Response of Prime Lambs to Dietary Omega-3-rich Oils: Impact on Meat Quality

by

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Declaration

I hereby declare that:

- The research presented and reported in this thesis was conducted in accordance with the University of Tasmania Animal Ethics Committee Guidelines, the 1993 Tasmanian Animal Welfare Act and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (Animal Ethics Permit Number A0015657).

- To the best of my knowledge and belief, this thesis contains no material which has been accepted for the award of a degree or diploma by the University or any other tertiary institution. The thesis contains no material previously published or written by any other person (s) except where background information duly acknowledged is made in the text of the thesis.

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Abstract

This thesis investigated the responses of confined and grazing weaner prime lambs to dietary omega-3 (n-3) polyunsaturated fatty acids (PUFA)-rich oil supplementation with regards to animal performance, carcass characteristics, feed conversion efficiency, feed costs and fatty acid profiles of *longissimus dorsi* muscle, liver, kidney and heart. The primary objectives were to systematically investigate and collect scientific evidence on the nutritional enhancement of health beneficial omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) content of confined and grazing weaner lambs supplemented with or without canola, rice bran, flaxseed, rumen-protected and safflower oil-infused pellets and to evaluate the cost of producing premium quality lamb relative to liveweight gain.

Two on-farm experiments representing both indoor lot-fed and outdoor grazing production systems, were conducted to achieve the overarching objectives of the thesis. In Experiment 1 (indoor confined production system), seventy-two, 6 months old, White Suffolk x Corriedale first-cross weaner prime lambs were randomly assigned to six treatment groups: (1) Control: Lucerne hay only; wheat-based pellets infused with 50 ml/kg dry matter (DM) of oil from (2) rice bran (RBO); (3) canola (CO); (4) rumen-protected (RPO), (5) flaxseed (FSO) and (6) safflower (SO) dietary sources in a completely randomized experimental design. All lambs had *ad libitum* access to lucerne hay and clean fresh water and supplemented lambs were fed 1 kg of pellet/head/day for 10 weeks after three weeks of adaptation. Data on daily dry matter feed intake, weekly liveweight and body conformation measurements were recorded. At the end of the feeding trial, all lambs were humanely sacrificed at a commercial abattoir, carcass characteristics evaluated and samples of the *longissimus dorsi* muscle, heart, liver and kidney

were taken. Fatty acid profiles of sampled tissues and organs were analysed by gas chromatography and gas chromatography mass spectrometry. All data were analysed in SAS utilising both general linear (PROC GLM) and mixed model (PROC MIXED) procedures with repeated measures that adjusted for fixed, random and interaction effects.

Supplementation of confined lambs resulted in improvement of dry matter feed intakes, lamb performance and carcass characteristics. RBO and CO treatments had lower feed costs and similar indices of lamb performance, carcass characteristics and over the hooks trade (OTH) incomes compared with other treatment groups. Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) contents of the *longissimus dorsi* muscle of lambs supplemented with CO, FSO, SO and RPO were above the 30 mg per standard serve threshold for omega-3 "source" claim under the Foods Standard Australia and New Zealand (FSANZ) guidelines. The $\geq C_{20}$ n-3 LC-PUFA contents of the *longissimus dorsi* muscle did not differ among supplemented confined lambs. However, variation in fatty acid contents among and between different organs and tissues was observed and the liver and kidney could be labelled as 'good source' (above 60 mg per standard serve threshold) of n-3 LC-PUFA.

From the results in Experiment 1, the following research questions needed to be answered:

1. Given that CO and RBO were the cheapest supplements, was there any added advantage of supplementing lambs grazing lucerne and cocksfoot pastures with CO and RBO oil-based pellets in enhancing EPA+DHA contents in the muscle tissue and organs?
2. Could animal performance, carcass traits, feed conversion efficiency and OTH trade incomes of grazing lambs be improved by additional supplementation with RBO and CO?

Experiment 2 was designed to answer these questions. Therefore, forty-eight White Suffolk x Corriedale first-cross weaners were randomly allocated to one of the following four treatments in a split-plot experimental design: (1) Cocksfoot cv. porto (CFP) or lucerne pastures only (control); (2) CFP or lucerne pastures supplemented with pellets infused with oil from (3) canola (CO); (4) rice bran (RBO) or no oil pellets (NOP). Lucerne and CFP pastures were considered as the main plot effects and pellet supplementation as a sub-plot effect in a feeding trial that lasted for nine weeks.

The findings demonstrated that animal performance and carcass characteristics of lucerne grazing lambs were not affected by pellet supplementation. However, lambs grazing CFP and supplemented with CO had lower feed conversion efficiency (FCE) and higher OTH trade income than CFP only grazing lambs. Lucerne grazing lambs had higher average daily gain, hot carcass weight and OTH trade income than CFP grazing lambs. The addition of pellets to the diet of grazing lambs generally decreased α-linolenic acid (ALA, 18:3n-3) and n-3 LC-PUFA contents and increased the n-6/n-3 ratio in the *longissimus dorsi* muscle. Different types of pastures and pellet supplementation affected the fatty acid profiles of organs and tissues of grazing lambs. For instance, ALA, 20:3n-6, EPA, PUFA, n-3 PUFA, and n-6 PUFA contents in *longissimus dorsi* muscle of lucerne grazing lambs were higher than in CFP grazing lambs. Variation in fatty acid contents in different organs of grazing lambs indicated that the liver and kidney can be used as 'good sources' of n-3 LC-PUFA. Cocksfoot cv. porto produced premium quality, healthy lambs with high contents of ALA and n-3 LC-PUFA.

Taken together, dry matter feed intake, animal performance, carcass characteristics and fatty acid profiles of confined lambs were improved by supplementation with PUFA-rich, oil-infused pellets. RBO and CO can be used to improve n-3 LC-PUFA contents in the *longissimus*

dorsi muscle of confined lambs at low feed costs, with comparable indices of animal performance and carcass characteristics with other sources of PUFA. Supplementation of CFP grazing lambs with CO can be used as a strategic nutrition tool for increasing OTH income with low FCE. CFP also demonstrated the potential for producing premium quality, healthy lambs.

Recommended future studies should focus on:

1) Whole farm production cost-benefit analysis;

2) Marketing omega-3 labelled lamb products;

3) Investigating potential new pasture varieties for improving n-3 LC-PUFA content of grazing lambs; and

4) Better understanding of the rumen biohydrogenation pathways in grazing lambs.

Thesis Publications

Peer-reviewed Journal Papers with 2018 Impact Factor (IF)

1. **Le HV**, Nguyen QV, Nguyen DV, Otto JR., Malau-Aduli BS, Nichols PD, Malau-Aduli AEO 2018. Enhanced omega-3 polyunsaturated fatty acid contents in muscle and edible organs of Australian prime lambs grazing lucerne and cocksfoot pastures. *Nutrients* 10(12):1985 **(IF 4.171**) DOI: 10.3390/nu10121985 https://doi.org/10.3390/nu10121985

2. **Le HV**, Nguyen DV, Nguyen QV, Malau-Aduli BS, Nichols PD, Malau-Aduli AEO 2019. Fatty acid profiles of muscle, liver, heart and kidney of Australian prime lambs fed different polyunsaturated fatty acids enriched pellets in a feedlot system. *Scientific Reports* 9(2): 1238 **(IF 4.011**) DOI: 10.1038/s41598-018-37956-y www.nature.com/articles/s41598-018-37956-y

3. **Le HV**, Nguyen QV, Nguyen DV, Malau-Aduli BS, Nichols PD, Malau-Aduli AEO 2018. Nutritional supplements fortified with oils from canola, flaxseed, safflower and rice bran improve feedlot performance and carcass characteristics of Australian prime lambs. *Animals* 8(12):231 **(IF 1.832**) DOI: 10.3390/ani8120231 https://doi.org/10.3390/ani8120231

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List of Abbreviations

ABARES = Australian Bureau of Agricultural and Resource Economics and Sciences

ABS = Australian Bureau of Statistics

ACIAR = the Australian Centre for International Agricultural Research

ADF = acid detergent fibre

ADG = average daily gain

ALA = alpha-linolenic acid

ARA = Arachidonic acid

ASBVs = Australian Sheep Breeding Values

BCS = body condition scores

BCTRC = boneless, closely trimmed retail cuts

BL = body length

BWT = body wall thickness

CFP = cocksfoot cv. porto

CG = chest girth

CLA = Conjugated linoleic acid

CO = canola oil

CP = crude protein

CSIRO = Commonwealth Scientific and Industrial Research Organization

DE = digestible energy

DHA = docosahexaenoic acid

DM = dry matter

DMI = dry matter intake

DP = dressing percentages

DPA = docosapentaenoic acid

EE = ether extract

EPA = eicosapentaenoic acid

FA = fatty acids

FAME = fatty acid methyl esters

FCE = feed conversion efficiency

FCPUG = feed cost per unit gain

FD = fat depth

FO = flaxseed oil

FSANZ = Food Standards Australia New Zealand

GC = gas chromatograph

HCW = hot carcass weight

IMF = intramuscular fat

JCU = James Cook University

LA = Linoleic acid

LSM = least square mean

LWT = liveweight

ME = metabolisable energy

MLA = Meat and Livestock Australia

MUFA = monounsaturated fatty acids

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

n-3 PUFA = omega-3 polyunsaturated fatty acids

n-6 PUFA = omega-6 polyunsaturated fatty acids

NDF = neutral detergent fibre

NOP = wheat-based pellet without infused oil

OM = organic matter

OTH = over the hook

PUFA = polyunsaturated fatty acids

RBO = rice bran oil

REA = ribeye area

RPO = rumen protected oil

SAS = Statistical Analysis System

SE = standard error

SFA = saturated fatty acids

SO = safflower oil

TDN = total digestible nutrients

UFA = unsaturated fatty acids

UTAS = the University of Tasmania

WH = wither height

WHO = World Health Organization

Δ = Change in

Σ = Sum of

Chapter 1: General Introduction

Omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) are essential for normal human cell functions (Farjadian et al., 2016), but cannot be synthesised by humans due to lack of delta-12 and delta-15 desaturase enzymes and must therefore be provided in the diet (Shahidi and Ambigaipalan, 2018). These n-3 PUFA have many double bonds in their molecular structure, with the first double bond on carbon number 3 from the methyl end (Calder and Yaqoob, 2009). There are four main n-3 LC-PUFA, namely; alpha-linolenic acid (ALA, 18:3n-3), docosapentaenoic acid (DPA, 22:5n-3), eicosapentaenoic acid (EPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Abedi and Sahari, 2014) that have a broad range of health benefits for humans in the prevention of chronic inflammatory diseases (Yates et al., 2014), protection, prevention and treatment of cardiovascular diseases (Cao et al., 2015; Kazemian et al., 2012; Yamagishi et al., 2008) and reduction in the risk of certain types of cancer (Witte and Hardman, 2015; Zarate et al., 2017). These n-3 LC-PUFA are found in brain and retinal structures where they play significant roles in both visual and neuronal functions associated with brain health and cognition in humans (Brenna and Carlson, 2014; Huhn et al., 2015; Nakamura and Nara, 2004; Querques et al., 2011).

In a global survey, Stark et al. (2016) found that most countries had very low to low range of blood $\geq C_{20}$ n-3 LC-PUFA, which may increase the risk of chronic diseases. In Australia, Meyer (2016) reported that approximately 80% of Australians did not meet the n-3 LC-PUFA recommended intakes for optimal health and 90% of childbearing women did not meet the recommendations for DHA intakes during pregnancy and lactation. Therefore, studies on increasing n-3 LC-PUFA intakes are topical and have drawn considerable nutrition research

interest (Tocher et al., 2019). There are many dietary sources of n-3 LC-PUFA which include marine, plant and animal derived products. Out of these sources, marine products are generally the richest in n-3 LC-PUFA content (Nichols et al., 2010). Considering the rapidly growing human population, marine products are under immense pressure in meeting the n-3 LC-PUFA needs for human nutrition, aquaculture feeds, and the nutraceutical and pharmaceutical industries (Amjad Khan et al., 2017; Nichols et al., 2010; Sprague et al., 2016). Plant-derived sources of the shorter chain ($\leq C_{18}$) n-3 PUFA are rich in alpha-linolenic acid (ALA). However, the low bioconversion of ALA to n-3 LC-PUFA due to the competition between n-3 and n-6 PUFA, restrict the health beneficial effects of plant-derived sources of n-3 LC-PUFA (Rajaram, 2014; Shahidi and Ambigaipalan, 2018).

Although animal products are regularly consumed by humans, they have low n-3 LC-PUFA content (Nichols et al., 2010). Notwithstanding, these animal products are an important source of high-quality nutrients for humans worldwide (Li et al., 2019). For example, the lipid intakes of the French population contains approximately 60% of animal-derived products (Schmitt et al., 2018). In 2018, Australia and the United States of America had similar meat consumption of approximately 115 kg per person, of which fish only accounted for 15 kg, with the remaining amount from poultry, pork, beef, veal and sheep meat (Whitnall and Pitts, 2019). Considering the current context of the dietary significance of omega-3 PUFA, animal products enhanced with n-3 LC-PUFA for regular human consumption, such as poultry, dairy and meat, have received considerable research interest in recent years (Lee et al., 2019). Out of the animal products, red meat, including lamb, constitutes a rich source of protein, micronutrients and n-3 LC-PUFA, which can be enhanced by dietary manipulation (Chikwanha et al., 2018; Howe et al., 2007; McNeill and Van Elswyk, 2012; Pethick et al., 2010).

Australia was the largest exporter of sheep meat in the world in 2017 (MLA, 2018c) having exported 61% of its total lamb production (532,000 tonnes carcass weight) and 96% of mutton production (204,000 tonnes carcass weight). Domestic lamb consumption was around 9 kg per capita and remained one of the largest per capita consumption of sheep meat in the world (MLA, 2018a). Consumers are more aware of the link between diet and health (Grasso et al., 2014). Consequently, meat products with health attributes are now more competitive in the global market (Font-i-Furnols and Guerrero, 2014; Resurreccion, 2004). Australian lamb faces strong competition on the global market due to the high price of lamb compared with pork or chicken and the rising health and environmental awareness of consumers (Montossi et al., 2013; Sañudo et al., 2013; Wong et al., 2015). Therefore, studies on enhancing n-3 LC-PUFA content of Australian lamb are expected to drive the potential increase in n-3 LC-PUFA intakes in addition to elevating the reputation of Australian lamb as a healthy food leading to high competitiveness on the global market.

Research on improving omega-3 PUFA and n-3 LC-PUFA content of lamb has achieved some promising results. In the confined feedlot system, many sources of PUFA-rich feeds such as fish oil, algae and plant-derived oils have been explored and tested for optimal supplementation levels. For example, Nguyen et al. (2017a) demonstrated that supplementation of confined lambs with 5% flaxseed or canola oil infused pellets increased n-3 LC-PUFA content in the *longissimus dorsi* muscle tissue. Annett et al. (2011a) revealed that supplementing indoor lambs with cereal-based concentrate enriched with fish oil increased EPA and DHA content in the muscle. However, there are a limited number of comparative studies between different PUFA-rich feeds under the same confined management conditions and their associated impacts on the effectiveness of n-3 LC-PUFA improvement in lamb. Similarly, the development of low feed costs to support lamb producers in better utilising their resources for producing premium

healthy lamb with high content of n-3 LC-PUFA are yet to be explored in any great detail. In lamb grazing production systems, some studies have shown that different types of pastures and PUFA-rich supplements affect lamb n-3 LC-PUFA content (De Brito et al., 2017a; Ponnampalam et al., 2012). Ponnampalam et al. (2014a) analysed EPA and DHA contents in the muscles of grazing lambs and concluded that finishing lambs on lucerne pasture had the potential to consistently result in high levels of omega-3 PUFA. However, there are still many other grasses and legumes currently being used in the prime lamb industry with the potential to improve n-3 LC-PUFA content of lamb, that have not yet been studied for their fatty acid composition and impacts on lamb performance and meat quality. Other than the use of raw PUFA-rich cracked flaxseed or flaxseed meal or grain and commercial feeds (Annett et al., 2011a; Boughalmi and Araba, 2016; Ponnampalam et al., 2012), there are no previous studies on the effect of supplementing grazing lambs with oil-infused pellets for improving n-3 LC-PUFA in lamb.

Therefore, the series of studies reported in this thesis were conducted for the purpose of answering the following key research question: What are the responses of prime lambs to dietary n-3 PUFA-rich oil supplementation and different types of pastures with regards to animal performance, carcass characteristics, feed conversion efficiency, feed costs, over the hooks trade income and fatty acid profiles of muscle, liver, kidney and heart in both indoor lot-fed and outdoor grazing systems?

The hypotheses tested to fill existing knowledge gaps and address the challenges to improving the quality of Australian lambs were as follows:

- In both indoor and outdoor production systems, the performance and carcass characteristics of confined and grazing prime lambs would be improved by

supplementation with n-3 PUFA-rich oil pellets in comparison to the control (grazing or only lucerne hay fed) lambs;

- The *longissimus dorsi* muscle, heart, kidney and liver tissues of prime lambs fed different n-3 PUFA-rich oil pellets would exhibit higher PUFA contents than only grazing or lucerne hay fed lambs; and
- Fatty acid profiles of confined and grazing prime lambs fed n-3 PUFA-rich oil pellets would vary according to type of pasture, tissue or organs.

The thesis is structured into the following chapters:

Chapter 1: General introduction

Chapter 1 gives a contextual background of benefits, intakes and sources of n-3 LC-PUFA for human consumption and changes in consumer perception towards healthy meat products, research questions, tested hypotheses and thesis structure.

Chapter 2: Literature review

Chapter 2 provides a narrative on comprehensive access, retrieval and review of the published literature focusing on the Australian sheep industry, prime lamb production, health benefits and sources of n-3 PUFA consumption and lipid metabolism in ruminants with emphasis of overcoming ruminal biohydrogenation. This chapter also summarises previous studies on the nutritional manipulation of fatty acid profiles in prime lambs for improving animal performance and meat quality using different PUFA-rich feeds in both confined and grazing systems.

Chapter 3: Nutritional supplements fortified with oils from canola, flaxseed, safflower and rice bran improve feedlot performance and carcass characteristics of Australian prime lambs

Chapter 3 investigates live animal performance and carcass characteristics of Australian prime lambs fed oil based PUFA enriched pellets in a feedlot system. It tested the hypothesis that supplementation of lambs with a variety of dietary oil based PUFA enriched pellets would enhance growth and carcass characteristics compared with control lambs fed only lucerne hay.

Chapter 4: Fatty acid profiles of muscle, liver, heart and kidney of prime lambs fed different polyunsaturated fatty acids enriched pellets in a feedlot system

Chapter 4 explores the hypothesis that the fatty acid profiles of the *longissimus dorsi* muscle, liver, kidney and heart of prime lambs would differ in response to supplementation with various dietary PUFA sources under a typical intensive, in-door feedlot management system.

Chapter 5: Performance and carcass characteristics of prime lambs grazing lucerne and cocksfoot pastures are enhanced by supplementation with plant oil infused pellets

Chapter 5 evaluates the effects of cocksfoot cv. porto (CFP) or lucerne pastures and supplementation with or without plant oil infused pellets on lamb performance and carcass characteristics. The tested hypothesis was that grazing lambs will respond differently to different pastures and additional supplementation of grazing lambs with plant oil infused pellets would improve performance and carcass characteristics.

Chapter 6: Enhanced omega-3 polyunsaturated fatty acid contents in muscle and edible organs of prime lambs grazing lucerne and cocksfoot pastures

Chapter 6 aims to (i) evaluate the potential of CFP grass to produce healthy, premium quality lamb with high content of health claimable n-3 LC-PUFA and (ii) to examine the effect of

different pasture types and supplementation of lambs with pellets with or without plant oil infusion, on the fatty acid contents of muscle and edible organs of prime lambs.

Chapter 7: General discussion, conclusions, implications, and recommendations

Chapter 7 presents a holistic and integrated summary of the findings of this thesis and their relation to the initial hypotheses. This chapter also provides key messages to lamb producers and readers and identifies areas warranting future research.

Appendix: Includes all supplementary data and hard copies of all published papers from this thesis.

Chapter 2: Literature Review

2.1. The Australian sheep industry

2.1.1. Historical background of the Australian sheep industry

The Australian sheep industry has a long history. The first flock of purebred Spanish Merino wool sheep arrived in Sydney Cove in 1797 (Evesson and Moor, 2000) from the Cape of Good Hope. John Macarthur and Reverend Samuel Marsden made a significant contribution to establishing the Australian wool industry using the Spanish Merinos (Henzell, 2007). The sheep industry expanded and driven by an increase in wool export demand, the sheep population peaked at 180 million in 1970 (East and Foreman, 2011), significantly declined in 1971-1982, recovered and peaked again in 1990 at 170 million (Figure 2.1). By 1990-2010, the Australian sheep industry had undergone irreversible transformation with a sustained decrease in the value and scale of the wool industry and a steady rise in production and price of lamb and mutton (Rowe, 2010). This transformation made the lamb industry no longer an add-on enterprise of the Merino wool industry. In other words, the Australian sheep industry became dual-purpose focussing on both meat and wool production.

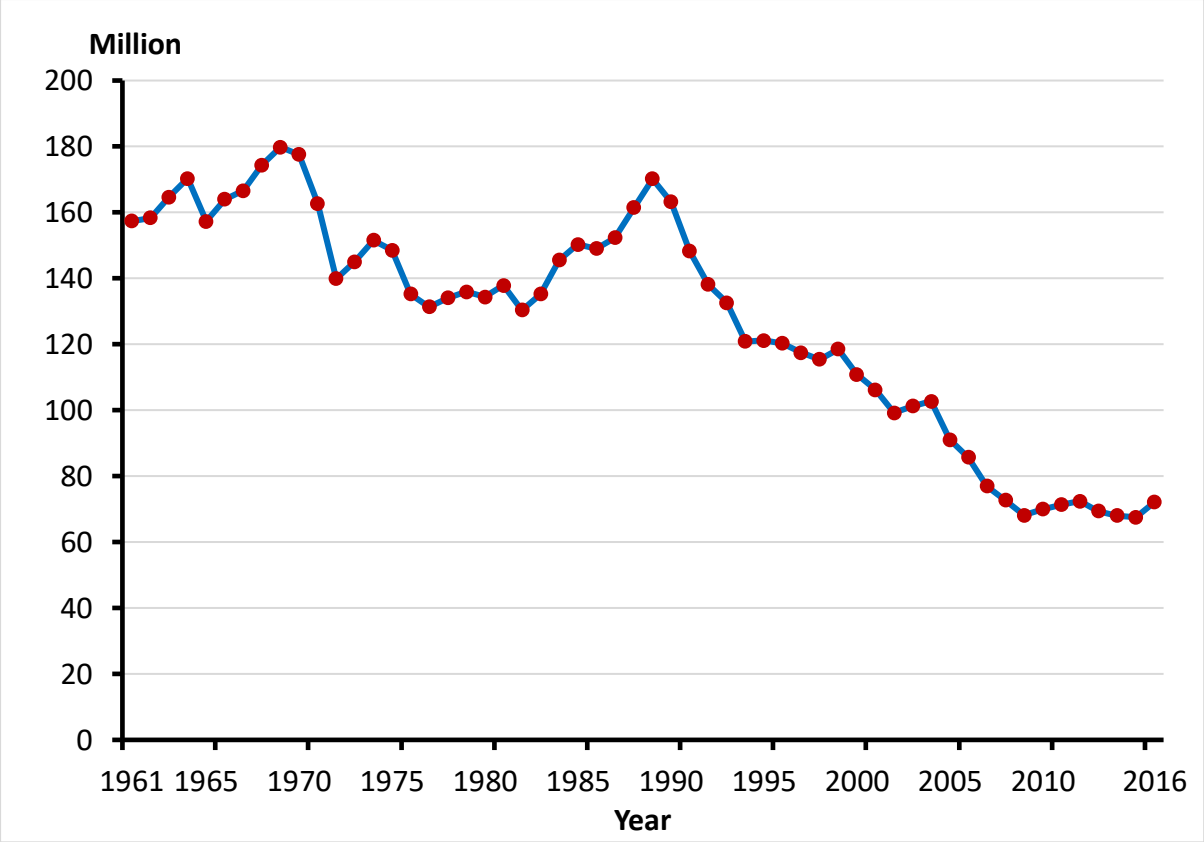

Figure 2. 1. The Australian sheep population during the period 1961-2019 (ABS, 2018).

2.1.2. Wool production

Wool production was the main focus of the Australian sheep industry right from the arrival of the first sheep flock until 1990 (Henzell, 2007). By 1970 when the sheep population peaked, wool production had contributed 15% to total gross value of national agricultural production (ABS, 2003). The use of wool fibre substitutes and the huge development of artificial fibres in the 1970s to 1980s, triggered negative impacts on the wool market (Valera et al., 2009).

With the establishment and operation of the Australian wool reserve price scheme from 1974 to 1990, the wool price stabilised and then increased (Bardsley, 1994). However, the collapse of the wool reserve price scheme in 1991 led to major structural adjustments to the Australian wool industry. The fall in wool prices relative to that of other commodities led to substantial movements away from wool production and sheep numbers fell (Ashton, 1998). Over the last two decades, the Australian wool industry witnessed a downward movement in wool

production and value (Figure 2.2), particularly from 2000-2009, but from 2009 to 2016, wool production remained stable and wool value increased again. The increase in wool value has been attributed to the high wool price in recent years. Although wool production significantly declined over the last decade, it is still an integral part of the Australian sheep industry with a farm gate value of $AUD 3.6 million in 2016 (ABARES 2016).

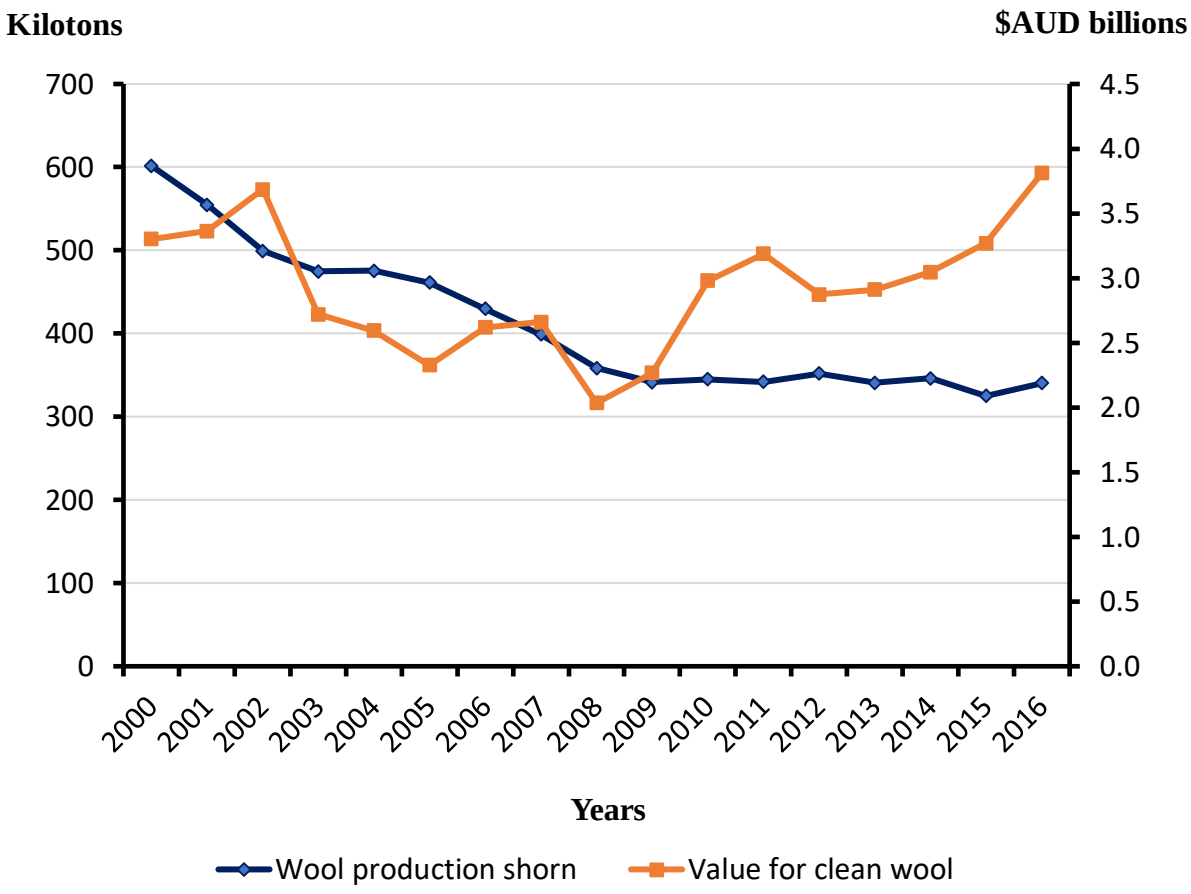

Figure 2. 2. Wool shorn and value for clean wool production from 2000 to 2016 (ABS, 2018)

2.1.3. Meat production

Sheep meat production had not been the main focus of the Australian sheep industry before its transformation about 30 years ago (Ashton, 1998; Rowe, 2010). The sheep meat industry has been integral to Australia's agricultural identity and had a gross value of $AUD 5.3 million in 2016-17 (MLA, 2017b). Australian sheep meat production is divided into lamb and mutton

production. Over the last two decades, lamb exports increased by 2.5 times, while mutton export highly fluctuated and fell by 30% as depicted in Figure 2.3. The reduction in mutton export originated from the declining number of slaughtered sheep due to drought and the decline in sheep population (MLA, 2017b). Despite the declining sheep population, Australian lamb production continued to increase by about 67% from 338 kilotons in 2002 to 506 kilotons in 2016 (Martin, 2012; MLA, 2017a). By 2016-2017, the lamb industry had contributed around 6% ($3.6 billion) of the gross value of agricultural production and around 4% ($1.9 billion) of agricultural export income (ABARES, 2017). This increase in Australian lamb production was driven by the high price of lamb due to increased demand in the global market, especially in China and USA. The price of lamb has increased by more than 50% over the last 20 years (MLA, 2018b).

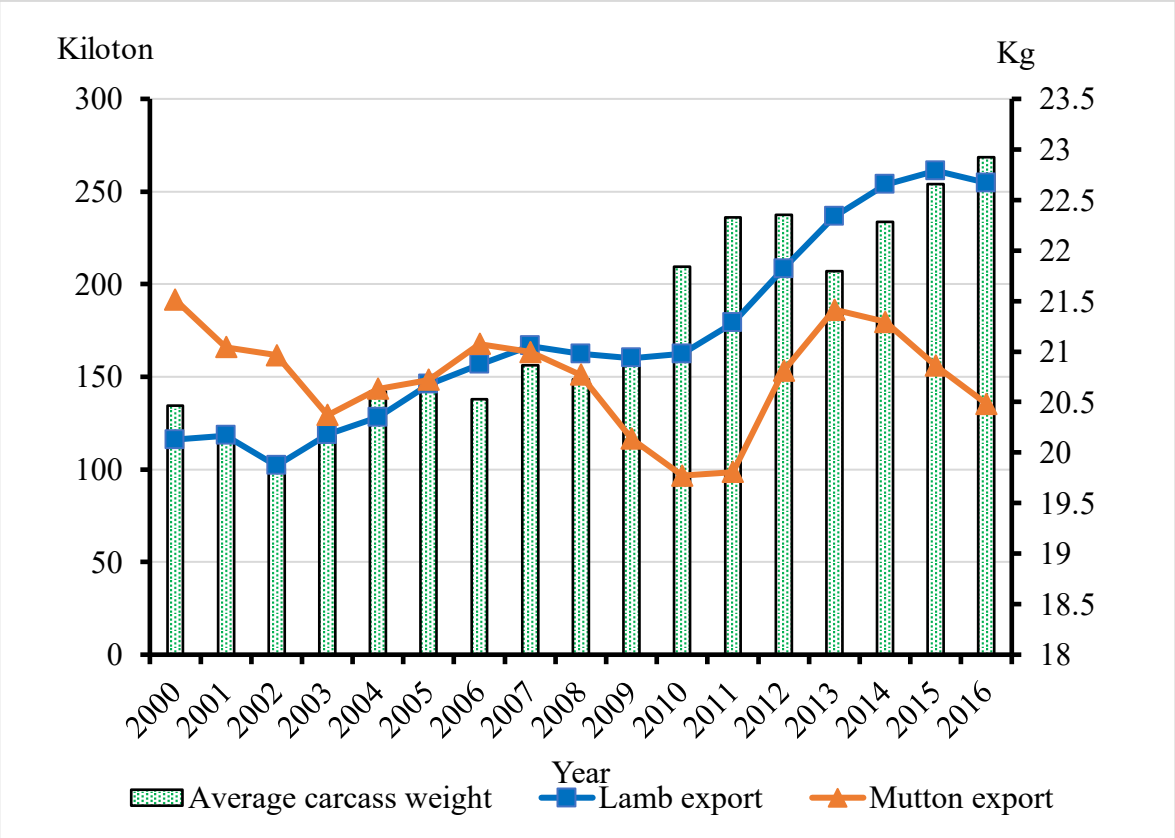

Figure 2. 3. Sheep meat export and average carcass weight in Australia during the period 2000-16 (ABS, 2018).

Although the Australian sheep meat industry suffered due to the negative impacts of drought, low price and decreased demand for wool in the global market in the past few decades, Australia was still the largest exporter of sheep meat in the world in 2017 (MLA, 2018c). The main export markets for Australian lamb in 2017 were China, USA and The Middle East, which accounted for approximately 70% (Figure 2.4). With the increase in lamb production, average carcass weight also rose from 20.5 kg in 2000 to 22.9 kg in 2016 (Figure 2.2). There were many reasons for the increase in average carcass weight; the most important being genetic improvement of lambs through the following programmes supported by Meat & Livestock Australia:

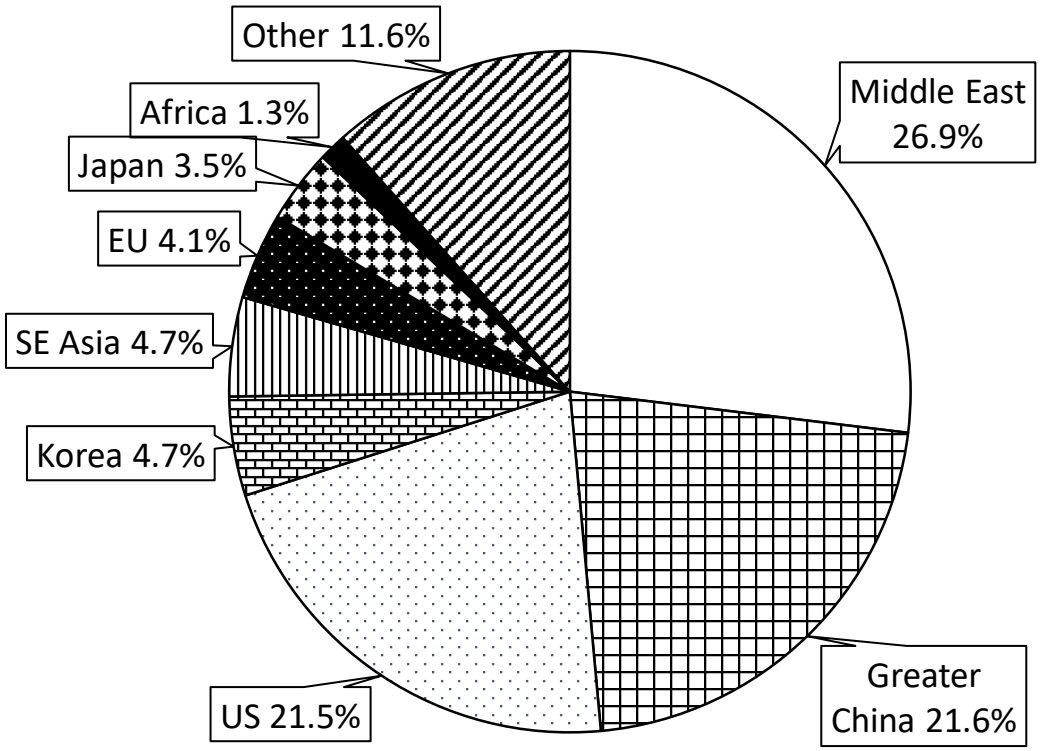

Figure 2. 4. Australian export markets for lamb in 2017 (Source: MLA, 2018c).

- *Genetic improvement for large and lean lamb carcass:*

During the 1980s, the consumption of lamb had significantly decreased due to a product that was considered too fat, reducing household sizes and increasing awareness of the impact of food choices on human health (Hopkins and Fowler, 2018). This drove lamb production

systems towards producing heavier, leaner lambs to meet the requirements of both the domestic and export markets (MLA, 2009). Considering the situation, Meat & Livestock Australia established and operated many programmes for lamb genetic improvement like LAMBPLAN, Terminal Sire Central Progeny Test and Maternal Sire Central Progeny Test.

