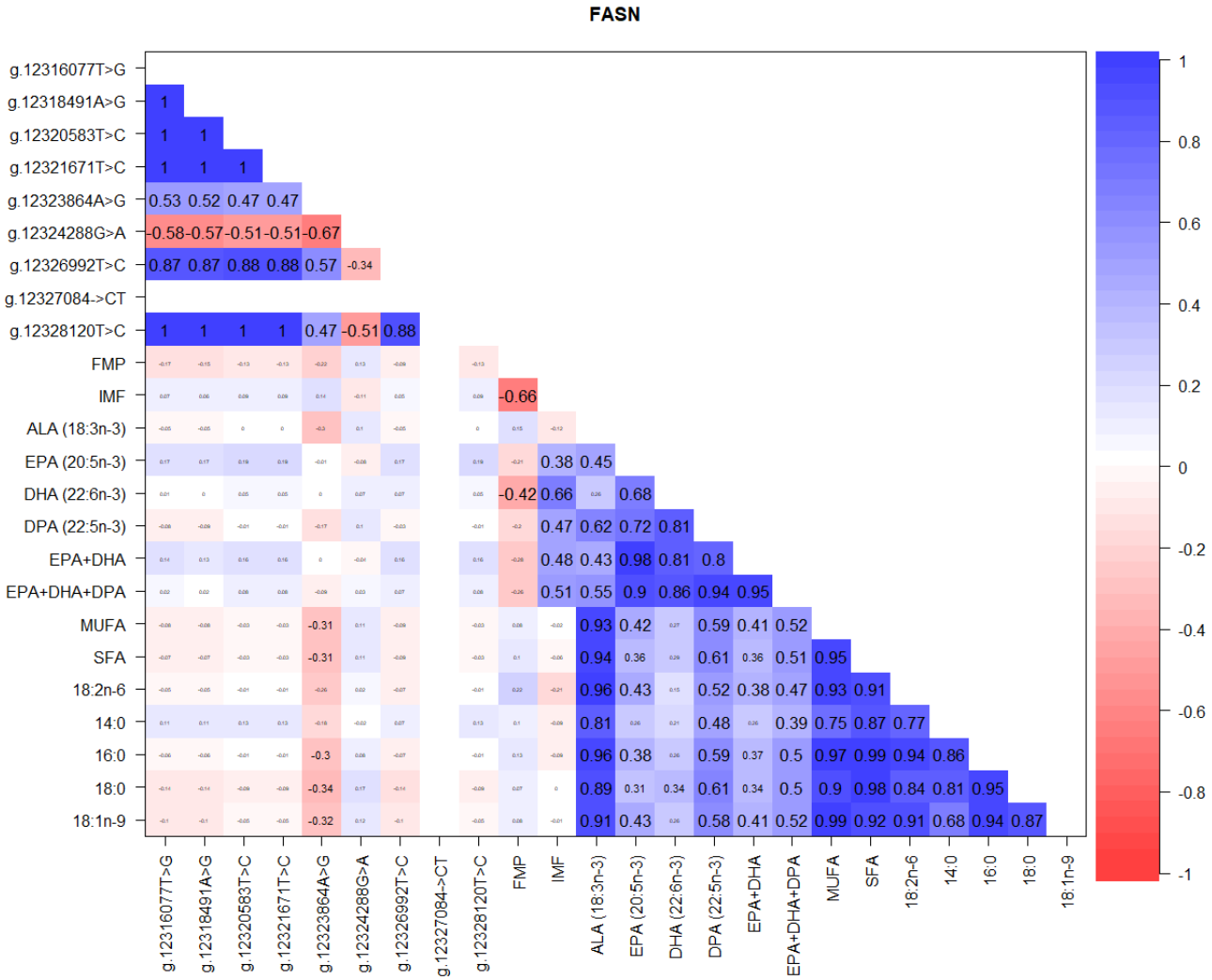

Figure 6.8. Correlations between *FASN* gene SNP loci, IMF, FMP and fatty acids in TAW lambs.

Figure 6.9 shows that among *FABP4* gene SNP, the highest correlation of 0.53 was between the loci g.62826965C>G and g.62826961T>C, while a negative correlation of -0.42 was observed between g.62826965C>G and g.62829478A>T. Consistently positive correlations between IMF and n-3 LC-PUFA of up to 0.66 with DHA, 0.47 with DPA and 0.38 with EPA were also observed, while the highest positive correlations were among the various fatty acids and their summations (Figure 6.9).

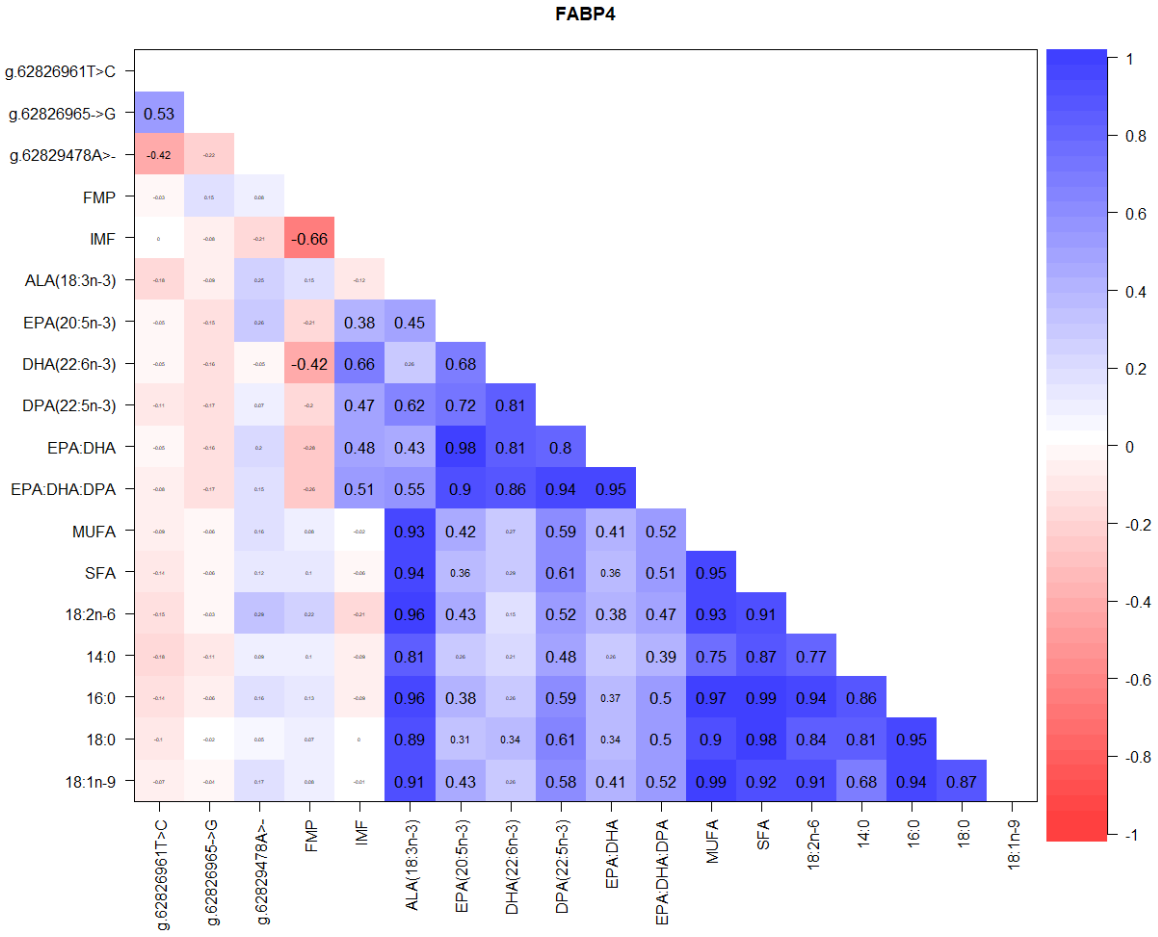

Figure 6.9. Correlations between *FABP4* gene SNP loci, IMF, FMP and fatty acids in TAW lambs.

6.3.3. Associations between SCD, FASN and FABP4 SNP, FMP, IMF and fatty acids

Descriptive statistics of mean, standard deviation, and coefficient of variation of the meat quality traits and full suite of fatty acids breakdown are presented in Table 6.5. FMP had a mean of 33.65 °C with a standard deviation of 2.74 and coefficient of variation of 8.14%, while IMF averaged 4.43% with a standard deviation of 1.31 and coefficient of variation of 29.58%.

Table 6.5 also shows that the *SCD* g.23881050T>C SNP was significantly associated with IMF ($p<0.0089$) and DHA ($p<0.0111$), while *FABP4* g.62829478A>G SNP was associated with only IMF ($p<0.0539$). The *FASN* g.12323864A>G SNP was associated with FMP ($p<0.0544$), ALA ($p<0.0033$), MUFA ($p<0.0025$), SFA ($p<0.0025$), C18:2n-6 ($p<0.0138$), C16:0 ($p<0.0039$), C18:0 ($p<0.0012$) and C18:1n-9 ($p<0.0023$) fatty acids (Table 6.5).

Table 6.5. Associations between SNP mutations and FMP, IMF and fatty acids in TAW lambs[#]

				SNP effect (p-values)		
				SCD	*FABP4*	*FASN*
Variable	**Mean**	**SD**	**CV (%)**	**g.23881050T>C**	**g.62829478A>T**	**g.12323864A>G**
FMP (°C)	33.65	2.74	8.14	0.2700	0.6115	0.0544*
IMF (%)	4.43	1.31	29.58	0.0089**	0.0539*	0.1915
Fatty acids (mg/100g)						
ALA (C18:3n-3)	163.03	192.27	117.94	0.7755	0.1419	0.0033**
EPA (C20:5n-3)	25.20	11.62	46.10	0.7683	0.1023	0.9810
DHA (C22:6n-3)	8.43	4.16	49.27	0.0111*	0.2145	0.9480
DPA (C22:5n-3)	23.85	13.70	57.44	0.0532*	0.3894	0.0927
EPA+DHA	33.64	14.75	43.84	0.2036	0.4794	0.9915
EPA+DHA+DPA	57.49	26.97	46.92	0.0728	0.8958	0.2004
MUFA	3694.70	4099.08	110.94	0.6824	0.3949	0.0025**
SFA	4392.18	5238.81	119.28	0.4000	0.5472	0.0029**
C18:2n-6	253.68	247.70	97.64	0.6781	0.0647	0.0138*
C14:0	287.92	437.58	151.98	0.0632	0.7354	0.1190
C16:0	2076.17	2419.46	116.53	0.5414	0.3751	0.0039**
C18:0	1683.83	2065.71	122.68	0.3891	0.9125	0.0012**
C18:1n-9	2901.10	3212.65	110.74	0.8555	0.3696	0.0023**

[#]*p<0.05, **p<0.01; ***p<0.001; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; SD, Standard Deviation; CV, Coefficient of variation.

6.3.4. Tukey-adjusted multiple comparison tests for significant SNP, FMP, IMF and fatty acids

As depicted in Table 6.6, Tukey-adjusted multiple genotype comparison tests at the *SCD* g.23881050T>C SNP locus confirmed significant differences where the homozygous TT genotype had the highest DHA (11.00 ± 2.34 mg/100g), IMF (5.43 ± 0.516%) and DPA (27.1 ± 3 .26 mg/100g) compared to CC genotype with the lowest DHA (7.00 ± 2.11 mg/100g), IMF (3.98 ± 0.312%) and DPA (17.9 ± 6.81mg/100g). The heterozygous genotype CT had intermediate DPA (7.64 ± 2.09 mg/100g), IMF (4.39 ± 0.287%) and DPA (19.4 ± 6.74 mg/100g) that were in-between the highest and lowest values (Table 6.6).

There were many more significant genotype variations at the *FASN* g.12323864A>G SNP mutation that were associated with FMP, ALA, MUFA, SFA, C18:2n-6, C18:1n-9, C18:0, and C16:0, in which the homozygous genotype GG had the highest values compared to the lowest values in AA genotype for all variables, with the exception of C18:2n-6 that was lowest in the heterozygous GA genotype (Table 6.6). In contrast, at the *FABP4* g.62829478A>G SNP locus, only IMF variation tended towards significance between the genotypes (p<0.06).