LAMBPLAN was the principal genetic improvement system used by the Australian lamb industry. Established in 1989, LAMBPLAN provided practical information for terminal, maternal and dual purpose sheep producers on the genetic potential of their sheep (MLA, 2019a). Harris and Ryce (2005) estimated that LAMBPLAN influenced about 70% of the gene pool of prime lamb production in Australia. Sheep registered to LAMBPLAN were ranked according to various production characteristics using Australian Sheep Breeding Values (ASBVs). The Terminal Sire and Maternal Sire Central Progeny Tests helped sheep producers to achieve a higher percentage of lambs that met carcass specifications or reached the targeted market specifications using ASBVs from LAMBPLAN (MLA, 2009). Anderson et al. (2015) reported that the use of ASBVs in the selection of Australian lambs for increased lean tissue and reduced carcass fatness resulted in decreased intramuscular fat percentage of the *longissimus lumborum*. Gardner et al. (2010) also revealed that selection of sires for producing prime lambs based on ASBVs remarkably improved dressing percentage and delivered larger leaner carcasses. The genetic improvement of lamb through the programmes conducted by Meat & Livestock Australia, has made a significant contribution to the success of Australian prime lamb in the last three decades. Although efforts geared at genetic improvement of lamb has enabled the industry to produce larger, leaner carcasses, meat eating quality traits of taste, tenderness, flavour and overall liking by consumers are inevitably compromised (Wood et al., 2004). Therefore, studies on improving lamb eating quality through increased intramuscular

fat (IMF) and lower fat melting points are currently receiving major interests from the prime lamb industry.

2.1.4. Finishing lamb systems in Australia

The Australian lamb industry has grown quickly over the last 30 years to become an important industry in the agricultural sector. One of the critical factors significantly contributing to the success of the Australian lamb industry is the sustainable development of the flexible lamb finishing system. The lamb finishing system in Australia is almost entirely based on a grazing system, with only a small number of lambs being finished in the feedlot system (Morris, 2017). The lamb finishing system in Australia can be classified into the following four main types (MLA, 2011):

- Traditional breeder-finishing
- Specialist pasture finishing
- Specialist grain-based finishing
- Opportunistic grain-based finishing

Traditional breeder-finisher

In the traditional breeder-finisher system, lamb producers use all finishing lambs from their own stock rather than purchasing lambs externally, and the lambs are finished on pastures, fodder crops or stubbles after weaning. This lamb finishing system helps lamb producers in reducing the expenses of purchasing lambs and feeds.

Specialist pasture/fodder crop finishing

In the specialist pasture/fodder crop finishing system, all lambs are purchased and finished on pastures, fodder crops or stubbles. This system utilises the advantages of seasonal conditions and high lamb prices to increase profits for lamb producers.

Specialist grain-based finishing systems

Here, lamb producers purchase lambs and finish them in well-structured feedlot conditions using purchased or home-grown grains, roughages, pellets or total mixed ration feed resources. This system is operated all year round with a number of finishing cycles completed within a year and aims to increase profitability by maximising the high throughput feeding system.

Opportunistic grain-based finishing systems

This finishing system is similar to the specialist grain based finishing system, but operates with small feedlots and may only run one cycle per year depending on favourable market conditions or lamb prices. Furthermore, the opportunistic grain-based finishing systems can complement other farm enterprises in which producers sacrifice paddocks to build feedlots. This finishing system is able to maximise profitability per head.

2.1.5. Challenges of the Australian lamb industry

2.1.5.1. Production

Australian lamb production has continuously grown over the last 20 years from 300,000 tonnes to 510,000 tonnes (MLA, 2019b). The continuous increase in lamb production could be attributed to the high price trend and genetic improvement of lamb growth and eating quality (Rowe, 2010). However, the Australian lamb industry was facing many challenges in managing continuous improvement for sustainable future development (Pethick et al., 2010). The fluctuation in herbage quantity and quality, which occurred within and between years and affected animal production and quality, was the first challenge in lamb production (De Brito et al., 2017b; Ferguson et al., 2011; Shakhane et al., 2013). The second challenge was climate change, which impacted livestock systems including lamb production via direct effects on animal physiology, behaviour, production and welfare and indirectly through feed availability, composition and quality (Gowane et al., 2017; Henry et al., 2018; Rojas-Downing et al., 2017). Furthermore, the ratio of the cost of sheep in relation to the cost of growing crops, which has

increased since 2001, would slow any swing out of cropping into lamb despite the attractive lamb prices compared to crop prices (MLA, 2017b).

2.1.5.2. Market

- Domestic market: Lamb had long been considered a traditional meal in Australia (Hopkins and Fowler, 2018). However, lamb only captured a 12% share of fresh meat retail sales in 2018 compared with 30% and 35% share of chicken and beef, respectively (MLA, 2019b). The lamb was priced higher than chicken and pork; therefore, lamb cannot compete on price alone with chicken and pork (MLA, 2019b; Pethick et al., 2010). As a result, pricing is a biggest challenge of lamb on domestic market. Wong et al. (2013) reported that chicken and pork had increased their share by 3 and 2 times, respectively, in the last 50 years at the expense of beef, lamb and mutton. Furthermore, the stagnant wage growth and increased living costs had driven many lamb consumers to become more price sensitive (MLA, 2019b). The another challenge for increasing domestic market share of lamb was that many migrants favoured pork and chicken as their traditional meat rather than lamb (Locke and O'Connor, 2017).
- International market: Sheep meat only captured about 5% of global protein consumption (Locke and O'Connor, 2017). Australia and New Zealand were two largest sheep meat exporters, which accounted for 70% of total global trade (MLA, 2019b). The biggest challenge for Australian sheep meat including lamb on international market was pricing because the price of lamb has been higher than chicken and pork over the last 10 years (MLA, 2017b). Furthermore, Australian lamb was exported to many countries with various market specifications (Colby, 2015). This is another challenge for Australian lamb industry due to that the lamb industry must maintain consistent quality and meet many specific market requirements.

2.1.5.3. Change in consumer perception towards healthy lamb

The economic development along with disposable income increase and the increase of non-communication diseases made people more aware of link between diets and health (Bruins et al., 2019; Kearney, 2010). Therefore, smart foods, or foods with functions that conferred health benefits, were the future of the food and nutrition sectors (Bermingham et al., 2008). In line with this trend, the lamb consumers were willing to pay more for high quality and healthy lamb (Montossi et al., 2013; Tighe et al., 2018). As a result, the lamb industry must produce lamb with health attribution for meeting emerging customer demand. This is a challenge for lamb industry; however, it also is an opportunity for lamb industry development because lamb can be manipulated to improve the health benefits for human (Ponnampalam et al., 2014a).

2.2. Structural features, health benefits and sources of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) for humans

2.2.1. Structural features of omega-3 polyunsaturated fatty acids

Omega-3 polyunsaturated fatty acids (PUFA) are classified as a family of PUFA with the first double bond on carbon number 3 from the methyl end as shown in Figure 2.5 (Calder and Yaqoob, 2009; Calder, 2017).

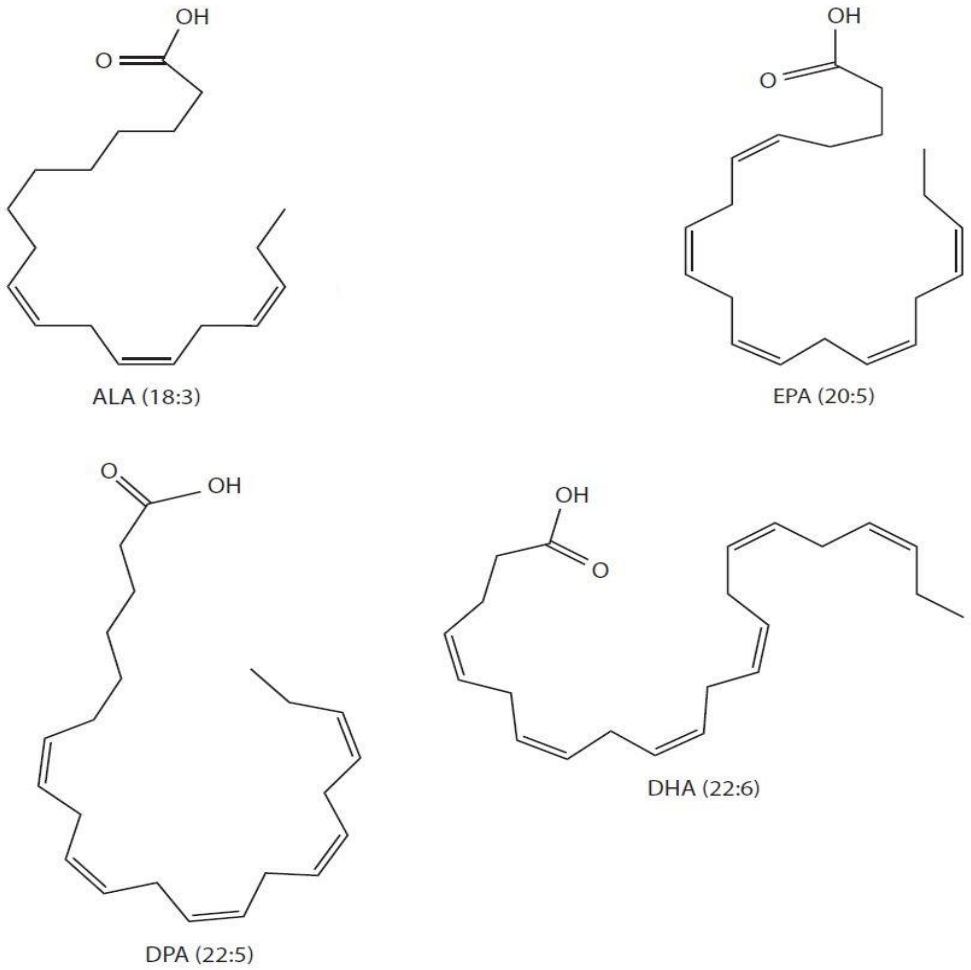

Figure 2. 5. Chemical structures of omega-3 polyunsaturated fatty acids and omega-3 long-chain polyunsaturated fatty acids (Source: Shahidi and Ambigaipalan, 2018)

Omega-3 PUFA (n-3 PUFA) play a crucial role in the prevention of non-communicable diseases and can contribute to a longer and healthier life (Li, 2015; Shahidi and Ambigaipalan, 2018; Xu, 2015). Therefore, many recent studies have focused on the benefits of omega-3 PUFA towards human health. The four important omega-3 PUFA include ALA, EPA, DHA and DPA. ALA is the precursor PUFA for the synthesis of n-3 LC-PUFA (EPA, DHA and DPA), through chain elongation and desaturation processes (Shahidi and Ambigaipalan, 2018). EPA and DHA are well recognised for many health benefits; however, in the recent years, DPA has also been demonstrated to have the same health benefits as EPA and DHA (Byelashov et

al., 2015). These n-3 LC-PUFA are termed essential fatty acids because mammals cannot synthesise them due to their lack of Δ12- and Δ15-desaturases (Burdge and Calder, 2014).

2.2.2 Omega-3 health benefits

2.2.2.1. Anti-inflammatory properties of omega-3 fatty acids

Inflammation is a normal host defence mechanism by which the host physiologically responds to infection and other harmful problems in the surrounding environment (Calder, 2009). This mechanism initiates pathogen killing as well as tissue repair processes and helps to restore homeostasis at infected or damaged sites. Most chronic diseases, such as diabetes and obesity, cancer, cardiovascular and neurodegenerative diseases, manifest some symptoms of inflammation at certain levels, i.e. pain, heat, redness, swelling and loss of function. Nevertheless, other disorders such as allergies, asthma, arthritis and autoimmune diseases are also classified as inflammatory diseases (Zarate et al., 2017). Tortosa-Caparrós et al. (2017) demonstrated that n-3 LC-PUFA had an anti-inflammatory role and gave a detailed explanation of n-3 LC-PUFA being precursors of eicosanoids with anti-inflammatory properties. It has been demonstrated that dietary supplementation with n-3 LC-PUFA reduced inflammation and therefore improved the health status in some diseases. For instance, Wan et al. (2015) revealed that the provision of n-3 LC-PUFA had a therapeutic effect on survival rate in patients with systemic inflammatory response syndrome. Yates et al. (2014) reported that supplementation with n-3 LC-PUFA was beneficial in some chronic inflammatory diseases. Klemens et al. (2011) did a systematic review and concluded that n-3 LC-PUFA supplementation during pregnancy decreases childhood asthma.

2.2.2.2. Cardiovascular diseases

Cardiovascular diseases are the leading cause of death worldwide (WHO, 2017). However, these diseases could be managed and prevented through healthy dietary habits and daily physical activities (Willett et al., 2006). The beneficial effect of n-3 LC-PUFA on cardiovascular diseases was first noticed in the mid-twentieth century when the British physiologist Hugh Sinclair, undertook his first visit to the Inuit and became convinced that the Inuit diet protected them against atherosclerosis and Western diseases (Kromhout et al., 2012). After that, the cardiometabolic effects of n-3 LC-PUFA continued to be extensively investigated and still remains an active area of research to this day.

Leslie et al. (2015) reported that n-3 PUFA play an important role in the maintenance of cardiovascular health and disease prevention. Yamagishi et al. (2008) conducted a nation-wide community-based cohort of Japanese men and women and suggested that there was a protective effect of n-3 LC-PUFA intake from fish on cardiovascular diseases. Cao et al. (2015) also revealed that n-3 LC-PUFA therapy was a useful tool in the primary and secondary prevention of cardiovascular diseases. Beyond the protective effect and prevention of cardiovascular diseases, dietary supplementation with n-3 LC-PUFA improved the result of disease treatments. Nestel et al. (2015) demonstrated that n-3 LC-PUFA supplements were effective in the treatment of hypertriglyceridaemia. Kazemian et al. (2012) concluded that the use of n-3 LC-PUFA supplementation was promising to improve clinical outcomes for heart failure patients with diabetes. While studies on the mechanisms of n-3 LC-PUFA effects on cardiovascular diseases are still on-going, the main effects of n-3 LC-PUFA in the human body involve increased arrhythmic thresholds, improved arterial and endothelial functions and reduced platelet aggregation and blood pressure (Kromhout et al., 2012).

2.2.2.3. Cancer

Cancer is defined as a large group of diseases related to uncontrolled growth of cells that can invade other normal parts and expand throughout the body (Jones and Baylin, 2007). The initiation and development of cancer processes are related to many factors such as inherited mutations, hormonal changes, alcohol consumption, smoking, pollution, etc (Anand et al., 2008; Ratna and Mandrekar, 2017). N-3 PUFA supplementation is mainly used as a supportive therapy in cancer treatments and for reducing the risk of certain types of cancer (Berquin et al., 2008; Gu et al., 2015). Zarate et al. (2017) reported that the increase in n-3 LC-PUFA consumption and an omega-6/omega-3 ratio of 2–4:1 were associated with a reduced risk of breast, prostate, colon and renal cancers. Witte and Hardman (2015) also concluded that the consumption of n-3 PUFA is associated with a reduced risk of breast cancer. The cause and progression of cancer are complicated, hence the mechanism of n-3 PUFA action on cancer is still not clearly understood and will be an enormous challenge for future research (D'Eliseo and Velotti, 2016; Martin et al., 2013). However, the promising effect of n-3 PUFA on certain types of cancer are related to their ability to modulate membrane-associated signal transductions and gene expressions involved in cancer pathogenesis that supress systemic inflammation (Nabavi et al., 2015).

2.2.2.4. Visual and brain functions

Omega-3 PUFA, especially DHA, are a major component of the human brain and have consistently been shown to have unique and indispensable roles in the neuronal membrane (Dyall, 2015; Lauritzen et al., 2016). Nakamura and Nara (2004) revealed that DHA was required in both visual and neuronal functions. The human brain requires an ample and sustained source of DHA from the diet in order to develop to its full potential (Brenna and Carlson, 2014). N-3 LC-PUFA also play an important role in cognitive performance throughout

all life stages (Stonehouse, 2014). Kuratko et al. (2013) reviewed studies on the relationship between DHA and learning and behaviour in healthy children which implicated problems in learning and behaviour as detrimental effects of DHA deficiency. Huhn et al. (2015) also found that consumption of n-3 LC-PUFA from fish or fish oil-supplements exerted positive effects on brain health and cognition in older humans. Furthermore, n-3 LC-PUFA supplementation could also benefit older adults with memory complaints, mild cognitive impairment and Alzheimer's disease (Cederholm, 2017). Besides cognitive effects, n-3 LC-PUFA have positive effects on visual function in humans. Sapieha et al. (2012) conducted a trial with an n-3 LC-PUFA diet using a mouse model with type 2 diabetes mellitus, and identified beneficial effects of dietary n-3 LC-PUFA on visual function. SanGiovanni and Chew (2005) concluded that n-3 LC-PUFA may act in a protective role against ischemia-, light-, oxygen-, inflammatory-, and age-associated pathology of the vascular and neural retina. Jacques et al. (2011a) conducted studies on the long-term effect of n-3 LC-PUFA intake during gestation on visual development and demonstrated beneficial effects of DHA intake during gestation on visual system function at school age.

2.2.3. Sources of omega-3 fatty acids for human consumption

The human body is unable to synthesise all n-3 PUFA as a result of the limitation of the enzyme responsible for inserting *cis* double bonds (Shahidi and Ambigaipalan, 2018). Therefore, humans must obtain these n-3 PUFA from their diets. There are diverse sources of n-3 PUFA and n-3 LC-PUFA for human consumption, which include marine sources for the key LC-PUFA (fish and algae), plant derived sources for the shorter chain ($\leq C_{18}$) PUFA (mainly some seeds, nuts and vegetable oils) and animal products (meat, milk and egg) containing both PUFA and LC-PUFA.

- *Marine sources:* Seafood has high contents of n-3 LC-PUFA (mainly EPA, DHA and DPA) and is generally regarded as the best and a safe source of LC omega-3 oils amongst the common food groups (Nichols et al., 2010). However, fisheries are currently producing the maximum fish stocks per annum in order to supply fish for human consumption, as well as supplying feed for industrial fish farms and fish oil containing nutraceutical and pharmaceutical products (Garcia and Rosenberg, 2010; Lenihan-Geels et al., 2013). Therefore, it is necessary to research for alternative and sustainable supplies of LC omega-3 oils.

- *Plant derived sources of n-3 PUFA:* The primary source of ALA in plants is mainly concentrated in some seeds, nuts and seed oils. There are a number of oil seeds such as flaxseed, canola, safflower, chia seeds, walnut, camelina and echium that are known to be good sources of ALA (Shahidi and Ambigaipalan, 2018). The oils extracted from these seeds have high ALA content and are good sources of n-3 PUFA for humans and animals. However, the bioconversion of ALA to the LC-PUFA - EPA and DHA, which have many beneficial health effects, is limited in humans (Calder, 2015). Therefore, there is the need to conduct research on ways to increase EPA and DHA intake in humans from these omega-3 PUFA sources. Many recent studies have shown that supplementation of domestic animals, especially ruminant livestock, with plant derived sources of n-3 PUFA enhances the n-3 LC-PUFA content of animal products (Chikwanha et al., 2018; Nguyen et al., 2019). Furthermore, with developments in genetic engineering technology, the feasibility of developing novel land plants with the ability to produce high amounts of LC omega-3 oils (Wijesundera et al., 2011) is now achievable. Positive results on improving the usage and production of plant derived sources of n-3 LC-PUFA would substantially reduce dependence on n-3 LC-PUFA sources from fish, introducing a much more sustainable and economically viable source (Lenihan-Geels et al., 2013).

- *Animal products:* Animal products including meat, milk and egg generally have low omega-3 PUFA content in comparison to marine sources (Nguyen et al., 2019). However, in Western countries, people consume more animal products as sources of n-3 PUFA (Nguyen et al., 2018a). Among animal products, red meat is an important source of n-3 PUFA in addition to providing humans with protein and micronutrients (Howe et al., 2007; McNeill and Van Elswyk, 2012). Studies on ruminants have demonstrated that the n-3 PUFA and n-3 LC-PUFA content of red meat can be manipulated using diets (Alvarenga et al., 2015; Chikwanha et al., 2018; Shingfield et al., 2013). This may help increase the intake of n-3 PUFA and n-3 LC-PUFA in countries with high consumption of red meat like Australia, New Zealand and the United States (Whitnall and Pitts, 2019). Furthermore, Coelho et al. (2016) revealed that consuming diets with improved n-3 LC-PUFA profiles could provide healthier diets without increasing environmental impacts or pressure on fish stocks.

2.3. **Nutritional manipulation of FA in lamb for human consumption and well-being**

2.3.1. Lipid metabolism in ruminants

2.3.1.1. Lipids

Lipids are generally defined as biological substances that are hydrophobic in nature and in many cases, soluble in organic solvents (Fahy et al., 2005). Lipids range in structure from simple short hydrocarbon chains to more complex molecules, including triacylglycerols (TAG), phospholipids (PL) and sterols and their esters (Burdge and Calder, 2014). The major components of TAG (aka triglycerides), PL, and other complex lipids are fatty acids. These lipids and their fatty acid components are widely dispersed in nature and play an important role in the cell structure and signal transduction processes in mammals (Calder, 2015; Müller et al., 2015).

2.3.1.2. Function of lipids in the body

- *Storage and provision of energy:* Lipids are a rich source of energy, yielding twice as many calories per gram than sugars owing to their high-energy bonds (Birsoy et al., 2013). Lipids are mainly stored in adipose tissue, and function as the body's major energy reserve in mammals. Unlike non-ruminants, the principal energy sources of ruminants are the short-chain fatty acids (less than 6 carbons), which are fermented in the rumen from dietary feedstuffs by rumen microbes (Dodson et al., 2010). However, in some cases, there is an insufficient energy intake caused by starvation, low quality feed or high productivity in animals; both ruminants and non-ruminants attempt to compensate for the energy deficit by mobilizing energy from adipose tissue depots (Bünemann et al., 2019; Gonzalez-Bulnes et al., 2013; Yu et al., 2016).

- *Hormonal roles and bioactive lipid mediators:* Hormones are chemicals secreted into the blood or extracellular fluids by cells that affect the functioning of other cells. Lipids and their fatty acids serve as precursors of various lipid derived hormones such as oestrogen and testosterone (Hiller, 2014; Van Tran et al., 2017). These hormones are vital to mammals because they regulate reproduction. Furthermore, many bioactive lipid mediators, especially eicosanoids (including prostaglandins, thromboxane and leukotrienes) which were synthesised from PUFA, are vital to animals in the prevention of inflammation and for disease treatment (Khanapure et al., 2007; Yokomizo et al., 2015).

- *Structural components of cells:* Lipids are the main components of cellular membranes. They have highly diverse structures and occur in different proportions in cellular membranes depending on the organism, cell type, organelle, membrane, bilayer-leaflet and membrane subdomain level (Harayama and Riezman, 2018). The major membrane lipids are phospholipids, glycosphingolipids and cholesterol. These lipids allow the membrane surface to be hydrophobic or hydrophilic depending on the orientation of the lipid

compounds into intra or extracellular spaces (Van Meer et al., 2008). Cellular membranes are vital to all living forms. They surround and protect cells and enable separation between the internal and external features of an organism, and control molecular movement through selective permeability deciding which substances enter or leave the cell (Watson, 2015).

2.3.1.3. Lipid metabolism in the rumen

- *Lipid digestion in the rumen*

The main types of dietary lipids entering the rumen are triacylglycerols, phospholipids, and galactolipids, which are then subjected to two major processes of lipolysis and biohydrogenation as depicted in Figure 2.6.

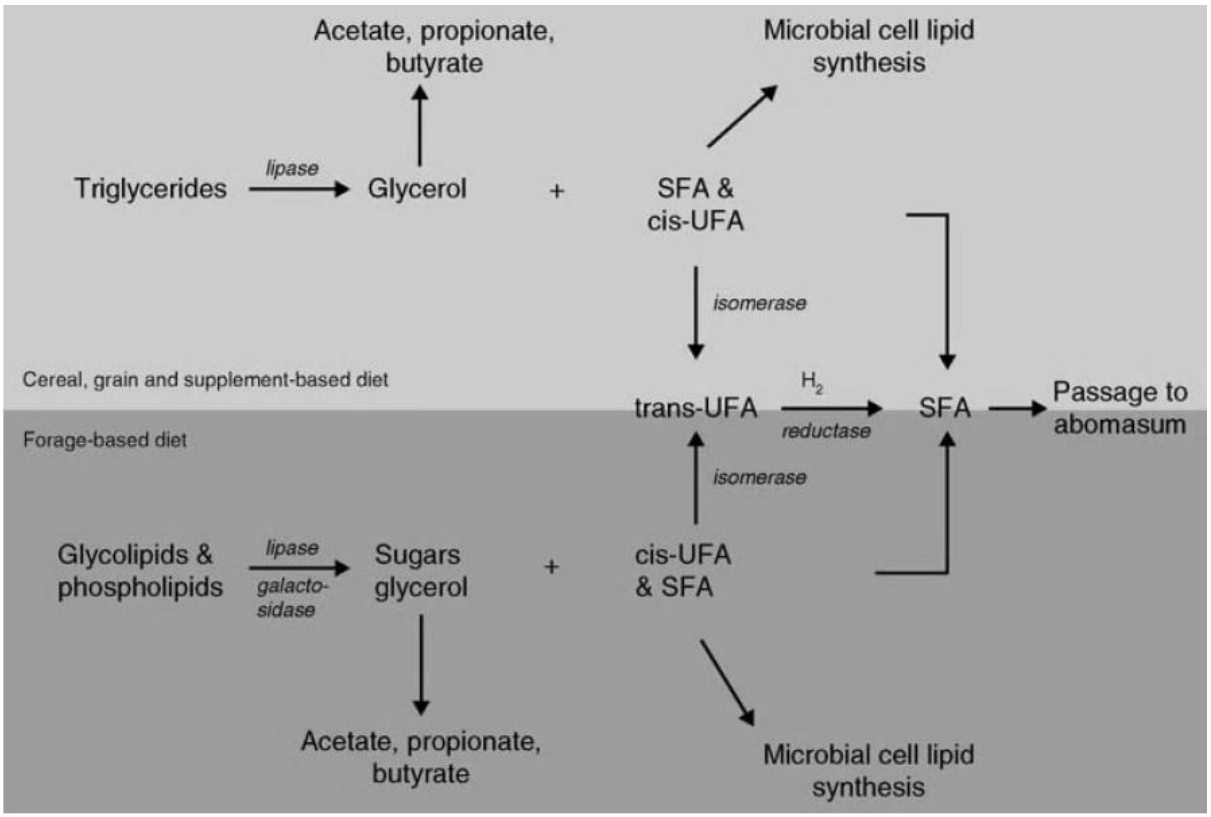

Figure 2. 6. Comparison of major lipid metabolic pathways in the rumen ecosystem of forage-fed and concentrate/supplement-fed ruminants (Source: Jarvis and Moore, 2010).

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The schematic diagram highlights the two major bacterially mediated lipid transformations in the rumen: (1) lipolysis, the hydrolysis of dietary lipids into constituent sugars, glycerol and fatty acids and (2) biohydrogenation, the conversion of unsaturated fatty acids into saturated fatty acids. UFA; unsaturated fatty acids, SFA; saturated fatty acids. These processes markedly change the fatty acid profile of lipids in the diet (mostly unsaturated fatty acids) compared to lipids leaving the rumen (mostly saturated fatty acids) (Jenkins et al., 2008). Lipolysis is a process in which dietary lipids are broken down, with free fatty acids and their constituent components, and glycerol and sugar moieties released (Jarvis and Moore, 2010).

Lipolysis of main types of dietary lipids in the rumen:

- *Triacylglycerols (TAGs):*

TAGs consist of a molecule of glycerol (a three-carbon alcohol) backbone and three fatty acid molecules (Fahy et al., 2005). Unlike in monogastric animals, dietary TAG are mostly broken down in the rumen of ruminants to release fatty acids and glycerol (Lourenço et al., 2010). Furthermore, the unsaturated fatty acids are largely modified by hydrogenation before reaching the small intestine (Jenkins et al., 2008). The glycerol component is fermented by rumen micro-organisms to carbon dioxide and acetic, propionic and butyric acids (Hiller, 2014; Trabue et al., 2007).

- *Glycolipids:*

The lipids in forages are primarily glycolipids (mostly galactolipids) and mainly exist in stems and leaves (Botté et al., 2011; Manan et al., 2017). The molecular structure of glycolipids is similar to TAG except that they have two or more sugars linked to one position of the glycerol backbone instead of the third fatty acid. The two remaining fatty acids in glycolipids are generally unsaturated (Fahy et al., 2005). The degradation of galactolipids in the rumen releases fatty acids, galactose and glycerol (Jarvis and Moore, 2010).

- *Phospholipids (PL)*

PL are an important part of the plant cell membrane (Maejima and Watanabe, 2014). PL consist of a glycerol backbone with two fatty acids attached, with the third position of glycerol attached to a phosphate group that links an organic base such as choline, ethanolamine, serine, or inositol to the molecule (Fahy et al., 2005). In the rumen, bacteria largely remove the base group and fatty acids from the phospholipids in dietary ingredients. However, protozoa and bacteria in the rumen also make their own PL for their cell membranes (Bainbridge et al., 2018). Therefore, the amount of PL leaving the rumen is more than the amount consumed in the diet.

- *Ruminal biohydrogenation*

Biohydrogenation (Figure 2.7) is the process in which unsaturated fatty acids are converted to saturated fatty acids via isomerization to trans fatty acid intermediates, followed by hydrogenation of the double bonds (Jenkins et al., 2008).

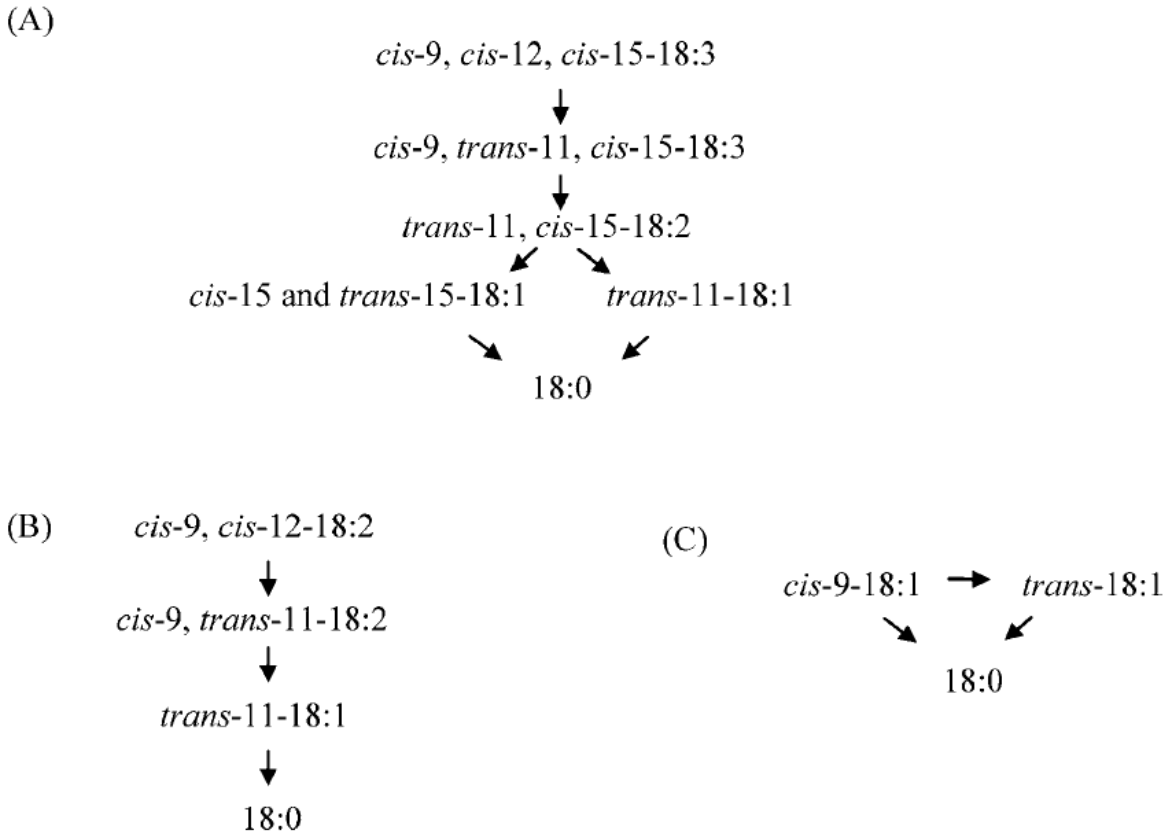

Figure 2. 7. Rumen biohydrogenation pathways of (A) α-linolenic, (B) linoleic, and (C) oleic acids (Source: Jenkins et al., 2008).

The biohydrogenation process occurs in the rumen and is mainly carried out by rumen bacteria (Harfoot and Hazlewood, 1997). There are a number of bacteria in the rumen which have the ability to hydrogenate unsaturated fatty acids. Many studies have explored rumen biohydrogenators. Kemp et al. (1975) identified five strictly anaerobic bacteria isolated from sheep rumen of which one was characterised as *Ruminococcus albus*, two as *Eubacterium* **spp**. and two as *Fusocillus* **spp**. (one of which is named as a new species) to have capability of hydrogenation of unsaturated fatty acids. Verhulst et al. (1985) revealed that several strains of *Clostridium bifermentans*, *Clostridium sporogenes* and *Clostridium sordellit* and one strain of *Bacteroides sp*. hydrogenate linoleic acid into trans-vaccenic acid *in vitro* following the same pathway. However, the total number of biohydrogenating species described is very small, largely because the isolation of such organisms is time-consuming (Harfoot and Hazlewood, 1997). Van de Vossenberg and Joblin (2003) demonstrated that *Butyrivibrio hungatei* played an important role in the transformation of unsaturated fatty acids in the rumen. With the advances of biotechnology, many microbial physiologists and ecologists have utilised specific gene probes in conjunction with amplification using the polymerase chain reaction to explore microbial populations in a wide variety of habitats. Jenkins et al. (2008) found that *Butyrivibrio* was of principal importance in ruminal biohydrogenation.

Impact of feeding factors on biohydrogenation:

- *Concentration of unsaturated fatty acid substrates*:

Many *in vitro* and *in vivo* studies have been shown that increasing the concentration of unsaturated fatty acids effects biohydrogenation in the rumen. Maia et al. (2006) concluded that dietary PUFA might be useful in suppressing the numbers of biohydrogenating ruminal bacteria, particularly *C. proteoclasticum*. Shingfield et al. (2003) revealed that feeding cows with fish oil inhibited the reduction of biohydrogenation intermediates in the rumen.

- *Protected oil or fat*:

Feeding ruminants with protected oil or fat has drawn the attention of many animal nutritionists. The aim is to reduce the biohydrogenation of lipids by microbes in the rumen and to increase the flow of unsaturated fatty acids into the small intestine. There are many methods to protect unsaturated fatty acids from microbial biohydrogenation. These include chemical treatment, encapsulation, and natural processing within the seed coat. Some studies have shown positive results in feeding protected unsaturated fatty acids from ruminal biohydrogenation. Sinclair et al. (2005b) showed that the pre-treatment of linseed with sodium hydroxide or formic acid, followed by formaldehyde treatment reduced ruminal microbial biohydrogenation in an *in vitro* study. In an *in vivo* study, Sinclair et al. (2005a) also reported that the provision of marine algae or fat encapsulated fish oil resulted in a lower biohydrogenation of DHA and EPA in comparison to fish oil. Wu et al. (1991) compared supplementation with fatty acids in calcium soap and in animal-vegetable blend fat and the result showed that the biohydrogenation of fatty acids in the former was lower than that in the latter (57% vs 87%).

- *Dietary composition:* Many recent studies have shown that components of the ruminant diet may effect ruminal biohydrogenation. Aurousseau et al. (2004) compared grass and concentrate feeding on the fatty acid composition of the *M. longissimus thoracis* muscle of lambs. The result revealed that PUFA contents were dependent upon only the type of feed and amount of

conjugated linoleic acid isomers (CLA) in lambs within a grass feeding system, which was higher than that in a concentrate feeding system. Kucuk et al. (2001) investigated whether or not the forage:concentrate ratio affected ruminal digestion and duodenal flow of fatty acids in ewes. The authors concluded that the highest-concentrate diet supported the greatest duodenal flows of dietary unsaturated fatty acids as well as the highest flow of 18:1trans-11.

Biohydrogenation remains a challenge for increasing the level of PUFA in ruminant products (meat & milk) for humans. However, this challenge can be overcome in part by genetic selection and dietary manipulation (Lourenço et al., 2010; Malau-Aduli et al., 2016).

2.3.1.4. Lipid metabolism in the ruminant body

Dietary lipids entering the rumen undergo extensive lipolysis and biohydrogenation by rumen microbes. The lipids leaving the rumen contain a variety of fatty acids and microbial phospholipids, which undergo further digestion and absorption in the small intestine as depicted in Figure 2.8. After absorption, fatty acids are transported to target tissues (e.g. muscle, adipose or udder tissue) for further metabolism and lipid synthesis, deposition and/or endocrine (e.g. muscle and adipose tissue) or exocrine (e.g. udder tissue) excretion (Hiller, 2014). Although the dietary lipids undergo massive changes in the rumen, they still play important roles in the fatty acid profiles of different ruminant tissues.

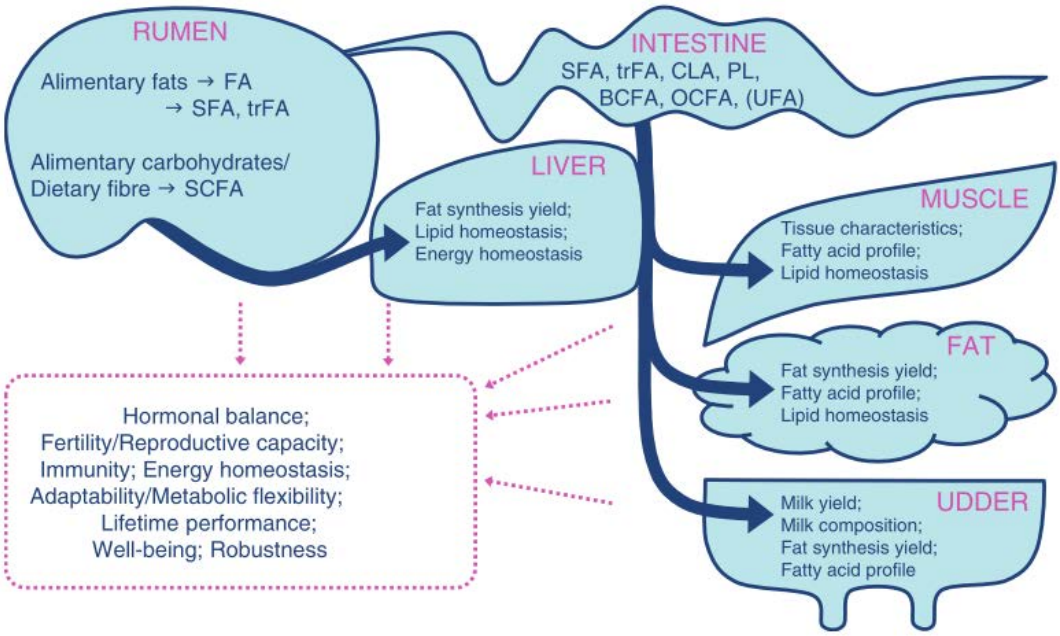

Figure 2. 8. Interrelations between lipid metabolism and ruminant health/performance in the lactating dairy cow (Source: Hiller, 2014).

Saturated fatty acids (SFA); trans fatty acids (trFA); conjugated linoleic acid isomers (CLA); short-chain fatty acids (SCFA); odd-branched-chain fatty acids (OCFA/BCFA) are absorbed, and also microbial phospholipids (PL); unsaturated fatty acids (UFA) that escape ruminal biohydrogenation.

2.3.2. PUFA-rich feed sources for ruminants

2.3.2.1. Forages

Forages such as grasses, legumes, hay and silage naturally contain high concentrations of ALA. However, the fatty acid profile as well as ALA concentration of forages depend on many factors such as form of forages (fresh, hay or silage), cultivar, cutting age and season (Garcia et al., 2016; Glasser et al., 2013; Meľuchová et al., 2008). Animals grazing green pastures generally have greater amounts of health-claimable n-3 LC-PUFA, such as EPA and DHA, than feeding systems based on feedlot pellets, grain, or dry pasture/straw (Annett et al., 2011b; Boughalmi

and Araba, 2016; De Brito et al., 2017b). Nevertheless, fluctuations in forage quantity and quality, especially under a climate change context, pose challenges for maintaining stable quality and animal productivity (Harle et al., 2007). Therefore, it is necessary to conduct research into supplementary feeds and high quality pastures in order to produce premium healthy animal products and increase animal productivity (Ponnampalam et al., 2014a).

2.3.2.2. Plant oil derived PUFA sources

Plant oils are sources of PUFA for human consumption. However, in recent years, they have been fed to animals, especially ruminants, to increase n-3 LC-PUFA and health-claimable omega-3 fatty acids in animal products (meat, cheese & milk). The following paragraphs summarise the results of studies that have been conducted on fatty acid characteristics and the use of common plant oils in ruminant production.

- *Canola oil:* Canola is classified under the botanical family of *Brassicaceae* and includes the species *Brassica napus*, *B. rapa* and *B. juncea* (Daun, 2011). The common canola varieties contain high oil content which ranges from 35% to 50% (Zum Felde et al., 2007). Furthermore, canola oil is rich in UFA and PUFA, mainly oleic acid (18:1n-9) (61%), linoleic acid (18:2n-6) (20%) and ALA (10%) (Bocianowski et al., 2012). Therefore, canola oil represents a 'good source' of PUFA which can be utilised for human consumption. Some recent studies in which canola oil had been used to supplement ruminant rations to increase the concentration of PUFA in meat had positive results. For instance, Flakemore et al. (2014) reported that supplementation of degummed crude canola oil into lamb rations induced softer fats with lower melting points in both visceral and subcutaneous fats of lambs; these are potentially healthier fats likely to contain higher levels of unsaturated fatty acids. Nguyen et al. (2017b) also revealed that lambs fed 5% canola oil supplements had higher n-3 LC-PUFA contents in

muscle in comparison to control lambs. In dairy cows, Otto et al. (2016) and Loor et al. (2002) revealed that cows supplemented with canola oil produced milk with higher conjugated linoleic and oleic acids.

- *Rice bran oil:* Rice (*Oryza sativa*) is a staple food for most of the world's population, especially Asian countries (Esa et al., 2013). The annual yield of milled rice globally is approximately 480 million metric tons (Muthayya et al., 2014). Rice bran is one of the main by-products (8%) of rice milling (Esa et al., 2013). Rice bran oil is normally produced from the extraction process of rice bran. According to Goffman et al. (2003), the oil content in rice bran varies from 17.3 to 27.4% (w/w). Rice bran oil is rich in oleic acid ranging from 32.4% to 43.4%, and linoleic acid ranging from 28.0% to 53.4% (Gopala Krishna et al., 2006). Although rice bran and rice bran oil are by-products of rice milling, they are utilised for both human and animal consumption. Many studies have been conducted using these by-products, especially for feeding or supplementing ruminant livestock. Flakemore et al. (2015) reported that rice bran can be utilised as a cost-effective supplementary feed source in genetically divergent sheep over a 49-day feeding period without detrimental effects on overall live animal performance or carcass characteristics. Bhatt et al. (2013) revealed that feeding Malpura lambs with rice bran oil in the form of calcium soap at 40 g/kg of concentrate improved growth, feed conversion efficiency and carcass quality compared to rice bran oil and control groups. Lunsin et al. (2012) showed that supplementing ruminants with rice bran oil should not exceed 4% to obtain the most beneficial effect on nutrient utilization, rumen fermentation and dairy cow performance.

- *Safflower oil:* Safflower (*Carthamus tintorius L.*) is a member of the family *Compositae* or *Asteracca* (Emongor, 2010). The oil content of safflower seeds ranges from 23.1% to 36.5%, of which the major fatty acid is linoleic acid (55.1–77.0%) (Matthaus et al., 2015). Safflower oil also consists of oleic acid which accounts for 12.2-19.8% (Sabzalian et al., 2008). Initially, safflower oil was utilised by the paint industry, now it is used as a multipurpose oil for cooking, making margarine and salad oil (Emongor, 2010). Recent studies have employed safflower oil as a feed supplement for animals due to its high concentration of PUFA. Boles et al. (2005) found that the addition of safflower oil to sheep rations elevated levels of UFA and CLA in the lean tissue without adversely affecting growth performance, carcass characteristics, or colour stability of lamb. Peng et al. (2010) revealed that supplementing mature Small Tail Han ewes with safflower oil increased the concentration of CLA in the tail, kidney, back, and muscle fat.

- *Flaxseed oil:* Flaxseed (*Linum usitatissimum*) or linseed has been used for food and textile fibre for over 5000 years and there are two main types of flax grown worldwide - fibre flax and seed flax (Singh et al., 2011). Seed flax is normally grown for oil production. Flaxseed oil is used to manufacture paints, varnishes, linoleum, oilcloths, printing inks, soaps, and numerous other products (Singh et al., 2011). The oil can be used for human consumption due to its high concentration of PUFA known to be beneficial to human health. Flaxseed has a high oil content which ranges from 36-40% (El-Beltagi et al., 2007). Popa et al. (2012) reported that linseed oil contained high levels of ALA (53.2%) followed by oleic (18.5%), and linoleic (17.3%). Flaxseed is also used for feeding animals. Kronberg et al. (2012) added treated flaxseed into the diets of forage-fed lambs which resulted in reduced hydrogenation of ALA by ruminal microbes and increased concentrations of n-3 LC-PUFA in the muscle of lambs. In another study, Nute et al. (2007) supplemented lambs with five sources of dietary oil (linseed oil, fish oil, a protected lipid supplement, fish oil/marine algae and a combination of a protected lipid

supplement and marine algae). The results revealed that the diet with linseed oil produced the highest proportion of ALA in muscle phospholipids, the highest ratings for lamb flavour intensity and overall liking and the lowest ratings for abnormal flavour intensity. Nguyen et al. (2017b) reported that the addition of 5% oil to pelleted lamb diets increased n-3 LC-PUFA contents in the muscle of supplemented lambs in comparison to control lambs. Furthermore, supplementing growing lambs with flaxseed flakes can increase dry matter intake, growth rate and GR fat depth (Burnett et al., 2017).

2.3.2.3 Marine products and rumen protected oils

- *Marine products:* There are a variety of marine oil containing products in the market. Marine products are well recognised for their high content of n-3 LC-PUFA (Nichols et al., 2010). Studies on the use of marine products as animal feed for increasing health-claimable omega-3 PUFA in animal products have gained positive results. In non-ruminant animals, feeding marine products such as fish oils and microalgae has been shown to increase DHA content of tissues and yolk (Lee et al., 2019). In ruminants, supplementation with marine products also increased n-3 LC-PUFA in meat (Hopkins et al., 2014; Kashani et al., 2015). Although the use of marine products as animal feed can raise the level of health-claimable n-3 LC-PUFA in animal products, the application of this feeding strategy under on-farm conditions is limited presently due to the high feeding cost, potentially deleterious odour and flavour reduction in animal products, i.e. fish smell (Lenihan-Geels et al., 2013; Urrutia et al., 2016).

- *Rumen protected oils:* It is well known that unsaturated fatty acids are subjected to biohydrogenation in the rumen by rumen bacteria and protozoa to a large extent (Sinclair et al., 2005a). Therefore, many studies have explored various methods for protecting unsaturated fatty acids from rumen biohydrogenation. Wood and Enser (1997) reported that the saturating effect of the rumen can be overcome by feeding PUFA which are protected either chemically,

by processing, or naturally e.g. within the seed coat. Demirel et al. (2004) showed that feeding lambs with different protected PUFA improved muscle PUFA to SFA ratios. In another in vitro study, Sinclair et al. (2005b) revealed that pre-treatment of linseed using sodium hydroxide or formic acid, followed by formaldehyde treatment, offered the best protection against ruminal microbial biohydrogenation.

2.4. Effect of PUFA-rich feed supplementation on lamb performance and product quality

2.4.1. Effect of PUFA-rich feed supplementation on growth rate, carcass characteristics and n-3 PUFA content of lot-fed lambs

2.4.1.1. Growth rate and carcass characteristics

PUFA-rich feeds currently being used in lamb feedlots originate from many sources such as oilseeds, terrestrial plant oils and marine products (Chikwanha et al., 2018; Nguyen et al., 2018a). Supplementation of ruminants with PUFA-rich feeds in an intensive production system may affect rumen microbes and fermentation leading to changes in animal performance, carcass characteristics and productivity (Kubelková et al., 2018; Mir et al., 2006; Vargas et al., 2017). Several studies have investigated the effect of PUFA-rich feed supplements on growth rate and carcass characteristics of lambs in confined production systems (Table 2.1). The different oils rich in PUFA formulated in isocaloric and isonitrogenous rations did not affect average daily gain (ADG) and carcass characteristics of confined lambs (Meale et al., 2015; Nguyen et al., 2018b). This result could be attributed to the fact that the percentage of PUFA rich oils included in the lamb ration in these studies was less than 6%. Therefore, it did not affect the growth rate and carcass characteristics of supplemented lambs (Nguyen et al., 2018a). However, in other studies, confined lambs supplemented with concentrates of different PUFA sources caused differences in ADG and carcass characteristics (Bhatt et al., 2013; De la Fuente-

Vazquez et al., 2014; Ponnampalam et al., 2015). There were two possible explanations for this result. Firstly, several PUFA-rich feed sources, besides providing unsaturated fatty acids for lambs, also provided protein sources. Holman et al. (2014) revealed that supplementation of lambs with *Spirulina* provided both dietary PUFA and protein sources, resulting in improved lamb growth rate. Secondly, the dry matter intake differences in lambs fed different PUFA-rich supplements lead to variations in results. For instance, a reduction in feed intake associated with a fish oil diet was observed by De la Fuente-Vazquez et al. (2014), while an increase in feed intake was reported in lambs fed rumen-protected PUFA oil or whole flaxseed (Bhatt et al., 2013; Ponnampalam et al., 2015). The increase or decrease in dietary intake directly affects the amount of nutrient digestion and absorption resulting in different changes in ADG and carcass characteristics of lamb.

Table 2.1. Lamb growth rate and carcass characteristics under feedlot production systems using PUFA-rich feed supplements

Basal diets	Supplements	ADG (g)	Days to slaughter (days)	Initial weight (kg)	Final weight (kg)	Carcass weight (kg)	Dressing percentage (%)	Fat thickness (mm)	Rib eye area (cm^2)	References
Pellets without safflower oil		180.0		36.8	52.5	28.7	54.4	3.6	15.5	Boles et al. (2005)
Pellets composed of 3% safflower oil (as-fed basis)		160.0		37.5	51.0	28.6	53.0	4.3	15.5	
Pellets composed of 6% safflower oil (as-fed basis)		210.0		37.6	55.4	29.2	54.4	4.1	16.1	
Roughage (*Prosopis cineraria* leaves)		110.4		8.6	26.9	14.5	58.1		11.2	Bhatt et al. (2013)
Roughage (*Prosopis cineraria* leaves)	Concentrate composed of 4% industrial grade rice bran oil	92.0		8.4	23.6	12.5	58.0		11.9	
Roughage (*Prosopis cineraria* leaves)	Concentrate composed of 4% Ca-soap rice bran oil	135.9		8.7	31.1	16.4	58.5		13.1	
Barley straw	3% palm oil concentrate	336.0	40	13.2	28.1	13.6	47.2	2.9		De la Fuente-Vazquez et al. (2014)
Barley straw	Concentrate composed of 12.5% linseed	355.0	40	13.0	29.1	13.6	45.5	3.2		
Barley straw	Concentrate composed of 12.5% linseed and 4% dehydrated microalgae	324.0	40	13.1	27.9	13.1	46.1	3.1		
Barley straw	3.3% fish oil concentrate	205.0	40	12.6	22.6	10.3	44.6	2.6		
Complete pelleted barley-based finishing diets composed of 2% canola oil		292.6		23.5	51.0	25.12	50.7			Meale et al. (2015)
Complete pelleted barley-based finishing diets composed of 2% flax oil		274.8		23.2	50.6	25.21	50.3			
Complete pelleted barley-based finishing diets composed of 2% high-linoleic safflower oil		256.9		23.3	50.5	25.32	49.9			
Complete pelleted barley-based finishing diets composed of 2% tasco		270.0		23.2	49.7	24.71	50.3			
Dry *Prosopis cineraria* and fresh *Azadirachta indica* leaves	Concentrate without oil	145.0	180	6.5	28.6		49.0		14.8	Bhatt et al. (2016)
Dry *Prosopis cineraria* and fresh *Azadirachta indica* leaves	Concentrate composed of 4% Ca-soap rice bran oil and	169.0	180	7.0	32.7		50.5		18.0	
Dry *Prosopis cineraria* and fresh *Azadirachta indica* leaves	Concentrate composed of 4% Ca-soap rice bran oil and DL-atocopherolacetate at 40 mg/kg feed	160.0	180	6.7	31.0		50.3		18.8	

Table 2.1. Lamb growth rate and carcass characteristics under feedlot production systems using PUFA-rich feed supplements (continued)

Basal diets	Supplements	ADG (g)	Days to slaughter (days)	Initial weight (kg)	Final weight (kg)	Carcass weight (kg)	Dressing percentage (%)	Fat thickness (mm)	Rib eye area (cm^2)	References
Lucerne hay	No oil pellets 1 kg/head/day	182.0	49	35.6	44.3	20.9	47.4	4.5	12.2	Nguyen et al. (2018b)
Lucerne hay	2.5% canola oil infused pellets 1 kg/head/day	189.0	49	37.1	46.1	22.0	47.7	4.6	12.4	
Lucerne hay	5% canola oil infused pellets 1 kg/head/day	205.0	49	35.5	45.3	22.0	48.6	4.4	12.8	
Lucerne hay	2.5% flaxseed oil infused pellets 1 kg/head/day	184.0	49	36.4	45.2	21.7	48.0	4.8	12.5	
Lucerne hay	5% flaxseed oil infused pellets 1 kg/head/day	204.0	49	36.0	45.8	21.9	47.8	4.8	12.2	
Annual ryegrass hay : clover hay (60:40)			56	35.0	45.6		43.2	10.7		Ponnampalam et al. (2015)
Annual ryegrass hay : clover hay (60:40)	Flaxseed supplementation (10.7%, dry matter basis)		56	35.0	49.2		45.6	15.1		
Annual ryegrass hay : clover hay (60:40)	Algae supplementation (1.8%, dry matter basis)		56	35.0	45.1		44.9	11.4		
Annual ryegrass hay : clover hay (60:40)	Flaxseed and algae supplementation (10.7% and 1.8%, dry matter basis, respectively)		56	35.0	46.9		45.3	13.3		
Lucerne hay	Wheat/barley-based pellets (1000 g/head/day)	177.3	49	35.8	44.5	21.3	46.2	4.5	15.0	Flakemore et al. (2015)
Lucerne hay	Wheat/barley-based pellets composed of 19% rice bran (1000 g/head/day)	216.0	49	33.9	44.5	20.4	44.4	4.3	15.3	
Lucerne hay	Wheat/barley-based pellets composed of 9.5% rice bran (1000 g/head/day)	159.0	49	35.6	43.4	19.4	43.5	3.8	14.5	
Concentrate composed of barley and soybean		382.0		16.2	26.9	11.8		2.6		Urrutia et al. (2016)
Barley and soybean with 10% linseed (dm basis)		350.0		16.3	26.9	12.0		3.3		
Barley and soybean with 5% linseed (dm basis) and 3.89% marine microalgae (dm basis)		234.0		16.4	26.2	11.6		2.5		
5% whole linseed concentrate		204.0		42.5	52.0	23.1	54.6	3.9		Guerrero et al. (2018)
10% whole linseed concentrate		165.0		43.1	51.1	22.8	52.9	3.6		
15% whole linseed concentrate		226.0		43.9	54.1	24.2	55.2	5.0		

2.4.1.2. Omega-3 fatty acid profile of lambs supplemented with PUFA-rich feeds in the feedlot

Some studies have shown that supplementation of confined lambs with PUFA-rich feeds resulted in an increase in omeg-3 PUFA in lamb (Chikwanha et al., 2018). Several PUFA-rich feed sources were reported to have the potential to increase n-3 PUFA content in lambs as depicted in Table 2.2. It was evident that supplementation with concentrate composed of different oils rich in PUFA increased the level of n-3 PUFA in lamb in comparison to unsupplemented lambs (Bhatt et al., 2016; Kitessa et al., 2012; Nguyen et al., 2017b). The increase in n-3 PUFA in supplemented lambs could be explained by the increase in PUFA intake and subsequent flow and absorption in the small intestine. Consequently, the PUFA absorption into lamb also increased and resulted in more n-3 PUFA deposition and synthesis in lamb muscle tissue. Liu et al. (2011) demonstrated that the injection of PUFA into the rumen of cows led to an increase in the amount of PUFA flowing through the small intestine and also increased PUFA concentration in milk.

The addition of different PUFA sources to the ration of confined lambs caused different responses in n-3 PUFA content of lamb (De la Fuente-Vazquez et al., 2014; Meale et al., 2015; Noci et al., 2011; Ponnampalam et al., 2015). The inclusion of marine products such as fish oils or algae in confined lamb rations significantly increased EPA and DHA contents in meat (De la Fuente-Vazquez et al., 2014; Ponnampalam et al., 2015; Urrutia et al., 2016). This result could be attributed to the differences in fatty acid profiles of various PUFA sources. Each PUFA source has its own FA profile with specific proportions of individual FA. For example, marine products such as fish oil or algae contain high EPA and DHA content, while vegetable oils are rich in ALA and LA content (Nguyen et al., 2018a). Therefore, lambs fed different PUFA sources will have different intake of individual FAs, especially n-3 PUFA or n-3 LC-PUFA leading to variation in the n-3 PUFA content of lambs. Furthermore, the increase in EPA and DHA in lambs fed marine products could be due to the ability of marine products to inhibit ruminal biohydrogenation (Lv et al., 2016). Supplementation of confined lambs with PUFA-rich feeds can increase n-3 PUFA content of meat. However, there is a limited number of

comparative studies on different PUFA-rich feed sources for helping lamb producers to reduce feed and labour costs and produce premium healthy lambs.

Table 2.2. Fatty acid profile of lambs reared in feedlot production system using PUFA-rich feed supplements

Basal diets	Supplements	Unit	Organs	ALA	EPA	DPA	DHA	n-3 PUFA	PUFA	n-6/n-3	References
Basal ration (oaten hay, barley grains, lupin grains, molasses, mineral mix)	Lambs abomasally infused with saline	mg/100 g muscle	*Longissimus dorsi* muscle	12.8	6.5	14.3	5.8	39.0			Kitessa et al. (2012)
Basal ration (oaten hay, barley grains, lupin grains, molasses, mineral mix)	Lambs abomasally infused with 25 ml of *echium* oil per day	mg/100 g muscle	*Longissimus dorsi* muscle	64.3	16.8	22.2	5.5	119.0			
Basal ration (oaten hay, barley grains, lupin grains, molasses, mineral mix)	Lambs abomasally infused with 50 ml of *echium* oil per day	mg/100 g muscle	*Longissimus dorsi* muscle	76.4	17.7	18.6	4.6	129.0			
Basal ration (oaten hay, barley grains, lupin grains, molasses, mineral mix)	Lambs abomasally infused with 25 ml of linseed oil per day	mg/100 g muscle	*Longissimus dorsi* muscle	79.9	13.5	18.2	7.1	121.0			
Basal ration (oaten hay, barley grains, lupin grains, molasses, mineral mix)	Lambs abomasally infused with 50 ml of linseed oil per day	mg/100 g muscle	*Longissimus dorsi* muscle	113.3	11.7	19.4	4.5	150.0			
Complete pelleted barley-based finishing diets composed of 2% canola oil		g/100 g FA	Skirt muscle	1.20	0.07	0.09	0.09	1.53	8.19	3.0	Meale et al. (2015)
Complete pelleted barley-based finishing diets composed of 2% flax oil		g/100 g FA	Skirt muscle	2.55	0.06	0.13	0.06	2.8	11.04	1.4	
Complete pelleted barley-based finishing diets composed of 2% high-linoleic safflower oil		g/100 g FA	Skirt muscle	0.72	0.09	0.07	0.13	1.02	8.13	5.8	
Complete pelleted barley-based finishing diets composed of 2% tasco		g/100 g FA	Skirt muscle	0.96	0.07	0.11	0.1	1.27	6.81	3.2	
Dry *Prosopis cineraria* and fresh *Azadirachta indica* leaves	Concentrate without oil	g/100 g FA	*Longissimus dorsi* muscle	0.41					2.92	6.4	Bhatt et al. (2016)
Dry *Prosopis cineraria* and fresh *Azadirachta indica* leaves	Concentrate composed of 4% Ca-soap rice bran oil	g/100 g FA	*Longissimus dorsi* muscle	0.51					6.53	10.8	
Dry *Prosopis cineraria* and fresh *Azadirachta indica* leaves	Concentrate composed of 4% Ca-soap rice bran oil and DL-atocopherolacetate at 40 mg/kg feed	g/100 g FA	*Longissimus dorsi* muscle	0.57					7.39	10.8	

Table 2.2. Fatty acid profile of lambs reared in feedlot production system using PUFA-rich feed supplements (continued)

Basal diets	Supplements	Unit	Organs	ALA	EPA	DPA	DHA	n-3 PUFA	PUFA	n-6/n-3	References
Lucerne hay	No oil concentrate	mg/100 g muscle tissue basis	*Longissimus thoracis et lumborum*	27.6	17.9	14.2	6.1	70.0	301.8	3.1	Flakemore et al. (2017b)
Lucerne hay	2.5% degummed crude canola oil concentrate	mg/100 g muscle tissue basis	*Longissimus thoracis et lumborum*	32.8	16.1	14.2	3.9	69.7	331.2	3.5	
Lucerne hay	5% degummed crude canola oil concentrate	mg/100 g muscle tissue basis	*Longissimus thoracis et lumborum*	33.1	21.0	16.3	7.2	80.3	326.9	2.9	
Lucerne hay	No oil pellets 1kg/head/day	mg/100 g wet tissue	*Longissimus thoracis et lumborum* muscle	24.9	11.3	10.8	2.8			2.6	Nguyen et al. (2017b)
Lucerne hay	2.5% canola oil infused pellets 1kg/head/day	mg/100 g wet tissue	*Longissimus thoracis et lumborum* muscle	39.2	13.1	13.4	4.5			2.3	
Lucerne hay	5% canola oil infused pellets 1kg/head/day	mg/100 g wet tissue	*Longissimus thoracis et lumborum* muscle	36.7	17.0	16.3	5.3			2.2	
Lucerne hay	2.5% flaxseed oil infused pellets 1kg/head/day	mg/100 g wet tissue	*Longissimus thoracis et lumborum* muscle	40.8	14.2	13.8	4.2			2.0	
Lucerne hay	5% flaxseed oil infused pellets 1kg/head/day	mg/100 g wet tissue	*Longissimus thoracis et lumborum* muscle	49.3	17.9	15.6	4.9			1.8	
Chopped hay (100 g/head/day)	Concentrate composed of 6% megalac (ruminally protected saturated fat)	g/100 g FA	*Longissimus dorsi* muscle	0.50	0.07	0.20	0.04	0.87	6.02	5.28	Noci et al. (2011)
Chopped hay (100 g/head/day)	Concentrate composed of 6% camelina seed oil	g/100 g FA	*Longissimus dorsi* muscle	1.27	0.11	0.20	0.05	1.75	6.41	2.14	
Chopped hay (100 g/head/day)	Concentrate composed of 6% linseed oil	g/100 g FA	*Longissimus dorsi* muscle	1.74	0.12	0.21	0.03	2.18	6.91	1.75	
Chopped hay (100 g/head/day)	Concentrate composed of 6% NAOH-treated camelina seed	g/100 g FA	*Longissimus dorsi* muscle	1.66	0.16	0.22	0.04	2.21	7.35	1.83	
Chopped hay (100 g/head/day)	Concentrate composed of 6% NAOH-treated linseed	g/100 g FA	*Longissimus dorsi* muscle	2.56	0.30	0.32	0.08	3.33	8.54	1.15	
Chopped hay (100 g/head/day)	Concentrate composed of 6% camelina oil amides	g/100 g FA	*Longissimus dorsi* muscle	1.90	0.12	0.20	0.03	2.36	7.20	1.72	
Barley straw	3% palm oil concentrate	g/100 g FA	*Longissimus dorsi* muscle	0.38	0.17	0.41	0.22	1.20	11.10		De la Fuente-Vazquez et al. (2014)
Barley straw	Concentrate composed of 12.5% linseed	g/100 g FA	*Longissimus dorsi* muscle	2.49	0.60	0.83	0.53	4.50	15.47		
Barley straw	Concentrate composed of 12.5% linseed and 4% dehydrated microalgae	g/100 g FA	*Longissimus dorsi* muscle	1.79	0.52	0.67	0.62	3.65	13.80		
Barley straw	3.3% fish oil concentrate	g/100 g FA	*Longissimus dorsi* muscle	0.29	1.80	1.18	2.65	6.07	14.05		

Table 2.2. Fatty acid profile of lambs reared in feedlot production system using PUFA-rich feed supplements (continued)

Basal diets	Supplements	Unit	Organs	ALA	EPA	DPA	DHA	n-3 PUFA	PUFA	n-6/n-3	References
Annual ryegrass hay : clover hay (60:40)		mg/100 g of meat	*Longissimus lumborum* muscle	34.30	17.60	13.90	7.60				Ponnampalam et al. (2015)
Annual ryegrass hay : clover hay (60:40)	Flaxseed supplementation (10.7%, dry matter basis)	mg/100 g of meat	*Longissimus lumborum* muscle	59.50	18.10	11.20	6.70				
Annual ryegrass hay : clover hay (60:40)	Algae supplementation (1.8%, dry matter basis)	mg/100 g of meat	*Longissimus lumborum* muscle	32.10	21.80	9.80	63.40				
Annual ryegrass hay : clover hay (60:40)	Flaxseed and algae supplementation (10.7% and 1.8%, dry matter basis, respectively)	mg/100 g of meat	*Longissimus lumborum* muscle	41.30	17.20	8.30	49.60				
Concentrate composed of barley and soybean		g/100 g FA	Intramuscular adipose tissue	0.40	0.19	0.23	0.05	1.04	12.2	10.5	Urrutia et al. (2016)
Barley and soybean with 10% linseed (DM basis)		g/100 g FA	Intramuscular adipose tissue	1.84	0.74	0.31	0.08	4.32	14.2	3.8	
Barley and soybean with 5% linseed (DM basis) and 3.89% marine microalgae (DM basis)		g/100 g FA	Intramuscular adipose tissue	0.89	1.01	0.32	0.99	3.21	13.9	4.4	
Alfalfa hay pellets or corn		mg /100 g of muscle	*Longissimus thoracis*	18.0	2.7	5.3	1.2	27.1	186.5	6.2	Realini et al. (2017)
Alfalfa hay pellets or corn	Extruded linseed	mg/100 g of muscle	*Longissimus thoracis*	32.0	4.0	6.3	1.4	43.7	217.5	3.7	
Alfalfa hay pellets or corn	Spices (aromatic spices: lemon albedo, thyme, garlic, salt).	mg/100 g of muscle	*Longissimus thoracis*	16.9	2.3	5.4	1.3	25.9	189.4	6.0	
5% whole linseed concentrate		g/100 g FA	*Longissimus thoracis* muscle	1.41	0.21	0.27	0.10	2.03	6.75	2.1	Guerrero et al. (2018)
10% whole linseed concentrate		g/100 g FA	*Longissimus thoracis* muscle	1.88	0.21	0.26	0.10	2.49	7.26	1.7	
15% whole linseed concentrate		g/100 g FA	*Longissimus thoracis* muscle	1.97	0.19	0.24	0.08	2.53	7.50	1.6	

2.4.2. Effect of supplementing lambs with PUFA-rich feeds and different pasture types on growth rate, carcass characteristics and omega-3 fatty acid profile in an extensive production system.

2.4.2.1. Growth rate and carcass characteristics

Lambs under grazing production systems are influenced by many more factors than lambs in confined indoor production systems, hence grazing lambs have different physical behavioural and feeding activities leading to different responses in terms of growth rate and carcass characteristics (De Brito et al., 2017b). Table 2.3 summarises growth rate and carcass characteristic results from grazing lambs supplemented with or without PUFA-rich feeds in several studies. The results showed that lambs grazing on pasture only required more time to reach slaughter weight and had lower ADG than grazing lambs receiving supplements and lot-fed lambs (Annett et al., 2011b; Gomez-Vazquez et al., 2011; Jacques et al., 2011b; Perlo et al., 2008; Ponnampalam et al., 2017). Lot-fed and grazing lambs receiving supplements had a constant supply of highly digestible nutrients from grains, total mixed rations or pellets, resulting in more dry matter intake and higher nutrient digestion and absorption, which taken together, shortened the required time to reach slaughter weight, in comparison to lambs only grazing (Riaz et al., 2014). Furthermore, unsupplemented grazing lambs had more physical activity than lot-fed and supplemented grazing lambs leading to more energy expense rather than using it for body tissue synthesis; the grazing lambs therefore required more time to reach slaughter weight due to their lower ADG (De Brito et al., 2017b; Fajardo et al., 2016). The different pasture types also caused differences in the ADG of grazing lambs (Fraser et al., 2004; Gallardo et al., 2011). However, there were no differences in carcass weight among unsupplemented lambs grazing different pasture types at the same slaughter weight (De Brito et al., 2016; Fraser et al., 2004; Gallardo et al., 2011). Pasture type has a close relationship with forage quantity and quality with profound subsequent effects on lamb ADG (Lee, 2018). For

example, legume pastures have higher forage quality than grass (Amiri and Abdul Rashid, 2012). As a result, lambs grazing different types of pastures have different ADG. The dressing percentage of grazing only lambs was lower than those of supplemented grazing lambs and feedlot lambs (Annett et al., 2011b; Boughalmi and Araba, 2016; Gomez-Vazquez et al., 2011; Karaca et al., 2016; Perlo et al., 2008). The dressing percentage of lambs also depends on diet composition (Gardner et al., 2015). It has been demonstrated that grazing only lambs consumed more fibre than feedlot and supplemented grazing lambs, leading to lower dressing percentages (Díaz et al., 2002; Sheridan et al., 2003).

Table 2.3. Effect of supplementing grazing lambs with PUFA-rich feeds on growth rate, carcass characteristics and FA profiles

Type of pasture	Supplements	ADG (g/d)	Days to slaughter (days)	Initial weight (kg)	Final weight (kg)	Carcass weight (kg)	Dressing percentage (%)	Fat thickness (mm)	Rib eye area ($cm^{2)}$	References
Lucerne		243	50			18.8				Fraser et al. (2004)
Red clover		305	38			18.4				
Perennial ryegrass		184	66			18.8				
Successional pasture		221	66	28.4	43	21.4	52.0			Gallardo et al. (2011)
Subterranean clover/ryegrass		259	66	29.8	46.9	22	52.0			
Red clover/ryegrass		296	66	30.1	49.6	24.5	53.0			
Bladder						21.7	49.2		17.9	De Brito et al. (2016)
Brassica						23.5	51.5		17.6	
Chicory + arrowleaf						25.1	54.7		17.9	
Lucerne + phalaris						22.1	51.3		17.2	
Lucerne						24.9	54.7		18.1	
Native grass pasture			165			16.2	39.5			Perlo et al. (2008)
Ground alfalfa diet			71			15.8	42.4			
Alfalfa hay : linseed (70:30)			71			20.7	49.0			
Stargrass (cynodonplectostachyus) pasture		66	90	18.5	24.4		45.0			Gomez-Vazquez et al. (2011)
Stargrass (cynodonplectostachyus) pasture	Concentrate 0.3 kg/head/day (13% crude protein)	78	90	18.8	25.8		47.4			
Stargrass (cynodonplectostachyus) pasture	Concentrate 0.3 kg/head/day (15% crude protein)	90	90	19.1	27.2		47.8			
Stargrass (cynodonplectostachyus) pasture	Concentrate 0.3 kg/head/day (17% crude protein)	92	90	19.9	28.2		48.1			
Stargrass (cynodonplectostachyus) pasture	Concentrate 0.3 kg/head/day (19% crude protein)	131	90	19.2	31.0		47.9			
Good quality hay (ad libitum access)	Commercial pellets (ad libitum access)	449	105	23.6	47.2	21.2	45.0	11.2		Jacques et al. (2011b)
Good quality hay : commercial pellets (60:40)		347	122	23.7	46.9	19.5	41.6	7.3		
Fresh grass cut (ad libitum access)		267	146	23.6	47	19.4	41.2	6.6		
Intensive rotational grazing		295	145	23.6	47.1	20.4	43.2	4.6		

Table 2.3. Effect of supplementing grazing lambs with PUFA-rich feeds on growth rate, carcass characteristics and FA profiles (continued)

Type of pasture	Supplements	ADG (g/d)	Days to slaughter (days)	Initial weight (kg)	Final weight (kg)	Carcass weight (kg)	Dressing percentage (%)	Fat thickness (mm)	Rib eye area ($cm^{2)}$	References
Perennial ryegrass		130	70	35.1	46.7	18.7	42.7	7.37		Annett et al. (2011b)
Perennial ryegrass	A cereal-based concentrate 0.4 kg/head/day	148	67.6	36.5	46.7	19.7	42.8	6.36		
Perennial ryegrass	A fish oil-enriched concentrate 0.4 kg/head/day	153	67.9	35	47.0	20.6	42.4	6.82		
Chopped straw (250 g/heard/day)	A cereal-based concentrate 0.4 kg/head/day	164	52.7	35.9	43.9	21.6	45.5	9.2		
Chopped straw (250 g/heard/day)	A fish oil-enriched concentrate 0.4 kg/head/day	162	58.3	34.6	44.4	21.5	45.0	8.91		
Hay	Commercial concentrate 0.625 kg/head/day (18% crude protein)	162	269	27	41.3	19.4	48.0			Boughalmi and Araba (2016)
Grazing on a natural pasture	Commercial concentrate 0.312 kg/head/day (18% crude protein)	149	276	25.9	40.4	19.6	47.5			
Grazing on a natural pasture		141	283	26.1	40.7	19.3	47.4			
Alfalfa hay	Concentrate (ad libitum access)					27.2	49.6			Karaca et al. (2016)
Grazing pasture						17.9	44.0			
Lucerne		267	56	31.4	47.4	22.5	47.2	11.6		Ponnampalam et al. (2017)
Ryegrass		286	56	31.6	49.1	21.6	44.2	10.5		
Ryegrass	Concentrate (ad libitum access)	217	56	31.6	44.7	19.5	43.6	9.2		
Phalaris hay (10% of total intake)	Concentrate (ad libitum access)	249	56	31.5	46.6	22.2	47.7	12.6		

2.4.2.2. Omega-3 fatty acids of lamb under PUFA rich dietary supplementation in an extensive system

The FA profile of lambs under an extensive production system is affected by many factors such as the type of pastures, supplementary feeds, composition of pasture and grazing time (De Brito et al., 2017b; Wang et al., 2015; Zhang et al., 2017). Studies investigating the effect of PUFA-rich feed supplements and different types of pastures on the n-3 PUFA content of lamb in an extensive production system is shown in Table 2.4. The results of these studies have been inconsistent and beset by several limitations. For instance, De Brito et al. (2017a) and Gallardo et al. (2014b) reported that different pasture types affected the level of n-3 PUFA and n-6/n-3 ratios in lambs, whereas Fraser et al. (2004), Gallardo et al. (2011) and Kliem et al. (2018) found that the different pasture types did not cause any differences. Boughalmi and Araba (2016) demonstrated that supplementation of lambs with PUFA-rich feeds altered n-3 PUFA content in meat, whilst the findings of Annett et al. (2011b) and Ponnampalam et al. (2012) revealed that supplementation of grazing lambs with PUFA-rich feeds did not alter n-3 PUFA content. The inconsistent results can be attributed to many factors. Firstly, designing an appropriate grazing trial is challenging due to largely diverse grazing production systems affected by severe climatic conditions (drought or temperature fluctuation), irrigation or soil types, with subsequent impact on pasture quantity and quality (Bell et al., 2012; Howden et al., 2008; Mitchell et al., 2015). Secondly, the lack of adequate replicates due to high labour costs all contribute to the inconsistencies in the outcomes emanating from extensive production systems (De Brito et al., 2017b).