Table 6.6. Tukey-adjusted multiple comparisons between SNP mutations and FMP, IMF and fatty acids in TAW lambs[#]

		Multiple genotype comparisons			
SNP locus	**Variable**	**Mean ± SE**	**Genotype**	**Difference ± SE**	**p-value**
***SCD* g.23881050T>C**	*DHA (C22:6n-3) (mg/100g)*				
	CC	7.00 ± 2.11	CC vs CT	-0.639 ± 0.834	0.7247
	CT	7.64 ± 2.09	CC vs TT	-3.998 ± 1.334	0.0105*
	TT	11.00 ± 2.34	CT vs TT	-3.359 ± 1.235	0.0223*
	IMF (%)				
	CC	3.98 ± 0.312	CC vs CT	-0.407 ± 0.323	0.4224
	CT	4.39 ± 0.287	CC vs TT	-1.446 ± 0.532	0.0222*
	TT	5.43 ± 0.516	CT vs TT	-1.038 ± 0.502	0.1041
	DPA (C22:5n-3) (mg/100g)				
	CC	17.9 ± 6.81	CC vs CT	-1.56 ± 2.65	0.8270
	CT	19.4 ± 6.74	CC vs TT	-9.19 ± 4.25	0.0850
	TT	27.1 ± 3 .26	CT vs TT	-7.63 ± 3.93	0.0356*
***FASN* g.12323864A>G**	*FMP (°C)*				
	GG	34.2 ± 0.4	GG vs GA	0.81 ± 0.64	0.4201
	GA	33.4 ± 0.3	GG vs AA	2.98 ± 1.61	0.0536*
	AA	31.5 ± 1.5	GA vs AA	2.16 ± 1.60	0.3685
	ALA (C18:3n-3) (mg/100g)				
	GG	188.7 ± 67.6	GG vs GA	114.7 ± 39.9	0.0149*
	GA	74.0 ± 66.7	GG vs AA	147.2 ± 100.1	0.3115
	AA	41.5 ± 113.7	GA vs AA	32.6 ± 99.8	0.9430
	MUFA (mg/100g)				
	GG	4524 ±1384	GG vs GA	2617 ± 867	0.0099**
	GA	1907 ±1361	GG vs AA	3089 ± 2175	0.3363
	AA	1436 ±2415	GA vs AA	472 ± 2168	0.9742
	SFA (mg/100g)				
	GG	5479 ± 1715	GG vs GA	3270 ± 1121	0.0132*

	GA	2208 ±1684	GG vs	AA	4162 ± 2812	0.3068
	AA	1317 ± 3086	GA vs	AA	892 ± 2803	0.9458
	C18:2n-6 (mg/100g)					
	GG	281 ± 84.8	GG vs	GA	142.5 ± 52.2	0.0216*
	GA	139 ± 83.4	GG vs	AA	105.8 ± 130.8	0.6988
	AA	175 ± 146.4	GA vs	AA	-36.7 ± 130.4	0.9573
	C16:0 (mg/100g)					
	GG	2539 ± 800	GG vs	GA	1475 ± 518	0.0158*
	GA	1063 ± 786	GG vs	AA	1826 ± 1298	0.3433
	AA	713 ± 1429	GA vs	AA	350 ± 1294	0.9604
	C18:0 (mg/100g)					
	GG	2227 ± 658	GG vs	GA	1419 ± 441	0.0056**
	GA	809 ± 646	GG vs	AA	1711 ± 1106	0.2756
	AA	516 ± 1205	GA vs	AA	292 ± 1102	0.9620
	C18:1n-9 (mg/100g)					
	GG	3589 ± 1078	GG vs	GA	2103 ± 679	0.0080**
	GA	1486 ± 1060	GG vs	AA	2353 ± 1704	0.3566
	AA	1236 ± 1892	GA vs	AA	250 ± 1698	0.9882
***FABP4* g.62829478A>T**	*IMF (%)*					
	A	4.57 ± 0.39	A vs	AA	0.07 ± 0.344	0.0556
	AA	3.92 ± 0.39				

[#]*p<0.05, **p<0.01; ***p<0.001; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; SD, Standard Deviation; CV, Coefficient of variation.

6.4. Discussion

It is well-established that DNA-based inheritance enables the transmission of selected phenotypes across generations either without changes in the DNA sequence through epigenetic inheritance (Khatib, 2021) or through functional mutations involving changes in only one base pair (single nucleotide polymorphisms - SNP). Through next-generation sequencing, SNP are valuable for detecting genetic variability and genomic prediction in sheep breeding

programmes (Sharifi et al., 2021), developing breed-specific DNA markers for breed identification (Deus et al., 2021; Xu et al., 2021), animal productivity (Krivoruchko et al., 2021), parentage assignment (Kumar et al., 2021; Long, 2021), forensics (Tao et al., 2021), and prediction of meat quality traits (Grochowska et al., 2021; Lopes et al., 2021; Marín-Garzón et al., 2021).

The prediction of meat-eating quality traits is highly challenging due to the hurdles associated with the low accuracy of estimated breeding values, inconsistency in technical ease of measurement in live animals, non-repeatable reproducibility of carcass data, and high costs of rapid generation of data from large scale consumer sensory panels (Holman and Hopkins, 2021). While the n-3 LC-PUFA profile of lamb and beef can be nutritionally enhanced using rumen-protected dietary supplements and pasture-based feeding (Maggiolino et al., 2021; Moloney et al., 2021; Perkins et al., 2021), already presented findings in Chapters 3 and 5 emphasised the need for the more permanent and cumulative genetic selection route for meat sheep producers to guarantee the consistency of their lamb products in order to meet consumer preferences and adapt to the dynamics of purchasing decisions based on meat eating quality. Consumers prefer meat with low FMP, moderate IMF and fatty acid composition with proportionately more of the health-promoting n-3 LC-PUFA (Realini et al., 2021; Stampa et al., 2020). Since humans and other vertebrates lack Δ15 desaturase enzyme to synthezise n-3 LC-PUFA, they must obtain these from dietary intake sources in order to meet their daily requirement of 500 mg of n-3 LC-PUFA (presented in Chapter 2). Therefore, lamb producers can tap into the omega-3 functional meat market niche through novel strategies for developing healthy meat products and reducing saturated fats (López-Pedrouso et al., 2021) by matching their sheep breeding and production system to meet this health-conscious consumer preference (presented in Chapters 2 and 3).

6.4.1 SCD gene polymorphism

The *SCD* gene increases the desaturation of stearic acid to oleic acid, and a functional variant in the *SCD* gene promoter affects fattening performance, carcass traits, meat quality, blood metabolites and gene expression in ovine muscle (Liu et al., 2020; Calvo et al., 2019). Our results herein showing that TAW lambs were all heterozygous at three *SCD* SNP loci g.23881050T>C, g.23883280G>A and g.23885910C>A in the parental, first and second generations (Table 6.2), presents a hereditary pattern and genetic divergence from the homozygous variants seen in the Poll Dorset, Texel and Rambouillet controls that can be used as molecular markers for breed-specific identification. The significant correlations (Figure 6.7) and associations (Tables 6.5 and 6.6) between detected *SCD* SNP loci, several fatty acids and other meat eating quality traits in TAW sheep is in consonance with other studies in Bashby x Argali (Wang et al., 2021), Rasa Aragonesa (Calvo et al., 2019), Iranian fat- and thin-tailed (Aali et al., 2016), Poll Dorset×Border Leicester×Merino (Alvarenga et al., 2016), Spanish, French, Egyptian and Israeli sheep breeds (García-Fernández et al., 2009) and Spanish goats (Avilés et al., 2016). In a comprehensive review of the genetics of n-3 LC-PUFA metabolism and meat eating quality in TAW lambs (presented in Chapter 3), it was reported that although they are renowned for an outstanding low fat melting point (28–39 °C), high n-3 LC-PUFA EPA+DHA content (33–69 mg/ 100 g), marbling (3.4–8.2 %), tenderness (20.0–38.5 N) and overall consumer liking (7.9–8.5), correlations between n-3 LC-PUFA profile, *SCD*, *FABP4*, *FASN* and other lipogenic genes and meat quality traits presented major knowledge gaps. Therefore, significant differences and associations were observed at the *SCD* g.23881050T>C SNP locus in the present study where TAW lambs with the TT genotype had the highest DHA, IMF and DPA compared to CC and CT genotypes (Table 6.6), have not only filled these knowledge gaps, but also equip lamb producers at the farmgate level to use this locus as a molecular marker for selection and breeding targeted at improving marbling and health-

beneficial n-3 LC-PUFA. Since IMF in lamb has a moderately high heritability of 0.32-0.48 (Mortimer et al., 2014), it has a direct relationship with tenderness, juiciness and flavour (Liu et al., 2020) and surpasses the minimum acceptable consumer satisfaction threshold of 4 % (Pannier et al., 2014), the TAW lamb is well positioned for a rapid genetic improvement for these meat-eating quality traits using the *SCD* gene g.23881050T>C SNP locus for identifying lambs at an early age.

6.4.2 FASN gene polymorphism

FASN catalyses the synthesis of fatty acids such as palmitate from acetyl-CoA and malonyl-CoA, in the presence of NADPH, into long-chain saturated fatty acids, hence its involvement with fat deposition and fatty acid synthesis (Raza et al., 2018). While novel genetic polymorphisms and gene expressions associated with carcass traits in Texel (Armstrong et al., 2018) and Rasa Aragonesa (Dervishi et al., 2011) sheep have been published, Sanz et al. (2015) reported that only a few studies have focused on genetic variation in 5′regulatory regions of genes involved in fat synthesis and metabolism pathways that could be good candidate genes. They identified *FASN* gene polymorphisms and the potential use of these variants as markers associated with fat-related traits in Assaf, Roja Mallorquina and Rasa Aragonesa sheep breeds (Sanz et al., 2015). In TAW sheep, our current study is the first to report significant genotype variations at the *FASN* g.12323864A>G SNP locus associated with FMP, ALA, MUFA, SFA, C18:2n-6, C18:1n-9, C18:0 and C16:0, in which the homozygous genotype GG had the highest values compared to the lowest values in AA genotype, with the exception of C18:2n-6 that was lowest in the heterozygous GA genotype (Figure 6.8, Tables 6.5 and 6.6). This finding fills in a significant knowledge gap in sheep where very little has been reported on *FASN* gene, in stark contrast to many publications on cattle (Fang et al., 2017; Bartoň et al., 2016; Papaleo-Mazzucco et al., 2016; Yeon et al., 2013) and pigs (Zappaterra et al., Zhang et al., 2019; Renaville et al., 2018; Zappaterra et al., 2016). Since fatty acid compositions determine the

melting point and quality of fat and are closely related to the nutrition and meat-eating quality of lambs (Ates et al., 2020), our findings will assist TAW lamb producers in selecting the *FASN* genotypes best suited to their environments, market specifications and processing needs in achieving efficiency in their management operations aimed at meeting consumer demand for healthy and nutritious lamb eating quality.

6.4.3 FABP4 gene polymorphism

The proteins of the *FABP4* family are small molecular-weight proteins that have a high binding affinity for long-chain fatty acids, participate in fatty-acid transportation from the plasma membrane to the sites of β-oxidation, triacylglycerol and phospholipid synthesis and variation in *FABP4* gene has been reported to affect fat deposition, growth and meat production in sheep (Bai et al., 2013; Yan et al., 2018). Several other research findings in sheep have demonstrated that dietary manipulation of omega-3 fatty acids can influence intramuscular fat deposition, growth, milk, wool and meat quality (Ponnampalam et al., 2001; 2002; 2014a; 2014b; Diaz et al., 2017; Flakemore et al., 2017; Nguyen et al., 2017a; 2017b; Álvarez-Rodríguez et al., 2018; Nguyen et al., 2018a; 2018b; 2018c; Fowler et al., 2019; Le et al., 2019; Malau-Aduli et al., 2019; Nguyen et al., 2019a; 2019b; Cardoso et al., 2021; Perkins et al., 2021). In comparison, only a handful of studies (Knight et al., 2014; Malau-Aduli et al., 2015; Alvarenga et al., 2016; Kashani et al., 2017) have validated independent associations of carcass quality, shear force, intramuscular fat percentage and omega-3 polyunsaturated fatty acid content with gene markers or the expression of genes encoding enzymes regulating fat metabolism in Australian lamb. Therefore, our current findings at the *FABP4* g.62829478A>- SNP locus show consistently positive correlations between IMF and n-3 LC-PUFA of up to 0.66 with DHA, 0.47 with DPA and 0.38 with EPA (Figure 6.9 and Tables 6.5 and 6.6) provides a novel molecular marker for TAW sheep producers to select and breed lambs that are not only of high meat-eating quality, but also provide a healthy product for brain growth and development. This stems from the fact

that IMF provides the needed marbling for taste, juiciness and tenderness, while DHA is the major prevalent fatty acid in the brain membrane and is vital for maintaining of healthy and functional brain development in infants and adults (Mallick et al., 2019). In pigs, Shang et al. (2019) identified 3 FABP gene SNP and demonstrated that the genotype C-1375G was associated with fat deposition, while Gao et al. (2011) reported that an association analysis of FABP SNP indicated that the polymorphism had a significant effect on marbling, in which pigs with the DD genotype had higher marbling than CD and CC genotypes, but the difference between CD and CC genotypes was not significant. They also reported that this FABP SNP had a highly significant effect on intramuscular fat content ($P < 0.01$). Our current study being the first report in TAW, provides foundational data for the selection and breeding of lambs for marbling and healthy n-3 LC-PUFA using the identified SNP herein.

6.5. Conclusions

This study has provided novel insights into the shared genetic control of fat melting point, intramuscular fat content and health-beneficial omega-3 long-chain fatty acid composition traits that are helpful in designing breeding strategies to genetically improve meat eating quality traits in TAW lambs while they are still alive. This innovative and minimally invasive *longissimus dorsi thoracis et lumborum* muscle biopsy sampling technique allows for early decision-making and quantifies the genetic worth of live lambs. This overcomes the problem of waiting to collect meat quality data after slaughter when selection decisions about the live animal are already too late. As the present data are laboratory-tested, personalised and customised to actual individual lamb performance and not based on estimated breeding values, precision problems due to low accuracy are minimised. The identified SNP of these lipid metabolism genes can also be used for breed-specific identification and marker-assisted selection of Tattykeel Australian White (TAW) sheep exclusive to MARGRA lamb brand for high-end meat-eating quality. Next-generation sequencing of the *FABP4*, *FASN* and *SCD* genes

also provides foundational data underpinning their roles in fatty acid metabolism unique to the TAW breed.

6.6. Summary

Meat quality data can only be obtained after slaughter when selection decisions about the live animal are already too late. Carcass estimated breeding values present major precision problems due to low accuracy and by the time an informed decision on the genetic merit for meat quality is made, the animal is already dead. We report for the first time, a targeted next generation sequencing (NGS) of single nucleotide polymorphisms (SNP) of lipid metabolism genes in TAW sheep of the MARGRA lamb brand, utilizing an innovative and minimally invasive muscle biopsy sampling technique for directly quantifying the genetic worth of live lambs for health-beneficial omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), intramuscular fat (IMF) and fat melting point (FMP). NGS of stearoyl-CoA desaturase (*SCD*), fatty acid binding protein-4 (*FABP4*) and fatty acid synthase (*FASN*) genes identified functional SNP with unique DNA marker signatures for TAW genetics. The *SCD g.23881050T>C* locus was significantly associated with IMF, C22:6n-3 and C22:5n-3; *FASN g.12323864A>G* locus with FMP, C18:3n-3, C18:1n-9, C18:0, C16:0, MUFA, and *FABP4 g.62829478A>T* locus with IMF. These add new knowledge, precision and reliability in directly making early and informed decisions on live sheep selection and breeding for health-beneficial n-3 LC-PUFA, FMP, IMF and superior meat-eating quality at the farm gate level. The findings prove that significant associations exist between SNP of lipid metabolism genes and n-3 LC-PUFA, IMF and FMP, thus underpinning potential marker-assisted selection for meat eating quality traits in TAW lambs.

Chapter 7: Differential expressions of *FASN*, *SCD*, and *FABP4* genes in the *Longissimus thoracis et lumborum* muscle of Tattykeel Australia White lambs supplemented with omega-3 oil

7.1 Introduction

The concept of a healthy and nutrient-dense diet is increasingly becoming a global and topical discourse amongst meat consumers. Improvements in medicine, science and technology have changed the lifestyles of the populace (Yeung et al., 2021). The fortification of livestock diets to increase the level of health-beneficial omega-3 fatty acids remains a viable strategy for improving meat quality and nutrient composition (Nudda et al., 2022). Fatty acids are the essential building molecules for cellular structures, tissues, and organs. They are also an integral part of synthesising essential biologically active elements, as well as coordinating the appropriate roles of metabolic processes (Sokoła-Wysoczańska et al., 2018). Omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) are also known as essential fatty acids required by mammals for various biological and physiological processes because mammals cannot synthesise them (Choudhary & Mishra, 2021; Patel et al., 2021; Wu et al., 2021). This is because they lack the required Δ12 (FADS12) and Δ15 (FADS15) fatty acid desaturase enzymes for their biosynthesis, thus necessitating that they must be obtained exclusively from the diet or nutritional supplements (Wu et al., 2021). The fatty acids alpha-linolenic (ALA) and linoleic (LA) acids are precursors of n-3 and n-6 LC-PUFA), respectively. ALA is converted to the more potent n-3 LC-PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) through the *de novo* synthesis metabolic pathway (Nigam et al., 2018). These omega-3 fatty acids are vital for memory improvement, elevation of visual acuity, depression of high blood pressure and prevention of heart disease (Pratiwy & Pratiwi, 2020). ALA is an essential n-3 fatty acid in the human diet. It plays diverse roles in reducing the danger of inflammatory and degenerative diseases such as cardiovascular diseases, cancer, skin conditions, metabolic syndrome, diabetic neuropathy, allergies, asthma, arthritis and immune

function (Silva et al., 2014; Silva et al., 2014; Sala-Vila et al., 2022). Similarly, LA is a precursor for synthesising the n-6 long-chain fatty acid known as arachidonic acid (ARA). ARA is converted to prostaglandins, leukotrienes and other associated compounds. Diets rich in omega-6 PUFA are linked with inflammation, blood vessel constriction, and platelet aggregation (Rogero & Calder, 2018; Corino et al., 2022). A high intake of LA relative to ALA has been reported to interfere with the desaturation and elongation pathways of ALA (Simopoulos, 2016). This is because LA and ALA utilise the same metabolic pathway for synthesising n-3 LC-PUFA (DHA and EPA) (Marangoni et al., 2020).

An animal's diet influences its meat fatty acid composition, nutritional quality, and gene expression patterns (Santos-Silva et al., 2022). Gene expression analysis can shed some light on the transcriptional pathway in the synthesis of functional gene products. Identifying the framework of gene expression is vital to unravelling the molecular mechanisms controlling complex traits (Lee, 2018). Only a few studies have evaluated dietary regulation of lipogenic gene expression in the ovine muscles (Dervishi et al., 2011; González-Calvo et al., 2017; Calvo et al., 2019). Hence, to better comprehend the genetic regulation of FA deposition in TAW lambs, *Longissimus thoracis et lumborum* muscles were utilised to provide a more detailed lipogenic gene expression pattern. To our current knowledge, there is dearth of available information on the influence of diet on the transcriptional expression and comparing mRNA expression of lipogenic enzymes and none in TAW lambs. Therefore, this study aimed to compare the lipogenic gene expression differences in *Longissimus thoracis et lumborum* muscles of Tattykeel Australian lambs. We hypothesised that dietary fortification with omega-3 oils influences the transcriptional expression of lipogenic genes in the *Longissimus thoracis et lumborum* muscle in TAW lambs.

7.2. Materials and Methods

7.2.1. Animals, housing, and feeding

The experimental animals, study design, and location were described previously in Chapter 5. In summary, the lamb finishing feeding trial was accomplished from April to June 2019 at Crown Agriculture's commercial feedlot complex at Borenore, New South Wales, Australia. The feedlot complex was well-ventilated, equipped with automated feeding and watering systems and had a concrete floor spacing of 5 m^2 per head. The feeding troughs were equipped with sensors capable of immediate data capture of every individual lamb's electronic ear tag number, body weight, rumination time, feed intake and other relevant parameters which were automatically recorded, cloud-stored and downloadable when required. The experimental animals comprised seventy-five six months old TAW lambs with a mean liveweight of 30 ± 1.2 kg randomly allocated into the following three dietary treatments of twenty-five animals per group: (a) Control grain pellets without omega-3 oil plus hay; (b) Commercial MSM whole grain pellets plus hay; and (c) Omega-3 oil supplemented grain pellets plus hay. Details of the nutrient compositions of these experimental diets have been presented in Chapter 5 and published[35]. Furthermore, all lambs had *ad libitum* access to basal hay diet and water. The study lasted 47 days including an initial 14-day adaptation period in a completely randomised design. At the expiration of the feeding trial, the lambs were transported in the cool hours of the evening to the Gundagai Meat Processing Plant, lairage-held until the following day, where they were sacrificed humanely as a single mob according to Meat Standards Australia regulations.

Samples of the *Longissimus thoracis et lumborum* muscles of all carcasses were taken between the 12^{th} and 13^{th} ribs 24 hours post-mortem, frozen in dry ice, transported to the laboratory and stored at -80 °C pending RNA extraction.

7.2.2. RNA extraction, cDNA synthesis, and Quantitative PCR

Total RNA was extracted from frozen *Longissimus thoracis et lumborum* muscle samples utilising TRIzol™ Plus RNA Purification Kit (Invitrogen, Thermo Fisher Scientific, Victoria, Australia), and subsequently purified and DNase treated with ezDNase™ Enzyme (Thermo Fisher Scientific, Victoria, Australia). Total RNA yield and quality were quantified with a NanoDrop ND-1000 spectrophotometer (NanoDrop, Analytical Technologies, Biolab) and QuantiFluor® dsDNA System (Promega, WI, USA). First-strand cDNA was synthesized from 100 ng using SuperScript™ IV VILO™ Master Mix Reverse Transcription Kit (random hexamers; Thermo Fisher Scientific, Victoria, Australia). Quantitative PCR (qPCR) reactions (20 µL) were run in duplicates utilising Fast SYBR Green Chemistry (Thermo Fisher Scientific, Victoria, Australia), 250 nM primer, and 10 µL template on a QuantStudio-3 Real-Time qPCR detection system (Applied Biosystem Inc.). This was carried out under fast-cycling settings (50°C for 2 min, 95°C for 2 min, and then 50 cycles at 95 °C for 15s and 65°C for 1min).

7.2.3. Primer design and housekeeping gene selection

All target and housekeeping gene primers (Table 2) were designed using the Geneious Prime Software Program 2020 v.2.2 (http://www.geneious.com). The suitability of all primers was ascertained by employing a serial dilution of pooled cDNA to generate a standard curve. The mRNA abundance was established utilising highly stable reference genes. All primer pairs established acceptable efficiency (90-110%) and R-value (99%). The expression of the unaffected technical reference gene EF-1α was used to normalize the expression data for *FASN*, *FABP4,* and *SCD*. Data normalisation for the target *FASN*, *FABP4* and *SCD* genes utilised two reference genes; the elongation factor 1A (EF1A, formerly termed *EF1α*) and Peptidyl-prolyl cis-trans isomerase A (*PPIA*) using an expression ratio that was constant amongst all samples as the key selection criterion.

Table 7.1: Primer sequences for *FABP4*, *FASN*, *SCD*, *EF1A*, and *PPIA* quantitative polymerase chain reaction assays

Gene	Primers		T_a	Amplicon (bp)
FABP4	Forward	Reverse		
	ATGAAAGAAGTGGGTGTGGGCTTT	TCCTGGCCCAATTTGAAGGACATC	65	149
FASN	CCACTTCCCACTGGAACAAGACAA	GGAGGCGTAATAGATGGTGCAGAG	65	166
SCD	AACACCCAGCTGTCAGAGAAAAGG	AACAGCAGGACACCAGGTTTGTAG	65	110
Reference				
EF1A	CGTGAAAACCACCGTTAAACCTAA	TCGTGGTAGACTTCCCTGAATCTA	65	100
PPIA	TCACACGCCATAATGGTACTGGTG	TGGCAGTGCAAATGAAAAACTGGG	65	153

FABP4: Fatty Acid Binding Protein-4, *FASN*: Fatty Acid Synthase, *SCD*: Stearoyl-CoA Desaturase, *EF1A*: Elongation Factor 1-Alpha, *PPIA*: Peptidyl-Prolyl Cis-Trans Isomerase A, T**a**: Annealing Temperature.

Fatty Acid Analysis

Details of the fatty acids analysis methodology will not be repeated herein because these had already been fully described in Chapter 5.

7.2.4. Statistical analyses

Data on gene expression, FMP, IMF, and FA profiles of TAW MARGRA lamb were analysed using nonparametric statistics in R version 4.0.1. Kruskal-Wallis tests with Bonferroni's adjusted p-values were used to test for differences in fold changes among dietary treatments. Relationships between variables were explored using Spearman correlation analysis. The effect of gene expression (fold change) on FMP, IMF, and fatty acids was investigated using a quantile (median) regression model. Median regression that is an extension of linear is often preferred to linear regression because it is "robust to outliers" (Bianchi & Salvita, 2015) and thus superior when linear regression is not met. The comparison between Quantile Regression and Linear regression models are depicted in Table 7.1 and Supplementary Figures S1-S3. Alpha was set to 0.05 for all statistical comparisons.

7.3. Results

The three lipogenic genes showed marked variation in expression levels in the *Longissimus thoracis et lumborum* muscle of lambs in all the three dietary treatment groups (Figure 7.1). The Kruskal-Wallis test showed that the *SCD* gene tended to be up-regulated ($p = 0.06$) in the omega-3 fortified diet compared to MSM whole grain and the control diets (Figure 7.1A). However, in Figure 7.1B, the expression of *FABP4* gene was significantly up-regulated 3-folds in the muscle of lambs fed MSM whole grain diet, while those on the omega-3 fortified diet had a significant down-regulation ($p< 0.018$). Irrespective of a down-regulation trend in Figure 7.1C, the differences in FASN gene expression between the control, MSM whole grain and omega-3 diets were insignificant ($p> 0.05$). Spearman correlations between the fold changes in *SCD*, *FABP4* and *FASN* gene expressions and meat quality traits (IMF, FMP and fatty acids) are summarized in Figure 7.2. It shows that for the most part, the relationships between *SCD* gene expression and meat quality traits did not attain statistical significance, but there were highly significant correlations ($p< 0.05$) among the fatty acids and meat quality traits. For *FABP4* gene as depicted in Figure 7.2, positive correlations ($p< 0.05$) were observed between fold changes and DHA, EPA, EPA +DHA, EPA +DHA + DPA and PUFA/ SFA ratio. For the *FASN* gene in Figure 7.2, there were some negative but significant correlations ($p< 0.05$) between IMF, MUFA, PUFA and n-6 PUFA and fold changes in the expression of *FASN*. The effects of PPIA and EFIA-corrected expressions of SCD, FABP4 and FASN genes on meat quality traits in the *Longissimus thoracis et lumborum* muscle of TAW lambs supplemented with omega-3 fortified diets are depicted in Table 7.1. The results clearly indicate that the effect of *SCD* gene expression on all the meat quality traits was negligible, whereas those of *FABP4* and *FASN* genes significantly influenced IMF, LA, ALA, EPA, DHA, DPA, EPA + DHA, n-3 PUFA and n-6 PUFA ($p <0.05$). The influence of fortification of diets with omega-3 on some meat quality traits IMF, FMP and FA in TAW lambs are presented in Figure 7.2.