Table 2.4. Fatty acid profiles of lamb in a grazing system supplemented with PUFA-rich feeds

Type of pasture	Supplements	Unit	Organs/tissues	ALA	EPA	DPA	DHA	n-3 PUFA	PUFA	n-6/n-3	References
Lucerne		g/100 g FA		2.7	0.9	0.8	0.3				Fraser et al. (2004)
Red clover		g/100 g FA		2.9	1.0	0.9	0.3				
Perennial ryegrass		g/100 g FA		2.1	0.9	0.8	0.2				
Successional pasture (naturalized pasture)		g/100 g FA		4.9					27.2	1.8	Gallardo et al. (2011)
Subterranean clover		g/100 g FA		5.3					26.0	2.1	
Red clover		g/100 g FA		4.4					23.0	1.9	
Calafatal pasture		g/100 g FA	*Longissimus dorsi* muscle	2.5	1.4		0.5	4.3	15.5	2.1	Gallardo et al. (2014b)
Naturalized pasture		g/100 g FA	*Longissimus dorsi* muscle	2.1	1.3		0.4	3.8	12.5	1.8	
Bladder		mg/100 g meat	*Longissimus lumborum* muscle	37.6	21.0	21.7	6.6	86.8	245.9	1.9	De Brito et al. (2017a)
Brassica		mg/100 g meat	*Longissimus lumborum* muscle	47.3	20.9	20.8	5.7	94.6	240.4	1.5	
Chicory + arrowleaf		mg/100 g meat	*Longissimus lumborum* muscle	51.2	21.2	21.9	6.8	101.1	250.4	1.5	
Lucerne + phalaris		mg/100 g meat	*Longissimus lumborum* muscle	42.7	20.8	21.8	7.4	92.6	237.7	1.6	
Lucerne		mg/100 g meat	*Longissimus lumborum* muscle	45.8	20.4	21.5	6.9	94.8	241.3	1.6	
Control (ryegrass)		mg/100 g meat	*Longissimus thoracis* muscle	21.8				37.7	130.0	2.5	Kliem et al. (2018)
Biodiverse (mixed pasture)		mg/100 g meat	*Longissimus thoracis* muscle	27.3				44.5	156.0	2.5	

Table 2.4. Fatty acid profiles of lamb in a grazing system supplemented with PUFA-rich feeds (continued)

Type of pasture	Supplements	Unit	Organs/tissues	ALA	EPA	DPA	DHA	n-3 PUFA	PUFA	n-6/n-3	References
Perennial ryegrass		g/kg total fatty acids	*Longissumus dorsi* muscle	9.1	2.5	1.1	1.9	16.0	47.0	2.6	Annett et al. (2011b)
Perennial ryegrass	A cereal-based concentrate 0.4 kg/head/day	g/kg total fatty acids	*Longissumus dorsi* muscle	8.9	0.9	0.6	1.1	12.0	39.0	2.6	
Perennial ryegrass	A fish oil-enriched concentrate 0.4 kg/head/day	g/kg total fatty acids	*Longissumus dorsi* muscle	8.4	1.8	1.0	0.9	11.0	52.0	4.4	
Chopped straw (250 g/heard/day)	A cereal-based concentrate 0.4 kg/head/day	g/kg total fatty acids	*Longissumus dorsi* muscle	6.8	2.1	1.5	0.3	10.0	62.0	3.9	
Chopped straw (250 g/heard/day)	A fish oil-enriched concentrate 0.4 kg/head/day	g/kg total fatty acids	*Longissumus dorsi* muscle	3.5	4.2	4.9	4.7	18.0	55.0	3.0	
Perennial pasture		mg/100 g meat	*Longissimus lumborum* muscle	61.5				117	333.0	1.8	Ponnampalam et al. (2012)
Annual pasture	Lucerne hay and oat grain pellet (700 g/head/day)	mg/100 g meat	*Longissimus lumborum* muscle	51.8				103	345.0	2.4	
Annual pasture	Lucerne hay, oat grain and cracked flaxseed pellet (700 g/head/day)	mg/100 g meat	*Longissimus lumborum* muscle	61.0				114	351.0	2.1	
Annual pasture	Lucerne hay, oat grain and flaxseed meal pellet (700 g/head/day)	mg/100 g meat	*Longissimus lumborum* muscle	58.9				109	348.0	2.2	
Hay	Commercial concentrate 0.625 kg/day (18% crude protein)	g/100 g FA	*Semimembranous* muscle	0.6				1.4	10.4	6.2	Boughalmi and Araba (2016)
Grazing on a natural pasture	Commercial concentrate 0.312 kg/day (18% crude protein)	g/100 g FA	*Semimembranous* muscle	1.5				2.7	13.8	3.8	
Grazing on a natural pasture		g/100 g FA	*Semimembranous* muscle	2.1				4.4	18.7	3.0	

2.5 Summary

The Australian prime lamb industry has rapidly developed over the last three decades in the face of negative impacts of climate change, with the sheep population decreasing and competition on the global market scene getting stronger and fiercer. It is no longer an add-on enterprise of the Merino wool industry but has now become the key driver of the sheep industry mainly due to the recorded success of significant contributions to genetic improvements in lambs. Furthermore, Australia has a range of diverse and flexible finishing lamb systems ranging from extensive to intensive operations, which allows the output of various products meeting different market specifications.

However, the industry is still facing many challenges such as fluctuation in product quality due to unstable quantity and quality of forages in grazing systems and increasing competition on the global market. Consumers are more aware and increasingly paying more attention to the consumption of healthy products, especially red meat. Therefore, there is an increasing need for research into improving lamb quality in order to meet the demands of consumers for healthy meat and to maintain the competitiveness of Australian lamb on the global market.

Lamb is considered as a rich source of proteins and micronutrients. Research findings have demonstrated that n-3 LC-PUFA content in lamb can be manipulated through diets. Supplementation of confined lambs with PUFA-rich feeds can increase the content of n-3 LC-PUFA in meat. Different pasture types and PUFA-rich supplements can affect lamb performance and n-3 LC-PUFA content in edible tissues and organs of grazing lambs.

Although positive results on the potentials of improving n-3 LC-PUFA content in lamb in both feedlot and grazing systems have been reported, some knowledge gaps still exist, which need to be filled. For instance, in the indoor feedlot system, there are no comparative studies between different dietary PUFA-rich sources available to lamb producers as options for matching their

production system with sheep genetics to produce premium quality lamb at reduced feed and labour costs. In the outdoor grazing systems, there are limited studies and inconsistent results on improving n-3 LC-PUFA content in lamb due to high experimental costs and limitations in experimental design.

Therefore, studies on the responses of prime lambs to dietary n-3 PUFA-rich oil supplementation and different types of pastures in terms of animal performance, carcass characteristics, feed costs and fatty acid profiles of muscle, liver, kidney and heart in both indoor lot-fed and outdoor grazing systems would prove beneficial. The outcomes will enable the production of premium, health beneficial, top quality lamb and also maximise profitability.

Chapter 3: Nutritional supplements fortified with oils from canola, flaxseed, safflower and rice bran improve feedlot performance and carcass characteristics of Australian prime lambs

3.1. Abstract

This study investigated live animal performance and carcass characteristics of Australian prime lambs fed oil based polyunsaturated fatty acid (PUFA) enriched pellets in a feedlot system. The tested hypothesis was that supplementation of lambs with a variety of dietary oil based PUFA enriched pellets would enhance growth and carcass characteristics compared with the control lambs fed only with lucerne hay. Seventy-two, 6 months old White Suffolk x Corriedale first-cross prime lambs with an average liveweight (LWT) of 35.7 0.9 kg were allocated to six treatment groups in a completely randomised experimental design. The treatments were: (1) control: lucerne hay only; or lucerne hay plus wheat-based pellets infused with 50 mL/kg dry matter (DM) of oils from (2) rice bran (RBO); (3) canola (CO); (4) rumen protected (RPO); (5) flaxseed (FO) and (6) safflower (SO) dietary sources. All lambs had ad libitum access to lucerne hay and clean fresh water. Supplemented lambs were fed 1 kg of pellet/head/day for 10 weeks. Feed intake, final LWT, average daily gain (ADG), body conformation and carcass characteristics of lambs in the supplemented groups were all greater than for the control group. SO lambs had the lowest ADG of 190.3 g/day. RBO and CO treatments had the lowest feed cost per unit gain of AU$3.0/kg. Supplemented lambs had similar over the hooks (OTH) incomes that were all higher than that of the control group. This empirical evidence-based data demonstrated that supplementation of lambs with RBO and CO had comparatively lower feed costs without compromising ADG, carcass characteristics and OTH income.

3.2. Introduction

The Australian sheep industry has undergone significant changes within the last decade and witnessed a sustained decrease in the value and scale of wool and a steady rise in production and price of lamb and mutton (Rowe, 2010). Australia was the second largest producer of lamb and mutton in the world from 2010 to 2016 (ABARES, 2018) and maintenance of the global competitiveness of Australian meat production ensured the sustainable development of its lamb industry. Pethick et al. (2010) revealed that meat with healthy nutritional composition is one of the five key attributes of modern meat products in a competitive market.

There are new emerging demands from meat consumers for high quality lamb meat, especially in developed countries (Montossi et al., 2013; Sañudo et al., 2013). Red meat contains natural omega-3 long-chain (C20) polyunsaturated fatty acids (n-3 LC-PUFA), the content of which can be manipulated by modifying the composition of livestock feeds (Howe et al., 2007). n-3 LC-PUFA are well known for human health benefits including anti-inflammatory, therapeutic and protective effects against cardiovascular diseases and various types of cancer (Calder, 2009; Cao et al., 2015; Leslie et al., 2015; Nabavi et al., 2015). However, it is challenging to increase n-3 LC-PUFA content in red meat because of lipolysis and extensive biohydrogenation that occurs in the rumen through microbial activity in ruminants (Bessa et al., 2015; Chikunya et al., 2004). Furthermore, in some instances, adding oil based PUFA supplements to ruminant rations resulted in reduced animal feed intake, animal performance and carcass muscle mass (Francisco et al., 2015; Wanapat et al., 2011) or a depression of ruminal fermentation (Beauchemin et al., 2009). High feed cost is another challenge in the on-farm application of oil based n-3 LC-PUFA supplementation because the

cost of nutrition can represent approximately 70% of the total cost of lamb production in confined systems (Bottje et al., 2017; Lima et al., 2017).

Supplementation of lamb diets with n-3 LC-PUFA has shown several positive results (Boles et al., 2005; Flakemore et al., 2015; Flakemore et al., 2017a; Flakemore et al., 2017b; Gallardo et al., 2014a; Kashani et al., 2015; Malau-Aduli et al., 2016; Nguyen et al., 2017a; Nguyen et al., 2017b; Nguyen et al., 2018b). The impact of fatty acids has been reported in a separate stand-alone manuscript (currently under review) where these oil supplements were shown to improve the health-beneficial n-3 LC-PUFA contents in the muscle, liver, heart and kidney of these supplemented prime lambs. However, there is still the need to examine other cost-effective and nutritionally viable oil-based PUFA dietary sources for quality prime lamb production. To our current knowledge, available published information is generally scarce on the impact of different supplemental oil based PUFA dietary sources on animal performance and associated feed costs in the Australian prime lamb feedlot industry. Therefore, the present study aims to determine the responses of Australian prime lambs, in terms of live animal performance, carcass characteristics as well as feed costs, to a variety of dietary supplemental oils from canola, rice bran, flaxseed, safflower and rumen-protected sources.

3.3. Materials and Methods

3.3.1. Animal ethics

This study was carried out at the Tasmanian Institute of Agriculture's Cressy Research and Demonstration Station, Burlington Road, Cressy, Tasmania, Australia from April to June 2016. The use of animals and procedures performed in this study were all approved by the University of Tasmania Animal Ethics Committee (Permit No. A0015657).

3.3.2. Animals, diets and experimental design

An a priori power analysis with repeated measures was conducted using G-Power to justify an appropriate sample and effect size. As depicted in Figure 1, a minimum total sample size of 36 lambs was sufficient for a large effect size, statistical power of 95% and two-sided significance level of 0.05 in an experimental design utilising 6 treatment groups and 7 repeated measurements. Therefore, the use of 72 animals in 6 treatment groups was a statistically robust experimental design.

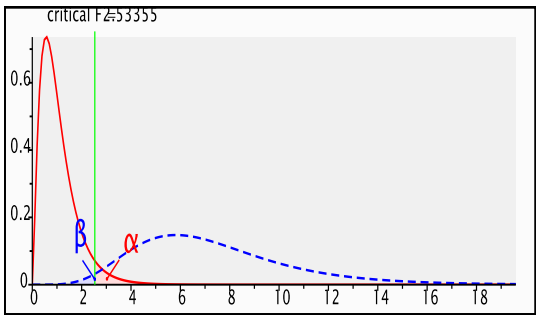

Figure 3. 1. G-Power analysis for statistical power, effect and sample size.

Seventy-two (White Suffolk x Corriedale first-cross) prime lambs with an average LWT of 35.7 ± 0.9 kg weaned at 6 months were randomly allocated into six treatments: (1) Control: lucerne hay only; or lucerne hay plus wheat-based pellet infused with 50 mL/kg DM of oil from (2) rice bran (RBO); (3) canola (CO); (4) rumen-protected (RPO), (5) flaxseed (FO) and (6) safflower (SO) sources in a completely randomised design balanced by equal number of ewe and wether lambs. The animals were kept in individual pens and had *ad libitum* access to clean fresh water and lucerne hay throughout the feeding trial. Each lamb in the supplemented treatments was fed 1 kg pellets/day. The feeding trial lasted 10 weeks including a 3-week adaptation period followed by 7 weeks of full supplementation. The

freshly weighed feed was offered every day at 9:00 h after the collection and weighing of the previous day's feed residues. Details of the experimental lamb management techniques and data collection had been extensively described in previous experiments (Flakemore et al., 2017a; Flakemore et al., 2017b; Malau-Aduli et al., 2016; Nguyen et al., 2017a; Nguyen et al., 2017b; Nguyen et al., 2018b).

3.3.3. Feed intake, body conformation and liveweight measurements

Lucerne hay and pellet intakes were separately calculated using the difference between daily amount of total feed offered and the residual feed measured. Feed conversion efficiency and feed cost per liveweight gain were computed as described in detail by Flakemore et al. (2015). Lucerne hay cost was calculated based on the market price during the experimental period (AU$0.575/kg) and concentrate price was based on the market ingredient costs of AU$0.160/kg, AU$0.194/kg, AU$0.534/kg, AU$0.486/kg, AU$0.778/kg for RBO, CO, RPO, FO and SO diets, respectively. Body conformation measurements including body length (BL), chest girth (CG) and withers height (WH) were performed weekly using a measuring tape and following the description by Holman et al. (2014). Body condition scores (BCS) were subjectively estimated at weekly intervals by the same researcher on a scale of 1 to 5. BCS measurement was as described in detail by Kenyon et al. (2014). Body conformation and BCS measurements were taken while lambs were restrained in a relaxed state with heads comfortably erect and standing stably upon all four legs on flat ground to minimise stress. Individual animal liveweight (LWT) was measured weekly after BCS estimation employing a calibrated Ruddweigh 3000XT Walkover weighing electronic scale with animals standing in a relaxed position.

3.3.4. Feed analysis

Feed samples from the supplements and lucerne hay were dried, ground and analysed as per standard laboratory methods of AOAC (AOAC, 1995) for DM, ash, neutral (NDF) and acid (ADF) detergent fibres, ether extract (EE) and crude protein (CP). Total digestible nutrients (TDN) and conversion of TDN to digestible (DE) and metabolisable (ME) energies were computed as per Bath and Marble (Bath and Marble, 1989) and Robinson et al. (Robinson et al., 2004).

3.3.5. Slaughter protocol and carcass characteristic measurements

All animals were fasted overnight before transporting them to a near-by commercial abattoir (Tasmanian Quality Meats, Cressy, Tasmania, Australia) adjacent to the experimental site in strict compliance with the slaughtering procedures prescribed by the Meat Standards of slaughter and the removal of non-edible carcass components (head, hide, intestinal tract, and internal organs). Dressing percentage (DP) was calculated as: DP (%) = (HCW/LWT) × 100. Thereafter, the carcasses were chilled for 24 h at 4 °C and transported to Robinson Meats, Glenorchy, Hobart, Tasmania, Australia for commercial boning out into retail cuts and carcass measurements. Carcass characteristic determination of fat thickness, body wall thickness and rib eye area were taken in accordance with the detailed description by Flakemore et al. (Flakemore et al., 2015). Percentage of boneless, closely trimmed retail cuts (BCTRC) was computed using the equation: %BCTRC = (49.936 − (0.0848 × 2.205 × HCW) − (4.376 × 0.3937 × FD) − (3.53 × 0.3937 × BWT) + (2.456 × 0.155 × REA) where HCW: hot carcass weight; FD: fat thickness; BWT: body wall thickness and REA: rib eye area (Neville et al., 2010). Over-the-hooks (OTH) trade value in Australian dollars was computed as HCW × 520 ¢/kg divided by 100 ¢ to produce an average total dollar value per carcass for animals from each treatment group. Five hundred and twenty Australian cents per kilogram (520 ¢/kg) was

the amount received for the sale of the lambs used in this study, and is within the range for OTH prices for 2016 (MLA, 2016).

3.3.6. Statistical analysis

All collected data were analysed using the General Linear Model procedure (PROC GLM) of Statistical Analysis System (SAS, 2009). The fixed effects of treatment and gender and their second order interactions on growth, body conformation and carcass traits were tested. The initial full statistical model used for the analysis was:

$$Y = \mu + O_i + G_j + (OG)_{ij} + (OG)^2_{ij} + (OG)^3_{ij} + e_{ijk}$$

where:

Y = dependent variable,

μ = overall mean,

O_i = oil supplementation treatment,

G_k = gender, brackets and superscripts represent linear and cubic second-order interactions and

e_{ijk} = residual error.

Linear and cubic orthogonal contrasts indicated that gender was not a significant factor, hence it was removed from the final model that assessed the impact of treatment only. Duncan's multiple range tests were used to determine the differences amongst treatments at a minimum threshold of $p < 0.05$ level.

3.4. Results

3.4.1. Chemical composition of experimental and basal feed

The chemical composition of experimental diets is presented in Table 3.1. DM of all supplemented pellets and basal feed were similar, ranging between 86.8% and 91%. Crude protein content of the different supplemented pellets ranged between 13.5% and 15.6%, which was lower than that in the basal feed (17.1%). ADF and NDF contents of the different supplemented pellets ranged from 7.5% to 10.4% and from 19.0% to 22.2%, respectively, while ADF and NDF content of the basal feed were 36.9% and 47.2%, respectively. In terms of EE content, the level in the supplemented pellets fluctuated between 5.1% and 5.6%, which was at least three-fold higher than the amount in the basal feed (1.5%). ME content of all supplemented pellets was approximately 12.2 (MJ/kg), whilst the basal feed contained 9.08 (MJ/kg) ME.

Table 3.1. Proximate analysis of the experimental and basal diets.

Chemical Composition (% DM)	Control Lucerne Hay	RBO	CO	RPO	FO	SO
DM	86.8	89.9	91.0	89.7	90.7	89.9
CP	17.1	14.8	14.0	15.6	14.6	14.5
ADF	36.9	7.5	9.4	8.2	10.4	10.0
NDF	47.2	19.0	19.1	20.4	22.2	21.1
EE	1.5	5.5	5.6	5.1	5.6	5.5
ASH	8.4	6.7	6.2	6.5	8.2	8.2
%TDN	60.2	83.1	81.6	82.5	80.8	81.1
DE (Mcal/kg)	2.65	3.65	3.59	3.63	3.56	3.57
ME (MJ/kg)	9.08	12.54	12.32	12.46	12.20	12.25

DM: dry matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; EE: ether extract; CP: crude protein; %TDN: total digestible nutrients; ME: metabolisable energy; RBO, CO, RPO, FO and SO was wheat-based pellet infused with 50 mL/kg DM of oil from rice bran, canola, rumen-protected, flaxseed and safflower sources, respectively. Total digestible nutrients (%TDN) were calculated as TDN (% of DM) = 82.38 − (0.7515 × ADF [% of DM]). Metabolisable energy (ME) was 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.

3.4.2. Liveweight, average daily gain, feed intake and feed cost

Liveweight, average daily gain, feed intake responses of prime lambs and feed costs, associated with the different PUFA enriched pellets are shown in Table 3.2. Liveweight of the animals in all treatments at the beginning of the experiment were similar. However, at the end of the experiment, all animals in the supplemented groups had a higher liveweight than their counterparts in the control group. Furthermore, there was no difference in liveweight among the supplemented groups. The ADG of the control group was only half of that recorded for the supplemented groups. ADG of the SO treatment had the lowest value among the supplemented

treatments (190.3 g/head/day). The ratio of hay to concentrate feed intake in lambs supplemented with PUFA enriched pellets was approximately 50:50. The total feed intake of the supplemented animals was 1.70 (kg/head/day), which was significantly higher than that of the control group (1.38 kg/head/day). Lucerne hay conversion efficiency (LCE) of all lambs in the supplemented groups was 4.20 (kg lucerne hay/kg LWT gain per animal), which was only approximately one fourth of the LCE in the control group (16.6 kg lucerne hay/kg LWT gain per animal). There was a significant difference among the supplemented treatments in terms of concentrate feed conversion efficiency (FCE) in which the RBO and SO groups had a higher mean FCE compared to the remaining supplemented treatments. The result also showed that the RBO and CO treatments had the lowest feed cost per unit gain (FCPUG) of AU$3.0/kg, which was only about one third of the FCPUG in the control group and one half of the FCPUG in the SO group. RPO and FO treatments had medium FCPUG of AU$4.1/kg and AU$4.2/kg, respectively.

Table 3.2. Liveweight, average daily gain, feed intake and feed costs per unit gain of prime lambs fed various PUFA enriched pellets*.

Parameters	Control	RBO	CO	RPO	FO	SO	SEM
Initial LWT (kg)	37.6	38.6	37.6	38.3	38.3	38.6	0.59
Final LWT (kg)	42.4 ^{b}	48.9 ^{a}	48.9 ^{a}	50.3 ^{a}	49.8 ^{a}	48.2 ^{a}	0.79
ADG (g)	94.3 ^{c}	205.7 ^{ab}	226.3 ^{a}	240.0 ^{a}	230.2 ^{a}	190.3 ^{b}	11.22
Lucerne hay intake (kg DM/head/day)	1.38 ^{a}	0.79 ^{b}	0.86 ^{b}	0.88 ^{b}	0.95 ^{b}	0.85 ^{b}	0.08
Concentrate intake (kg DM/head/day)	-	0.86	0.82	0.83	0.84	0.86	0.05
Total intake (kg DM/head/day)	1.38 ^{b}	1.64 ^{a}	1.68 ^{a}	1.71 ^{a}	1.79 ^{a}	1.71 ^{a}	0.07
LCE	16.6 ^{a}	4.0 ^{b}	3.9 ^{b}	3.8 ^{b}	4.2 ^{b}	4.6 ^{b}	0.79
FCE	-	4.3 ^{ab}	3.7 ^{b}	3.6 ^{b}	3.7 ^{b}	4.7 ^{a}	0.24
FCPUG (AU$/kg)	9.5 ^{a}	3.0 ^{d}	3.0 ^{d}	4.1 ^{c}	4.2 ^{c}	6.3 ^{b}	0.23

* Values within the same row not bearing a common superscript differ ($p < 0.05$). LWT: liveweight; ADG: average daily gain; LCE: lucerne hay conversion efficiency (kg DM lucerne hay/kg gain per animal); FCE: concentrate feed conversion efficiency (kg DM concentrate/kg gain per animal); FCPUG: feed cost per unit gain (concentrate and lucerne hay cost of feed/kg; LWT: gain (AU$/kg) per animal); SEM: standard error of the means. All other abbreviations as explained in Table 3.1.

3.4.3. Body conformation traits

All body conformation traits and BCS in all experimental animals did not differ at the beginning of the experiment (Table 3.3). However, at the end of the experiment, all animals in the supplemented treatments had significantly greater changes in all body conformation traits and BCS than animals in the control group. There was no difference in any of the changes in

body conformation traits and BCS among the supplemented treatments throughout the whole experimental period.

Table 3.3. Changes in body conformation traits of prime lambs fed various PUFA enriched pellets*.

Body Conformation Traits	Control	RBO	CO	RPO	FO	SO	SEM
Initial CG (cm)	77.3	79.0	77.3	78.8	78.4	78.6	0.63
ΔCG (cm)	4.9 [b]	8.6 [a]	9.3 [a]	8.8 [a]	9.1 [a]	8.5 [a]	0.63
Initial WH (cm)	61.6	60.9	61.2	61.4	62.0	61.1	0.44
ΔWH (cm)	3.9 [b]	5.2 [a]	5.6 [a]	5.6 [a]	5.5 [a]	5.2 [a]	0.35
Initial BL (cm)	61.8	62.7	62.1	62.7	62.9	62.3	0.39
ΔBL (cm)	4.1 [b]	5.2 [a]	5.1 [a]	5.2 [a]	5.4 [a]	5.5 [a]	0.33
Initial BCS	2.63	2.67	2.58	2.63	2.63	2.67	0.07
ΔBCS	−0.21 [b]	0.96 [a]	1.00 [a]	1.13 [a]	1.21 [a]	1.08 [a]	0.13

Δ: change in; CG: chest girth; WH: withers height; BL: body length; BCS: body condition score. All other abbreviations as in Tables 3.1 and 3.2. * Values within the same row bearing different superscripts differ ($p < 0.05$).

3.4.4. Carcass characteristics

Carcass characteristics of experimental lambs are demonstrated in Table 3.4. All the parameters related to carcass characteristics of the supplemented treatments were greater than that of the control group, with the exception of BCTRC% which was greater in the control animals than in the other treatments. There was no difference in pre-slaughter weight, HCW, body wall thickness, rib eye area, BCTRC%, GR fat score and OTH trade among supplemented treatments. The mean dressing percentage significantly differed among supplemented treatments, with the highest value found in the RBO (50.9%) group. Similarly, the mean fat thickness also significantly differed among the supplemented treatments, with the highest value

recorded in the lambs supplemented with RPO (6.4 mm) and the lowest value in those supplemented with SO (5.2 mm).

Table 3.4. Carcass characteristics of prime lambs fed various PUFA enriched pellets*.

Items	Control	RBO	CO	RPO	FO	SO	SEM
Pre-slaughter weight (kg)	40.4 ^{b}	47.0 ^{a}	46.1 ^{a}	47.6 ^{a}	47.7 ^{a}	47.3 ^{a}	0.72
HCW (kg)	19.4 ^{b}	24.9 ^{a}	23.7 ^{a}	24.6 ^{a}	24.5 ^{a}	23.9 ^{a}	0.47
Dressing percentage (%)	45.7 ^{c}	50.9 ^{a}	48.4 ^{b}	48.9 ^{b}	49.1 ^{b}	49.5 ^{ab}	0.53
Fat thickness (mm)	4.0 ^{c}	5.7 ^{ab}	5.5 ^{ab}	6.4 ^{a}	6.2 ^{ab}	5.2 ^{b}	0.38
Body wall thickness (mm)	16.4 ^{b}	21.8 ^{a}	21.8 ^{a}	22.8 ^{a}	20.4 ^{a}	21.7 ^{a}	1.03
Rib eye area (cm2)	14.8 ^{b}	17.0 ^{a}	15.8 ^{ab}	16.4 ^{a}	16.1 ^{ab}	16.1 ^{ab}	0.44
BCTRC%	49.0 ^{a}	47.7 ^{b}	47.5 ^{b}	47.3 ^{b}	47.6 ^{b}	47.7 ^{b}	0.27
GR fat score (1–5)	2.5 ^{b}	3.7 ^{a}	3.4 ^{a}	3.7 ^{a}	3.5 ^{a}	3.3 ^{a}	0.17
OTH trade (AU$)	100.6 ^{b}	129.3 ^{a}	123.1 ^{a}	127.8 ^{a}	127.2 ^{a}	124 ^{a}	2.43

Pre-slaughter weight: the weight of animals prior to transport for slaughter; HCW: hot carcass weight; BCTRC%: boneless, closely trimmed retail cuts; OTH: over the hooks trade (this was based on 520 AU¢ return per kg of HCW). All other abbreviations as in Tables 3.1 and 3.2. * Values within the same row bearing different superscripts differ ($p < 0.05$).

3.5. Discussion

3.5.1. Chemical composition of experimental and basal feed

The ME content of the basal feed was 9.08 MJ/kg which is lower than the 12.0 MJ/kg proposed for the ideal growth rate (Salah et al., 2014). Therefore, concentrate supplementation is needed to maximise lamb growth potential. The CP, ME, NDF and EE content of all supplemented pellets were similar, therefore any differences in lamb growth indicators could be attributed to the different oil sources. The CP content (from 14.0% to 17.1%) in both basal feed and supplemented pellets were well above the 10.7%CP requirement for maintenance and growth

(Salah et al., 2014). The high CP content in pellets and the increased NDF levels (47.2%) in basal feed provide good fibre and nitrogen sources for rumen microbial growth and contribute to the high growth performance of lambs.

3.5.2. Liveweight, average daily gain, feed intake and feed cost

Supplementation of prime lambs with PUFA enriched pellets significantly increased LWT and doubled the ADG in the supplemented groups compared to the control group at the end of the feeding trial. Although better protein nutrition can improve lamb performance, we argue that this was not the case in our study. The reason for this is that the supplements were deliberately formulated to have a very similar protein content of approximately 15% and a similar metabolisable energy content of approximately 12 MJ/kg to provide a level playing field for all the supplements. In other words, the supplements were isonitrogenous (similar protein content) and isocaloric (similar energy content). Therefore, this eliminates or minimises any potential differences due to protein or energy. This significant increase in LWT and ADG over and above those in the control group could be explained by the input of supplemented nutrients contained in the pellets, especially ME, minerals and vitamins which better matched the nutrient requirements for growing lambs and increased the total DM and ME intake. This result was in line with the observations in fat-tailed lambs and goats (Papi et al., 2011; Safari et al., 2009), which showed that an increase in the level of concentrate resulted in improved ADG. Although there was no significant difference in final LWT between the supplemented treatments, the ADG of the SO treatment was the lowest among the supplemented treatments. Peng et al. (2010) investigated the use of different oilseed supplements (sunflower seed, safflower seed, rapeseed, and linseed) in adult ewes and their findings closely agree with the present study in which animals in safflower supplementation group had the lowest ADG in comparison with other treatments. The low ADG and high lucerne intake resulted in the

markedly high value of LCE (16.6) in the control group. The poor feed conversion also led to the high FCPUG (AU$9.5/kg) in the control group. The lower ADG in SO treatment resulted in higher FCE value in comparison with the remaining supplemented treatments. The total of LCE and FCE in all the supplemented treatments ranged from 7.44 to 9.3, which is similar to the results of Papi et al. (2011), in which lambs had a feed conversion ratio in the range of 7.35 and 9.53. The lowest FCPUG in the RBO and CO treatments can be explained by the lower price of the oils used in preparation of the pellets. Safflower oil was the most expensive ingredient (AU$0.778/kg) which resulted in a higher feed expense in the SO treatment. Similarly, the groups of RPO and FO, with prices of 0.534 and 0.486 AU$/kg, respectively, were in a medium level of feed costs. Feed cost may be an important factor that could influence lamb producers in their decision to supplement lambs with a new feed source or not. However, feed cost depends on many factors such as price and availability of the different feed sources, as well as the supplementation proportion and quality. This study showed that RBO and CO could be used as alternative supplements for lambs, with low feed cost occurring, whilst maintaining comparable lamb ADG value to other treatments.

3.5.3. Body conformation traits

The magnitude of changes in body conformation and body condition score parameters in supplemented lambs were higher than those in the control group. As explained previously, the result is likely due to the intake of feed with higher nutrient content by lambs fed diets supplemented with concentrates. The observation herein of no differences occurring in any body conformation traits and body condition score changes between the supplemented treatments, closely aligns with the results of Nguyen et al. (2018b) who reported that the inclusion of different levels of canola and flaxseed oils in prime lamb diets did not cause any significant differences in body conformation and body condition scores.

3.5.4. Carcass characteristics

The lower values associated with carcass characteristics of the control lambs could be due to the unbalanced diet and low ME content. Although lucerne hay fed to the lambs in this trial had high CP content (17.1%), it had a relatively lower ME (9.08 MJ/kg) than the ME of 12 MJ/kg required for optimal lamb growth (Salah et al., 2014). All the other supplemented treatments had a higher ME of approximately 12.2 MJ/kg which is above the ME requirement for growth and also contained a better roughage-concentrate ratio (approximately 50:50). This trend was observed in the study on different hay-to-concentrate ratios in which the results showed that hot carcass weight, pre-slaughter weight, dressing percentage, and backfat thickness increased when the proportion of concentrates in the ration increased from 30% to 70% (Papi et al., 2011). The findings in the present study of no differences in carcass characteristics and OTH among the supplemented treatments agree with the results of Flakemore et al. (2015) that examined different levels of oil-infused rice bran supplement in their feeding trial. Those authors also did not find any differences between treatments in terms of carcass characteristics and OTH in the supplemented lambs. Manso et al. (2009) also showed that the inclusion of 4% hydrogenated palm oil or sunflower oil in the concentrate did not affect any of the carcass characteristics of lambs. In summary, with the exception of a higher dressing percentage and fat thickness in the RBO and RPO treatments, respectively, the inclusion of different oils in feed pellets did not change the carcass characteristics and OTH of lamb meat.

3.6. Conclusions

Increases in total feed intake, final LWT, ADG, body conformation traits and carcass characteristics were found in prime lambs fed a lucerne-basal and wheat-based pelleted diet enriched with PUFA-containing oil compared to lambs that were fed lucerne alone. Therefore, supplementation of lucerne fed lambs with PUFA enriched pellets is recommended to increase

animal production. The use of RBO and CO in fattening prime lambs had comparatively lower feeding costs and did not affect live animal performance, carcass characteristics and OTH trade income in comparison to other sources of PUFA.

Chapter 4: Fatty acid profiles of muscle, liver, heart and kidney of Australian prime lambs fed different polyunsaturated fatty acids enriched pellets in a feedlot system

4.1. Abstract

We investigated the effect of various dietary polyunsaturated fatty acid (PUFA) sources on the fatty acid profiles of muscle, liver, heart and kidney of Australian prime lambs. Seventy-two White Suffolk x Corriedale first-cross lambs weaned at 6 months of age were randomly allocated to the following six treatments: (1) Control: Lucerne hay only; wheat-based pellets infused with 50 ml/kg dry matter (DM) of oil from (2) rice bran (RBO); (3) canola (CO); (4) rumen-protected (RPO), (5) flaxseed (FSO) and (6) safflower (SO) sources in a completely randomized experimental design. Lambs in CO, FSO, SO and RPO treatments achieved contents of eicosapentaenoic acid (EPA, 22:5n-3) plus docosahexaenoic acid (DHA, 22:6n-3) in the *longissimus dorsi* muscle ranging from 31.1 to 57.1 mg/135 g, over and above the 30 mg per standard serve (135 g) threshold for "source" claim under the Australian guidelines. There was no difference in n-3 LC-PUFA contents in *longissimus dorsi* muscle of lambs fed dietary oils of plant origin. The highest 18:3n-3 (ALA) contents achieved with FSO diet in the muscle, liver and heart were 45.6, 128.1 and 51.3 mg/100 g, respectively. Liver and kidney contained high contents of n-3 LC-PUFA (ranging from 306.7 to 598.2 mg/100 g and 134.0 to 300.4 mg/100 g, respectively), with all values readily exceeding the 'good source' status (60 mg per serve under Australian guidelines). The liver and kidney of PUFA fed lambs can be labelled as 'good source' of n-3 LC-PUFA based on EPA and DHA contents stipulated by the Food Standards of Australia and New Zealand guidelines. Therefore, if lamb consumers consider eating the liver and kidney as their dietary protein sources, they can adequately obtain the associated health benefits of n-3 LC-PUFA.