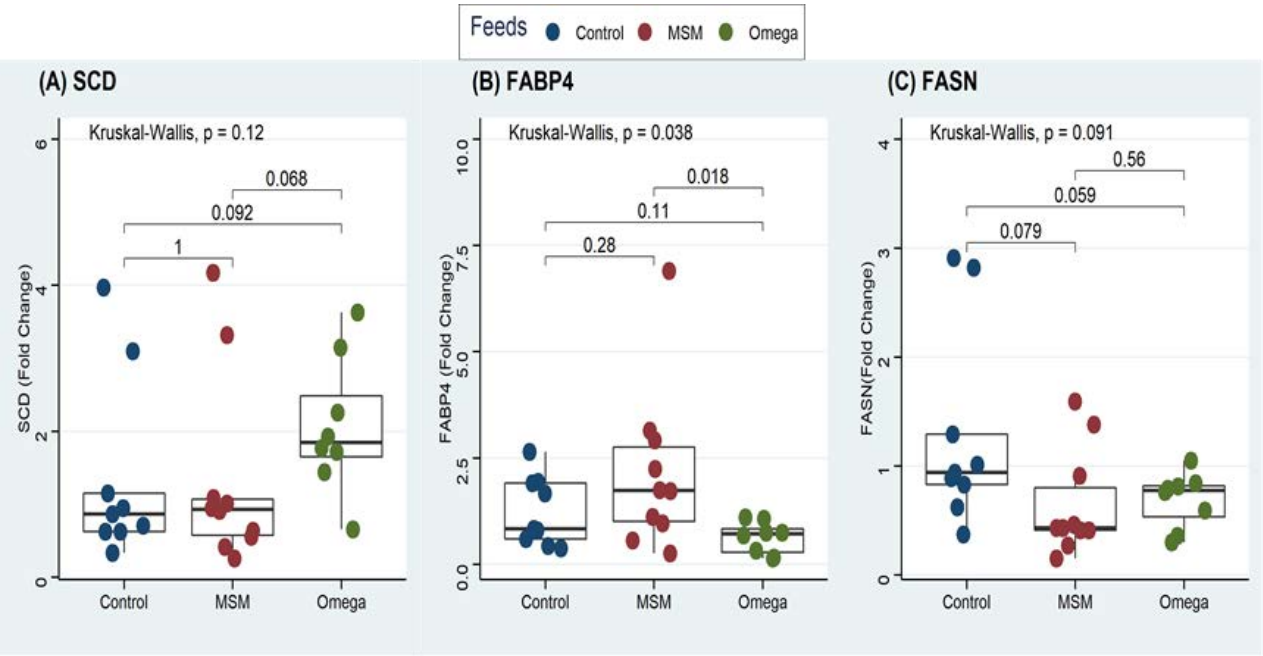

Figure 7.1. Gene expression of *SCD, FASN* and *FABP4* genes in the *Longissimus thoracis et lumborum muscle* of TAW lambs fortified with omega-3 oils. Significant differences ($P < 0.05$).

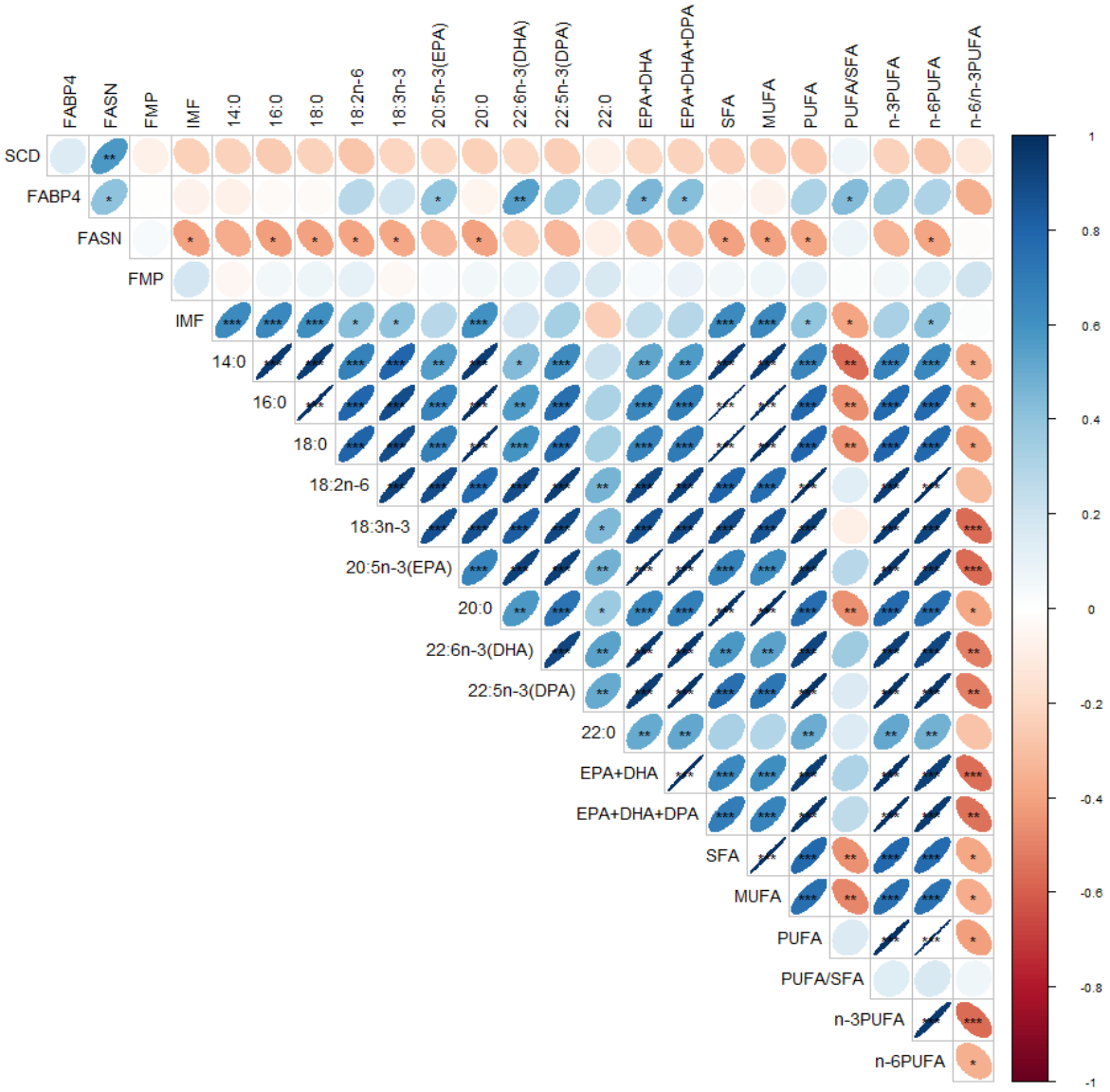

Figure 7.2. Correlations of *SCD, FASN* and *FABP4* expression with meat quality traits in the *Longissimus thoracis et lumborum* muscle of TAW lambs on omega-3 fortified diets (*= 0.05, **= 0.01, ***= 0.001)

Table 7.2. Effect of gene expression (*SCD, FABP4* and *FASN*) on meat quality traits in the *Longissimus thoracis et lumborum* muscle of TAW MARGRA lambs fortified with omega-3 oils.

Variable	*SCD*			*FABP4*			*FASN*		
	Est.	Lower	Upper	Est.	Lower	Upper	Est.	Lower	Upper
FMP	0	-0.473	0.333	0	-0.528	0.686	0.262	-0.728	0.312
IMF	-0.184	-0.473	0.067	0.045	-0.567	0.2	**-0.586**	**-1.443**	**-0.367**
14:00	-0.538	-4.761	0.679	-0.297	-3.261	2.732	-5.184	-16.185	0.065
16:00	6.327	-67.754	12.902	-5.951	-18.975	27.225	-65.862	-157.254	14.556
18:00	3.982	-36.763	12.405	-2.009	-15.966	19.41	**-37.923**	**-104.016**	**-0.077**
18:2n-6	-2.903	-16.197	5.597	**8.657**	**6.214**	**13.435**	**-17.773**	**-50.658**	**-0.104**
18:3n-3	0.13	-2.435	0.282	**1.057**	**0.293**	**2.314**	-2.839	-8.764	0.322
20:5n-3(EPA)	-0.708	-1.757	0.617	**1.263**	**0.116**	**3.24**	**-1.451**	**-8.645**	**-0.659**
20:00	0.009	-0.261	0.114	-0.03	-0.112	0.185	**-0.302**	**-0.896**	**-0.006**
22:6n-3(DHA)	0.181	-1.048	0.593	**0.905**	**0.402**	**1.347**	-0.647	-5.007	0.237
22:5n-3(DPA)	0.164	-1.182	0.555	**0.749**	**0.547**	**1.833**	-1.508	-5.914	0.498
22:00	0.027	-0.081	0.06	**0.053**	**0.026**	**0.144**	-0.016	-0.294	0.048
EPA+DHA	-0.281	-2.687	1.338	**2.006**	**0.564**	**4.621**	**-1.834**	**-13.628**	**-0.136**
EPA+DHA+DPA	0.099	-3.783	1.501	**2.765**	**1.425**	**6.254**	-3.488	-23.758	0.357
SFA	18.009	-111.898	21.973	-6.806	-36.818	48.051	-115.571	-287.41	24.38
MUFA	8.727	-124.707	36.206	-19.197	-64.078	64.313	-137.149	-324.607	33.141
PUFA	-2.593	-32.448	6.184	13.377	-1.081	32.037	**-31.311**	**-118.191**	**-1.849**
PUFA/SFA	0.002	-0.016	0.06	0.048	-0.034	0.081	-0.016	-0.023	0.264
n-3PUFA	0.66	-6.219	2.108	**3.152**	**2.042**	**9.309**	-6.86	-32.513	0.965
n-6PUFA	-3.034	-26.771	5.225	**9.832**	**2.582**	**22.603**	**-22.415**	**-81.32**	**-1.496**
n-6/n-3PUFA	-0.074	-0.161	0.249	**-0.158**	**-0.454**	**-0.045**	0.109	-0.235	0.658

Note: Estimate of effect (Est.), lower 95% Confidence interval (Lower), Upper 95% Confidence interval (Upper), Bold, $p < 0.05$.

7.4. Discussion

To the best of our knowledge, this is the first study describing *SCD, FABP4* and *FASN* lipogenic gene expressions in the *Longissimus thoracis et lumborum* muscles of TAW lambs. The processes of fatty acid metabolism in ruminants are complex. Due to microbial biohydrogenation in the rumen, unsaturated fatty acids (UFA) are converted into saturated fatty acids (SFA) forms (Deng et al., 2018). The increased levels of these SFA increase the risks of atherosclerosis and coronary heart disease in meat consumers (Virtanen, 2018). The modification of fatty acid composition in meat and its products by the inclusion of n-3 LC-PUFA is therefore, an excellent approach to the promotion and improvement of human health (Nong, 2020). This study utilised a lamb finishing feeding trial with omega-3 fortified, conventional grain and control diets to quantify the expression levels of *SCD*, *FABP4* and *FASN* lipogenic genes in the *Longissimus thoracis et lumborum* muscle.

7.4.1. Stearoyl-CoA desaturase *(SCD)* gene

The *SCD* gene enhances meat quality traits by modifying fat deposition and fatty acid composition (Li et al., 2018). It achieves this by catalysing the synthesis of cis-vaccenic acid from the c9,t11 isomer of conjugated linoleic acid (CLA) (Urrutia et al., 2015) and converting SFA to MUFA by inserting a double bond between carbon atoms Δ9 and Δ10 of stearic acid (C18: 0) to generate oleic acid (C18:1 c-9) (Fan et al., 2019; Al-Thuwaini & Al-Shuhiab, 2022). Wang et al. (2009) and Cedernaes et al. (2013) described the expression of the *SCD* gene as an indicator of IMF development, hence, it could be an essential regulator of muscle metabolism as it aids lipid biosynthesis, depresses FA degradation (Iommelli et al., 2021) and low-density lipoprotein cholesterol, thus playing a crucial role in the pathogenesis of atherosclerosis (DuBroff & de Lorgeril, 2021). Dietary fortification with omega-3 oil in this study did not lead to any significant change in the expression of the *SCD* gene in the *Longissimus thoracis et lumborum* muscle in agreement with a previous study that reported no change in *SCD* gene

expression in Italian Simmental and Holstein bulls fed linseed (Corazzin et al., 2013). This was in sharp contrast to other studies that reported significant down-regulation of *SCD* expression when soybean oil was substituted with 2.7 % of linseed oil (Jacobs et al., 2011) in cattle and in lambs fed alfalfa Dervishi et al., 2011). Another study reported that dietary supplementation with oil depressed *SCD* gene expression in cattle muscle tissue (Joseph et al., 2010). These results also align with other researchers who argued that the *SCD* gene stimulates fat deposition in Duolang sheep in probably similar ways as in cattle and pigs (Liu et al., 2022b). Conversely, an up-regulation of the *SCD* gene was reported in Guangling large-tailed sheep fed alfalfa, grass hay, silage, carrots, and mixed concentrates indoors (Liu et al., 2022b). Taken together, dietary fortification with omega-3 in this current study did not influence *SCD* gene expression in muscle tissues. As reported in Chapter five, the diets fed to these lambs had high levels of ALA, hence the reduction in SFA. This agrees with the report of Joseph et al. (2010) who reported the depression of SCD gene expression by PUFA.

7.4.2. Fatty acid-binding protein 4 gene

The *FABP4* gene influences lipid synthesis, feed intake, and growth (Yan et al., 2018) and drives the absorption, transport, lipolysis, lipogenesis, storage of long chain fatty acids and regulation of gene expression (Gan et al., 2015; Pećina & Ivanković, 2021) hence, it is a metabolic indicator of an animal's capacity to store IMF (Jurie et al., 2007). It is also associated with the regulation of lipid metabolic syndrome, insulin resistance, diabetes, and obesity (Poulos te al., 2010; Wei et al., 2013). Transcription factors, including PPARα, -β, -γ, are triggered by fatty acids or other hydrophobic ligands and are responsible for stimulating *FABP4* gene expression, which occurs mainly in the adipocytes. In the current study, the expression of the *FABP4* gene was up-regulated in the *Longissimus thoracis et lumborum* muscle tissue of lambs fed MSM whole grain diet, but down-regulated in lambs fed omega-3 diet in line with an observation with steers fed concentrates and roughage (Kim et al., 2022). Other researchers

reported a significant influence of the *FABP4* genotype in Wagyu (Michal et al., 2006) and Limousin crossbred cattle, which accounted for the FA content of the IMF (Narukami et al., 2011). Another study found that the *FABP4* gene was significantly correlated with marbling and fat depth in cattle (Bartoň et al., 2016) and in regulating the tenderness of meat in ovine species Xu et al., 2011).

In the current study, *FABP4* gene expression in the muscle of lambs fed the MSM whole grain diet was significantly more than for the control and omega-3 fortified diets, indicating that *FABP4* up-regulation is driven by supplementation with MSM whole grains rather than omega-3 oil. Diets regulate the mechanisms governing IMF deposition, and *PPARγ* (peroxisome proliferator-activated receptor γ) influences the expression of genes that encode proteins involved in fat accumulation and differentiation of adipocytes in muscle tissues (Lee et al., 2019). This gene is responsible for the expression of some adipocyte proteins, *FABP4* and *FASN* (Rosa et al., 2014). Studies by Yang et al. (2017) on the influence of diets with varied levels of energy on the efficiency of fat deposition and FA profile of the *Longissimus dorsi* muscle of yak, revealed dense energy diets boosted the deposition and partial FA content of this muscle primarily by the up-regulation of mRNA expression of lipogenic genes including *FABP4.* The results presented in this current study showed that *FABP4* gene expression are associated with higher levels of FA deposition in TAW lambs fed MSM whole grain diets than those lambs fed control and omega-3 fortified diets. The higher expression levels of mRNA due to *FABP4* gene show the higher FA deposition in the *Longissimus thoracis et lumborum* muscle of lambs fed MSM whole grains. Some studies recounted the association between *FABP4* gene expression or protein activity and intramuscular fat content in ruminants (Fernyhough et al., 2005; Jurie et al., 2007), and backfat depth (Michal et al., 2007).

7.4.3. Fatty acid synthase gene

The *FASN* gene encodes the fatty acid synthase enzyme, a rate-limiting enzyme in *de novo* long chain fatty acid synthesis from acetyl-CoA and malonyl-CoA precursors (Smith et al., 2003; Berndt et al., 2007). Hence, the degree of *FASN* expression plays a key role in fat deposition (Liu et al., 2020). Previous reports of down-regulation of the *FASN* gene expression in the *Longissimus dorsi* muscle of Italian Large White and Duroc pigs (Braglia et al., 2014) and an up-regulation in cattle fed corn compared to those on a corn oil treatment diet had been reported (Cedernaes et al., 2013). These reports agree with the findings of the current study where the *FASN* gene was suppressed in the *Longissimus thoracis et lumborum* muscle of lambs fed MSM whole grain and omega-3 diets, but up-regulated in the control diet. This outcome implies that fortifying the diet with omega-3 oils decreases the expression levels of the *FASN* gene in the *Longissimus thoracis et lumborum* muscle. This possibly reduced the n-6 and n-9 LC PUFA by using palmitate, the first fatty acid produced during fatty acid synthesis (Malau-Aduli & Kashani, 2015). Studies have shown the inhibitory influence of ALA and possibly products of biohydrogenation due to *de novo* FA synthesis that resulted to the reduction of SFA in intramuscular tissue of goats fed flaxseed oil (Ebrahimi et al., 2014). The down-regulation of the FASN gene could depress the levels of SFA and provide a healthier meat product (Malau-Aduli et al., 2015).

7.5. Conclusion

Dietary treatment influences the fatty acid content and lipogenic gene expression in the *Longissimus thoracis et lumborum* muscle in TAW lambs. The three lipogenic genes under investigation in this study showed marked variation in expression levels in the *Longissimus thoracis et lumborum* muscle of lambs in all the three dietary treatment groups. The *SCD* gene in this study tended towards up-regulation in the omega-3 fortified diet compared to MSM whole

grain and control diets. The *FABP4* gene was significantly up-regulated in 3 folds in the muscles of lambs fed MSM whole grain diets, conversely its *SCD* counterpart was significantly down-regulated in lambs fed omega-3 fortified diets. Furthermore, FABP4 gene had positive correlations between fold changes and DHA, EPA, EPA +DHA, EPA +DHA + DPA and PUFA/ SFA ratio. For the *FASN* gene in Figure 2, there were some negative but significant correlations ($p< 0.05$) between IMF, MUFA, PUFA and n-6 PUFA and fold changes in the expression of *FASN*. The findings herein presented buttress that *FABP4* was concomitant with the main fatty acids in the *Longissimus thoracis et lumborum* muscle in TAW lambs. These results reinforce the significant role of *FABP4* gene in the development of intramuscular fat in ruminants.

7.6. Summary

The primary objective of this study was to evaluate the expression of fatty acid synthase (*FASN*), stearoyl-CoA desaturase (*SCD*) and fatty acid binding protein 4 (*FABP4*) lipogenic genes in the *Longissimus thoracis et lumborum* muscles of Tattykeel Australia White (TAW) lambs supplemented with omega-3 fortified diets and correlations with with some unsaturated fatty acids (UFA). To answer the research question "*are there differences in the expression of lipogenic genes between control and omega-3 supplemented lambs*?", we tested the hypothesis that *fortification of lamb diets with omega-3 will lead to a down-regulation and a three-fold up-regulation of the FABP4 gene in the conventional MSM whole grain diet compared to the control.* Seventy-five six months old TAW lambs were randomly assigned to the following three dietary treatments of twenty-five animals each over a 47-day feeding trial: (1) control diet of pelleted hay without omega-3 oil, (2) MSM whole grain diet without omega-3 oil, and (3) pelleted hay fortified with omega-3 oil. Total RNA was extracted from *Longissimus thoracis et lumborum* muscle samples using TRIzol™ Plus RNA Purification Kit, and subsequently purified and DNase treated with ezDNase™ Enzyme. First-strand cDNA was synthesized, and quantitative DNA (qDNA) reactions were run using Faster

SYBR Green chemistry on a Quant Studio-3 Real-Time qPCR detection system. A serial dilution of pooled cDNA was employed to plot a standard curve after establishing the fitness of all primers. Results showed a striking disparity in the expression of the lipogenic genes tested in the muscles from lambs in the three dietary treatments. Utilising the Kruskal- Wallis test, the *SCD* gene tended to be up-regulated in lambs fed omega-3 fortified diets in comparison to MSM whole grain and the control diets. FABP4 gene was significantly up-regulated by 3-folds in the muscles of lambs fed MSM whole grain diet. Conversely, a significant down-regulation was detected for lambs fed omega-3 fortified diet. Positive and significant correlations ($p< 0.05$) were observed between fold changes of α-linolenic acid (ALA), and some UFA and dietary treatments for the *FABP4* gene. In contrast, a significant negative correlation was seen between the *FABP4* gene and the ratio between omega-6 and omega-3 polyunsaturated fatty acid (n-6/ n-3 PUFA). A significant negative correlation was observed between *FASN* and intramuscular fat (IMF), eicosapentaenoic acid (EPA), polyunsaturated fatty acid (PUFA), monounsaturated fatty acid (MUFA), and omega-6 polyunsaturated fatty acid (n-6 PUFA).

Chapter 8: General Discussion, Recommendations and Conclusions

Meat quality is impacted by genetic and non-genetic factors including species, production, and environment (Womack et al., 2012). Meat quality is a significant index of sheep breeding programmes aimed at meeting the market specifications and demands of consumers. Previous reports indicated that most meat quality traits were low-to-moderately heritable, hence, enhancing meat quality through standard selection methods remains challenging (Gao et al., 2021), especially because the data are collected after death as carcass traits. Therefore, marker-assisted selection is the preferred route of choice for such difficult to measure traits using genome-wide and targeted probing of polymorphisms associated with economically significant livestock production parameters (Kowalczyk et al., 2022). Genomic selection is aimed at raising precision, shortening generation intervals, and accelerating genetic gains in animals approaching maturity through early selection with a potentially favourable outcome on reproduction, growth rate, carcass, and meat quality traits (Duchemin et al., 2012; Noya et al., 2022).

As meat quality data are mostly obtained after slaughter, by the time an informed decision on the genetic merit for meat quality is made, the animal is already dead. This makes selection decisions about the live animal too late. Carcass estimated breeding values as the next best alternative, present major precision problems due to low accuracy. This thesis reports for the first time, a targeted NGS of lipogenic genes SNP in TAW sheep of the MARGRA lamb brand, utilizing an innovative and minimally invasive muscle biopsy sampling technique. This unique technique aided the direct quantification of the genetic worth of live lambs for health-beneficial n-3 LC-PUFA, IMF, and FMP primarily for enhancing meat eating quality. The thesis also established significant differential gene expression patterns and associations between lipogenic genes SNP and n-3 LC-PUFA, IMF and FMP, hence underpinning potential marker-assisted selection for meat eating quality traits in TAW lambs.

Intramuscular fat (IMF) denotes marbling is a key trait influencing meat quality and palatability (Park et al., 2018) and has direct influence on several aspects of ruminant meat, including tenderness, juiciness, flavour and antioxidant status (Reuben et al., 2022). In sheep husbandry practice, IMF is difficult to measure in live lambs. With the novel biopsy technique developed for sheep in this thesis, it is possible to determine the IMF in lambs shortly before they attain maturity. The IMF content is essential for meat eating quality and consumer acceptability (Clelland et al., 2014). The average IMF content of 4.4% ± 0.2% reported in this thesis exceeds the minimum suggested palatability limit of 4% for Australian lambs (Pannier et al., 2014) and other values reported in the literature. Take-home message is that TAW lamb is a top-end high eating quality meat that meets consumer acceptability and aligns with the low SFA, high IMF, MUFA, n-3 LC-PUFA and low FMP. The lambs on ryegrass had low dry matter, high contents of phenolic antioxidants, crude protein, low and high metabolisable energy. This agrees with the report of Montenegro et al. (2022), where ryegrass (*Lolium multiflorum)* fed grass carp (*Ctenopharyngodon idella*) improved the quality of meat by increasing the composition of health beneficial fatty acids such as EPA, DHA and DPA to human health. This also increased the content of antioxidant fat-soluble vitamins, thereby increasing the stability of lipids. Results obtained from the study also revealed no significant difference because of gender and inbreeding coefficient in total phenolics and antioxidant enzyme activities**.**

It was pertinent to investigate if there was any potential to further improve meat eating quality and health beneficial EPA, DHA and DPA levels by fortifying feedlot-finishing diets with omega-3 oils. Primarily the melting points of its constituent fatty acids affect the FMP of meat. Stearic acid (C18:0) is known to melt at 69.6 $^{\circ}$C, while its elongated counterpart, Oleic acid (C18:1), melts at a far lesser temperature of 13.4 $^{\circ}$C. Therefore, the contents of these FA have significant effect on the FMP, hence firmness of ruminant meat (Turk and Smith, 2009).