4.2. Introduction

Accumulating scientific evidence demonstrates the important role that ≥C20 n-3 LC-PUFA play in conditions such as cardiovascular disease, certain cancers and other diseases (Xu, 2015; Zarate et al., 2017). Chen et al. (2016) suggested that both dietary and circulating LC-PUFA were inversely associated with all-cause mortality. In another study, Stark et al. (2016) found that most of the countries and regions of the world having very low to low range of blood n-3 LC-PUFA were associated with an increased risk of chronic diseases. Therefore, the consumption of adequate n-3 LC-PUFA is crucial to maintaining a healthy body and for the prevention of chronic diseases. However, humans like all other mammals, cannot synthesise n-3 LC-PUFA because they lack the necessary Δ12 and Δ15-desaturase enzymes. Therefore, these health-benefitting fatty acids must be supplied in the diet (Nakamura and Nara, 2004). Humans can obtain n-3 LC-PUFA or their C_{18} PUFA precursors from various sources including aquatic (fish, crustaceans, and molluscs), animal sources (meat, egg, and milk), seeds and seed oils, fruits, herbs, cyanobacteria; and microorganisms (bacteria, fungi, microalgae, and diatoms) (Abedi and Sahari, 2014). Ruminant meat research has drawn considerable attention because ruminant meat contains some bioactive lipids (including n-3 LC-PUFA) and the fatty acid profiles of ruminant meat can be enhanced through dietary supplementation (Chikwanha et al., 2018; Vargas-Bello-Perez and Larrain, 2017). In this regard, the manipulation of fatty acid profiles in sheep meat via dietary n-3 LC-PUFA supplementation has yielded some promising results. Flakemore et al. (2017b) reported that supplementation of degummed crude canola oil to lambs improved n-3 LC-PUFA contents in meat. Supplementing prime lambs with 5% flaxseed or canola oil pellets increased n-3 LC-PUFA in the liver, heart, kidney and *longissimus thoracis et lumborum* muscle tissue (Nguyen et al., 2017a; Nguyen et al., 2017b). Boles et al. (2005) found that supplementation of safflower oil at up to 6% of sheep diet on as-fed basis, increased levels of PUFA and conjugated linoleic acids (especially the cis-9, trans-11 isomer)

in the lean tissue. However, the studies described above emphasised the need to find new n-3 LC-PUFA feed sources and associated optimal supplementation levels. There are only a handful of published comparative studies into different dietary oil sources and their ability to elevate the level of n-3 LC-PUFA in lamb, especially under feedlot finishing systems. Feeding lambs in an intensive finishing system, such as a feedlot, has rapidly become a specialised component of the prime lamb industry with the number of lambs being grain finished steadily increasing. This increase in intensive feeding can be mainly attributed to the export demand for a consistent supply of lambs that meet market specifications. This is particularly so when quality pasture feed is unavailable or during drought conditions. Therefore, we hypothesised herein that the fatty acid profiles of the *longissimus dorsi* muscle, liver, kidney and heart of Australian prime lambs would differ in response to supplementation with various dietary PUFA sources under a typical intensive, in-door feedlot management system. It was intended that this study would provide outcomes that can be used as guidelines for lamb producers for a better utilisation of available dietary n-3 LC-PUFA sources for enhancing lamb nutritional quality and beneficial human health outcomes.

4.3. Materials and methods

This study was carried out at the Tasmanian Institute of Agriculture's Cressy Research and Demonstration Station, Burlington Road, Cressy, Tasmania, Australia (41° 43′S, 147° 03′E) from April to June, 2016. The use of animals and procedures performed in this study were all approved by the University of Tasmania Animal Ethics Committee (Permit No A0015657).

*Animals, diets and experimental design***.** Seventy-two White Suffolk x Corriedale first-cross prime lambs with an average liveweight (LWT) of 35.7 ± 0.9 kg weaned at 6 months were randomly allocated into six treatments: (1) Control: lucerne hay only; wheat-based pellet infused with 50 ml/kg dry matter (DM) of oil from (2) rice bran (RBO); (3) canola (CO); (4)

rumen-protected (RPO), (5) flaxseed (FSO) and (6) safflower (SO) sources in a completely randomized design balanced by equal number of ewe and wether lambs. The animals were kept in individual pens and had *ad libitum* access to clean fresh water and lucerne hay throughout the duration of the feeding trial. The lambs were sheltered in a roofed experimental shed with adequate ventilation and individual lamb pen dimension was 6 m^2 (1.2 m in width and 5 m in length). The average minimum and maximum temperatures during the experimental period were 6.5 °C and 19.7 °C in April and 3.4 °C and 12.6 °C in June, respectively. Average rainfall figures during the experimental period were 21.4 mm and 147 mm in April and June, respectively. Each lamb in the supplemented treatments was fed 1 kg pellets/day. The feeding trial lasted 10 weeks including a 3-week adaptation period and followed by 7 weeks of full supplementation. Freshly offered feed was given every day at 9.00 h after residual feed had been collected and weighed. The level of refusal of the concentrate supplement ranged from 0.14–0.18 kg DM/head/day. The chemical composition of the experimental and basal diets is presented in Table 4.1.

Procedures of feed sample and chemical composition determination. Every week, feed samples were taken from each pellet bag and bale of lucerne hay and bulked. The bulked samples were stored at −20 °C until the end of the feeding trial. The feed samples were dried at 60 °C over 72 h, ground to pass through a 1 mm sieve using a Laboratory Mill (Thomas Model 4 Wiley® Mill; Thomas Scientific) and analysed using the standard laboratory analytical methods of AOAC (1995) for DM and ash. Neutral Detergent (NDF) and Acid Detergent (ADF) fibre contents were determined using an Ankom Fibre Analyzer (ANKOM2000; ANKOM Technology, USA). Nitrogen content was quantified using a Thermo Finnigan EA 1112 Series Flash Elemental Analyzer and the values multiplied by 6.25 to give the estimated crude protein

(CP) percentage. Ether extract (EE) was analysed using an ANKOMXT15 fat/oil extractor (ANKOM Technology, USA).

Table 4.1. Proximate analysis of the experimental and basal diets

Chemical composition (% DM)	Lucerne hay	RBO	CO	FSO	SO	RPO
DM	86.8	89.9	91.0	90.7	89.9	89.7
Protein	17.1	14.8	14.0	14.6	14.5	15.6
ADF	36.9	7.5	9.4	10.4	10.0	8.2
NDF	47.2	19.0	19.1	22.2	21.1	20.4
EE	1.5	5.5	5.6	5.6	5.5	5.1
ASH	8.4	6.7	6.2	8.2	8.2	6.5
%TDN	60.2	83.1	81.6	80.8	81.1	82.5
DE (Mcal/kg)	2.65	3.65	3.59	3.56	3.57	3.63
ME (MJ/kg)	9.08	12.54	12.32	12.20	12.25	12.46

RBO, CO, RPO, FSO and SO were wheat-based pellets infused with 50 ml/kg DM of oil from rice bran, canola, rumen-protected, flaxseed and safflower sources, respectively; Dry matter (DM), Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Ether extract (EE) and crude protein (CP), Total digestible nutrients (%TDN), Metabolisable energy (ME). Total digestible nutrients (%TDN) were calculated as TDN (% of DM) = 82.38 - (0.7515 x ADF [% of DM]). Metabolisable energy (ME) was 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.

Slaughter protocol and fatty acid analysis. All animals were fasted overnight before transporting them to a near-by commercial abattoir (Tasmanian Quality Meats, Cressy, Tasmania) adjacent to the experimental site in strict compliance with the slaughter procedures prescribed by the Meat Standards of Australia guidelines (Australia and New Zealand Food Regulation Ministerial Council. Food Regulation Standing, 2007). Heart, kidney and liver samples were taken immediately after evisceration. All samples were vacuum-sealed, code-labelled and stored at −20 °C until fatty acid analysis. Thereafter, the carcasses were chilled for 24 h at 4 °C and *longissimus dorsi* muscle sampled at the 12/13th rib of each carcass as a commercial loin chop (approximately 200 g) for FA analysis.

FA analyses of meat, organs and feed samples were conducted at the Commonwealth Scientific and Industrial Research Organization (CSIRO) Food Nutrition & Bio-based Products, Oceans & Atmosphere Laboratory in Hobart, Tasmania, Australia. The procedure of FA analysis was described in detail previously by Malau-Aduli et al. (2016). In short, total lipids in 1 gram of un-homogenised and wet liver, kidney, heart and muscle tissue and feed samples were extracted overnight using a modified Bligh and Dyer (1959). The first step of the process was a single-phase overnight extraction using $CHCl_3$:MeOH:H_2O (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) and followed by rotary evaporation of the lower chloroform phase at 40 °C to obtain total lipids. An aliquot from each total lipid extract was used in transmethylation process with MeOH:$CHCl_3$:HCl (10:1:1 v/v) for 2 h at 80 °C and then 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 1500 µL vial containing the extracted FAME. The FAME was analysed on a 7890B gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an EquityTM-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 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 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 was of similar polarity to the column described above. The carrier gas was helium and GC conditions were according to the description of Miller et al. (2006). FA percentages were computed as follows: FA% = (individual fatty acid area) ∗ (100)/(sum total area of fatty acids). FA contents were calculated as follows: FA mg/100 g = (Total lipid) ∗ (LCF [0.916]) ∗ ([%FA]/100) ∗ 1000 (Flakemore et al., 2017a), where 0.916 was the lipid conversion factor cited by Clayton (Clayton, 2014).

Statistical analysis. Fatty acid data were initially transformed into fatty acid contents (mg/ 100 g). These data were then analysed using the General Linear Model procedure (PROC GLM) of Statistical Analysis System (SAS, 2009). The fixed effects of treatment and gender and their second order interaction effects on fatty acid contents were tested. The initial full statistical model used for the analysis was: $Y = \mu + O_i + G_j + (OG)_{ij} + (OG)^2_{ij} + (OG)^3_{ij} + e_{ijk}$ where Y = dependent variable, μ = overall mean, O_i = oil supplementation treatment, G_j = gender, brackets and superscripts represent linear and cubic second-order interactions and e_{ijk} = residual error. Linear and cubic orthogonal contrasts indicated that gender was not a significant factor, hence it was removed from the final model that assessed the impact of treatment only. Duncan's multiple range tests were used to determine the differences amongst treatments at a minimum threshold of $p < 0.05$ level.

4.4. Results

4.4.1 Fatty acid profiles in basal and supplementary feed

Fatty acid composition (as % total fatty acids) of both basal and supplementary feeds are presented in Table 4.2. Lucerne hay contained high proportions of PUFA and n-3 PUFA (48.7 and 28.2%, respectively). The major fatty acids in lucerne hay were 18:2n-6 (LA) and 18:3n-3 (ALA), with relatively high levels of 19.0 and 27.5%, respectively. Similarly, flaxseed oil infused pellets (FSO) contained relatively high levels of PUFA and n-3 PUFA (62.2 and 20.7%, respectively) and the major fatty acids in FSO were LA (41.0%), ALA (20.5%) and 18:1n-9 (18.8%).

Table 4.2. Fatty acid composition (% total fatty acids) of basal and supplementary feeds.

Fatty acid	Lucerne hay	RBO	CO	FSO	SO	RPO
14:0	0.4	0.1	0.1	0.1	0.2	2.4
15:0	0.5	0.1	0.1	0.1	0.1	0.4
16:1n-9c	0.0	0.0	0.0	0.0	0.0	0.0
16:1n-7c	0.5	0.2	0.3	0.3	0.2	4.0
16:0	26.6	17.4	12.4	11.0	14.0	19.4
17:0	0.7	0.1	0.1	0.1	0.1	0.2
18:2n-6 LA	19.0	41.1	33.7	41.0	56.5	35.7
18:3n-3 ALA	27.5	2.3	4.8	20.5	1.7	2.7
18:1n-9	2.7	31.8	41.8	18.8	20.3	20.9
18:1n-7c	1.0	1.1	2.5	1.8	1.5	1.9
18:1n-7t	0.1	0.0	0.0	0.0	0.0	0.0
18:0	5.5	2.3	1.1	3.4	2.3	2.9
20:4n-6 ARA	0.2	0.0	0.0	0.0	0.0	0.2
20:5n-3 EPA	0.2	0.1	0.1	0.1	0.1	2.5
20:3n-6	0.4	0.2	0.1	0.3	0.5	0.4
20:4n-3	0.5	0.0	0.0	0.0	0.0	0.1
20:2n-6	0.3	0.1	0.1	0.1	0.1	0.1
20:0	1.6	0.6	0.6	0.4	0.5	0.4
22:5n-6 DPA-6	0.0	0.0	0.0	0.0	0.0	0.2
22:6n-3 DHA	0.0	0.0	0.0	0.0	0.0	1.6
22:5n-3 DPA-3	0.0	0.0	0.0	0.0	0.0	0.5
22:0	2.0	0.5	0.3	0.3	0.2	0.4
23:0	1.0	0.1	0.1	0.1	0.1	0.1
24:0	2.3	0.6	0.4	0.2	0.3	0.4
∑SFA	40.9	21.9	15.1	16.0	17.9	26.7
∑MUFA	9.7	34.0	45.7	21.8	22.9	28.3
∑PUFA	48.7	43.9	39.0	62.2	59.0	44.6
∑n-3 LC-PUFA	0.7	0.1	0.1	0.1	0.1	4.8
∑n-3 PUFA	28.2	2.4	4.9	20.7	1.7	7.4
∑n-6 PUFA	20.2	41.4	34.0	41.4	57.0	37.1
∑other FA	0.8	0.2	0.2	0.1	0.2	0.4
n-6/n-3	0.7	17.3	6.9	2.0	33.5	5.0

LA, linoleic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; and total polyunsaturated fatty acids (ΣPUFA); ΣSFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ΣMUFA 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; ΣPUFA 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; Σn-3 LC-PUFA is the sum of 20:5n-3, 20:4n-3, 22:6n-3, 22:5n-3; Σn-3 PUFA is the sum of 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:6n-3, 22:5n-3; Σn-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; Σother FA is the sum of other individual FA present at <0.1% except ARA, DHA, EPA, and DPA. All other abbreviations are as defined in Table 4.1.

The remaining treatments were rich in PUFA and n-6 PUFA with relatively high levels ranging from 39 to 59 (%) and 34 to 57(%), respectively, in which the major fatty acids were LA and 18:1n-9. The highest n-6 PUFA level was found in safflower oil infused pellets (SO) (mainly LA: 56.5%). The rumen protected oil infused pellets (RPO) had the highest proportions of n-3 LC-PUFA comprising 4.8% eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3).

4.4.2. Fatty acid profiles in longissimus dorsi muscle tissue

Fatty acid contents (mg/100 g) of the *longissimus dorsi* muscle tissue is depicted in Table 4.3. There was no difference among treatments in total fatty acids (FA), saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). The PUFA, n-3 LC-PUFA, n-3 PUFA and 18:1n-7t of supplemented treatments were significantly higher than that of control. The RPO treatment had the highest contents of n-3 LC-PUFA (66 mg/100 g); mainly EPA, DPA and DHA. The highest mean of ALA was found in the FSO treatment (45.6 mg/100 g) and the lowest value was in the control. All supplemented treatments, except for RPO, had significantly higher values of LA than that of control. The SO treatment contained the highest contents of n-6 PUFA and n-6/n-3 ratios of all treatments varied from 1.8 to 3.5.

4.4.3 Fatty acid profiles in edible organs

Fatty acid profiles in the liver. Table 4.4 shows the fatty acid contents (mg/100 g) of liver tissue. The means of all fatty acid components in the liver were markedly higher than those in the muscle and significant differences were evident in both saturated and unsaturated FA. The CO treatment had the highest total FA (3775.0 mg/100 g) and MUFA (1018.0 mg/100 g) contents. The lowest SFA and 18:0 contents were found in the RPO group (1086.0 mg/100 g and 595.5 mg/100 g, respectively).

Table 4.3. Fatty acid contents (mg/100 g) of *longissimus dorsi* muscle tissue in lambs fed various dietary PUFA enriched pellets.

Fatty acid	Control	RBO	CO	FSO	SO	RPO	SEM	P
14:0	24.1^{ab}	34.6^{a}	30.8^{ab}	23.5^{ab}	26.3^{ab}	18.4^{b}	4.81	0.047
15:0	4.6	5.9	4.9	3.5	4.2	3.5	0.77	0.259
16:1n-9c	3.1^{ab}	4.0^{a}	4.0^{a}	2.6^{ab}	3.1^{ab}	2.4^{b}	0.49	0.041
16:1n-7c	16.6	23.1	21.0	16.8	18.8	16.3	2.64	0.380
16:0	375.7	498.8	484.9	386.2	460.7	342.0	55.69	0.266
17:0	20.6	25.1	20.3	16.8	21.0	17.2	3.04	0.444
18:2n-6 LA	87.0^{c}	158.5^{ab}	140.0^{b}	148.7^{b}	196.8^{a}	122.9^{bc}	14.58	0.000
18:3n-3 ALA	23.9^{c}	31.2^{bc}	35.8^{b}	45.6^{a}	29.5^{bc}	25.6^{c}	2.95	0.000
18:1n-9	601.6	794.6	776.2	558.3	720.9	538.6	92.86	0.227
18:1n-7c	23.8^{b}	36.6^{a}	40.0^{a}	31.1^{ab}	36.1^{a}	31.6^{ab}	3.73	0.009
18:1n-7t	22.1^{b}	50.3^{a}	47.6^{a}	50.4^{a}	48.4^{a}	42.2^{a}	6.68	0.041
18:0	323.5	386.4	387.3	299.6	382.5	247.5	47.89	0.236
20:4n-6 ARA	27.9^{b}	36.1^{ab}	33.7^{ab}	30.1^{b}	45.2^{a}	29.1^{b}	4.45	0.046
20:5n-3 EPA	12.6^{c}	16.0^{bc}	18.1^{bc}	19.8^{b}	18.1^{bc}	28.9^{a}	2.02	0.000
20:3n-6	5.9^{ab}	3.9^{b}	5.6^{ab}	5.3^{ab}	6.7^{a}	6.9^{a}	0.74	0.048
20:4n-3	1.9^{bc}	2.8^{ab}	1.2^{bc}	1.1^{bc}	1.0^{c}	4.2^{a}	0.55	0.002
20:2n-6	1.4	1.6	1.7	1.4	2.2	2.3	0.30	0.168
20:0	2.4	3.1	3.0	2.2	2.9	2.2	0.39	0.383
22:5n-6 DPA-6	0.6^{ab}	0.7^{a}	0.5^{ab}	0.3^{b}	0.6^{ab}	0.9^{a}	0.13	0.042
22:6n-3 DHA	3.7^{b}	4.3^{b}	4.9^{b}	5.4^{b}	5.4^{b}	13.3^{a}	0.95	<.0001
22:5n-3 DPA-3	10.1^{b}	15.2^{ab}	16.8^{a}	14.4^{ab}	18.0^{a}	19.6^{a}	1.97	0.033
22:0	1.2	1.3	1.2	1.1	1.2	0.9	0.17	0.605
23:0	1.9	1.5	1.4	1.3	1.5	1.3	0.23	0.462
24:0	1.9	1.8	1.6	1.3	1.7	1.8	0.22	0.534
Total FA	1748.0	2297.0	2241.0	1821.0	2218.0	1658.0	239.60	0.247
∑SFA	755.9	958.4	935.5	735.5	902.0	634.9	110.76	0.259
∑MUFA	717.0	966.8	947.5	713.3	883.2	677.1	109.81	0.254
∑PUFA	183.4^{b}	279.2^{a}	266.1^{a}	279.7^{a}	332.9^{a}	260.8^{a}	25.55	0.012
∑n-3 LC-PUFA	28.4^{c}	38.3^{b}	41.0^{b}	40.7^{b}	42.5^{b}	66.0^{a}	4.51	<.0001
∑n-3 PUFA	52.2^{d}	69.6^{c}	76.7^{abc}	86.9^{ab}	72.7^{bc}	91.6^{a}	6.55	0.003
∑n-6 PUFA	124.8^{c}	204.2^{ab}	183.9^{bc}	188.1^{b}	254.9^{a}	164.3^{bc}	19.71	0.003
∑other FA	91.7	92.6	91.9	92.1	100.1	84.9	8.75	0.908
n-6/n-3	2.4^{c}	3.0^{b}	2.4^{c}	2.2^{cd}	3.5^{a}	1.8^{d}	0.16	<.0001

* Values within the same row bearing different superscripts differ (P<0.05). Total FA is the combined FA contents. All other abbreviations are as defined in Table 4.1 and 4.2

Table 4.4. Fatty acid contents (mg/100 g) of the liver

Fatty acid	Control	RBO	CO	FSO	SO	RPO	SEM	P
14:0	10.0^{a}	10.0^{a}	10.2^{a}	8.5^{ab}	7.7^{ab}	4.7^{b}	1.43	0.043
15:0	12.0^{a}	7.3^{bc}	9.0^{b}	6.9^{bc}	6.4^{bc}	5.6^{c}	0.86	0.000
16:1n-9c	10.5^{ab}	11.1^{ab}	12.9^{a}	8.0^{bc}	9.2^{ab}	4.9^{c}	1.31	0.004
16:1n-7c	20.4^{a}	18.1^{ab}	17.9^{ab}	11.7^{c}	12.8^{bc}	13.4^{bc}	1.94	0.017
16:0	526.9^{a}	520.5^{a}	541.8^{a}	446.9^{ab}	453.0^{ab}	396.0^{b}	35.51	0.044
17:0	56.9^{a}	39.2^{b}	44.7^{b}	42.6^{b}	41.5^{b}	42.9^{b}	2.61	0.001
18:2n-6 LA	209.5^{b}	407.0^{a}	389.3^{a}	411.1^{a}	434.5^{a}	227.4^{b}	30.30	<.0001
18:3n-3 ALA	61.6^{c}	70.2^{bc}	104.3^{ab}	128.1^{a}	72.5^{bc}	48.5^{c}	11.83	0.000
18:1n-9	566.8^{ab}	585.4^{ab}	668.0^{a}	423.6^{cd}	459.1^{bc}	313.1^{d}	46.77	0.000
18:1n-7c	36.8^{b}	48.1^{ab}	52.4^{ab}	41.3^{b}	93.5^{a}	64.3^{ab}	15.95	0.041
18:1n-7t	57.1^{b}	118.8^{ab}	147.8^{a}	172.9^{a}	115.0^{ab}	146.5^{a}	22.77	0.023
18:0	748.8^{a}	718.2^{a}	792.8^{a}	743.0^{a}	737.8^{a}	595.5^{b}	30.43	0.002
20:4n-6 ARA	153.0^{b}	243.3^{a}	234.7^{a}	189.5^{b}	253.3^{a}	109.8^{c}	13.62	<.0001
20:5n-3 EPA	55.3^{c}	53.5^{c}	81.0^{b}	85.1^{b}	57.6^{c}	125.0^{a}	7.68	<.0001
20:3n-6	26.2	22.7	24.0	27.4	30.8	25.5	2.53	0.303
20:4n-3	7.7	9.8	9.6	6.1	5.2	11.4	2.55	0.515
20:2n-6	3.0^{b}	4.9^{a}	5.9^{a}	5.2^{a}	6.0^{a}	4.5^{ab}	0.55	0.008
20:0	3.7^{ab}	3.1^{b}	5.2^{a}	2.8^{b}	3.2^{b}	4.5^{ab}	0.57	0.045
22:5n-6 DPA-6	5.4^{b}	9.9^{a}	8.7^{a}	4.6^{b}	7.4^{ab}	4.7^{b}	1.05	0.004
22:6n-3 DHA	124.6^{bc}	108.7^{c}	147.1^{b}	152.5^{b}	132.2^{bc}	289.5^{a}	12.19	<.0001
22:5n-3 DPA-3	147.7^{ab}	134.8^{b}	161.9^{ab}	169.6^{a}	143.7^{ab}	172.4^{a}	9.52	0.050
22:0	6.1^{ab}	5.2^{b}	7.3^{a}	6.1^{ab}	6.0^{ab}	5.9^{ab}	0.55	0.032
23:0	21.2	16.4	19.4	18.5	16.6	18.1	1.67	0.355
24:0	12.6	11.0	13.9	12.2	12.0	13.0	1.20	0.642
Total FA	3093.0^{b}	3373.0^{ab}	3775.0^{a}	3325.0^{ab}	3296.0^{ab}	2776.0^{b}	188.40	0.024
∑SFA	1398.0^{a}	1331.0^{a}	1444.0^{a}	1287.0^{a}	1284.0^{a}	1086.0^{b}	63.30	0.008
∑MUFA	798.3^{bc}	878.4^{ab}	1018.9^{a}	775.0^{bc}	784.2^{bc}	615.7^{c}	66.48	0.007
∑PUFA	829.8^{b}	1110.0^{a}	1234.1^{a}	1206.5^{a}	1180.5^{a}	1034.9^{a}	64.34	0.001
∑n-3 LC-PUFA	335.3^{cd}	306.7^{d}	399.6^{bc}	413.2^{b}	338.7^{cd}	598.2^{a}	24.52	<.0001
∑n-3 PUFA	396.9^{d}	378.3^{d}	503.9^{bc}	542.9^{b}	412.4^{cd}	647.1^{a}	32.47	<.0001
∑n-6 PUFA	412.8^{b}	716.4^{a}	712.7^{a}	653.6^{a}	755.4^{a}	382.5^{b}	43.05	<.0001
∑other FA	66.2^{a}	53.5^{ab}	70.8^{a}	55.7^{ab}	47.2^{ab}	38.8^{b}	7.49	0.045
n-6/n-3	1.0^{c}	1.9^{a}	1.4^{b}	1.3^{bc}	1.9^{a}	0.6^{d}	0.11	<.0001

* Values within the same row bearing different superscripts differ (P<0.05). All other abbreviations are as defined in Table 4.1 and 4.2.

Contents of n-6 PUFA and LA in all supplemented treatments, except for RPO, were significantly higher than in the control. RPO had the highest n-3 PUFA and n-3 LC-PUFA which mainly consisted of EPA, DPA and DHA. The ratio of n-6/n-3 in all treatments ranged from 0.6 to 1.9. The highest value of ALA was found in the FSO treatment (128.1 mg/100 g).

Fatty acid profiles in the heart. Table 4.5 depicts fatty acid contents (mg/100 g) of the heart. There was no difference in total FA, SFA and MUFA; similar to the same trend observed in the *longissimus dorsi* muscle. PUFA and 18:1n-7t in all supplemented treatments were significantly higher than in the control treatment. The control had the lowest contents of n-6 PUFA and LA. The RPO treatment had the highest n-3 LC-PUFA and n-3 PUFA, whilst the lowest contents of these FA were in the control treatment. FSO had the highest ALA content (51.3 mg/100 g). SO and rice bran infused pellets (RBO) treatments had the highest n-6/n-3 ratio and RPO had the lowest. Generally, the n-6/n-3 ration in all treatments ranged from 2.7 to 6.6.

Fatty acid profiles in the kidney. Table 4.6 shows the fatty acid contents (mg/100 g) in the kidney. SFA contents did not differ among treatments. All supplemented treatments had significantly higher PUFA and LA contents than the control. RPO treatment had the highest n-3 LC-PUFA and n-3 PUFA contents. The highest total FA, n-6 PUFA and LA contents were in the SO treatment (2305.0; 752.6 and 375.8 mg/100 g, respectively). The observed trend of n-3 LC-PUFA content was similar to the pattern observed in the liver where only FSO treatment had greater n-3 LC-PUFA content than the control. The range in n-6/n-3 ratio in all treatments was from 1.3 to 4.0 in which the SO treatment had the highest ratio.

Table 4.5. Fatty acid contents (mg/100 g) of the heart

Fatty acid	Control	RBO	CO	FSO	SO	RPO	SEM	P
14:0	8.5	6.6	9.2	9.6	9.7	9.5	2.54	0.949
15:0	4.3	2.8	3.5	3.7	3.7	3.9	0.50	0.444
16:1n-9c	2.8	2.3	3.4	2.6	2.5	2.4	0.40	0.507
16:1n-7c	5.9^{ab}	4.4^{b}	5.6^{ab}	5.3^{ab}	4.9^{ab}	7.7^{a}	0.93	0.042
16:0	206.9	214.4	234.5	225.7	228.2	221.9	22.60	0.962
17:0	21.8^{a}	14.5^{b}	16.6^{ab}	17.7^{ab}	17.9^{ab}	18.5^{ab}	2.16	0.043
18:2n-6 LA	267.2^{c}	489.2^{a}	427.9^{ab}	439.0^{ab}	467.5^{a}	365.9^{b}	25.02	<.0001
18:3n-3 ALA	23.2^{c}	29.9^{bc}	38.4^{b}	51.3^{a}	24.6^{c}	27.5^{bc}	3.73	<.0001
18:1n-9	245.6	202.6	265.2	229.7	223.8	217.8	32.90	0.812
18:1n-7c	34.3^{b}	38.8^{b}	52.8^{a}	37.3^{b}	41.8^{ab}	51.5^{a}	4.03	0.011
18:1n-7t	21.0^{c}	42.6^{b}	44.3^{b}	66.1^{a}	53.2^{ab}	51.6^{ab}	6.42	0.001
18:0	309.0	312.2	327.2	332.8	342.1	271.5	35.17	0.769
20:4n-6 ARA	81.8^{b}	128.6^{a}	126.5^{a}	101.7^{ab}	135.0^{a}	81.1^{b}	10.82	0.002
20:5n-3 EPA	17.2^{d}	25.0^{bcd}	33.1^{bc}	37.0^{b}	21.2^{cd}	65.6^{a}	3.98	<.0001
20:3n-6	9.6^{b}	9.5^{b}	9.9^{b}	8.8^{bc}	6.7^{c}	12.5^{a}	0.84	0.002
20:4n-3	2.4^{bc}	1.3^{c}	1.7^{c}	1.5^{c}	4.3^{ab}	5.2^{a}	0.78	0.004
20:2n-6	1.3^{c}	1.9^{bc}	2.2^{ab}	1.9^{bc}	2.5^{ab}	2.7^{a}	0.23	0.003
20:0	4.3	3.7	4.3	3.7	4.1	3.5	0.35	0.452
22:5n-6 DPA-6	1.0^{b}	1.6^{a}	1.3^{ab}	1.0^{b}	1.6^{a}	1.5^{ab}	0.17	0.042
22:6n-3 DHA	10.1^{c}	14.8^{bc}	17.2^{b}	17.9^{b}	15.8^{bc}	39.8^{a}	2.17	<.0001
22:5n-3 DPA-3	25.3^{c}	29.1^{bc}	32.4^{abc}	34.3^{ab}	28.9^{bc}	36.8^{a}	2.39	0.024
22:0	6.0	6.1	5.8	5.4	5.8	5.2	0.31	0.336
23:0	11.6^{a}	9.7^{b}	9.0^{bc}	9.3^{b}	7.4^{c}	8.9^{bc}	0.59	0.001
24:0	6.4^{ab}	6.7^{a}	5.7^{ab}	5.7^{ab}	5.4^{b}	5.9^{ab}	0.39	0.045
Total FA	1611.0	1913.0	1992.0	1960.0	1961.0	1802.0	145.20	0.438
∑SFA	580.0	577.3	616.5	614.2	625.0	549.4	62.73	0.951
∑MUFA	360.8	344.0	431.7	406.1	384.0	386.8	46.16	0.803
∑PUFA	453.5^{b}	745.7^{a}	704.4^{a}	705.4^{a}	720.4^{a}	646.4^{a}	41.02	0.000
∑n-3 LC-PUFA	54.8^{c}	70.2^{bc}	84.4^{b}	90.6^{b}	70.1^{bc}	147.5^{a}	8.13	<.0001
∑n-3 PUFA	78.2^{d}	101.7^{cd}	123.6^{bc}	143.2^{b}	95.7^{cd}	175^{a}	10.37	<.0001
∑n-6 PUFA	363.9^{c}	636.5^{a}	572.6^{a}	556.4^{ab}	618.8^{a}	467^{b}	33.61	<.0001
∑other FA	215.5	244.9	238.2	233.3	229.8	218.9	15.27	0.742
n-6/n-3	4.7^{b}	6.3^{a}	4.7^{b}	4.1^{b}	6.6^{a}	2.7^{c}	0.32	<.0001

* Values within the same row bearing different superscripts differ (P<0.05). All other abbreviations are as defined in Table 4.1 and Table 4.2.

Table 4.6. Fatty acid contents (mg/100 g) of the kidney

Fatty acid	Control	RBO	CO	FSO	SO	RPO	SEM	P
14:0	3.7^{a}	3.3^{ab}	3.7^{a}	2.2^{b}	3.0^{ab}	3.2^{ab}	0.42	0.046
15:0	4.9^{a}	2.8^{b}	3.9^{ab}	3.4^{b}	4.0^{ab}	3.6^{b}	0.40	0.018
16:1n-9c	3.1^{b}	2.7^{bc}	4.1^{a}	2.0^{c}	2.9^{bc}	2.2^{bc}	0.31	0.001
16:1n-7c	5.8^{a}	3.8^{bc}	5.0^{ab}	3.2^{c}	3.8^{bc}	5.3^{a}	0.42	0.001
16:0	270.1	285.3	309.1	264.5	341.0	276.6	25.50	0.293
17:0	24.0^{a}	18.2^{b}	19.7^{ab}	18.8^{b}	22.3^{ab}	19.7^{ab}	1.54	0.041
18:2n-6 LA	167.0^{c}	282.0^{b}	272.3^{b}	293.6^{b}	375.8^{a}	238.4^{b}	23.95	<.0001
18:3n-3 ALA	24.6^{a}	16.8^{b}	27.7^{a}	30.0^{a}	18.2^{b}	16.3^{b}	1.85	<.0001
18:1n-9	238.8^{a}	198.1^{ab}	244.2^{a}	165.6^{b}	215.2^{ab}	159.6^{b}	17.83	0.006
18:1n-7c	25.5^{ab}	27.6^{ab}	34.5^{ab}	23.6^{b}	35.6^{a}	30.7^{ab}	3.47	0.043
18:1n-7t	13.8^{c}	26.8^{bc}	30.4^{ab}	45.1^{a}	43.0^{ab}	39.0^{ab}	5.35	0.002
18:0	305.9^{b}	319.5^{ab}	349.1^{ab}	331.8^{ab}	399.1^{a}	300.6^{b}	28.37	0.038
20:4n-6 ARA	132.4^{c}	255.1^{b}	247.5^{b}	208.6^{bc}	334.7^{a}	136.2^{c}	27.11	<.0001
20:5n-3 EPA	54.0^{bc}	34.5^{c}	59.9^{bc}	71.0^{b}	45.2^{bc}	139.3^{a}	11.20	<.0001
20:3n-6	10.8	11.7	12.3	17.9	16.6	16.6	2.46	0.214
20:4n-3	7.7	5.1	3.0	2.6	4.5	4.8	1.86	0.467
20:2n-6	3.4^{d}	8.8^{ab}	6.2^{c}	7.5^{bc}	10.9^{a}	5.5^{cd}	0.78	<.0001
20:0	6.5^{b}	6.3^{b}	6.5^{b}	6.0^{b}	8.1^{a}	5.0^{b}	0.52	0.011
22:5n-6 DPA-6	0.3^{d}	2.6^{a}	1.6^{b}	0.7^{cd}	2.5^{a}	1.1^{bc}	0.19	<.0001
22:6n-3 DHA	27.7^{c}	42.8^{bc}	52.2^{b}	51.2^{b}	56.8^{b}	97.3^{a}	6.59	<.0001
22:5n-3 DPA-3	32.2^{c}	51.7^{b}	63.9^{ab}	70^{a}	64.5^{ab}	59^{ab}	5.14	0.000
22:0	31.0^{b}	35.4^{ab}	36.3^{ab}	34.4^{b}	44.0^{a}	29.8^{b}	2.89	0.025
23:0	13.4	10.7	11.0	11.0	12.7	11.3	0.85	0.158
24:0	38.9^{ab}	38.7^{ab}	40.3^{ab}	36.9^{ab}	46.7^{a}	35.5^{b}	3.13	0.032
Total FA	1611.0^{b}	1855.0^{ab}	2015.0^{ab}	1857.0^{ab}	2305.0^{a}	1772.0^{b}	160.60	0.038
∑SFA	699.4	720.9	780.4	709.9	882.1	686.2	61.24	0.232
∑MUFA	358.8^{ab}	330.7^{ab}	400.9^{a}	315.2^{ab}	383.4^{ab}	301.1^{b}	28.89	0.031
∑PUFA	475.5^{c}	730.7^{b}	765.2^{ab}	764.6^{ab}	951.7^{a}	721.8^{b}	66.99	0.002
∑n-3 LC-PUFA	121.4^{c}	134.0^{bc}	179.1^{bc}	194.7^{b}	171.0^{bc}	300.4^{a}	20.81	<.0001
∑n-3 PUFA	146.1^{c}	150.8^{c}	206.8^{bc}	224.6^{b}	189.2^{bc}	316.8^{a}	21.66	<.0001
∑n-6 PUFA	317.7^{d}	571.2^{b}	548.5^{bc}	533.8^{bc}	752.6^{a}	401.3^{cd}	51.39	<.0001
∑other FA	76.9^{ab}	72.0^{ab}	68.3^{ab}	66.7^{ab}	87.7^{a}	62.7^{b}	6.62	0.041
n-6/n-3	2.2^{c}	3.8^{a}	2.7^{b}	2.4^{bc}	4.0^{a}	1.3^{d}	0.17	<.0001

* Values within the same row bearing different superscripts differ (P<0.05). All other abbreviations are as defined in Table 4.1 and Table 4.2

4.5. Discussion

Past studies had used raw oil or oilseeds as supplements in ruminant feeding trials. In order to minimise oxidative rancidity, improve stability and shelf life of both feeds and meat products, we chose to use oil-infused pellets as an innovative means of delivering n-3 LC-PUFA to lambs. Besides, available information on the effect of oil-infused pellets on the fatty acid profile of lamb is scanty in the published literature. Furthermore, the use of oil-infused pellets is less bulky than raw oil or oilseeds, hence our strategy minimises transportation and storage issues in commercial lamb feed production. The fatty acid profile of lucerne hay was similar to that reported by Nguyen et al. (2017b). Many past studies had reported that flaxseed oil contained a high concentration of ALA (El-Beltagi et al., 2007; Gutte et al., 2015; Noci et al., 2011; Popa et al., 2012). Our study showed that FSO had the highest concentration of ALA among the supplemented treatments. The high concentration of LA in SO observed in this study was consistent with other previous studies (Boles et al., 2005; Sabzalian et al., 2008). The RPO treatment contained high concentrations of EPA, DPA and DHA because of its fish oil content.