The health benefits of n-3 LC-PUFA in human diets range from ante- to post-natal stages of growth, healthy aging, and the maintenance of an efficient immune system, neurological and cardiac functions (Schl et al., 2022). Pastures are natural sources of antioxidants and LC-PUFA especially ALA (C18:3) (Santa et al., 2022), which is the precursor for EPA and DHA. Mammals in general, cannot synthesise these FA and must be supplied in the diets in order to meet daily nutritional requirements. Adequate PUFA intake reduces the risks of inflammatory disorders, mental health, cardiovascular disorders, and certain cancer types like breast, colorectal and prostatic cancers (Herter-Aeberli et al., 2019; Kapoor et al., 2021). Le et al. (2019) reported that FSANZ (Food Standards of Australia and New Zealand) guidelines specify that any food or meat product may be termed as 'source' if its n-3 LC-PUFA is at least 30 mg/ 100g /serve. The findings of this thesis showed that the *Longissimus thoracis et lumborum* muscle of TAW MARGRA lambs exceeded this 'source' limit as set by FSANZ. An increase in inbreeding coefficient from 0 to 15.6 % did not in any way, affect FMP, IMF and FA proportions indicating that linebreeding has no detrimental effect on the consistency of health-beneficial n-3 LC-PUFA in TAW MARGRA lambs. As demonstrated in **Chapter 4**, the FMP from lambs on n-3 LC-PUFA fortified diet had the lowest mean of 30.15 $^{\circ}$C, as opposed to the control and MSM whole grains with 34.75 $^{\circ}$C and 36.8 $^{\circ}$C, respectively.

The ADG was higher for lambs fed fortified n-3 LC-PUFA feed but this did not have a significant influence on dressing percentage. This finding was in agreement with earlier results by Dong et al. (2020) and Jin et al. (2021). Furthermore, there were significant effects of supplementation on feed intake, ADG, LWT, French rack and bones, with omega-3 oil fortified dietary treatment recording the highest performance. These outcomes concur with other findings by Nguyen et al. (2017) and Van Le et al. (2019). In this thesis, strong correlations ($p < 0.05$) between liveweight, temperature, pH, FMP and IMF were observed. Similarly, significant correlations between carcass characteristics of total saleable meat yield, lean trim,

fat trims, bones, and leg shank were evident ($p < 0.05$). Lot-fed lambs on the omega-3 diet had the highest ADG, indicating the greatest feed efficiency since it had the best growth response with minimal feed intake. The efficient usage of resources is viewed as a serious part of importance for livestock production (Kenny et al., 2018; Brito et al., 2021). Feed costs may account for 70% of total livestock production costs (Becker 2008), feed efficiency remains a target today in beef producers' efforts toward sustainability (Vaughn et al., 2022). The persistent increase in feed prices has made it mandatory to improve livestock feed efficiency (Ellison et al., 2022). The results in this thesis established that omega-3 could be utilised efficiently in TAW lambs in the feedlot. This is without any unfavourable consequence on animal performance, meat quality and carcass traits.

Fatty acid analyses can offer insights into the sensory and nutritional qualities of meat, which have health implications (Wood et al., 2008; Woloszyn et al., 2020). The edible tissues and organs were evaluated in **Chapter 5**. The *Longissimus thoracis et lumborum* muscle, heart, kidney and liver, were examined. The Σn-3 PUFA, PUFA/ SFA, and *n-6*/ *n-3* PUFA ratios are useful indices for appraising the nutritional value of healthy food (Attia et al., 2017). The n-6/ n-3 proportions in the measured tissues and organs except for the heart, were less than 4/1, thus falling within the dietary requirements for human consumption (WHO, 2003). Besides, n-6 and n-3 compete for the identical enzymes (Δ-4, 5, 6 desaturases and elongase) that convert fatty acids into biologically active forms (Njuricic and Calder, 2021). Adding 5 % omega-3 oil to the feedlot diet significantly reduced the n-6/ n-3 ratios in tissues and organs. Furthermore, lambs fed omega-3 fortified diets, unlike those fed control and MSM whole grain diets, had a significant increase in ALA, EPA+DHA+`DPA, total FA, total PUFA, total n-3 PUFA contents, PUFA/ SFA ratio and reduced n-6/ n-3 PUFA in the tissues evaluated. This is attributable to the higher proportions of ALA and lower LA levels in the omega-3 fortified dietary treatment. Gómez-Cortés et al. (2017) and Parente et al. (2020) reported that ruminant

fats could be augmented with PUFA biohydrogenation intermediates when vegetable or omega-3 oil is supplemented in ruminant diets. Thus, fortifying ruminant diets with omega-3 can alter the meat's fatty profile depending on the lipid, fatty acid content, and feeding duration (Shingfield et al., 2013; de Araújo et al., 2020; Dos Santos et al., 2022). Therefore, including omega-3 oil augmented the total n-3 PUFA proportions in the tissues and organs evaluated with a remarkable increase in the proportion of total n-3 LC-PUFA compared to the control and MSM whole grain diets.

The proportions of EPA + DHA + DPA and total n-3 LC-PUFA of the *Longissimus thoracis et lumborum* muscle improved significantly in lambs fed omega-3 diets compared to those fed control and MSM whole grain diets. Similarly, the contents of EPA + DHA + DPA and total n-3 LC-PUFA increased in the liver, kidneys and heart of the lambs feed diets fortified with omega-3 compared with those on control and MSM whole grain diets. This implies that sufficient levels of dietary ALA are required to provide the needed EPA + DHA + DPA in the blood and tissue systems (Ponnampalam et al., 2021). It was also evident that the liver from lambs fed all the three diets satisfied the claimable 'source' and 'good source' levels of these n-3 LC-PUFA (EPA + DHA), but omega-3 far exceeded the control and MSM whole grain as recommended by FSANZ in Le et al. (2019). In the kidney, lambs fed omega-3 fortified diets met the 'source' and 'good source', while control and MSM whole grains met only the 'source' recommendation. This shows that the visceral organs, liver, kidneys and heart are alternative 'good sources' of omega-3. As demonstrated by Nguyen et al. (2017), this thesis's findings provide validation that fortifying the diet with omega-3 lifts the EPA + DHA profiles of sheep meat and edible visceral organs.

DPA functions as an intermediary between EPA and DHA, performs a vital function in the synthesis pathway from ALA and can be converted to either APA or DPA (Ahmmed et al., 2020). Several studies, including those of Batetta et al. (2009), Vakhapova et al. (2011) and

Lapointe (2019), found no side effects with the consumption of omega-3. Vakhapova et al. (2011) stated that consuming DHA-containing phosphatidylserine for 30 weeks at 100 mg/ day or 300 mg/ day was safe. The liver and skeletal muscles in ruminants have high DPA content, more than five-fold greater than DHA (Crawford et al., 1976). Hence, for exclusive red meat consumers devoid of any fish source, DPA remains the critical source of dietary n-3 LC-PUFA (Fard et al., 2021). DPA has a more remarkable similarity in function with DHA than EPA. Notwithstanding, its low concentration relative to DHA + EPA in most tissues is vital in resolving inflammation-related cardiovascular, gut, joint, skin, and neural diseases (Fard et al., 2021). Fard et al. (2021) also reported that DPA improves cardiovascular and metabolic disease risks, and serves as a marker, particularly in plasma lipid indices, platelet aggregation, insulin sensitivity and cellular plasticity. Furthermore, in the brain, DHA and DPA are the most abundant and essential n-3 LC-PUFA, and they might be of benefit for aged neuroprotection and early-life growth (Drouin et al., 2019). Results in this thesis show that, fortification with omega-oils did not have any detrimental effect on meat quality due to high levels of healthy fatty acids in comparison to other diets.

Global meat production is increasing to meet the expected rise in world human population (Hunter et al., 2017), and several strategies have been put in place to achieve the goal of excellent meat production using genetically superior animals. In farm animal husbandry practice, the genetic value of an animal is known after it has been slaughtered, making it late to make any selection decisions. Therefore, EBVs are commonly used as indicators of an animal's genetic potential expressed relative to the population mean (Altınçekiç et al., 2022). These are not always accurate since they are estimates based on averages, hence poor precision. Genome-Wide Association Studies (GWAS) are costly and take longer to accomplish. Therefore, the use of targeted SNP for marker-assisted selection is cheaper, and its outcomes are faster than GWAS. In **Chapter 6**, this thesis examined a targeted

NGS of SNP of lipogenic genes in TAW lamb. It is a pioneering and marginally invasive *Longissimus thoracis et lumborum* muscle biopsy sampling technique intended to gauge the genetic value of live lambs for meat quality traits such as health-beneficial n-3 LC-PUFA, IMF, and FMP. According to Robert & Pelletier (2018), SNP is a single base-pair alteration in the DNA sequence that arises at high rate within the genome.

The NGS of stearoyl-CoA desaturase (*SCD*), fatty acid-binding protein-4 (*FABP4*), and fatty acid synthase (*FASN*) genes detected functional SNP with exclusive DNA marker signatures for TAW genetics. Assessing genetic tendencies for sustainable and economic traits in animal breeding is vital for developing effective future breeding programmes (Khanal et al., 2022; Altınçekiç et al., 2022). Nicolazzi et al. (2015) stated that the detection and utilisation of genome sequences had freed numerous studies on genetic traits of livestock, including sheep leading to the availability of SNP arrays, presenting many applications in some areas of livestock production, including genetic improvement, breeding and conservation. It provides more rapid access to vastly accurate genome-wide data on individuals at a moderately low cost. Genomic selection leads to additive, cumulative and permanent genetic change over generations (Rowe et al., 2019; Lambe, 2022).

Di Giorgio et al. (2022) opined that the FA profile of meat has implications for the production's flavour, functional properties, and shelf life. The proportion between SFA, MUFA and PUFA affects chemical constitution, sensory features of carcass fat and the shelf life of meat products (Webb, 2014). High levels of SFA are associated with cardiovascular disorders, cancers and obesity (Martins et al., 2020); PUFA confers health-promoting roles for the cardiovascular, neurological and immunological systems (de Melo Ramos et al., 2021). Fat deposition is a multifaceted economic attribute controlled by genetic and environmental factors. It is an essential trait that controls feed efficiency and meat quality, thereby influencing flavour, juiciness, and tenderness (Du et al., 2022). In this thesis, three candidate

genes, *FASN* (de novo FA synthesis), *SCD* (FA desaturation) and *FABP4* (FA transport), which influence FA deposition via tasks involved in FA synthesis, oxidation, and metabolism, were studied (Du et al., 2022). *FASN* primarily controls *the de novo* synthesis of long-chain SFA and fat deposition (Yeon et al., 2013). Ameer et al., 2014 described it as the critical rate-limiting enzyme that converts acetyl-coenzyme A (CoA) and malonyl-CoA as the initiating substrate into palmitate and yielding stearate and shorter fatty acids. The current study found significant genotype differences of the *FASN* gene at g.12323864A>G SNP locus and was correlated with FMP, ALA, MUFA, SFA, C18:2n-6, C18:1n-9, C18:0, and C16:0. Furthermore, the homozygous genotype GG recorded the uppermost values in comparison with the least values in AA genotype, except for C18:2n-6 that was least in the heterozygous GA genotype. This study also observed a negative correlation of -0.66 between FMP and IMF. Similarly, moderate but positive correlations were observed between IMF and essential fatty acids EPA, DPA, DHA, EPA + DHA + DPA and EPA + DHA. This relationship clearly illustrates *FASN*'s central role in fatty acid metabolism and fat deposition. In pigs, AA genotypes of the *FASN* gene presented significantly lesser arachidonic acid (C20:4n6) but greater linoleic acid (C18:2n6), linolenic acid (C18:3n3), and PUFA content than CC genotype. Zappaterra et al. (2019) stated that c.265T > C *FASN* SNP significantly altered the compositions of stearic, arachidonic, dihomo-γ-linolenic (DGLA) and arachidonic fatty acids in the *Longissimus thoracis et lumborum* muscle of Italian Large White pigs. The g.16024G > A in *FASN* was highly correlated with SFA, principally C14:0 and C16:0, and C18:1 n-9 concentrations in the Fleckvieh breed of cattle (Bartoň et al., 2016). Additionally, Raza et al. (2018) observed that in Chinese Qinchuan cattle, the g.13232C > T SNP in the *FASN* gene of the TT genotype was associated with greater IMF.

In ovine species, the *SCD* gene is located on chromosome 22, and encodes the *SCD* enzyme accountable for the transformation of SFA into MUFA (Al-Thuwaini & Al-

Shuhaib, 2022). The *SCD* SNP loci detected in TAW showed significant association with some fatty acids and meat-eating qualities. The homozygous TT genotype had higher values of DHA (C22:6n-3), DPA (C22:5n-3) and IMF; heterozygous CT and homozygous CC genotypes were the least. Several researchers have described relations between the *SCD* gene polymorphisms and fat composition in livestock. In Auraucano Creole sheep, Quiñones et al. (2017) reported that polymorphisms of *SCD* gene in the coding region g.878 T > C were correlated with differences in MUFA and CLA concentrations in milk and meat. A total of 8 SNP of the *SCD* gene were described by Alwiyah et al. (2016) in Bali cattle, where the g.10428C > T was significantly correlated with marbling score and the degrees of intramuscular lipids.