In the *longissimus dorsi* muscle, the higher contents of n-3 LC-PUFA in the supplemented treatments can be explained by the higher levels of by-pass lipids and protection from excessive lipolysis and extensive biohydrogenation in the rumen (Bessa et al., 2015; Chikunya et al., 2004) as observed in previous studies (Flakemore et al., 2017b; Nguyen et al., 2017b; Peng et al., 2010). On the other hand, it would seem that the levels and extent of biohydrogenation and conversion of stearate (18:0) into oleate (18:1n-9) were similar, hence the observed result where no difference was found in the 18:1n-9 content of the *longissimus dorsi* muscle between the supplemented and control treatments. This observation could also be indicative of similar delta-nine desaturase enzyme activities among treatment groups since it is the primary enzyme responsible for the conversion of stearate to oleate in the biochemical pathway of *in vivo* lipid

synthesis. One of the biohydrogenation intermediates of ALA and LA is 18:1n-7t (Lanier and Corl, 2015). The increased contents of ALA with flaxseed supplementation has also been reported in a previous study by Wachira et al. (2002) who reported that supplementation with flaxseed doubled the proportion (x100) of 18:3n-3 in the *longissimus dorsi* muscle from 1·4 to 3·1. Demirel et al. (2004) reported that feeding protected linseed resulted in 3.0-fold higher concentration of ALA in lamb muscles compared with Megalac (calcium soap of palm fatty acid distillate). The observed trend in our current study where the ALA content in the *longissimus dorsi* muscle of lambs in the FSO treatment was 1.9 times that of the control but similar EPA and DHA contents, could be due to the low elongase enzyme activity for the elongation of C18 to ≥C20 FA and subsequent desaturation of ALA into more potent n-3 LC-PUFA, and the capacity of muscle lipids to incorporate ALA into n-3 LC-PUFA (Bessa et al., 2015).

In contrast, our finding that the RPO treatment had double EPA and triple DHA contents in *longissimus dorsi* muscle tissue in comparison with the control was in line with the results of Wachira et al. (2002) who reported that feeding fish oil increased the muscle proportion (x100) of EPA from 0·7 to 2·3 and DHA from 0·3 to 0·8. The greatest n-6 to n-3 ratio in SO treatment could be explained by high n-6 PUFA contents of safflower oil (57%) as shown in Table 4.2. According to Pannier et al. (2010), a standard serve was 135 g of meat. Food Standards of Australia and New Zealand (FSANZ) regulate that a given food can be claimed as a 'source' of omega-3 fatty acids if the EPA + DHA contents of that food is greater than 30 mg per serve (Zealand, 2012). Lambs in the CO, FSO, SO and RPO treatments had EPA + DHA content in the *longissimus dorsi* muscle exceeding the 30 mg per serve cut-off point for "source" claim under the FSANZ guidelines (Zealand, 2012) (as depicted in Fig. 4.1) and the n-6/n-3 ratio within the desirable level of less than 4.0 (Simopoulos, 2008). The DPA content is not currently

considered as an omega-3 content claim under current regulations (Howe et al., 2007); however, Australia and New Zealand have guidelines for total EPA + DHA + DPA consumption (NHMRC, 2015). Byelashov et al. (2015) revealed that DPA was related to various improvements in human health and served as a storage depot for EPA and DHA in the human body. Howe et al. (2007) suggested that the importance of DPA in delivering the health benefits associated with n-3 LC-PUFA was equal to that of EPA and DHA. Therefore, if DPA was considered as an omega-3 content claim, a significant amount of n-3 LC-PUFA intake (~39%) would be added to lamb meat consumers as demonstrated in Fig. 4.1.

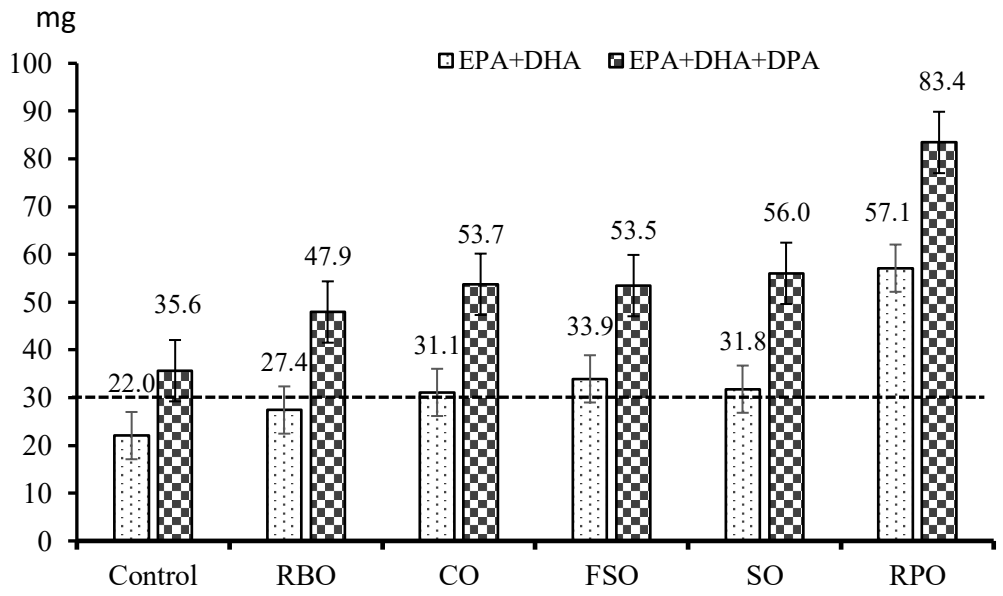

Figure 4. 1. The contents of EPA+DHA and EPA+DHA+DPA per a standard serve (135 g) in *longissimus dorsi* muscle tissue of prime lambs fed various PUFA sources

(EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid and DHA: docosahexaenoic acid; RBO, CO, RPO, FSO and SO were wheat-based pellet infused with 50 ml/kg DM of oil from rice bran, canola, rumen-protected, flaxseed and safflower sources, respectively).

Edible by-products (organ meats) from cattle, pigs and lambs range from 20 to 30% of the animal's liveweight (Umaraw et al., 2015). The findings of Bester et al. (2018) showed that lamb organ meats are nutrient-dense animal source foods that provide protein, zinc and iron to meet the nutritional requirements of humans. Furthermore, the edible internal organs of prime lambs (heart, liver and kidney) are rich in essential fatty acids (Malau-Aduli et al., 2016). Edible internal organs are widely consumed in many countries as special ingredients in traditional foods such as kidney pie in the United Kingdom, cooked and diced delicacies in South America, sheep liver in Iran and paté in many countries (Toldrá et al., 2012). Therefore, knowledge of the fatty acid profiles of lamb edible internal organs could contribute innovations in value-addition of edible meat by-products and generate extra income for lamb producers and slaughterhouses.

The liver is the first organ to receive and metabolise fatty acids after lipid digestion and absorption. As with previously reported studies (Malau-Aduli et al., 2016; Nguyen et al., 2017a), individual fatty acid content was markedly high in the liver. Our observation of the lowest SFA and 18:0 contents in RPO treatment is supported by the work of of Gallardo et al. (2014a) who found that supplementation of ewes with 3% Ca soap FA in fish oil based diets dramatically reduced 18:0 content in milk. Shingfield et al. (2010) also observed that increased fish oil percentage in the diet linearly decreased 18:0 contents in the duodenum. The highest MUFA in CO (mainly 18:1n-9 contents) can be explained by the high MUFA (45.7%) input of CO into the diet. Similarly, the high LA and ALA contents (128.1 mg/100 g) in the liver of FSO fed lambs indicates that despite rumen biohydrogenation, a significant amount of ALA had escaped and was absorbed in the intestines and metabolised in the liver. Demirel et al. (2004) reported that feeding linseed more than doubled the concentration of ALA in the liver compared to Megalac diet. Out of all the PUFA of plant oil origin in this study, only FSO

treatment had an n-3 LC-PUFA content that was significantly higher than the control. This could be explained in part, by the high ALA content in FSO being converted to n-3 LC-PUFA. Our result was in line with the findings of Alhazzaa et al. (2013) who reported that the gradual increase in ALA concentration led to a proportional increase in EPA in hepatocytes. The liver EPA and DHA contents of PUFA fed lambs ranged from 162.2 to 414.0 mg/100 g which was similar to EPA and DHA contents of wild Australian seafoods such as fish (containing 350 mg/150 g (~233 mg/100 g), shellfish (containing 225 mg/150 g (~150 mg/100 g) (see the review by Nichols et al. (2010)). Furthermore, the n-6/n-3 ratio in the liver is within desirable level (Simopoulos, 2008). Therefore, the n-3 LC-PUFA content in the liver of PUFA fed lambs can be labelled as reaching the claimable 'good source' based on EPA + DHA threshold of the Food Standards of Australia and New Zealand guidelines (Zealand, 2012). Therefore, if lamb consumers consider the liver as their dietary protein source, they would obtain the associated dietary health benefits. In the heart, Kashani et al. (2015) reported that supplementation of lambs with Spirulina at the medium-level increased both n-3 and n-6 PUFA compositions. The results from our study showed that total PUFA, n-3 LC-PUFA and n-6 PUFA contents of heart were all enhanced by supplementation with various PUFA enriched pellets. Furthermore, n-3 LC-PUFA contents in the heart of supplemented lambs ranged from 70.1 to 147.5 mg/100 g; indicating that the heart could be a 'good source' of n-3 LC-PUFA (Zealand, 2012). However, the n-6/n-3 ratio of the heart in all lambs with the exception of RPO, was much higher than the minimum desirable level of 4.0 (Simopoulos, 2008). The kidney samples in the present study had high contents of key health-benefitting n-3 LC-PUFA in agreement with the findings of Malau-Aduli, et al.(Malau-Aduli et al., 2016). Specifically, our current findings showed that the contents of n-3 LC-PUFA ranged from 121.4 to 300.4 mg/100 g which is equal to the n-3 LC-PUFA content in Australian wild fish or shellfish (see details in the review by Nichols et al. (2010)). The result of the present study demonstrated that supplementation of lambs with

PUFA enriched pellets substantially increased kidney n-3 LC-PUFA contents. Nguyen et al. (2017a) also reported that supplementation of lambs with canola and flaxseed oils at 5% enhanced n-3 LC-PUFA contents of the kidney.

4.6. Conclusion

Supplementation of lambs with PUFA enhanced pellets in a feedlot system is needed to boost the contents of n-3 LC-PUFA in the *longissimus dorsi* muscle. Lambs in CO, FSO, SO and RPO treatments had contents of EPA + DHA in *longissimus dorsi* muscle that were over the claimable 'source' level of omega-3 as stipulated by the Foods Standards of Australia and New Zealand guidelines. Supplementation of lambs with oils of plant origin including canola, rice bran, flaxseed and safflower had similar effects on the contents of n-3 LC-PUFA in lamb *longissimus dorsi* muscle. FSO had the highest contents of ALA in the muscle, liver and heart tissues. Liver and kidney of PUFA fed lambs can be utilised as omega-3 rich foods on the basis of their high n-3 LC-PUFA contents and desirable n-3/n-6 ratios. In line with the tested hypothesis in this study, it is proven that canola, flaxseed, safflower and rumen protected oil infused pellets can be used in feedlot systems for producing healthy lamb meat as a source of omega-3 food. The cost-efficacy of supplementing lambs with these oil-infused pellets has been presented in detail in a separate stand-alone manuscript (currently under review) and details will not be duplicated herein. Suffice it to say that the feed cost per unit gain in Australian dollars/kg (AU$/kg) for RBO and CO supplements were 3.0 AU$/kg each, 4.1, 4.2 and 6.3 AU$/kg for RPO, FO and SO supplements, respectively.

Chapter 5: Performance and carcass characteristics of Australian prime lambs grazing lucerne and cocksfoot pastures are enhanced by supplementation with plant oil infused pellets

5.1. Abstract

The aim of this study was to determine the effect of cocksfoot cv. porto (CFP) and lucerne pastures and supplementation of grazing lambs with pellets with or without plant oil infusion on performance and carcass characteristics. Forty-eight White Suffolk x Corriedale first-cross weaners were randomly assigned to one of the following four treatments in a split-plot experimental design: (1) CFP or lucerne pastures only (control); (2) CFP or lucerne pastures supplemented with pellets infused with oil from (3) canola (CO); (4) rice bran (RBO) or no oil pellets (NOP). Lucerne and CFP pastures were considered as the main plot effect and played the role of basal pastures. Lambs grazing lucerne or CFP pastures with pellet supplementation achieved carcass weights of >22 kg at 9 weeks, which met the specific requirements of Asian and United States export markets. Pellet supplementation did not affect final liveweight, average daily gain, body length, wither height and chest girth of grazing lambs. Dressing percentage of lambs grazing CFP pasture with pellet supplementation and lambs grazing lucerne pasture with RBO supplementation increased compared with lambs on pasture grazing only. Supplementation with CO to lambs on CFP pasture had low feed conversion efficiency and significantly increased over the hook trade value compared with lambs grazing CFP pasture only. In conclusion, lucerne or CFP pasture plus pellet supplementation produced >22 kg lamb carcasses suitable for the export market. CO could also be used as a tactical tool for increasing the carcass weight of lambs grazing CFP pasture.

5.2. Introduction

The lamb industry plays an important role in the Australian economy with 6% ($3.2 billion) of the gross value of agricultural production and around 4% ($1.8 billion) of agricultural export income in 2015-16(ABARES, 2017). The Australian lamb industry benefits from the large grazing land area (approximately 341 million hectares)(ABARES Land management and farming in Australia) because animal production based on grazing systems is relatively low cost compared to more intense production systems (Pembleton et al., 2015). Secondly, lamb meat finished on green pastures is generally regarded as a good source of iron, zinc and omega 3 polyunsaturated fatty acids (n-3 PUFA)(Jacob and Pethick, 2014). However, lamb production based on grazing systems is dependent on pasture quantity and quality, which in turn, is dependent on seasonal patterns and climate (Shakhane et al., 2013). In addition, lamb meat export for specific markets such as Asia and the USA which represent more than 50% of the total Australian lamb export volume (MLA, 2017a), prefer carcasses of heavier weights (22 kg or more) (Ponnampalam et al., 2017). Therefore, there is a need to evaluate the effect of legume feeds, improved pastures and specialized forages currently being used in lamb finishing systems on growth rate, carcass characteristics and fatty acid profiles of grazing lambs. The additional use of supplementation with diverse oils of plant origin and subsequent effects on growth rate and carcass traits also needs to be evaluated in order to support lamb producers in the production of high quality and heavier carcasses for meeting the requirements of specific export markets (De Brito et al., 2017b).

Cocksfoot cv. porto (*Dactylis glomerata* L. cv. Porto) (CFP) was released in Tasmania in 1972 (Lolicato and Rumball, 1994). This grass has the potential to produce healthy, premium quality meat with high contents of n-3 PUFA and n-3 long-chain ($\geq C_{20}$) PUFA (n-3 LC-PUFA) (Le et

al., 2018b). However, there is currently no available published information on the effect of CFP on lamb performance and carcass characteristics. Lucerne dominant pastures have been shown to consistently produce premium lamb carcasses for both the Australian local and export markets (Ponnampalam et al., 2017). Nevertheless, available knowledge on the effect of lucerne pasture on prime lamb production and carcass characteristics in Tasmania characterized by a cool, temperate climate and considered as Australia's coolest state with a 'clean, green image'(ACECRC, 2010; Australia101, 2019), is generally limited.

Canola oil is commercially extracted from canola seeds (Gaber et al., 2018). Rice bran oil is a by-product of rice processing (Esa et al., 2013). Canola and rice bran oils have been studied for their effect on prime lamb performance and carcass characteristics in a confined system. Supplementation of lambs with rice bran oil and canola oil in confined systems had comparatively lower feed costs, without compromising average daily gain (ADG), carcass characteristics and over the trade (OTH) income (Le et al., 2018a). Supplementation of canola oil infused pellets to prime lambs in a confined system did not cause significant differences in daily feed intake, growth performance and carcass characteristics compared with supplementation with a no oil containing pellet (Nguyen et al., 2018b). However, there is no published information on the effect of rice bran and canola oil supplementation on the performance and carcass characteristics of prime lambs managed on cocksfoot and lucerne dominated grazing systems. Therefore, the objectives of this study were to evaluate the effects of CFP and lucerne pastures and supplementation with pellets with or without plant oil infusion on lamb performance and carcass characteristics.

5.3. Materials and methods

5.3.1. Animal ethics

The research was carried out at the Tasmanian Institute of Agriculture's Cressy Research and Demonstration Station, Burlington Road, Cressy, Tasmania, Australia from October to December, 2016. The use of animals and procedures performed in this study were all approved by the University of Tasmania Animal Ethics Committee (Permit No A0015657).

5.3.2. Animals, experimental design, diets and feed sampling procedures

The animals, experimental design and fatty acid profiles in muscle and edible organs of pasture grazing lambs have previously been described elsewhere (Le et al., 2018b). In brief, forty-eight White Suffolk x Corriedale first-cross, weaned prime lambs, with an average liveweight (LWT) of 38.7 ± 0.7 kg were used in a split-plot design. The main plot in this trial included cocksfoot cv. porto (CFP) and lucerne pastures, which played the role of basal pastures. There was a total of 1.5 ha for each pasture split into 12 equal 0.125 ha subplots as main plot units. These 24 plots of basal pastures were used for rotational grazing. All lambs were randomly assigned to 12 groups of four lambs balanced by gender. Lambs in each group were allocated to one of the following four treatments: (1) CFP or lucerne pastures only as the control treatment; (2) CFP or lucerne pastures supplemented with: no oil pellets (NOP); (3) canola oil infused pellets (CO); and (4) rice bran oil infused pellets (RBO). Thereafter, the 12 groups of lambs were allocated to six CFP pasture subplots and six lucerne pasture subplots. The experimental lambs were grazed daily on pastures from 07:00 to 18:00 h and rotated on fresh pasture subplots every 14 days throughout the trial. Fresh water was provided *ad libitum* during the grazing trial. Before being transferred to pastures, lambs in supplemented groups were individually fed pellets at the rate of 1 kg/head/day. The total duration of the grazing trial was nine weeks, including three weeks of adjustment prior to commencement of the study and six weeks of post-adjustment

data collection. The pasture samples were collected weekly from five sites within an area of 50 cm x 50 cm of each subplot. These samples were bulked, thoroughly mixed and subsamples taken for laboratory analyses. Samples of the pelleted feeds were taken from each bag and stored at -20℃ until the end of the trial before subjected to laboratory analyses. The composition of experimental pellets is shown in Table 5.1.

Table 5.1. Ingredient composition of the experimental pellets*.

Ingredient (g/kg)	NOP	CO	RBO
Wheat	575	465	525
Paddy rice	220	280	220
Lupins	148	148	148
Canola oil, mL/kg	-	50	-
Rice bran oil, mL/kg	-	-	50
Ammonium sulphate	12.6	12.6	12.6
Salt	10	10	10
Limestone	20.9	20.9	20.9
Sheep premix	1	1	1
Acid buff	6.25	6.25	6.25
Sodium bicarbonate	6.25	6.25	6.25

*NOP: wheat-based pellet without infused oil;

CO: wheat-based pellets infused with 50 ml/kg DM of canola oil;

RBO: wheat-based pellets infused with 50 ml/kg DM of rice bran oil.

5.3.3. Feed intake, body conformation measurements and liveweight.

Pellet intake was calculated using the difference between daily amount of total feed offered and the residual feed refused. Feed conversion efficiency (FCE) was calculated according to Flakemore et al. (2015): the average daily feed intake (g)/1000 × 42 [days of supplementation]/Total weight gain (kg) over the full trial period. Feed cost per kg of live animal weight gain was calculated as follows: FCE × ($/kg) of supplementary pellet. Pellet price was based on the market ingredient costs of AU$ 0.147/kg, AU$ 0.160/kg and

AU$ 0.194/kg for NOP, RBO and CO diets, respectively. Body conformation parameters including body length (BL), chest girth (CG) and wither height (WH) were measured weekly according to the description by Holman et al. (2014). Body condition scores (BCS) were recorded at the same time by the same researcher on a scale of 1 to 5 using the method described by Kenyon et al. (2014). Body conformation and BCS parameters were measured while lambs were restrained in a relaxed state with heads comfortably erect and standing stably on all four legs on flat ground to minimise stress(Malau-Aduli et al., 2019). LWT was recorded weekly after measuring body condition score using a calibrated Ruddweigh 3000XT Walkover weighing electronic scale (Gallagher Group Limited, Hamilton, New Zealand) with animals standing in a relaxed position.

5.3.4. Analysis of pellet and pasture samples

Representative samples of pellets and pastures were dried at 60℃ for 72 h, ground to pass through a 1 mm sieve using a Laboratory Mill (Thomas Model 4 Wiley® Mill; Thomas Scientific) and analysed using standard methods of AOAC (1995) for dry matter (DM) and ash. Neutral Detergent (NDF) and Acid Detergent (ADF) fibre contents were determined using an Ankom Fibre Analyzer (ANKOM2000; ANKOM Technology, USA). Nitrogen content was quantified using a Thermo Finnigan EA 1112 Series Flash Elemental Analyzer and the values multiplied by 6.25 to give the crude protein (CP) percentage. Ether extract (EE) was analysed using an ANKOMXT15 fat/oil extractor (ANKOM Technology, USA). Total digestible nutrients (%TDN) were calculated as TDN (% of DM) = 82.38 - (0.7515 x ADF [% of DM])(Bath and Marble, 1989). Metabolisable energy (ME) was 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(Robinson et al., 2004).

5.3.5. Slaughter protocol and carcass characteristics measurements

All experimental lambs were fasted for 12 h with free access to water before transportation to a near-by commercial abattoir (Tasmanian Quality Meats, Cressy, Tasmania) adjacent to the experimental site in strict compliance with Meat Standards of Australia guidelines(Australia and New Zealand Food Regulation Ministerial Council. Food Regulation Standing, 2007). Pre-slaughter weight of lambs was taken and hot carcass weights (HCW) recorded immediately after slaughter and removal of non-edible carcass components (head, hide, intestinal tract, and internal organs). Dressing percentage (DP) was computed as DP (%) = (HCW/LWT) x 100. The carcasses were chilled for 24 h at 4℃ before conveyance to Robinson Meats, Glenorchy, Hobart, Tasmania, Australia for commercial boning out into retail cuts and for carcass measurements. Carcass characteristics including fat thickness, body wall thickness and rib eye area were determined according to Flakemore et al. (2015). The following equation was used to calculate the percentage boneless, closely trimmed retail cuts (BCTRC): %BCTRC = (49.936 – (0.0848 x 2.205 x HCW) - (4.376 x 0.3937 x FT) – (3.53 x 0.3937 x BWT) + (2.456 x 0.155 x REA) where HCW is hot carcass weight; FT is fat thickness; BWT is body wall thickness and REA is rib eye area(Neville et al., 2010). OTH trade value in Australian dollars was calculated as HCW × 500¢/kg divided by 100¢ to make an average total dollar value per carcass for animals from each treatment group. The 500¢/kg used in the present study was the amount received per kg for the sale of the lambs in 2016, and is within the range for OTH prices for 2016 (MLA, 2016).

5.3.6. Statistical analysis

All data were analysed using the split-plot model in General Linear Model procedures (PROC GLM) of the Statistical Analysis System software(SAS, 2009). The main plot and subplot

effects were pasture types and supplementation of pellets with or without oil infusion, respectively. Initial descriptive summary statistics were computed with least square means, standard errors, and scrutinized for data entry errors and outliers. Non-significant interactions between fixed effects were dropped from the analytical model and treatment differences were declared significant at $P \leq 0.05$ using Bonferroni probabilities.

5.4. Results

5.4.1. Chemical composition of pastures and supplementary feeds

The proximate analysis of experimental diets is presented in Table 5.2. Dry matter (DM) of pastures was 20.6%, which was considerably lower than supplemented pellets (~90%). Lucerne pasture had the highest crude protein (CP) of 18.6%, which was higher than that of the supplemented pellets, while CFP had the lowest CP (13.3%). The acid detergent fibre (ADF) and neutral detergent fibre (NDF) of pastures were triple and double those of supplemented pellets, respectively. Canola (CO) and rice bran (RBO) oil infused pellets had higher ether extract (EE) than no oil pellets (NOP) and pastures. The metabolisable energy (ME) of the supplemented pellets was similar and higher than that of the pastures.

Table 5.2. Proximate analysis of supplementary feeds and pastures*

Chemical composition (% DM)	Lucerne	CFP	NOP	CO	RBO
DM	20.7	20.5	89.1	91.1	90.2
CP	18.6	13.3	15.7	15.3	14.7
ADF	25.6	26.7	6.8	7.4	8.0
NDF	35.9	43.8	18.3	19.9	18.7
EE	1.8	3.0	2.1	4.6	3.9
ASH	6.8	6.4	4.0	6.5	5.0
%TDN	63.2	62.3	77.2	76.8	76.4
DE (Mcal/kg)	2.8	2.7	3.4	3.4	3.4
ME (MJ/kg)	9.5	9.4	11.7	11.6	11.5

*CFP: cocksfoot cv. porto pasture; DM: Dry matter; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; EE: Ether extract; CP: crude protein; %TDN: total digestible nutrients, calculated as (% of DM) = 82.38 - 0.7515 x ADF [% of DM]). ME: metabolisable 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. All other abbreviations as in Table 5.1.

*5.4.2. Effect of pellet supplementation on liveweight, concentrate intake, body conformation and carcass characteristic of prime lamb*s

Liveweight, concentrate intake and body conformation: The final LWT and average daily gain (ADG) of grazing lambs were unaffected by pellet supplementation as depicted in Table 5.3. Lucerne grazing lambs supplemented with RBO had significantly greater concentrate intake than those lambs supplemented with NOP or CO. Lambs grazing CFP with no oil pellet (NOP) supplementation had higher FCE than CFP grazing lambs with CO and RBO supplementation. CFP grazing lambs supplemented with RBO had lower feed cost per unit gain (FCPUG) than those lambs grazing CFP with NOP or CO supplementation. Pellet supplementation did not affect changes in the body length, withers height and chest girth of grazing lambs.

Supplementation of RBO to CFP grazing lambs increased change in body condition score (Δ BCS) in comparison to grazing lambs with NOP supplementation or without supplement.

Carcass characteristics: Carcass characteristics of grazing lambs are shown in Table 5.4. Pellet supplementation did not affect pre-slaughter weight, fat thickness, body wall thickness and % boneless, and closely trimmed retail cuts (BCTRC%) of grazing lambs. Supplementation with NOP and CO significantly increased the HCW of CFP grazing lambs compared with CFP grazing only. Lambs grazing CFP with CO and RBO supplementation had greater GR (Girth Rib) fat score than CFP only grazing lambs. Pellet supplementation significantly increased the dressing percentage of CFP grazing lambs. Only RBO supplementation increased the dressing percentage of lucerne grazing lambs. The grazing lambs with NOP supplementation had greater rib eye area than lambs grazing only without supplementation. NOP and CO supplementation to CFP grazing lambs significantly increased over the hooks trade (OTH trade) in comparison to the only CFP grazing lambs.

Table 5.3. Effect of pellet supplementation on liveweight, concentrate intake and body conformation of prime lambs (LSM±SE)*.

Items	Control		NOP		CO		RBO	
	CFP (n = 6)	**Lucerne (n = 6)**	**CFP (n = 6)**	**Lucerne (n = 6)**	**CFP (n = 6)**	**Lucerne (n = 6)**	**CFP (n = 6)**	**Lucerne (n = 6)**
Initial LWT (kg)	41.9 ± 0.8	41.0 ± 0.8	42.6 ± 0.8	40.9 ± 0.8	41.4 ± 0.8	41.0 ± 0.8	40.9 ± 0.8	41.6 ± 0.8
Final LWT (kg)	49.3 ± 1.2	50.1 ± 1.2	48.1 ± 1.2	50.5 ± 1.2	48.5 ± 1.2	50.8 ± 1.2	49.5 ± 1.2	51.1 ± 1.2
ADG (g)	181.3 ± 21.9^{abc}	221.9 ± 21.9^{ab}	134.2 ± 21.9^{c}	234.1 ± 21.9^{ab}	172.4 ± 21.9^{bc}	240.7 ± 21.9^{a}	209.4 ± 21.9^{ab}	231.7 ± 21.9^{ab}
Concentrate intake (kg DM/head/day)	-	-	0.9 ± 0.04^{a}	0.7 ± 0.04^{b}	0.8 ± 0.04^{a}	0.6 ± 0.04^{b}	0.8 ± 0.04^{a}	0.9 ± 0.04^{a}
FCE	-	-	6.6 ± 0.4^{a}	3.0 ± 0.4^{c}	4.9 ± 0.4^{b}	2.9 ± 0.4^{c}	4.3 ± 0.4^{b}	3.9 ± 0.4^{bc}
FCPUG ($AU/kg)	-	-	1.0 ± 0.1^{a}	0.4 ± 0.1^{c}	0.9 ± 0.1^{a}	0.6 ± 0.1^{bc}	0.7 ± 0.1^{b}	0.6 ± 0.1^{bc}
Initial BL (cm)	64.8 ± 0.4	64.0 ± 0.4	64.7 ± 0.4	64.8 ± 0.4	64.7 ± 0.4	64.3 ± 0.4	64.3 ± 0.4	64.8 ± 0.4
Δ BL (cm)	3.5 ± 0.5	4.5 ± 0.5	4.2 ± 0.5	4.3 ± 0.5	3.3 ± 0.5	4.3 ± 0.5	3.7 ± 0.5	3.8 ± 0.5
Initial WH (cm)	62.8 ± 0.6	62.2 ± 0.6	62.8 ± 0.6	62.5 ± 0.6	62.5 ± 0.6	62.7 ± 0.6	62.3 ± 0.6	61.8 ± 0.6
Δ WH (cm)	2.8 ± 0.6^{bc}	4.7 ± 0.6^{a}	2.7 ± 0.6^{c}	4.5 ± 0.6^{ab}	3.2 ± 0.6^{abc}	3.5 ± 0.6^{abc}	3.5 ± 0.6^{abc}	4.7 ± 0.6^{a}
Initial CG (cm)	80.8 ± 0.7	81.2 ± 0.7	81.7 ± 0.7	80.7 ± 0.7	81.0 ± 0.7	80.5 ± 0.7	81.7 ± 0.7	81.2 ± 0.7
Δ CG (cm)	3.3 ± 0.8^{c}	6.2 ± 0.8^{ab}	3.7 ± 0.8^{c}	6.8 ± 0.8^{a}	4.5 ± 0.8^{bc}	6.3 ± 0.8^{ab}	5.3 ± 0.8^{abc}	7.0 ± 0.8^{a}
Initial BCS	3.0 ± 0.04	3.0 ± 0.04	3.1 ± 0.04	3.0 ± 0.04	3.0 ± 0.04	3.0 ± 0.04	3.1 ± 0.04	3.0 ± 0.04
Δ BCS	0.4 ± 0.1^{c}	0.8 ± 0.1^{ab}	0.4 ± 0.1^{c}	0.9 ± 0.1^{a}	0.6 ± 0.1^{bc}	0.7 ± 0.1^{abc}	0.8 ± 0.1^{ab}	0.8 ± 0.1^{ab}

*LWT: liveweight;
ADG: average daily gain;
FCE: concentrate feed conversion efficiency (kg DM concentrate/kg gain per animal);
FCPUG: feed cost per unit gain (concentrate cost/kg LWT gain);
Δ: change in;
CG: chest girth;
WH: withers height;
BL: body length;
BCS: body condition score;
LSM: least square mean;
SE: standard error.
All other abbreviations as in Tables 5.1 and 5.2.
Values within the same row bearing different superscripts differ (P<0.05).

Table 5.4. Effect of pellet supplementation on carcass characteristics of prime lambs (LSM±SE)*.

Items	Control		NOP		CO		RBO	
	CFP (n = 6)	Lucerne (n = 6)	CFP (n = 6)	Lucerne (n = 6)	CFP (n = 6)	Lucerne (n = 6)	CFP (n = 6)	Lucerne (n = 6)
Pre-slaughter weight (kg)	45.6 ± 1.1	46.9 ± 1.1	47.9 ± 1.1	47.5 ± 1.1	46.9 ± 1.1	47.9 ± 1.1	46.7 ± 1.1	48.3 ± 1.1
HCW (kg)	22.5 ± 0.7^{c}	24.1 ± 0.7^{abc}	24.4 ± 0.7^{ab}	25.4 ± 0.7^{ab}	24.6 ± 0.7^{ab}	25.2 ± 0.7^{ab}	23.8 ± 0.7^{bc}	25.7 ± 0.7^{a}
Dressing percentage (%)	45.6 ± 0.8^{c}	48.2 ± 0.8^{b}	50.8 ± 0.8^{a}	50.3 ± 0.8^{ab}	50.8 ± 0.8^{a}	49.7 ± 0.8^{ab}	48.1 ± 0.8^{b}	50.4 ± 0.8^{a}
Fat thickness (mm)	6.0 ± 1.0^{c}	11.0 ± 1.0^{a}	8.3 ± 1.0^{abc}	8.3 ± 1.0^{abc}	8.2 ± 1.0^{abc}	8.7 ± 1.0^{abc}	7.8 ± 1.0^{bc}	9.2 ± 1.0^{ab}
Body wall thickness (mm)	16.5 ± 1.5^{b}	21.7 ± 1.5^{a}	18.3 ± 1.5^{ab}	21.2 ± 1.5^{a}	20.7 ± 1.5^{ab}	20.8 ± 1.5^{a}	18.8 ± 1.5^{ab}	22.0 ± 1.5^{a}
Rib eye area (cm^2)	13.6 ± 0.7^{c}	14.7 ± 0.7^{bc}	15.8 ± 0.7^{ab}	16.8 ± 0.7^{a}	15.3 ± 0.7^{abc}	16.0 ± 0.7^{ab}	15.0 ± 0.7^{abc}	15.8 ± 0.7^{ab}
BCTRC%	48.0 ± 0.3^{a}	46.9 ± 0.3^{b}	48.0 ± 0.3^{a}	47.8 ± 0.3^{ab}	47.4 ± 0.3^{ab}	47.5 ± 0.3^{ab}	47.8 ± 0.3^{ab}	47.1 ± 0.3^{ab}
GR fat score (1–5)	2.7 ± 0.2^{b}	3.5 ± 0.2^{a}	3.2 ± 0.2^{ab}	3.3 ± 0.2^{a}	3.3 ± 0.2^{a}	3.3 ± 0.2^{a}	3.3 ± 0.2^{a}	3.5 ± 0.2^{a}
OTH trade ($AU)	112.3 ± 3.3^{c}	120.5 ± 3.3^{abc}	122.2 ± 3.3^{ab}	127.0 ± 3.3^{ab}	123.2 ± 3.3^{ab}	126.2 ± 3.3^{ab}	119.2 ± 3.3^{bc}	128.7 ± 3.3^{a}

*Pre-slaughter weight: the weight of animals prior to transport for slaughter;

HCW: hot carcass weight;

BCTRC%: boneless, closely trimmed retail cuts;

OTH: over the hooks trade (this was based on 500AU¢ return per kg of HCW).

All other abbreviations as in Tables 5.1, 5.2 and 5.3.

Values within the same row bearing different superscripts differ (P<0.05).

5.4.3. Effect of pastures on liveweight, concentrate intake, body conformation and carcass characteristics of prime lambs

Table 5.5 shows liveweight, concentrate intake and body conformation, while Table 5.6 depicts carcass characteristics of prime lambs as impacted by different pastures. There was no significant difference in initial and final LWT, change in body length (ΔBL) between lucerne and CFP grazing lambs. The ADG, change in withers height (ΔWH), chest girth (ΔCG) and body condition score (ΔBCS) of lucerne grazing lambs was greater than that of lambs that grazed pastures containing CFP. Concentrate intake, FCE and FCPUG of lucerne grazing lambs was lower than that of CFP grazing lambs. The different pasture types did not affect pre-slaughter weight, dressing percentage, rib eye area, BCTRC% and GR fat score. Lucerne grazing lambs had higher HCW, fat thickness, body wall thickness and OTH trade than CFP grazing lambs.

Table 5.5. Effect of different pasture types on liveweight, concentrate intake and body conformation of prime lambs (LSM±SE)*.

Items	Pastures		P value
	CFP (n = 24)	Lucerne (n = 24)	
Initial LWT (kg)	41.7 ± 0.37	41.1 ± 0.37	0.057
Final LWT (kg)	48.9 ± 0.62	50.6 ± 0.62	0.247
ADG (g)	174.3 ± 10.93[b]	232.1 ± 10.93[a]	0.001
Concentrate intake (kg DM/head/day)	0.85 ± 0.02[a]	0.72 ± 0.02[b]	0.001
FCE	5.25 ± 0.24[a]	3.26 ± 0.24[b]	<.0001
FCPUG ($AU/kg)	0.87 ± 0.04[a]	0.54 ± 0.04[b]	<.0001
Initial BL (cm)	64.6 ± 0.18	64.5 ± 0.18	0.630
Δ BL (cm)	3.7 ± 0.27	4.3 ± 0.27	0.137
Initial WH (cm)	62.6 ± 0.29	62.3 ± 0.29	0.427
Δ WH (cm)	3.0 ± 0.31[b]	4.3 ± 0.31[a]	0.005
Initial CG (cm)	81.3 ± 0.37	80.9 ±0.37	0.426
Δ CG (cm)	4.2 ± 0.39[b]	6.6 ± 0.39[a]	0.001
Initial BCS	3.0 ± 0.02	3.0 ± 0.02	0.167
Δ BCS	0.5 ± 0.05[b]	0.8 ± 0.05[a]	0.001

*All other abbreviations as in Tables 5.1, 5.2, and 5.3
Values within the same row bearing different superscripts differ (P<0.05).

Table 5.6. Effect of different pasture types on carcass characteristics of prime lambs (LSM±SE)*.

Items	Pastures		P value
	CFP (n = 24)	Lucerne (n = 24)	
Pre-slaughter weight (kg)	46.8 ± 0.56	47.7 ± 0.56	0.247
HCW (kg)	23.8 ± 0.33[b]	25.1 ± 0.33[a]	0.009
Dressing percentage (%)	48.8 ± 0.39	49.6 ± 0.39	0.143
Fat thickness (mm)	7.6 ± 0.50[b]	9.3 ± 0.50[a]	0.021
Body wall thickness (mm)	18.6 ± 0.74[b]	21.4 ± 0.74[a]	0.011
Rib eye area (cm^2)	14.9 ± 0.35	15.8 ± 0.35	0.080
BCTRC%	47.8 ± 0.16	47.3 ± 0.16	0.051
GR fat score (1–5)	3.1 ± 0.11	3.4 ± 0.11	0.061
OTH trade ($AU)	119.2 ± 1.63[b]	125.6 ± 1.63[a]	0.009

*All abbreviations as in Tables 5.1, 5.2, and 5.4
Values within the same row bearing different superscripts differ (P<0.05).

5.5. Discussion

5.5.1. Chemical composition of pastures and supplementary feeds

The CP and ME content of supplemented pellets were over the 10.7% CP and 11.5 MJ/kg requirements for ideal lamb growth (Salah et al., 2014). The chemical compositions of CO and RBO pellets were similar to our previous findings in indoor systems (Gaber et al., 2018; Le et al., 2019; Malau-Aduli and Kashani, 2015; Malau-Aduli et al., 2016; Malau-Aduli et al., 2019). Although, the CP content of the pastures in this study was well over the CP requirement for ideal lamb growth, the ME content of the pastures was lower than ME requirement for ideal lamb growth (Salah et al., 2014). The DM and CP contents of lucerne in the present study were similar to the results of Robertson et al. (2015).

5.5.2. Effect of pellet supplementation on liveweight, concentrate intake, body conformation and carcass characteristics of prime lambs

Live weight, concentrate intake and body conformation: There was no significant difference in the final LWT and ADG of grazing lambs with and without pellet supplementation. This result is in contrast to Fajardo et al. (2016) who reported that supplementation with concentrates at 2.5% of lamb body weight significantly increased the ADG of grazing lambs. Although, the pastures in this study had ME that was lower than the requirement for ideal lamb growth as mentioned above, lambs that ingested only pastures still had similar growth rates to lambs that were supplemented with pellets. This could be due to the grazing behaviour of lambs, which allowed them to obtain enough ME for growth, resulting in no significant differences due to supplementation in the final LWT and ADG of grazing lambs. Douglas et al. (1995) demonstrated that the diet selected by grazing lambs was mainly leaf, which had lower NDF content and higher organic matter digestibility than pre-grazing herbage samples. The result of our study was in line with the findings of Santos-Silva et al. (2003) who found no significant

differences in liveweight and ADG when unprotected, unsaturated fat was used as a supplement for grass-fed lambs. The FCPUG in our study ranged from 0.4 to 1.0 ($AU/kg), which was considerably lower than FCPUG of RBO and CO supplementation in the indoor system (3 ($AU/kg) (Le et al., 2018a). The higher FCE in CFP grazing lambs supplemented with NOP could be the reason for greater FCPUG of the NOP treatment compared with that of the RBO treatment. The higher price of CO in comparison to RBO (AU$ 0.194/kg vs AU$ 0.160/kg) resulted in the greater FCPUG of the CO treatment.

The BL, WH and CG of grazing sheep has been shown to be highly correlated with body weight (Fourie et al., 2002). Our finding of no significant differences in the BL, WH and CG of grazing lambs in this study could be associated with the similar weight of the experimental lambs irrespective of treatment. This finding also was in agreement with the results of Holman et al. (2014); Malau-Aduli and Kashani (2015) and Malau-Aduli et al. (2019) who reported that the BL, WH and CG of prime lambs were not affected by dietary protein-rich *Spirulina* supplements. The BCS can be used to assess the subcutaneous fat and muscle reserves of sheep (Kenyon et al., 2014). It is likely that the lambs that grazed CFP pasture with RBO supplementation had better nutrient utilization than the grazing CFP lambs with NOP supplementation as demonstrated by the low FCE and high changes in BCS of RBO supplemented lambs.

Carcass characteristics: The carcass weight of lucerne grazing lambs with or without pellet supplementation was similar and well over the 22 kg threshold that is required for export (Ponnampalam et al., 2017). The unchanged carcass weight of lucerne grazing lambs is likely due to the quality of the lucerne pasture available, with its nutritional composition, and in

particular, high protein content, being adequate to support the growth of fast growing lambs. Ponnampalam et al. (2017) also demonstrated that the carcass weight of lambs grazing lucerne pasture was similar to that of lambs in a feedlot system (>22 kg), and was sufficient to meet carcass requirements for the export market. Supplementation of lambs that grazed CFP pasture with NOP and CO also increased their carcass weight to over 22 kg, which met market requirements for export. The findings of this study are in line with the results of Moron-Fuenmayor and Clavero (1999) who reported that concentrate supplementation significantly increased the hot carcass weight of grazing lambs. Furthermore, De Brito et al. (2017b) revealed that lambs under extensive systems with supplementation had heavier carcass weights than lambs that were grazing only without supplementation.

Sheep dressing percentage is affected by a range of factors including nutrition, maturity, wool growth, and breed (Gardner et al., 2015). The observed higher dressing percentage in lambs grazing CFP with pellet supplementation than that of lambs grazing lucerne with RBO supplementation could have been as a result of their increased concentrate intake in comparison to their un-supplemented peers. Supplementation with concentrate to grazing lamb may have reduced grazing time and increased idle time resulting in higher forage intake of grazing lambs without concentrate supplementation (Fajardo et al., 2016). Sheridan et al. (2003) found that lambs on a diet with an increased roughage component had higher associated gut-fill and lower dressing percentages. In addition, Díaz et al. (2002) also reported that pasture only grazing lambs had lower dressing percentages than concentrate fed lambs. The higher REA of grazing lambs supplemented with NOP in this study in comparison with pasture only grazing lambs is in agreement with the findings of Turner et al. (2014a).

5.5.3. Effect of pastures on liveweight, concentrate intake, body conformation and carcass characteristics of prime lambs

Liveweight, concentrate intake and body conformation: Lambs grazing on lucerne had higher ADG than CFP grazing lambs. This could be due to the fact that legume fed lambs have more efficient dietary protein utilisation and faster rate of digestion (De Brito et al., 2017b). Higher ADG in legume fed lambs compared with grass fed lambs was also observed by Fraser et al. (2004). The low FCE of lucerne grazing lambs also indicated that lambs grazing lucerne pasture were more efficient at utilising dietary nutrients than lambs grazing CFP pasture. The higher change in WH and BCS of lucerne grazing lambs in comparison to CFP grazing lambs may have resulted from increased fat thickness.

Carcass characteristics: Lambs grazing lucerne pastures in this study had higher carcass weight, fat thickness and body wall thickness than lambs grazing CFP. This result could be due to increased protein intake in the lambs grazing legume pastures than lambs grazing grass pastures (Ponnampalam et al., 2017). In an indoor management system, Estrada-Angulo et al. (2018) demonstrated that lambs fed with increasing amounts of protein in isocaloric diets had linear increases in protein intake, carcass weight and fat thickness. The findings of the present study are in line with the results of Turner et al. (2014b) who reported that goat kids grazing on legumes (alfalfa or red clover pastures) produced heavier carcasses than goat kids grazing orchard grass (*Dactylis glomerata* L.). The heavier carcasses of lucerne grazing lambs in our study compared with lambs grazing CFP resulted in higher OTH trade of these lambs.

5.6. Conclusion

The carcasses of lambs grazing on lucerne pasture or CFP pasture with pellet supplementation were over 22 kg, which was suitable for meeting local and export market requirements. Lambs

grazing lucerne pasture had higher performance and carcass traits than lambs grazing CFP pasture. Pellet supplementation did not change the final LWT, ADG, BL, WH and CG of grazing lambs. Cocksfoot grazing lambs with pellet supplementation and lucerne grazing lambs with RBO supplementation had higher dressing percentages than lambs grazing on pasture only. Supplementation with NOP significantly increased rib eye area of grazing lambs. Supplementation of CFP grazing lambs with CO can be utilised as a strategic nutritional tool to increase the carcass weight and OTH trade of lambs with low FCE.

Chapter 6: Enhanced omega-3 polyunsaturated fatty acid contents in muscle and edible organs of Australian prime lambs grazing lucerne and cocksfoot pastures

6.1. Abstract

The enhancement of health-beneficial omega-3 long–chain (≥C20) polyunsaturated fatty acid (n-3 LC-PUFA) contents in the muscle, liver, heart, and kidney of Australian prime lambs through pasture grazing and supplementation with oil infused pellets was investigated. Forty-eight first-cross prime lambs were randomly assigned into a split-plot design with pasture type as the main plot effect and pellet supplementation as a sub-plot effect in a feeding trial that lasted for nine weeks. The n-3 LC-PUFA content in *longissimus dorsi* muscle of all lambs was well above the 30 mg threshold for "omega-3 source" nutrition claim under the Australian Food Standards and Guidelines. Pasture type impacted the fatty acid contents in muscle, heart, and kidney of prime lambs. Lambs grazing cocksfoot grass only had high 18:3n-3 (ALA) and n-3 LC-PUFA contents (67.1 mg/100 g and 55.2 mg/100 g, respectively) in the *longissimus dorsi* muscle, which was not significantly different ($p > 0.8990$) from the contents of lambs grazing only lucerne. Supplementation of pellets with or without oil infusion to grazing lambs generally decreased the ALA and n-3 LC-PUFA contents and increased the n-6/n-3 ratio in the *longissimus dorsi* muscle. The fatty acid content in the internal organs of grazing lambs was also affected by pellet supplementation. The liver and kidney of grazing lambs were both "good sources" (60 mg/100 g) of omega-3. The cocksfoot grass showed considerable potential for producing healthy, premium quality meat with high contents of n-3 and n-3 LC-PUFA, which may consequently enhance the omega-3 intake of Australian lamb consumers.

6.2. Introduction

Research on increasing the content of n-3 long-chain (≥C20) polyunsaturated fatty acids (n-3 LC-PUFA) in red meat has gained considerable attention because of their beneficial impact on human health. Omega-3 (n-3) fatty acids have potent anti-inflammatory and inflammation resolving properties in model systems (Calder, 2009) and n-3 fatty acid supplementation may be used as an effective tool in the primary and secondary prevention of cardiovascular disease (Cao et al., 2015; Leslie et al., 2015). In effects on many types of cancers (breast, colorectal, leukaemia, gastric, pancreatic, oesophageal, prostate, lung, colon, head, and neck) (Cabo-Garcia et al., 2015; Fu et al., 2015; Manzi et al., 2015; Nabavi et al., 2015).

Against the increasing recognition of health benefits derived from increased n-3 fatty acid consumption, recent studies have generally revealed that consumers do not obtain sufficient n-3 LC-PUFA for their daily recommended requirement. Sheppard and Cheatham (Sheppard and Cheatham, 2018) revealed that very few American children met even the lowest recommendations for eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) intake. The findings of Pittaway et al. (2015) showed that most healthy older adults in Tasmania, Australia, who participated in the observational study were unlikely to meet the recommended daily intake of 0.5 g EPA and DHA combined, without the use of fish oil supplements. In another study, Nichols et al. (2010) found that future supplies of the beneficial n-3 LC-PUFA containing oils may be insufficient for the predicted increasing demands for their inclusion in livestock and aquaculture feeds, human foods, and nutraceutical products. Therefore, the utilization of alternative omega-3 LC-PUFA sources (such as red and other meat, egg, and milk) beyond marine products is of increasing importance in order to enhance n-3 LC-PUFA intake in humans.

According to Howe et al. (2007), lean red meat is an important natural food source of omega-3 LC-PUFA, the content of which can be manipulated by modifying the composition of livestock feeds. Australia was the world's largest exporter of sheep meat and second largest producer of lamb and mutton in 2016–2017 (ABARES, 2018). Furthermore, Australians are among the highest lamb meat consumers in the world (9 kg of lamb per person in a year) (Wong et al., 2015). Therefore, increasing omega-3 LC-PUFA content in lamb meat is one potential way to boost intake levels of omega-3 LC-PUFA among Australians, thereby meeting the daily-recommended requirements of these health-benefitting ingredients. In addition, lamb meat with high omega-3 LC-PUFA content helps to improve the reputation and competitiveness of Australian lamb meat in terms of healthy products.

Lamb production based on a grazing system has been reported to incur lower cost with respect to inputs, is more sustainable, and had greater amounts of health-claimable n-3 fatty acids such as EPA plus DHA than lamb production based on feedlot pellets, grain, or dry pasture/straw (De Brito et al., 2017b). A number of current forage-types are presently used by the lamb industry; however, limited scientific information is available on how they influence n-3 LC-PUFA levels in lamb meat. There is a clear need to investigate the effect of these forage-types on n-3 LC-PUFA content in meat in order to assist lamb producers in selecting the optimal forage-type for producing premium lamb meat with health claimable sources of n-3 fatty acids.

Cocksfoot cv. porto (Dactylis glomerata L. cv. Porto) was released in 1972 by the Tasmanian Department of Agriculture (Lolicato and Rumball, 1994). This grass grows actively in summer under low rainfall areas (Clark et al., 2016) and contains a high proportion of linolenic acid (ALA, 18:3n-3) (Casey et al., 1988; Clapham et al., 2005), which is a precursor to the synthesis of the health claimable long-chain fatty acids in lambs (Chikwanha et al., 2018; Ponnampalam et al., 2014a). Furthermore, cocksfoot cv. porto has a growth pattern which is better adapted to

the Australian temperate climate and could replace ryegrass in relatively drier areas (Lolicato and Rumball, 1994). To the best of our knowledge, there is no published information on the fatty acid (FA) profile of cocksfoot cv. porto and its impact on the FA profile of lamb meat. Lucerne is a deep-rooted herbaceous perennial legume and is adapted to a broad range of agro-ecological environments (Humphries, 2012). Lucerne pasture has the potential to consistently produce premium grade carcasses and quality lamb meat with high levels of n-3 PUFA (Fraser et al., 2004; Ponnampalam et al., 2014a; Ponnampalam et al., 2017).

Supplementation of grains or a feedlot ration to grazing lambs in late spring and early summer is often necessary due to a decline in feed nutritive characteristics of annual pasture that occur as the plants mature (Ponnampalam et al., 2017) and also, in particular, under drought conditions. However, supplementation of grain or a feed concentrate to grazing lambs can affect the FA profile of lamb meat (Boughalmi and Araba, 2016; Turner et al., 2014a). Canola oil was used for fattening lambs in an indoor system and successfully increased the n-3 LC-PUFA content in lamb meat (Nguyen et al., 2017b). Rice bran oil is rich in PUFA (Goffman et al., 2003), and rice bran oil supplementation in an indoor system increased PUFA concentration in milk of dairy cows (Lunsin et al., 2012) and in adipose tissue of lambs (Bhatt et al., 2016). Nevertheless, no published work is available on canola and rice bran oil supplementation in an external grazing system.

The internal organs including liver, heart, and kidney of lambs are nutrient dense animal-derived foods, which can provide protein, minerals (copper, iron, and zinc), and vitamins for humans (Bester et al., 2018; Umaraw et al., 2015). Furthermore, these organs are also rich in the essential n-3 LC-PUFA content (Malau-Aduli et al., 2016; Nguyen et al., 2017c). These

edible organs can be directly consumed in some developing countries as cheap protein sources or processed to become traditional foods like liver pasties in developed countries such as France and Spain (Amaral et al., 2015). The study and potential enhancement of the nutritional composition of such organs could add value to animal by-products and earn extra income for the farming and slaughter sectors.

On the basis of the need in lamb production and emerging demand by consumers for healthy lamb meat as mentioned above, this study was designed to: (i) evaluate the potential of cocksfoot cv. porto grass to produce healthy, premium quality lamb with high content of health claimable n-3 LC-PUFA and (ii) to examine the effect of different pasture types and supplementation of lambs with pellets with or without plant oil infusion, on FA content in muscle and edible organs of Australian prime lambs.

6.3. Materials and methods

The study was conducted at the Tasmanian Institute of Agriculture's Cressy Research and Demonstration Station, Burlington Road, Cressy, Tasmania, Australia from October to December, 2016. The use of animals and procedures performed in this study were all approved by the University of Tasmania Animal Ethics Committee (Permit No A0015657).

6.3.1. Animals, Diets, Experimental Design, and Feed Sample Collection

Experimental design: The experiment was a split-plot design with the basal pastures being cocksfoot cv. porto and lucerne. Main plot: Total of 1.5 ha of Cocksfoot cv. porto pasture with three equal 0.5 ha plots. Each 0.5 ha plot was split into four equal 0.125 ha sub-plots. Therefore, cocksfoot cv. porto pasture had 12 equal 0.125 ha sub-plots units in the main plot. Similarly, there were 12 equal 0.125 ha subplots as main plot units for lucerne pasture. These plots were

used for rotational grazing. Sub-plots: Lambs were divided into four groups. Lambs in each group were allocated to one of the following four treatments: (1) cocksfoot cv. porto or lucerne pastures only as the control treatment; (2) cocksfoot cv. porto or lucerne pastures supplemented with: no oil pellets (NOP); (3) canola oil infused pellets (CO); and (4) rice bran oil infused pellets (RBO). Thus, each main plot unit had sub-plots.

Forty-eight White Suffolk x Corriedale first-cross prime lambs with an average liveweight (LWT) of 38.7 ± 0.7 kg weaned at six months were randomly allocated to 12 groups of four lambs balanced by gender. The 12 groups of lambs were allocated to six cocksfoot pasture subplots and six lucerne pasture subplots. The experimental lambs were grazed daily on pastures from 07:00 to 18:00 h and rotated to fresh pasture subplots every 14 days during the trial. Fresh water was available at all times throughout the grazing trial. The lambs in the supplemented treatments were individually offered oil infused pellets at 1 kg/head/day before going to pastures. The feeding trial lasted for nine weeks comprising three weeks of adjustment and six weeks of post-adjustment data collection.

Basal pasture and feed samples: The pasture samples were taken weekly from five area sites 50 cm × 50 cm of each subplot and then homogenized for withdrawing subsamples. The oil infused pellets were sampled from each bag and stored at -20 ºC until the end of the trial.

6.3.2. Slaughter and fatty acid analysis

All lambs were slaughtered at a commercial abattoir (Tasmanian Quality Meats, Cressy, Tasmania) adjacent to the experimental site after staying in the animal house for 12 h without feed and free access to fresh water. The slaughter procedures prescribed by the Meat Standards of Australia guidelines was strictly applied. Samples of liver, heart, and kidney were taken at

the abattoir and immediately vacuum-sealed, code-labelled, and stored at -20 ºC until FA analysis. Carcasses were chilled for 24 h at 4 ºC before transporting to Robinson Meats, Glenorchy, Hobart, Tasmania, Australia. Thereafter, the *longissimus dorsi* muscle were sampled at the 12/13th rib of each carcass as a commercial loin chop (approximately 200 g) for subsequent FA analysis.

FA analysis was as described by Malau-Aduli et al. (2016). Briefly, the FA analysis included three processes. 1. A single-phase overnight extraction using CH_2Cl_2:MeOH:H_2O (1:2:0.8 v/v) to extract total lipids from 1 gram of un-homogenised and wet liver, kidney, heart, and muscle tissues and feed samples according to a modified Bligh and Dyer protocol (Bligh and Dyer, 1959). Phase separation with the addition of CH_2Cl_2:saline Milli-Q H_2O (1:1 v/v) was carried out and followed by rotary evaporation of the lower CH_2Cl_2 phase at 40 ℃ to obtain the total lipids. 2. Methylation: An aliquot was taken from each total lipid extract for transmethylation with MeOH:CH_2Cl_2:HCl (10:1:1 v/v) for 2 h at 80 ºC and Milli-Q H_2O (1 mL) was then added before the FA methyl esters (FAME) extraction process with hexane:CH_2Cl_2 (4:1 v/v); extraction was performed three times. 3. Fatty acid quantification: Extracted FAME in glass vials were made up to a volume of 1500 _L with a known concentration of an internal injection standard (19:0). A 7890B gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA, USA) equipped with an EquityTM-1 fused 15 m silica capillary column with 0.1 mm internal diameter and 0.1-_m film thickness (Supelco, Bellefonte, PA, USA), a flame ionisation detector, a split/splitless injector and an Agilent Technologies 7683 B Series autosampler was employed to analyse the FAME. The GC conditions were: splitless mode injection; carrier gas He; initial oven temperature 120 ºC and then increased to 270 ºC at 10 ºC /min and to 310 ºC at 5 ºC/min. Peak quantification was performed using Agilent Technologies ChemStation software (Palo Alto, CA, USA). FA identifications were confirmed by GC-mass spectrometric

(GC/MS) analysis with a Thermo Scientific 1310 GC coupled with a TSQ triple quadropole (Thermo Fisher Scientific, Milan, Italy) PTV injector and Thermo Scientific XcaliburTM software (Austin, Texas USA). The GC was equipped with a HP-5 cross-linked methyl silicone-fused silica capillary column (50 m × 0.32 mm internal diameter) which was of similar polarity to the column described above. The operating conditions was previously described by Miller et al. (2006) and helium served as the carrier gas. FA percentages (FA%) and contents (FA mg/100 g) were calculated as follows (Flakemore et al., 2017a), where 0.916 was the lipid conversion factor as cited by Clayton (2014).

(a) FA% = (individual fatty acid area) × (100)/(sum total area of fatty acids)

(b) FA mg/100 g = (Total lipid) × (LCF (0.916)) × ((%FA)/100) × 1000

6.3.3. Statistical analysis

FA data were initially transformed into FA contents (mg/100 g). Thereafter, the data were analysed using the split-plot model in General Linear Model procedures (PROC GLM) of the Statistical Analysis System software (SAS Institute, North Carolina, USA) (SAS, 2009). Pasture types were considered as the main plot effects and supplementation of pellets with or without oil infusion as subplot effects. Non-significant interactions between fixed effects were dropped from the analytical model and treatment differences were declared significant at $p < 0.05$ using Bonferroni probabilities. Probability values ranging between $p > 0.056$ and $p < 0.059$ were deemed as "tending towards significance".

6.4. Results

The chemical composition of experimental diets is presented in Table 6.1. Dry matter (DM) of lucerne and cocksfoot were similar ($p > 0.7880$), while those of the pelleted supplements were much higher ($p < 0.05$) and ranged from 89.1% to 91.1%.

Table 6.1. Proximate analysis of supplementary feed and pasture.

Chemical Composition (% DM)	Cocksfoot cv. Porto	Lucerne	NOP	CO	RBO
DM	20.5	20.7	89.1	91.1	90.2
CP	13.3	18.6	15.7	15.3	14.7
ADF	26.7	25.6	6.8	7.4	8.0
NDF	43.8	35.9	18.3	19.9	18.7
EE	3.0	1.8	2.1	4.6	4.9
Ash	6.4	6.8	4.0	6.5	5.0
%TDN	62.3	63.2	77.2	76.8	76.4
DE (Mcal/kg)	2.7	2.8	3.4	3.4	3.4
ME (MJ/kg)	9.4	9.5	11.7	11.6	11.5

Dry matter (DM), Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Ether extract (EE) and crude protein (CP), Total digestible nutrients (%TDN), Metabolisable energy (ME). NOP was wheat-based pellet without infused oil. CO and RBO was wheat-based pellet infused with 50 mL/kg DM of oils from canola and rice bran sources, respectively. Total digestible nutrients (%TDN) were calculated as TDN (% of DM) = 82.38 − (0.7515 × ADF (% of DM)). Metabolisable energy (ME) was 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.

Crude protein content of the different supplemented pellets ranged between 13.3% and 15.7%, which was lower ($p < 0.05$) than that in the basal lucerne feed (18.6%), but higher than in cocksfoot (13.3%). Acid detergent fibre (ADF) and Neutral detergent fibre (NDF) contents of the different supplemented pellets ranged from 6.8% to 8.0% and from 18.3% to 19.9%, respectively, while ADF and NDF content of the basal feed were 35.9% and 43.8%, respectively. In terms of EE content, the level in the supplemented pellets fluctuated between 4.6% and 4.9%, which was at least three-fold higher than the amount in the basal feed (1.8%).

ME content of all supplemented pellets was approximately 12 MJ/kg, whilst the basal feed contained 9.5 (MJ/kg) ME.

6.4.1. FA composition of pastures and supplementary feeds

Table 6.2 shows the FA composition of supplementary feed and pastures. Cocksfoot cv. porto and lucerne pasture contained high proportions of ALA at 57.6% and 51.9%, respectively. Supplementary feeds including NOP, CO and RBO had high levels of linoleic acid (LA, 18:2n-6) (50.3%, 32.9%, and 42.2%, respectively). A high relative level of 18:1n-9c was found in the NOP, CO and RBO treatments ranging from 24.5% to 44.5%. Cocksfoot cv. porto and lucerne pasture contained 73.1% and 69.7% of PUFA, respectively, while the PUFA proportion of the supplementary feeds varied from 39.2% to 54.5%. The n-3 PUFA levels of cocksfoot cv. porto and lucerne pastures were 58.0% and 52.2%, respectively, which were considerably higher than the n-3 PUFA levels of the three supplementary feeds. In contrast, NOP, CO, and RBO contained high relative levels of n-6 PUFA, ranging from 3.1% to 50.6%. The n-6/n-3 ratio of NOP and RBO diet treatments was similar and double the ratio of the CO treatment. The cocksfoot cv. porto and lucerne pastures had the lowest n-6/n-3 ratio (0.3) among all treatments.

Table 6.2. Fatty acid composition (as % total fatty acids) of supplementary feeds and pastures.

% Lipid	Cocksfoot cv. Porto	Lucerne	NOP	CO	RBO
14:0	0.6	1.2	0.2	0.2	0.3
15:0	0.2	0.4	0.1	0.1	0.1
16:1n-9c	0.0	0.0	0.0	0.1	0.0
16:1n-7c	0.2	0.1	0.3	0.3	0.2
16:0	15.7	17.1	16.3	9.9	15.5
17:0	0.5	0.5	0.1	0.1	0.1
18:2n-6 LA	14.8	17.1	50.3	32.9	42.2
18:3n-3 ALA	57.6	51.9	3.5	5.7	2.7
18:1n-9c	1.0	0.5	24.5	44.5	32.3
18:1n-7c	0.2	0.1	1.0	2.6	1.2
18:1n-7t	0.0	0.0	0.0	0.0	0.0
18:0	0.1	2.2	0.3	0.5	0.3
20:4n-6 ARA	0.0	0.1	0.0	0.0	0.0
20:5n-3 EPA	0.0	0.0	0.1	0.1	0.1
20:3n-6	0.1	0.1	0.1	0.1	0.1
20:4n-3	0.1	0.1	0.2	0.1	0.1
20:2n-6	0.1	0.1	0.1	0.1	0.1
20:0	1.6	1.2	0.5	0.6	0.6
22:5n-6 DPA-6	0.0	0.0	0.1	0.1	0.1
22:6n-3 DHA	0.0	0.0	0.0	0.0	0.0
22:5n-3 DPA-3	0.0	0.0	0.1	0.0	0.0
22:0	1.0	1.3	0.6	0.3	0.4
23:0	0.3	0.5	0.1	0.0	0.1
24:0	0.9	1.4	0.3	0.2	0.3
∑SFA	20.9	25.8	18.5	11.8	17.6
∑MUFA	4.9	3.9	26.9	48.7	34.6
∑PUFA	73.1	69.7	54.5	39.2	45.5
∑*n*-3 LC-PUFA	0.1	0.1	0.3	0.3	0.3
∑*n*-3 PUFA	58.0	52.2	3.8	6.0	3.0
∑*n*-6 PUFA	15.2	17.5	50.6	33.1	42.5
∑other FA	1.0	0.4	0.1	0.2	2.3
n-6/*n*-3	0.3	0.3	13.3	5.5	14.2

All abbreviations are as defined in Table 6.1

6.4.2. Effect of pellet supplements on the fatty acid contents in longissimus dorsi muscle, liver, heart, and kidney

FA of longissimus dorsi muscle: Supplementation with pellets as depicted in Table 6.3, did not affect the total FA, MUFA, and PUFA contents in *longissimus dorsi* muscle of grazing lambs. However, supplementation with pellets tended to decrease the ALA and n-3 PUFA contents in *longissimus dorsi* muscle of grazing lamb, and the lowest values occurred in the RBO treatment. Lambs grazing on cocksfoot cv. porto or lucerne pastures only had similar ALA content (67.1 mg/100 g and 68.1 mg/100 g, respectively) in the *longissimus dorsi* muscle ($p > 0.899$). Supplementation with NOP and RBO pellets increased the LA content in *longissimus dorsi* muscle of lambs grazing on lucerne pasture. Lucerne grazing lambs supplemented with RBO had lower EPA and Docosapentaenoic acid (DPA, 22:5n-3) contents in their *longissimus dorsi* muscle than lambs grazing on lucerne pasture only. Pellet supplementation tended to decrease the total n-3 LC-PUFA and EPA+DHA+DPA contents (as demonstrated in Figure 6.1) in *longissimus dorsi* muscle of Lucerne grazing lambs and lowest value occurred in the RBO treatment. Supplementation with pellets decreased the 18:0 content in the *longissimus dorsi* muscle of lambs grazing cocksfoot cv. Porto. The additional access to pellets by grazing lambs increased the n-6/n-3 ratio in the *longissimus dorsi* muscle.

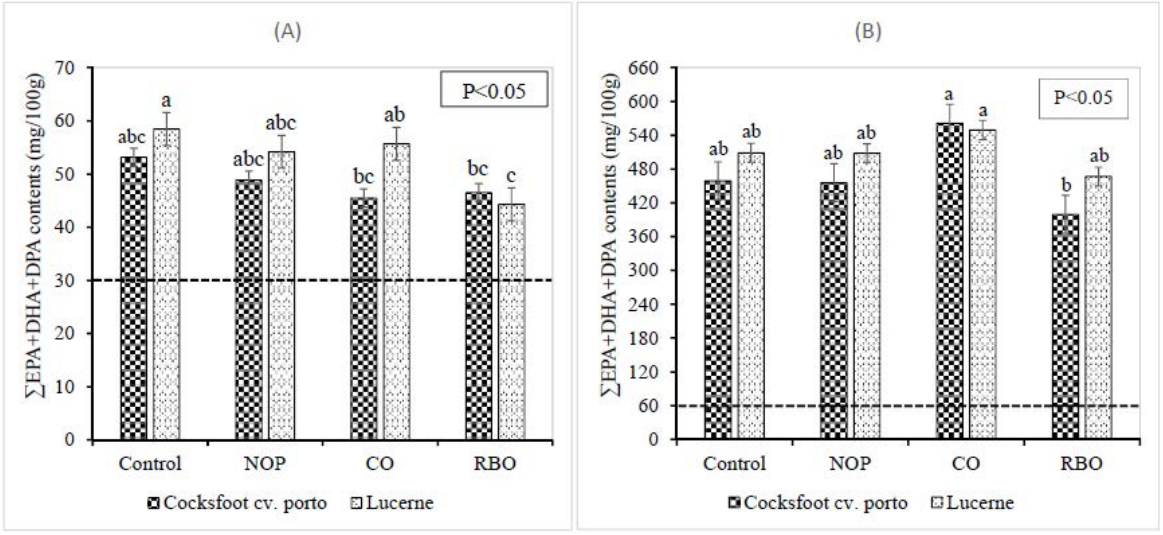

Figure 6. 1. Effect of pellet supplementation on the contents of ∑EPA + DPA + DHA

(EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid) in *longissimus dorsi* muscle (A) and liver (B) of grazing lambs. Control: grazing on cocksfoot cv. porto or lucerne pastures only as basal pastures; NOP: basal pastures plus no oil pellets; CO: basal pastures plus canola oil infused pellets; RBO: basal pastures plus rice bran oil infused pellets. Different letters (a, b, c) indicate significant differences between treatments ($p < 0.05$).

FA of liver: FA content of the liver are shown in Table 6.4. Pellet supplementation did not affect the SFA, MUFA, PUFA, n-3 PUFA, and n-3 LC-PUFA contents of grazing lambs. Supplementation of cocksfoot cv. porto with NOP and CO to grazing lambs tended to increase the ALA content in the liver. However, supplementation of pellets to lucerne grazing lambs did not change the ALA content in liver. Supplementation of CO to cocksfoot cv. porto grazing lambs resulted in higher EPA, DPA, PUFA, n-3 LC-PUFA, and EPA + DHA + DPA contents in liver in comparison with RBO supplementation. There was no difference in the EPA + DHA + DPA content of liver between grazing lambs with and without pellet supplementation (Figure 6.1).

FA of heart: FA contents of the heart are demonstrated in Table 6.5. Pellet supplementation did not change the FA content of lucerne grazing lambs. Nevertheless, supplementation of NOP and CO significantly decreased the ARA and DPA contents in the heart of cocksfoot cv. porto grazing lambs. Furthermore, NOP supplementation to cocksfoot cv. porto grazing lambs lowered the DHA and PUFA contents in heart tissues. There was no difference in the EPA + DHA + DPA content in heart of grazing lambs.

FA of kidney: Table 6.6 demonstrates the FA contents of the kidney. The FA contents in kidney of cocksfoot cv. porto grazing lambs were not affected by pellet supplementation. However, supplementation of NOP to lucerne grazing lambs significantly increased the n-6 PUFA, PUFA,

and total FA of kidney tissues. Pellet supplementation did not change the EPA + DHA + DPA content in kidney of grazing lambs.

Table 6.3. Effect of pellet supplementation on IMF percentage (g fat/ 100 g) and fatty acid contents (mg/ 100 g) in *longissimus dorsi* muscle tissue of grazing prime lambs* (LSM ± SE).