Fatty acid (FA) metabolism in ruminants is complicated by hydrogenation by ruminal microbes, where unsaturated fatty acids are converted to SFA (Beam et al., 2000; Hoashi et al., 2008). Increased levels of SFA are associated with health risks already mentioned in this thesis. To reduce the contents of these SFA in meat, various strategies, including fortification of the diet with n-3 LC-PUFA, are employed. It is essential to manipulate fat deposition to produce high quality lambs. Fat deposition is a multifaceted process that is regulated by several pathways and genes (Wang et al., 2021). The influence of omega-3 supplements on the expression of genes engaged in lipogenesis in the *Longissimus thoracis et lumborum* muscle of TAW MARGRA lambs remains unknown. This informed the need to investigate the response to dietary fortification with omega-3 oils and the expression patterns of the three lipogenic genes (*FASN, FABP4* and *SCD*) considered in **Chapter 7**. These genes play different but complementary roles in FA metabolism, and these have been highlighted in Chapters 2 and 7 of this thesis. Zhang et al. (2013) recounted that the *SCD* is a rate-limiting enzyme involved in the desaturation of SFA from principally palmitic acid, C16:0 and stearic acid, C18: 0 to yield palmitoleic acid (C16:1) and oleic acid (C18:1), respectively.

This study shows that *SCD* gene was significantly suppressed the expression of this gene in diets supplemented with omega-3. The results obtained from this study revealed that the expression of the *SCD* gene was significantly reduced in the *Longissimus thoracis et lumborum* muscle of TAW lambs fed diets fortified with omega-3 oils. It implies that the *SCD* gene in TAW lambs is down-regulated.

FABP4 plays an active role in the uptake and transport of long chain FA (Osorio et al., 2016). The *FABP4* gene is a principal metabolic pointer of IMF deposition as it is situated inside the quantitative trait loci area that adds to serum leptin, a protein contributing to body fat regulation (Jurie et al., 2007; Hoashi et al., 2008). IMF content is essential in improving meat quality traits (Hausman et al., 2009). In this study, lambs fed MSM whole grain diet had significant ($p< 0.05$) up-regulation of the *FABP4* gene while those fed control and omega-3 diets were not up-regulated ($p> 0.05$). The *FASN* gene is involved in the *de novo* synthesis of FA and fat deposition (Tian et al., 2022). According to Guo et al. (2021), this gene requires two molecules of NADPH (nicotinamide adenine dinucleotide phosphate), with two precursors, acetyl-CoA and malonyl-CoA to catalyse the *de novo* synthesis of palmitic acid. In the current study, none of the lipogenic genes evaluated was significantly ($p> 0.05$) up-regulated in any of the lambs fed the three dietary treatments. Lee et al. (2019) reported up-regulation of the mRNA of *FABP4* gene in Korean native steers fed rumen protected L-tryptophan supplementation. The findings of this thesis will be of immense benefit to TAW lamb producers and researchers for:

a. Early selection decisions while the animals are still young and alive;
b. Breed-specific detection and utilisation of identified lipogenic gene SNP for marker-assisted selection of TAW lambs for improved meat quality with high precision, validity and reliability;
c. Improvement of lamb eating quality and health-beneficial omega-3 in lamb;

d. Identifying alternative sources of n-3 PUFA-rich supplements influence animal health, productivity and sensory meat quality traits;
e. Cost-effective fortification of feedlot diets with n-3 LC-PUFA for the early attainment of target finishing weights and 'source' levels of healthy n-3 LC-PUFA content in muscles and other edible tissues at finishing phases;
f. Establishing that the liver and kidney are 'very good sources' of healthy n-3 LC-PUFA with low n-6/n-3 ratio.

Further investigation is essential to better elucidate the:

1). Exploration of more sources of omega-3 oils and their utilisation in feedlot finishing of lambs to increase profitability and health-beneficial levels of n-3 LC-PUFA content in meat;

2). Serum metabolite profiles in TAW sheep to unravel their physiological and immune roles since metabolomics offers an opportunity to quantify endogenous metabolic changes in biological systems in response to genetic or environmental pertubations.

3). Gene expression analyses in the visceral organs (liver, kidney and heart).

4). In-depth comprehension of the rumen biohydrogenation pathways in lambs.

5). Sensory evaluation of TAW lamb eating quality by consumers.

In conclusion, the study has unravelled novel perception regarding shared genetic control of the intramuscular fat, fat melting point and health-promoting omega-3 long-chain polyunsaturated fatty acid profile traits that could be utilised in designing breeding schemes important to genetically upgrade meat-eating quality traits in TAW MARGRA lambs just before they are used for mating purposes.

Limitations of the study:

1). Serum metabolite profiles as indicators of physiological immune function and nutritional status were not analysed, hence future studies could fill this knowledge gap.

2). This study was conducted in the Australian State of New South Wales where climatic conditions and production systems may not be applicable to the rest of the country. Therefore, comparative studies across the country under diverse climatic settings and production systems may better explain and confirm some of the findings herein.

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Appendices

Appendix 1

genes

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Review

Genetics of Omega-3 Long-Chain Polyunsaturated Fatty Acid Metabolism and Meat Eating Quality in Tattykeel Australian White Lambs

Shedrach Benjamin Pewan [1,2], John Roger Otto [1], Roger Huerlimann [3], Alyssa Maree Budd [3], Felista Waithira Mwangi [1], Richard Crawford Edmunds [1], Benjamin William Behrens Holman [4], Michelle Lauren Elizabeth Henry [5,6], Robert Tumwesigye Kinobe [1], Oyelola Abdulwasiu Adegboye [7] and Aduli Enoch Othniel Malau-Aduli [1,*]

1 Animal Genetics and Nutrition, Veterinary Sciences Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, Queensland 4811, Australia; shedrach.pewan@my.jcu.edu.au (S.B.P.); john.otto@jcu.edu.au (J.R.O.); felista.mwangi@my.jcu.edu.au (F.W.M.); richard.c.edmunds@gmail.com (R.C.E.); robert.kinobe@jcu.edu.au (R.T.K.)
2 National Veterinary Research Institute, Private Mail Bag 01, Vom, Plateau State, Nigeria
3 Centre for Sustainable Tropical Fisheries and Aquaculture and Centre for Tropical Bioinformatics and Molecular Biology, College of Science and Engineering, James Cook University, Townsville, Queensland 4811, Australia; roger.huerlimann@jcu.edu.au (R.H.); alyssa.budd@jcu.edu.au (A.M.B.)
4 Centre for Red Meat and Sheep Development, NSW Department of Primary Industries, Cowra, New South Wales 2794, Australia; benjamin.holman@dpi.nsw.gov.au
5 Gundagai Meat Processors, 2916 Gocup Road, South Gundagai, New South Wales 2722, Australia; MHenry@gmpgundagai.com.au
6 Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Melbourne, VIC 3010, Australia
7 Australian Institute of Tropical Health and Medicine, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, Queensland 4811, Australia; oyelola.adegboye@jcu.edu.au
* Correspondence: aduli.malauaduli@jcu.edu.au; Tel.: +61-747-815-339

Received: 4 March 2020; Accepted: 21 May 2020; Published: 25 May 2020

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Abstract: Meat eating quality with a healthy composition hinges on intramuscular fat (IMF), fat melting point (FMP), tenderness, juiciness, flavour and omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) content. These health-beneficial n-3 LC-PUFA play significant roles in optimal cardiovascular, retinal, maternal and childhood brain functions, and include alpha linolenic (ALA), eicosapentaenoic (EPA), docosahexaenoic (DHA) and docosapentaenoic (DPA) acids. The primary objective of this review was to access, retrieve, synthesise and critically appraise the published literature on the synthesis, metabolism and genetics of n-3 LC-PUFA and meat eating quality. Studies on IMF content, FMP and fatty acid composition were reviewed to identify knowledge gaps that can inform future research with Tattykeel Australian White (TAW) lambs. The TAW is a new sheep breed exclusive to MARGRA brand of lamb with an outstanding low fat melting point (28–39°C), high n-3 LC-PUFA EPA+DHA content (33–69mg/100g), marbling (3.4–8.2%), tenderness (20.0–38.5N) and overall consumer liking (7.9–8.5). However, correlations between n-3 LC-PUFA profile, stearoyl-CoA desaturase (SCD), fatty acid binding protein 4 (FABP4), fatty acid synthase (FASN), other lipogenic genes and meat quality traits present major knowledge gaps. The review also identified research opportunities in nutrition–genetics interactions aimed at a greater understanding of the genetics of n-3 LC-PUFA, feedlot finishing performance, carcass traits and eating quality in the TAW sheep. It was concluded that studies on IMF, FMP and n-3 LC-PUFA profiles in parental and progeny generations of TAW sheep will be foundational for the genetic selection of healthy lamb eating qualities and provide useful insights into their correlations with SCD, FASN and FABP4 genes.

Appendix 2

antioxidants

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Artide

MARGRA Lamb Eating Quality and Human Health-Promoting Omega-3 Long-Chain Polyunsaturated Fatty Acid Profiles of Tattykeel Australian White Sheep: Linebreeding and Gender Effects

Shedrach Benjamin Pewan [1,2], John Roger Otto [1], Robert Tumwesigye Kinobe [1], Oyelola Abdulwasiu Adegboye [3] and Aduli Enoch Othniel Malau-Aduli [1,*]

1 Animal Genetics and Nutrition, Veterinary Sciences Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia; shedrach.pewan@my.jcu.edu.au (S.B.P.); john.otto@jcu.edu.au (J.R.O.); robert.kinobe@jcu.edu.au (R.T.K.)

2 National Veterinary Research Institute, Private Mail Bag 01 Vom, Plateau State, Nigeria

3 Australian Institute of Tropical Health and Medicine, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia; oyelola.adegboye@jcu.edu.au

* Correspondence: aduli.malauaduli@jcu.edu.au; Tel.: +61-747-815-339

Received: 16 October 2020; Accepted: 11 November 2020; Published: 12 November 2020

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Abstract: Health-conscious consumers increasingly demand healthier, tastier, and more nutritious meat, hence the continuous need to meet market specifications and demand for high-quality lamb. We evaluated the *longissimus dorsi* muscle of 147 Tattykeel Australian White (TAW) sheep fed on antioxidant-rich ryegrass pastures exclusive to MAGRA lamb brand for meat eating quality parameters of intramuscular fat (IMF) content, fat melting point (FMP) and omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). The aim was to assess the impact of linebreeding and gender on pasture-fed lamb eating quality and to test the hypothesis that *variation in healthy lamb eating quality is a function of lamb gender and not its antioxidant status or inbreeding coefficient* (IC). After solid-phase extraction and purification, phenolics and antioxidant enzyme activities were analysed by high-performance liquid chromatography and mass spectrometry. IMF and fatty acid composition were determined using solvent extraction and gas chromatography, respectively. IC was classified into low (0–5%), medium (6–10%) and high (>10%) and ranged from 0–15.6%. FMP and IMF ranged from 28 to 39 °C and 3.4% to 8.2%, with overall means of 34.6 ± 2.3 °C and 4.4 ± 0.2%, respectively, and n-3 LC-PUFA ranged from "source" to "good source" levels of 33–69 mg/100 g. Ewes had significantly ($P < 0.0001$) higher IMF, C22:5n-3 (DPA), C22:6n-3 (DHA), C18:3n-6, C20:3, C22:4n-6, C22:5n-6, total monounsaturated (MUFA), PUFA and Σn-3 fatty acids and lower total saturated fatty acids (SFA) and FMP, than rams. As IC increased, there were no differences in FMP and IMF. Folin–Ciocalteu total phenolics, ferric reducing antioxidant power and antioxidant activities of glutathione peroxidase, catalase and superoxide dismutase enzymes did not differ by either gender or IC. This study provides evidence that IC is inconsequential in affecting antioxidant status, IMF, FMP and n-3 LC-PUFA in linebred and pasture-fed TAW sheep because the observed variation in individual fatty acids was mainly driven by gender differences between ewes and rams, hence the need to accept the tested hypothesis. This finding reinforces the consistent healthy eating quality of MARGRA lamb brand from TAW sheep regardless of its linebred origin.

Keywords: antioxidants; Tattykeel Australian White; MARGRA lamb; meat quality; *longissimus dorsi* muscle; omega-3 LC-PUFA; fat melting point; intramuscular fat; inbreeding coefficient; gender

Appendix 3

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 12 September 2022
DOI 10.3389/fvets.2022.933038

OPEN ACCESS

EDITED BY
Xuezhao Sun,
Jilin Agricultural Science and Technology University, China

REVIEWED BY
Panagiotis Simitzis,
Agricultural University of Athens, Greece
Maghsoud Besharati,
University of Tabriz, Iran

*CORRESPONDENCE
Aduli Enoch Othniel Malau-Aduli
aduli.malauaduli@jcu.edu.au

SPECIALTY SECTION
This article was submitted to Animal Nutrition and Metabolism, a section of the journal Frontiers in Veterinary Science

RECEIVED 30 April 2022
ACCEPTED 22 August 2022
PUBLISHED 12 September 2022

CITATION
Pewan SB, Otto JR, Kinobe RT, Adegboye OA and Malau-Aduli AEO (2022) Fortification of diets with omega-3 long-chain polyunsaturated fatty acids enhances feedlot performance, intramuscular fat content, fat melting point, and carcass characteristics of Tattykeel Australian White MARGRA lambs. *Front. Vet. Sci.* 9:933038. doi: 10.3389/fvets.2022.933038

Fortification of diets with omega-3 long-chain polyunsaturated fatty acids enhances feedlot performance, intramuscular fat content, fat melting point, and carcass characteristics of Tattykeel Australian White MARGRA lambs

Shedrach Benjamin Pewan[1,2], John Roger Otto[1], Robert Tumwesigye Kinobe[1], Oyelola Abdulwasiu Adegboye[3] and Aduli Enoch Othniel Malau-Aduli[1]*

[1]Animal Genetics and Nutrition, Veterinary Sciences Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD, Australia, [2]National Veterinary Research Institute, Vom, Plateau State, Nigeria, [3]Public Health and Tropical Medicine Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD, Australia

Meat eating quality indices such as intramuscular fat content (IMF) and fat melting point (FMP) of the *Longissimus thoracis et lumborum* muscle and the feedlot performance, carcass traits, and commercial wholesale cuts of lot-fed Tattykeel Australian White (TAW) MARGRA lambs as a result of dietary fortification of the diet with omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) were evaluated. A total of 75 TAW MARGRA lambs at 6 months of age with an average liveweight of 30 ± 1.2 kg were used. The lambs were randomly allocated to the following three dietary treatments of 25 lambs each in a 47-day feeding trial using a completely randomized experimental design: (1) control diet of hay plus pellets without omega-3 oil, (2) hay plus commercial whole grain pellets (MSM) without omega-3 oil, and (3) hay plus pellets fortified with omega-3 oil. It was hypothesized that dietary supplementation with omega-3 fortified pellets will improve feedlot performance, meat-eating quality indices of IMF, FMP, and carcass characteristics. Lot-fed lambs on the MSM whole grain had the highest feed intake of 1.69 kg/day, followed by the control at 1.57 kg/day and the lowest in the omega-3 diet at 1.01 kg/day ($p = 0.0001$). However, the omega-3 diet had the highest average daily gain of 230 g/head/day ($p = 0.0001$), indicating the greatest feed efficiency since it had the best growth response with minimal feed intake. Post-slaughter evaluation of the *Longissimus thoracis et lumborum* muscle revealed significant treatment variations in IMF ($p = 0.0001$), FMP ($p = 0.0001$), pH ($p = 0.0380$), and wholesale French rack primal cut ($p = 0.0001$). Strong correlations ($p < 0.05$) between liveweight, temperature, pH, FMP, and IMF were observed. Similarly, significant

Appendix 4

biology

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Article

Nutritional Enhancement of Health Beneficial Omega-3 Long-Chain Polyunsaturated Fatty Acids in the Muscle, Liver, Kidney, and Heart of Tattykeel Australian White MARGRA Lambs Fed Pellets Fortified with Omega-3 Oil in a Feedlot System

Shedrach Benjamin Pewan [1,2], John Roger Otto [1], Robert Tumwesigye Kinobe [1], Oyelola Abdulwasiu Adegboye [3] and Aduli Enoch Othniel Malau-Aduli [1,*]

[1] Animal Genetics and Nutrition, Veterinary Sciences Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia; shedrach.pewan@my.jcu.edu.au (S.B.P.); john.otto@jcu.edu.au (J.R.O.); robert.kinobe@jcu.edu.au (R.T.K.)
[2] National Veterinary Research Institute, Private Mail Bag 01 Vom, Plateau State, Nigeria
[3] Public Health and Tropical Medicine Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia; oyelola.adegboye@jcu.edu.au
* Correspondence: aduli.malauaduli@jcu.edu.au; Tel.: +61-747-815-339

Simple Summary: The problem addressed in this research was the possibility of enhancing the nutritional value and health beneficial omega-3 long-chain fatty acid content of lamb and its edible components. The aims and objectives were to evaluate the omega-3 contents of muscle, liver, kidney, and heart of lot-fed Tattykeel Australian White lambs of the MARGRA brand, in response to dietary supplementation with or without omega-3 oil fortified pellets. The findings demonstrate that the inclusion of omega-3 oil in feedlot diets of lambs enhances the human health beneficial omega-3 long-chain polyunsaturated fatty acid profiles of edible muscle tissue and organs without compromising meat quality or shelf life. These results are valuable to society because of increased functionality, health benefits, micro-marbling, tender, mouth-melting taste, and high-end eating quality experience of MARGRA lamb tissues and organs.

Abstract: The aim of this research was to evaluate the nutritional enhancement of omega-3 long-chain polyunsaturated fatty acid (n-3 LC-PUFA) composition of edible lamb *Longissimus thoracis et lumborum* muscle, heart, kidney, and liver in response to dietary supplementation of lot-fed lambs with or without omega-3 oil fortified pellets. The hypothesis tested was that fortifying feedlot pellets with omega-3 oil will enhance the human health beneficial n-3 LC-PUFA composition of edible lamb muscle tissue and organs. Seventy-five Tattykeel Australian White lambs exclusive to the MARGRA brand, with an average body weight of 30 kg at six months of age, were randomly assigned to the following three dietary treatments of 25 lambs each, and lot-fed as a cohort for 47 days in a completely randomized experimental design: (1) Control grain pellets without oil plus hay; (2) Omega-3 oil fortified grain pellets plus hay; and (3) Commercial whole grain pellets plus hay. All lambs had *ad libitum* access to the basal hay diet and water. Post-slaughter fatty acid composition of the *Longissimus thoracis et lumborum* muscle, liver, kidney, and heart were determined using thee gas chromatography–mass spectrophotometry technique. Results indicated significant variations ($p < 0.05$) in fatty acid profiles between tissues and organs. Omega-3 oil fortified pellets significantly ($p < 0.05$) increased ≥C20 n-3 LC-PUFA (C20:5n-3 eicosapentaenoate, EPA + C22:5n3 docosapentaenoate, DPA + C22:6n3 docosahexanoate DHA); C18:3n-3 alpha-linolenate, ALA; C18:2 conjugated linoleic acid, CLA; total monounsaturated fatty acids, MUFA; polyunsaturated fatty acids, PUFA contents; and reduced the ratio of omega-6 to omega-3 fatty acids in all lamb organs and tissues without impacting shelf-life. The findings demonstrate that the inclusion of omega-3 oil in

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Citation: Pewan, S.B.; Otto, J.R.; Kinobe, R.T.; Adegboye, O.A.; Malau-Aduli, A.E.O. Nutritional Enhancement of Health Beneficial Omega-3 Long-Chain Polyunsaturated Fatty Acids in the Muscle, Liver, Kidney, and Heart of Tattykeel Australian White MARGRA Lambs Fed Pellets Fortified with Omega-3 Oil in a Feedlot System. *Biology* **2021**, *10*, 912. https://doi.org/10.3390/biology10090912

Academic Editors: Zipei Zhang, Quancai Sun and Xian Wu

Received: 31 August 2021
Accepted: 10 September 2021
Published: 14 September 2021

Appendix 5

foods

MDPI

Article

Next Generation Sequencing of Single Nucleotide Polymorphic DNA-Markers in Selecting for Intramuscular Fat, Fat Melting Point, Omega-3 Long-Chain Polyunsaturated Fatty Acids and Meat Eating Quality in Tattykeel Australian White MARGRA Lamb

Shedrach Benjamin Pewan [1,2], John Roger Otto [1], Roger Huerlimann [3,4], Alyssa Maree Budd [4], Felista Waithira Mwangi [1], Richard Crawford Edmunds [1], Benjamin William Behrens Holman [5], Michelle Lauren Elizabeth Henry [6,7], Robert Tumwesigye Kinobe [1], Oyelola Abdulwasiu Adegboye [8] and Aduli Enoch Othniel Malau-Aduli [1,*]

1 Animal Genetics and Nutrition, Veterinary Sciences Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia; shedrach.pewan@my.jcu.edu.au (S.B.P.); john.otto@jcu.edu.au (J.R.O.); felista.mwangi@my.jcu.edu.au (F.W.M.); richard.edmunds@jcu.edu.au (R.C.E.); robert.kinobe@jcu.edu.au (R.T.K.)
2 National Veterinary Research Institute, Private Mail Bag 01 Vom, Plateau State, Nigeria
3 Marine Climate Change Unit, Okinawa Institute of Science and Technology, 1919-1 Tancha, Onna-son, Okinawa 904-0495, Japan; roger.huerlimann@jcu.edu.au
4 Centre for Sustainable Tropical Fisheries and Aquaculture and Centre for Tropical Bioinformatics and Molecular Biology, College of Science and Engineering, James Cook University, Townsville, QLD 4811, Australia; alyssa.budd@jcu.edu.au
5 Centre for Red Meat and Sheep Development, NSW Department of Primary Industries, Cowra, NSW 2794, Australia; benjamin.holman@dpi.nsw.gov.au
6 Gundagai Meat Processors, 2916 Gocup Road, South Gundagai, NSW 2722, Australia; MHenry@gmpgundagai.com.au
7 Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Melbourne, VIC 3010, Australia
8 Public Health and Tropical Medicine Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia; oyelola.adegboye@jcu.edu.au
* Correspondence: aduli.malauaduli@jcu.edu.au; Tel.: +61-747-815-339

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Citation: Pewan, S.B.; Otto, J.R.; Huerlimann, R.; Budd, A.M.; Mwangi, F.W.; Edmunds, R.C.; Holman, B.W.B.; Henry, M.L.E.; Kinobe, R.T.; Adegboye, O.A.; et al. Next Generation Sequencing of Single Nucleotide Polymorphic DNA-Markers in Selecting for Intramuscular Fat, Fat Melting Point, Omega-3 Long-Chain Polyunsaturated Fatty Acids and Meat Eating Quality in Tattykeel Australian White MARGRA Lamb. *Foods* **2021**, *10*, 2288. https://doi.org/10.3390/foods10102288

Academic Editors: Yongkang Luo and Hui Hong

Received: 27 August 2021
Accepted: 17 September 2021
Published: 27 September 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Meat quality data can only be obtained after slaughter when selection decisions about the live animal are already too late. Carcass estimated breeding values present major precision problems due to low accuracy, and by the time an informed decision on the genetic merit for meat quality is made, the animal is already dead. We report for the first time, a targeted next-generation sequencing (NGS) of single nucleotide polymorphisms (SNP) of lipid metabolism genes in Tattykeel Australian White (TAW) sheep of the MARGRA lamb brand, utilizing an innovative and minimally invasive muscle biopsy sampling technique for directly quantifying the genetic worth of live lambs for health-beneficial omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), intramuscular fat (IMF), and fat melting point (FMP). NGS of stearoyl-CoA desaturase (*SCD*), fatty acid binding protein-4 (*FABP4*), and fatty acid synthase (*FASN*) genes identified functional SNP with unique DNA marker signatures for TAW genetics. The *SCD g.23881050T>C* locus was significantly associated with IMF, C22:6n-3, and C22:5n-3; *FASN g.12323864A>G* locus with FMP, C18:3n-3, C18:1n-9, C18:0, C16:0, MUFA, and *FABP4 g.62829478A>T* locus with IMF. These add new knowledge, precision, and reliability in directly making early and informed decisions on live sheep selection and breeding for health-beneficial n-3 LC-PUFA, FMP, IMF and superior meat-eating quality at the farmgate level. The findings provide evidence that significant associations exist between SNP of lipid metabolism genes and n-3 LC-PUFA, IMF, and FMP, thus underpinning potential marker-assisted selection for meat-eating quality traits in TAW lambs.

Keywords: SNP; *FASN*; *SCD*; *FABP4*; IMF; FMP; eating quality; TAW MARGRA lamb; biopsy; n-3 LC-PUFA

Appendix 6

Differential expression of *FASN*, *SCD*, and *FABP4* genes in the *Longissimus thoracis et lumborum* muscle of Tattykeel Australia White lambs supplemented with omega-3 oil

Shedrach B. Pewan[1, 2], John R. Otto[1], Richard C. Edmunds[1], Robert T. Kinobe[1], Oyelola A. Adegboye[3] and Aduli E. O. Malau-Aduli[1,*].

[1] Animal Genetics and Nutrition, Veterinary Sciences Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, Queensland 4811, Australia; shedrach.pewan@my.jcu.edu.au (S.B.P.); J.R.O.); (R.T.K.)

[2] National Veterinary Research Institute, Private Mail Bag 01 Vom, Plateau State, Nigeria.

[3] Public Health and Tropical Medicine Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, Queensland 4811, Australia; oyelola.adegboye@jcu.edu.au (O.A.A.)

*Correspondence:

ABSTRACT

The primary objective of this study was to evaluate the expression of fatty acid synthase (*FASN*), stearoyl-CoA desaturase (*SCD*) and fatty acid binding protein 4 (*FABP4*) lipogenic genes in the *Longissimus thoracis et lumborum* muscles of Tattykeel Australia White (TAW) lambs supplemented with omega-3 fortified diets and correlations with with some unsaturated fatty acids (UFA). To answer the research question "*are there differences in the expression of lipogenic genes between control and omega-3 supplemented lambs*?", we tested the hypothesis that *fortification of lamb diets with omega-3 will lead to a down-regulation and a three-fold up-regulation of the FABP4 gene in the conventional MSM whole grain diet compared to the control.* Seventy-five six months old TAW lambs were randomly assigned to the following three dietary treatments of twenty-five animals each over a 47-day feeding trial: (1) control diet of pelleted hay without omega-3 oil, (2) MSM whole grain diet without omega-3 oil, and (3) pelleted hay fortified with omega-3 oil. Total RNA was extracted from *Longissimus thoracis et lumborum* muscle