Items	Control		NOP		CO		RBO	
	CFP	Lucerne	CFP	Lucerne	CFP	Lucerne	CFP	Lucerne
IMF percentage	3.5 ± 0.5	3.4 ± 0.5	2.5 ± 0.5	2.5 ± 0.5	3.1 ± 0.5	2.7 ± 0.5	2.6 ± 0.5	3.6 ± 0.5
14:0	68.9 ± 13.9	54.5 ± 13.9	43.7 ± 13.9	65.7 ± 13.9	52.5 ± 13.9	39.5 ± 13.9	30.3 ± 13.9	49.6 ± 13.9
15:0	10.1 ± 1.7 ^{a}	7.4 ± 1.7 ^{ab}	5.5 ± 1.7 ^{ab}	8.9 ± 1.7 ^{a}	6.4 ± 1.7 ^{ab}	5.9 ± 1.7 ^{ab}	3.9 ± 1.7 ^{b}	7.9 ± 1.7 ^{ab}
16:1n-9c	9.0 ± 1.3 ^{a}	6.2 ± 1.3 ^{ab}	5.3 ± 1.3 ^{ab}	8.4 ± 1.3 ^{a}	5.6 ± 1.3 ^{ab}	5.6 ± 1.3 ^{ab}	4 ± 1.3 ^{b}	7.3 ± 1.3 ^{ab}
16:1n-7c	37.1 ± 7.7	31.8 ± 7.7	27.0 ± 7.7	41.9 ± 7.7	32.1 ± 7.7	30.7 ± 7.7	22.4 ± 7.7	35.0 ± 7.7
16:0	712.9 ± 113.8 ^{ab}	541.3 ± 113.8 ^{ab}	433.5 ± 113.8 ^{ab}	753.2 ± 113.8 ^{a}	545.9 ± 113.8 ^{ab}	592.1 ± 113.8 ^{ab}	416.6 ± 113.8 ^{b}	663.9 ± 113.8 ^{ab}
17:0	32.6 ± 5.5 ^{ab}	24.9 ± 5.5 ^{ab}	19 ± 5.5 ^{bc}	36.3 ± 5.5 ^{a}	21.2 ± 5.5 ^{ab}	25.7 ± 5.5 ^{ab}	16.6 ± 5.5 ^{c}	32.9 ± 5.5 ^{ab}
18:2n-6 LA	119.3 ± 8.7 ^{bc}	122.8 ± 8.7 ^{bc}	123.4 ± 8.7 ^{bc}	156.8 ± 8.7 ^{a}	110.6 ± 8.7 ^{c}	144.2 ± 8.7 ^{ab}	119.8 ± 8.7 ^{bc}	157.2 ± 8.7 ^{a}
18:3n-3 ALA	67.1 ± 5.5 ^{ab}	68.1 ± 5.5 ^{a}	33.9 ± 5.5 ^{d}	57.3 ± 5.5 ^{ab}	35.8 ± 5.5 ^{cd}	51.6 ± 5.5 ^{bc}	35.5 ± 5.5 ^{cd}	39.5 ± 5.5 ^{cd}
18:1n-9c	1169.0 ± 197.0 ^{ab}	902.9 ± 197.0 ^{ab}	767.5 ± 197.0 ^{b}	1351.9 ± 197.0 ^{a}	925.8 ± 197.0 ^{ab}	1024.5 ± 197.0 ^{ab}	759.6 ± 197.0 ^{b}	1153.8 ± 197.0 ^{ab}
18:1n-7c	41.2 ± 7.5 ^{ab}	33.4 ± 7.5 ^{b}	38.4 ± 7.5 ^{ab}	54.1 ± 7.5 ^{ab}	43.2 ± 7.5 ^{ab}	50.3 ± 7.5 ^{ab}	34.2 ± 7.5 ^{ab}	55.5 ± 7.5 ^{a}
18:1n-7t	83.6 ± 18.3 ^{ab}	57 ± 18.3 ^{b}	56 ± 18.3 ^{b}	102.5 ± 18.3 ^{ab}	67.5 ± 18.3 ^{b}	82.9 ± 18.3 ^{ab}	57.2 ± 18.3 ^{b}	122.5 ± 18.3 ^{a}
18:0	592.4 ± 75.4 ^{a}	364.8 ± 75.4 ^{bc}	328.4 ± 75.4 ^{bc}	533.4 ± 75.4 ^{ab}	362.5 ± 75.4 ^{bc}	402.1 ± 75.4 ^{abc}	307.3 ± 75.4 ^{c}	515.2 ± 75.4 ^{abc}
20:4n-6 ARA	33.7 ± 2.9	35.7 ± 2.9	38.2 ± 2.9	36 ± 2.9	34.8 ± 2.9	37.2 ± 2.9	34.5 ± 2.9	36.9 ± 2.9
20:5n-3 EPA	24.7 ± 1.9 ^{abc}	26.9 ± 1.9 ^{a}	22.1 ± 1.9 ^{abc}	24.2 ± 1.9 ^{abc}	19.8 ± 1.9^{bc}	25 ± 1.9 ^{ab}	20.2 ± 1.9 ^{bc}	19.1 ± 1.9 ^{c}
20:3n-6	6.1 ± 0.5 ^{c}	6.7 ± 0.5 ^{bc}	6.6 ± 0.5 ^{bc}	8.0 ± 0.5 ^{a}	5.8 ± 0.5 ^{c}	7.7 ± 0.5 ^{ab}	6.4 ± 0.5 ^{bc}	7.5 ± 0.5 ^{ab}
20:4n-3	2.0 ± 0.3	1.9 ± 0.3	2.2 ± 0.3	1.9 ± 0.3	1.7 ± 0.3	2.1 ± 0.3	1.9 ± 0.3	2.1 ± 0.3
20:2n-6	1.4 ± 0.2 ^{bc}	1.1 ± 0.2 ^{c}	1.5 ± 0.2 ^{bc}	2.5 ± 0.2 ^{a}	1.2 ± 0.2^{c}	1.7 ± 0.2 ^{bc}	1.9 ± 0.2 ^{abc}	2.1 ± 0.2 ^{ab}
20:0	4.4 ± 0.7	3 ± 0.7	3.3 ± 0.7	4.4 ± 0.7	3.9 ± 0.7	3.4 ± 0.7	2.7 ± 0.7	4.2 ± 0.7
22:5n-6 DPA-6	1.2 ± 0.2	1 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2
22:6n-3 DHA	6.7 ± 0.8	7.1 ± 0.8	5 ± 0.8	7.1 ± 0.8	7 ± 0.8	7.1 ± 0.8	6.1 ± 0.8	5.7 ± 0.8
22:5n-3 DPA-3	21.8 ± 1.1 ^{abc}	24.5 ± 1.1 ^{a}	21.7 ± 1.1 ^{abc}	22.9 ± 1.1 ^{ab}	18.8 ± 1.1^{c}	23.7 ± 1.1 ^{a}	20.2 ± 1.1 ^{bc}	19.5 ± 1.1 ^{c}
22:0	1.5 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.1
23:0	2.1 ± 0.1 ^{a}	2 ± 0.1 ^{a}	1.3 ± 0.1 ^{c}	1.8 ± 0.1 ^{ab}	1.4 ± 0.1 ^{bc}	1.8 ± 0.1 ^{ab}	1.5 ± 0.1 ^{bc}	1.7 ± 0.1 ^{abc}
24:0	2.3 ± 0.1 ^{a}	2.2 ± 0.1 ^{a}	1.8 ± 0.1 ^{b}	2.1 ± 0.1 ^{ab}	1.9 ± 0.1 ^{ab}	2.1 ± 0.1 ^{ab}	1.9 ± 0.1 ^{ab}	2.1 ± 0.1 ^{ab}
Total FA	3291.8 ± 467.8 ^{ab}	2514.6 ± 467.8 ^{ab}	2162.5 ± 467.8 ^{ab}	3513.2 ± 467.8 ^{a}	2496 ± 467.8 ^{ab}	2762 ± 467.8 ^{ab}	2070.8 ± 467.8 ^{b}	3161.8 ± 467.8 ^{ab}
∑SFA	1427.0 ± 208.0 ^{a}	1001.5 ± 208.0 ^{ab}	837.6 ± 208.0 ^{ab}	1407.3 ± 208.0 ^{a}	997 ± 208.0 ^{ab}	1073.8 ± 208.0 ^{ab}	782 ± 208.0 ^{b}	1278.9 ± 208.0 ^{ab}
∑MUFA	1469.1 ± 242.9 ^{ab}	1138.6 ± 242.9 ^{ab}	977.0 ± 242.9 ^{ab}	1685.7 ± 242.9 ^{a}	1162.8 ± 242.9 ^{ab}	1297.6 ± 242.9 ^{ab}	952.2 ± 242.9 ^{b}	1485.8 ± 242.9 ^{ab}
∑PUFA	294.7 ± 17.3 ^{abcd}	306.2 ± 17.3 ^{abc}	268.3 ± 17.3 ^{bcd}	329.8 ± 17.3 ^{a}	247.4 ± 17.3 ^{d}	312.0 ± 17.3 ^{ab}	257.4 ± 17.3 ^{cd}	301.6 ± 17.3 ^{abc}
∑*n*-3 LC-PUFA	55.2 ± 3.6 ^{abc}	60.4 ± 3.6 ^{a}	51.0 ± 3.6 ^{abc}	56.0 ± 3.6 ^{abc}	47.2 ± 3.6 ^{c}	57.8 ± 3.6 ^{ab}	48.3 ± 3.6 ^{bc}	46.4 ± 3.6 ^{c}
∑*n*-3 PUFA	123.2 ± 7.7 ^{a}	129.8 ± 7.7 ^{a}	85.5 ± 7.7 ^{b}	114.4 ± 7.7 ^{a}	83.1 ± 7.7 ^{b}	110.2 ± 7.7 ^{a}	84.1 ± 7.7 ^{b}	86.1 ± 7.7 ^{b}
∑*n*-6 PUFA	164.9 ± 10.8 ^{bc}	170.9 ± 10.8 ^{bc}	175 ± 10.8 ^{bc}	208.8 ± 10.8 ^{a}	157.4 ± 10.8 ^{c}	196 ± 10.8 ^{ab}	167.4 ± 10.8 ^{bc}	209.2 ± 10.8 ^{a}
∑other FA	99.6 ± 12.8	67.0 ± 12.8	78.3 ± 12.8	89.4 ± 12.8	87.6 ± 12.8	78.1 ± 12.8	78.8 ± 12.8	94.4 ± 12.8
n-6/*n*-3	1.4 ± 0.1 ^{c}	1.3 ± 0.1 ^{c}	2.1 ± 0.1 ^{b}	1.9 ± 0.1 ^{b}	1.9 ± 0.1 ^{b}	1.8 ± 0.1 ^{b}	2.0 ± 0.1 ^{b}	2.4 ± 0.1 ^{a}

* Values within the same row bearing different superscripts differ ($p < 0.05$); total FA is the combined FA contents; aside from Cocksfoot cv. Porto (CFP), least square mean (LSM), standard error (SE) and intramuscular fat (IMF), all other abbreviations are as defined in Tables 6.1 and 6.2. Different letters (a, b, c, d) indicate significant differences between treatments ($p < 0.05$).

Table 6.4. Effect of pellet supplementation on total lipid percentage (g fat/100 g) and fatty acid contents (mg/100 g) in liver of grazing prime lambs (LSM ± SE) *.

Items	Control		NOP		CO		RBO	
	CFP	Lucerne	CFP	Lucerne	CFP	Lucerne	CFP	Lucerne
Lipid percentage	6.8 ± 0.4	6.9 ± 0.4	6.3 ± 0.4	7.0 ± 0.4	6.7 ± 0.4	6.7 ± 0.4	6.5 ± 0.4	6.5 ± 0.4
14:0	23.8 ± 5.0 ^{ab}	21.4 ± 5.0 ^{b}	38.4 ± 5.0 ^{a}	22.7 ± 5.0 ^{b}	28.4 ± 5.0 ^{ab}	26.3 ± 5.0 ^{ab}	22.6 ± 5.0 ^{b}	17.7 ± 5.0 ^{b}
15:0	12.9 ± 1.2 ^{bc}	12.2 ± 1.2 ^{c}	18.3 ± 1.2 ^{a}	13.4 ± 1.2 ^{bc}	15.9 ± 1.2 ^{ab}	14.1 ± 1.2 ^{bc}	13.4 ± 1.2 ^{bc}	13.0 ± 1.2 ^{bc}
16:1n-9c	22.2 ± 4.0	21.8 ± 4.0	31.1 ± 4.0	23.1 ± 4.0	24.8 ± 4.0	21.5 ± 4.0	21.2 ± 4.0	19.4 ± 4.0
16:1n-7c	33.9 ± 10.4 ^{ab}	32.2 ± 10.4 ^{ab}	60.2 ± 10.4 ^{a}	29.6 ± 10.4 ^{b}	36.5 ± 10.4 ^{ab}	32.5 ± 10.4 ^{ab}	28.0 ± 10.4 ^{b}	26.4 ± 10.4 ^{b}
16:0	723.7 ± 83.3	686.9 ± 83.3	879.1 ± 83.3	703.5 ± 83.3	786.6 ± 83.3	835.2 ± 83.3	660.6 ± 83.3	665.8 ± 83.3
17:0	51.3 ± 3.8 ^{ab}	55.8 ± 3.8 ^{ab}	57.4 ± 3.8 ^{ab}	57.4 ± 3.8 ^{ab}	56.5 ± 3.8 ^{ab}	61.7 ± 3.8 ^{a}	47.2 ± 3.8 ^{b}	58.4 ± 3.8 ^{ab}
18:2n-6 LA	370.2 ± 48.4	384.9 ± 48.4	394.2 ± 48.4	396.2 ± 48.4	419.6 ± 48.4	412.1 ± 48.4	327.0 ± 48.4	402.4 ± 48.4
18:3n-3 ALA	108.0 ± 22.8 ^{b}	167.5 ± 22.8 ^{ab}	180.0 ± 22.8 ^{a}	162.1 ± 22.8 ^{ab}	164.1 ± 22.8 ^{ab}	184.8 ± 22.8 ^{a}	106.2 ± 22.8 ^{b}	109.0 ± 22.8 ^{b}
18:1n-9c	928.1 ± 148.5	850.4 ± 148.5	1174.3 ± 148.5	800.9 ± 148.5	977.1 ± 148.5	950.8 ± 148.5	864.2 ± 148.5	807.2 ± 148.5
18:1n-7c	75.4 ± 15.0	67.7 ± 15.0	90.9 ± 15.0	66.3 ± 15.0	72.1 ± 15.0	73.0 ± 15.0	69.8 ± 15.0	71.8 ± 15.0
18:1n-7t	230.7 ± 43.5	237.5 ± 43.5	238.4 ± 43.5	213.8 ± 43.5	254.0 ± 43.5	240.4 ± 43.5	228.4 ± 43.5	217.2 ± 43.5
18:0	876.1 ± 65.8 ^{ab}	859.9 ± 65.8 ^{ab}	923.4 ± 65.8 ^{ab}	808.2 ± 65.8 ^{b}	918.5 ± 65.8 ^{ab}	1023.0 ± 65.8 ^{a}	786.0 ± 65.8 ^{b}	869.3 ± 65.8 ^{ab}
20:4n-6 ARA	260.6 ± 23.1	203.5 ± 23.1	211.9 ± 23.1	204.4 ± 23.1	240.2 ± 23.1	238.7 ± 23.1	200.1 ± 23.1	249.0 ± 23.1
20:5n-3 EPA	75.4 ± 14.4 ^{bc}	117.2 ± 14.4 ^{ab}	105.1 ± 14.4 ^{abc}	108.8 ± 14.4 ^{abc}	114.7 ± 14.4 ^{ab}	117.7 ± 14.4 ^{a}	68.1 ± 14.4 ^{c}	82.2 ± 14.4 ^{abc}
20:3n-6	31.6 ± 3.3 ^{abc}	37.7 ± 3.3 ^{abc}	30.3 ± 3.3 ^{bc}	36.8 ± 3.3 ^{abc}	39.4 ± 3.3 ^{abc}	39.7 ± 3.3 ^{ab}	29.7 ± 3.3 ^{c}	40.4 ± 3.3 ^{a}
20:4n-3	9.4 ± 1.3 ^{b}	9.6 ± 1.3 ^{b}	9.8 ± 1.3 ^{b}	10.8 ± 1.3 ^{ab}	11.4 ± 1.3 ^{ab}	14.0 ± 1.3 ^{a}	9.4 ± 1.3 ^{b}	11.7 ± 1.3 ^{ab}
20:2n-6	5.5 ± 0.8 ^{b}	6.3 ± 0.8 ^{ab}	5.0 ± 0.8 ^{b}	5.8 ± 0.8 ^{b}	8.1 ± 0.8 ^{a}	7.1 ± 0.8 ^{ab}	5.2 ± 0.8 ^{b}	6.0 ± 0.8 ^{ab}
20:0	6.0 ± 0.7 ^{ab}	5.5 ± 0.7 ^{ab}	6.0 ± 0.7 ^{ab}	4.6 ± 0.7 ^{b}	7.0 ± 0.7 ^{a}	6.6 ± 0.7 ^{ab}	5.4 ± 0.7 ^{ab}	4.8 ± 0.7 ^{b}
22:5n-6 DPA-6	9.2 ± 2.2	4.8 ± 2.2	9.3 ± 2.2	3.9 ± 2.2	5.1 ± 2.2	8.6 ± 2.2	9.7 ± 2.2	4.8 ± 2.2
22:6n-3 DHA	173.1 ± 26.2	132.8 ± 26.2	149.5 ± 26.2	156.8 ± 26.2	196.5 ± 26.2	207.8 ± 26.2	159.8 ± 26.2	165.9 ± 26.2
22:5n-3 DPA-3	210.5 ± 20.2 ^{ab}	258.9 ± 20.2 ^{a}	201.1 ± 20.2 ^{ab}	242.5 ± 20.2 ^{a}	249.9 ± 20.2 ^{a}	223.8 ± 20.2 ^{ab}	171.5 ± 20.2 ^{b}	218.5 ± 20.2 ^{ab}
22:0	8.5 ± 0.4 ^{ab}	7.9 ± 0.4 ^{b}	8.4 ± 0.4 ^{b}	7.4 ± 0.4 ^{b}	9.8 ± 0.4 ^{a}	8.2 ± 0.4 ^{b}	7.6 ± 0.4 ^{b}	8.3 ± 0.4 ^{b}
23:0	16.4 ± 2.2	18.3 ± 2.2	20.2 ± 2.2	21.3 ± 2.2	20.3 ± 2.2	22.1 ± 2.2	18.5 ± 2.2	22.2 ± 2.2
24:0	15.6 ± 0.9 ^{ab}	15.1 ± 0.9 ^{ab}	16.5 ± 0.9 ^{ab}	14.4 ± 0.9 ^{b}	17.7 ± 0.9 ^{a}	16.2 ± 0.9 ^{ab}	14.6 ± 0.9 ^{b}	16.0 ± 0.9 ^{ab}
Total FA	4662.0 ± 446.0	4580.7 ± 446.0	5334.2 ± 446.0	4476.4 ± 446.0	5075.9 ± 446.0	5183.5 ± 446.0	4246.8 ± 446.0	4480.4 ± 446.0
∑SFA	1734.2 ± 143.1 ^{ab}	1682.7 ± 143.1 ^{ab}	1967.7 ± 143.1 ^{ab}	1652.8 ± 143.1 ^{ab}	1860.5 ± 143.1 ^{ab}	2013.2 ± 143.1 ^{a}	1575.7 ± 143.1 ^{b}	1675.3 ± 143.1 ^{ab}
∑MUFA	1490.4 ± 231.0	1406.3 ± 231.0	1844.3 ± 231.0	1343.8 ± 231.0	1578.8 ± 231.0	1527.9 ± 231.0	1417.2 ± 231.0	1344.9 ± 231.0
∑PUFA	1318.3 ± 96.5 ^{ab}	1376.1 ± 96.5 ^{ab}	1369.8 ± 96.5 ^{ab}	1376.9 ± 96.5 ^{ab}	1509.0 ± 96.5 ^{a}	1510.4 ± 96.5 ^{a}	1137.4 ± 96.5 ^{b}	1356.4 ± 96.5 ^{ab}
∑*n*-3 LC-PUFA	468.4 ± 49.6 ^{ab}	518.4 ± 49.6 ^{ab}	465.4 ± 49.6 ^{ab}	518.8 ± 49.6 ^{ab}	572.6 ± 49.6 ^{a}	563.3 ± 49.6 ^{a}	408.7 ± 49.6 ^{b}	478.3 ± 49.6 ^{ab}
∑*n*-3 PUFA	582.6 ± 68.2 ^{ab}	691.3 ± 68.2 ^{ab}	658.0 ± 68.2 ^{ab}	687.3 ± 68.2 ^{ab}	744.4 ± 68.2 ^{a}	753.2 ± 68.2 ^{a}	520.5 ± 68.2 ^{a}	593.5 ± 68.2 ^{ab}
∑*n*-6 PUFA	708.9 ± 76.1	662.8 ± 76.1	685.7 ± 76.1	673.4 ± 76.1	741.1 ± 76.1	733.8 ± 76.1	597.5 ± 76.1	740.5 ± 76.1
∑other FA	118.5 ± 16.3	114.8 ± 16.3	150.2 ± 16.3	102.6 ± 16.3	126.7 ± 16.3	131.3 ± 16.3	115.0 ± 16.3	103.6 ± 16.3
n-6/*n*-3	1.2 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.2

* Values within the same row bearing different superscripts differ ($p < 0.05$); all other abbreviations are as defined in Tables 6.1–6.3.

Table 6.5. Effect of pellet supplementation on total lipid percentage (g fat/100 g) and fatty acid contents (mg/100 g) in heart of grazing prime lambs (LSM ± SE) *.

Items	Control		NOP		CO		RBO	
	CFP	Lucerne	CFP	Lucerne	CFP	Lucerne	CFP	Lucerne
Lipid percentage	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1
14:0	6.0 ± 2.1	5.7 ± 2.1	9.5 ± 2.1	5.7 ± 2.1	3.9 ± 2.1	6.7 ± 2.1	7.7 ± 2.1	5.9 ± 2.1
15:0	2.6 ± 0.5	2.6 ± 0.5	3.2 ± 0.5	3.0 ± 0.5	2.5 ± 0.5	3.1 ± 0.5	3.3 ± 0.5	2.7 ± 0.5
16:1n-9c	2.7 ± 0.4	2.8 ± 0.4	3.1 ± 0.4	2.7 ± 0.4	2.4 ± 0.4	2.6 ± 0.4	3.4 ± 0.4	2.4 ± 0.4
16:1n-7c	5.2 ± 0.9	5.1 ± 0.9	6.4 ± 0.9	5.0 ± 0.9	4.2 ± 0.9	4.8 ± 0.9	6.7 ± 0.9	4.9 ± 0.9
16:0	184.4 ± 13.4	197.6 ± 13.4	193.9 ± 13.4	196.5 ± 13.4	168.5 ± 13.4	187.7 ± 13.4	202.1 ± 13.4	194.9 ± 13.4
17:0	13.3 ± 1.6	15.6 ± 1.6	14.6 ± 1.6	15.4 ± 1.6	12.2 ± 1.6	15.3 ± 1.6	15.9 ± 1.6	14.0 ± 1.6
18:2n-6 LA	390.0 ± 24.4	416.4 ± 24.4	357.8 ± 24.4	415.7 ± 24.4	392.6 ± 24.4	401.2 ± 24.4	371.0 ± 24.4	400.8 ± 24.4
18:3n-3 ALA	33.0 ± 10.2	55.2 ± 10.2	53.9 ± 10.2	53.8 ± 10.2	33.5 ± 10.2	35.0 ± 10.2	54.4 ± 10.2	52.6 ± 10.2
18:1n-9c	208.2 ± 34.6	205.4 ± 34.6	239.7 ± 34.6	192.0 ± 34.6	200.8 ± 34.6	195.4 ± 34.6	257.4 ± 34.6	187.3 ± 34.6
18:1n-7c	40.6 ± 3.4	37.3 ± 3.4	31.8 ± 3.4	38.8 ± 3.4	36.8 ± 3.4	36.4 ± 3.4	38.0 ± 3.4	33.0 ± 3.4
18:1n-7t	44.3 ± 6.3	46.2 ± 6.3	41.8 ± 6.3	46.7 ± 6.3	51.8 ± 6.3	49.4 ± 6.3	43.5 ± 6.3	43.7 ± 6.3
18:0	278.7 ± 26.1	267.3 ± 26.1	312.6 ± 26.1	277.4 ± 26.1	269.5 ± 26.1	284.4 ± 26.1	303.2 ± 26.1	268.2 ± 26.1
20:4n-6 ARA	124.4 ± 8.1 ^{a}	94.8 ± 8.1 ^{b}	90.1 ± 8.1 ^{b}	105.7 ± 8.1 ^{ab}	100.9 ± 8.1 ^{b}	111.2 ± 8.1 ^{ab}	105.9 ± 8.1 ^{ab}	99.0 ± 8.1 ^{b}
20:5n-3 EPA	33.7 ± 4.5	37.2 ± 4.5	31.2 ± 4.5	38.4 ± 4.5	27.0 ± 4.5	28.3 ± 4.5	38.1 ± 4.5	36.9 ± 4.5
20:3n-6	11.5 ± 0.5 ^{a}	11.5 ± 0.5 ^{a}	9.8 ± 0.5 ^{b}	11.5 ± 0.5 ^{a}	10.9 ± 0.5 ^{ab}	11.7 ± 0.5 ^{a}	10.9 ± 0.5 ^{ab}	11.2 ± 0.5 ^{ab}
20:4n-3	2.1 ± 0.3	2.0 ± 0.3	2.2 ± 0.3	1.9 ± 0.3	2.1 ± 0.3	1.5 ± 0.3	1.9 ± 0.3	2.1 ± 0.3
20:2n-6	2.0 ± 0.2 ^{ab}	2.3 ± 0.2 ^{a}	1.7 ± 0.2 ^{b}	1.9 ± 0.2 ^{ab}	2.0 ± 0.2 ^{ab}	2.0 ± 0.2 ^{ab}	1.8 ± 0.2 ^{b}	1.9 ± 0.2 ^{ab}
20:0	3.5 ± 0.3 ^{ab}	3.4 ± 0.3 ^{ab}	3.6 ± 0.3 ^{ab}	3.1 ± 0.3 ^{b}	3.3 ± 0.3 ^{ab}	3.4 ± 0.3 ^{ab}	4.0 ± 0.3 ^{a}	3.2 ± 0.3 ^{b}
22:5n-6 DPA-6	0.9 ± 0.2 ^{ab}	1.1 ± 0.2 ^{ab}	0.7 ± 0.2 ^{b}	1.1 ± 0.2 ^{ab}	1.4 ± 0.2 ^{a}	1.1 ± 0.2 ^{ab}	1.2 ± 0.2 ^{ab}	0.9 ± 0.2 ^{ab}
22:6n-3 DHA	18.7 ± 1.9 ^{a}	13.1 ± 1.9 ^{b}	12.7 ± 1.9 ^{b}	16.7 ± 1.9 ^{ab}	17.3 ± 1.9 ^{ab}	16.0 ± 1.9 ^{ab}	17.8 ± 1.9 ^{ab}	17.9 ± 1.9 ^{ab}
22:5n-3 DPA-3	37.5 ± 2.3 ^{a}	36.2 ± 2.3 ^{abc}	26.8 ± 2.3 ^{d}	36.8 ± 2.3 ^{ab}	29.8 ± 2.3 ^{cd}	32.1 ± 2.3 ^{abcd}	36.8 ± 2.3 ^{ab}	30.3 ± 2.3 ^{bcd}
22:0	5.7 ± 0.3	5.6 ± 0.3	5.4 ± 0.3	5.7 ± 0.3	5.5 ± 0.3	5.7 ± 0.3	6.0 ± 0.3	5.7 ± 0.3
23:0	7.4 ± 0.8 ^{b}	8.2 ± 0.8 ^{ab}	8.5 ± 0.8 ^{ab}	9.8 ± 0.8 ^{a}	9.2 ± 0.8 ^{ab}	9.4 ± 0.8 ^{ab}	8.5 ± 0.8 ^{ab}	9.6 ± 0.8 ^{ab}
24:0	5.4 ± 0.3	5.9 ± 0.3	5.6 ± 0.3	6.1 ± 0.3	6.0 ± 0.3	6.2 ± 0.3	5.8 ± 0.3	6.2 ± 0.3
Total FA	1743.8 ± 87.7	1768.3 ± 87.7	1719.2 ± 87.7	1777.5 ± 87.7	1655.1 ± 87.7	1721.1 ± 87.7	1823.7 ± 87.7	1682.4 ± 87.7
∑SFA	506.8 ± 42.9	511.9 ± 42.9	557.0 ± 42.9	522.7 ± 42.9	480.4 ± 42.9	521.8 ± 42.9	556.4 ± 42.9	510.2 ± 42.9
∑MUFA	387.9 ± 41.3	374.7 ± 41.3	405.1 ± 41.3	366.7 ± 41.3	387.0 ± 41.3	375.6 ± 41.3	435.7 ± 41.3	361.7 ± 41.3
∑PUFA	671.6 ± 22.4 ^{a}	685.2 ± 22.4 ^{a}	602.0 ± 22.4 ^{b}	697.5 ± 22.4 ^{a}	634.7 ± 22.4 ^{ab}	656.1 ± 22.4 ^{ab}	654.3 ± 22.4 ^{ab}	667.2 ± 22.4 ^{ab}
∑*n*-3 LC-PUFA	92.0 ± 7.7	88.4 ± 7.7	72.8 ± 7.7	93.8 ± 7.7	76.2 ± 7.7	77.9 ± 7.7	94.7 ± 7.7	87.2 ± 7.7
∑*n*-3 PUFA	125.2 ± 17.0	143.8 ± 17.0	127.2 ± 17.0	147.6 ± 17.0	110.1 ± 17.0	113.1 ± 17.0	149.2 ± 17.0	140.2 ± 17.0
∑*n*-6 PUFA	534.1 ± 29.3	530.8 ± 29.3	464.4 ± 29.3	541.1 ± 29.3	512.2 ± 29.3	532.7 ± 29.3	495.2 ± 29.3	518.4 ± 29.3
∑other FA	177.5 ± 18.4	196.5 ± 18.4	155.1 ± 18.4	190.4 ± 18.4	153.0 ± 18.4	167.5 ± 18.4	177.3 ± 18.4	143.3 ± 18.4
n-6/*n*-3	4.4 ± 0.7	3.9 ± 0.7	4.3 ± 0.7	3.8 ± 0.7	4.7 ± 0.7	4.8 ± 0.7	3.6 ± 0.7	4.3 ± 0.7

* Values within the same row bearing different superscripts differ ($p < 0.05$); all other abbreviations are as defined in Tables 6.1–6.3.

Table 6.6. Effect of pellet supplementation on total lipid percentage (g fat/100 g) and fatty acid contents (mg/100 g) in kidney of grazing prime lambs (LSM ± SE) *.

Items	Control		NOP		CO		RBO	
	CFP	Lucerne	CFP	Lucerne	CFP	Lucerne	CFP	Lucerne
Lipid percentage	2.8 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	3.1 ± 0.1
14:0	6.4 ± 1.3	3.7 ± 1.3	5.9 ± 1.3	3.9 ± 1.3	5.0 ± 1.3	4.0 ± 1.3	7.1 ± 1.3	5.2 ± 1.3
15:0	4.5 ± 0.4 ^{ab}	3.7 ± 0.4 ^{b}	4.6 ± 0.4 ^{ab}	4.1 ± 0.4 ^{ab}	4.3 ± 0.4 ^{ab}	4.4 ± 0.4 ^{ab}	4.8 ± 0.4 ^{a}	4.3 ± 0.4 ^{ab}
16:1n-9c	4.9 ± 0.7	3.2 ± 0.7	4.4 ± 0.7	3.5 ± 0.7	4.2 ± 0.7	3.8 ± 0.7	4.4 ± 0.7	3.7 ± 0.7
16:1n-7c	6.4 ± 0.8	4.7 ± 0.8	6.2 ± 0.8	4.4 ± 0.8	4.8 ± 0.8	4.9 ± 0.8	6.3 ± 0.8	5.1 ± 0.8
16:0	318.9 ± 14.6 ^{a}	272.2 ± 14.6 ^{b}	304.4 ± 14.6 ^{ab}	305.8 ± 14.6 ^{ab}	286.8 ± 14.6 ^{ab}	291.2 ± 14.6 ^{ab}	305.2 ± 14.6 ^{ab}	311.7 ± 14.6 ^{ab}
17:0	18.4 ± 0.8 ^{abc}	18.1 ± 0.8 ^{abc}	18.0 ± 0.8 ^{bc}	20.4 ± 0.8 ^{a}	17.3 ± 0.8 ^{c}	19.5 ± 0.8 ^{abc}	18.9 ± 0.8 ^{abc}	19.7 ± 0.8 ^{ab}
18:2n-6 LA	228.0 ± 14.2	215.3 ± 14.2	221.0 ± 14.2	250.0 ± 14.2	240.5 ± 14.2	250.3 ± 14.2	229.2 ± 14.2	228.1 ± 14.2
18:3n-3 ALA	23.8 ± 4.7	29.4 ± 4.7	33.0 ± 4.7	28.0 ± 4.7	22.2 ± 4.7	23.0 ± 4.7	34.7 ± 4.7	31.7 ± 4.7
18:1n-9c	240.8 ± 15.5	200.1 ± 15.5	226.3 ± 15.5	213.8 ± 15.5	226.3 ± 15.5	219.5 ± 15.5	232.9 ± 15.5	211.9 ± 15.5
18:1n-7c	34.0 ± 2.6	27.8 ± 2.6	27.5 ± 2.6	30.1 ± 2.6	30.1 ± 2.6	30.5 ± 2.6	27.5 ± 2.6	27.2 ± 2.6
18:1n-7t	37.1 ± 6.6	34.3 ± 6.6	31.6 ± 6.6	36.5 ± 6.6	39.6 ± 6.6	37.9 ± 6.6	39.2 ± 6.6	36.6 ± 6.6
18:0	319.0 ± 16.0	291.3 ± 16.0	308.4 ± 16.0	336.3 ± 16.0	310.7 ± 16.0	301.9 ± 16.0	324.5 ± 16.0	318.6 ± 16.0
20:4n-6 ARA	236.9 ± 16.9 ^{a}	184.8 ± 16.9 ^{b}	202.9 ± 16.9 ^{ab}	221.7 ± 16.9 ^{ab}	214.6 ± 16.9 ^{ab}	218.0 ± 16.9 ^{ab}	210.7 ± 16.9 ^{ab}	218.6 ± 16.9 ^{ab}
20:5n-3 EPA	57.2 ± 11.7	70.2 ± 11.7	70.1 ± 11.7	76.2 ± 11.7	49.9 ± 11.7	49.8 ± 11.7	75.5 ± 11.7	68.9 ± 11.7
20:3n-6	15.5 ± 1.4 ^{b}	16.2 ± 1.4 ^{b}	15.2 ± 1.4^{b}	20.4 ± 1.4 ^{a}	15.1 ± 1.4 ^{b}	16.2 ± 1.4 ^{b}	15.5 ± 1.4 ^{b}	18.3 ± 1.4 ^{ab}
20:4n-3	2.7 ± 0.6	3.2 ± 0.6	4.2 ± 0.6	2.8 ± 0.6	2.5 ± 0.6	3.0 ± 0.6	3.8 ± 0.6	4.3 ± 0.6
20:2n-6	4.1 ± 0.6	4.6 ± 0.6	4.9 ± 0.6	5.4 ± 0.6	4.6 ± 0.6	5.8 ± 0.6	4.2 ± 0.6	5.8 ± 0.6
20:0	5.4 ± 0.4	5.0 ± 0.4	5.5 ± 0.4	5.5 ± 0.4	4.9 ± 0.4	5.8 ± 0.4	5.4 ± 0.4	5.6 ± 0.4
22:5n-6 DPA-6	1.5 ± 0.1 ^{a}	0.9 ± 0.1 ^{b}	1.1 ± 0.1 ^{ab}	1.0 ± 0.1 ^{b}	1.5 ± 0.1 ^{a}	1.0 ± 0.1 ^{b}	1.1 ± 0.1 ^{ab}	1.0 ± 0.1 ^{b}
22:6n-3 DHA	51.4 ± 5.4 ^{ab}	37.7 ± 5.4 ^{b}	47.3 ± 5.4 ^{ab}	49.9 ± 5.4 ^{ab}	55.6 ± 5.4 ^{a}	45.8 ± 5.4 ^{ab}	53.0 ± 5.4 ^{ab}	53.7 ± 5.4 ^{ab}
22:5n-3 DPA-3	66.1 ± 5.8 ^{b}	72.4 ± 5.8 ^{ab}	71.6 ± 5.8 ^{ab}	84.4 ± 5.8 ^{a}	63.7 ± 5.8 ^{b}	64.5 ± 5.8 ^{b}	68.5 ± 5.8 ^{ab}	72.1 ± 5.8 ^{ab}
22:0	29.5 ± 1.9 ^{ab}	26.2 ± 1.9 ^{b}	30.1 ± 1.9 ^{ab}	30.7 ± 1.9 ^{ab}	28.2 ± 1.9 ^{ab}	31.8 ± 1.9 ^{a}	29.2 ± 1.9 ^{ab}	29.8 ± 1.9 ^{ab}
23:0	9.0 ± 0.7 ^{ab}	8.5 ± 0.7 ^{b}	8.9 ± 0.7 ^{ab}	10.3 ± 0.7 ^{ab}	9.3 ± 0.7 ^{ab}	10.4 ± 0.7 ^{a}	9.6 ± 0.7 ^{ab}	10.0 ± 0.7 ^{ab}
24:0	31.5 ± 2.6	30.1 ± 2.6	32.4 ± 2.6	33.8 ± 2.6	32.8 ± 2.6	32.1 ± 2.6	30.9 ± 2.6	33.0 ± 2.6
Total FA	1924.5 ± 75.8 ^{ab}	1710.2 ± 75.8 ^{b}	1852.6 ± 75.8 ^{ab}	1953.3 ± 75.8 ^{a}	1840.2 ± 75.8 ^{ab}	1850.3 ± 75.8 ^{ab}	1905.0 ± 75.8 ^{ab}	1904.2 ± 75.8 ^{ab}
∑SFA	742.6 ± 32.2	658.6 ± 32.2	718.1 ± 32.2	750.9 ± 32.2	699.3 ± 32.2	701.2 ± 32.2	735.6 ± 32.2	737.8 ± 32.2
∑MUFA	413.2 ± 25.2	347.6 ± 25.2	379.0 ± 25.2	383.5 ± 25.2	392.6 ± 25.2	385.1 ± 25.2	393.0 ± 25.2	372.4 ± 25.2
∑PUFA	706.0 ± 26.3 ^{ab}	650.1 ± 26.3 ^{b}	688.5 ± 26.3 ^{ab}	755.2 ± 26.3 ^{a}	685.8 ± 26.3 ^{ab}	696.6 ± 26.3 ^{ab}	712.2 ± 26.3 ^{ab}	722.4 ± 26.3 ^{ab}
∑*n*-3 LC-PUFA	177.3 ± 19.6	183.6 ± 19.6	193.1 ± 19.6	213.2 ± 19.6	171.6 ± 19.6	163.2 ± 19.6	200.7 ± 19.6	202.2 ± 19.6
∑*n*-3 PUFA	201.1 ± 23.2	213.0 ± 23.2	226.2 ± 23.2	241.3 ± 23.2	193.8 ± 23.2	186.2 ± 23.2	235.5 ± 23.2	234.0 ± 23.2
∑*n*-6 PUFA	493.2 ± 26.7 ^{ab}	427.2 ± 26.7 ^{b}	451.9 ± 26.7 ^{ab}	505.4 ± 26.7 ^{a}	482.6 ± 26.7 ^{ab}	500.4 ± 26.7 ^{ab}	466.4 ± 26.7 ^{ab}	479.0 ± 26.7 ^{ab}
∑other FA	62.7 ± 4.3 ^{ab}	53.9 ± 4.3 ^{b}	67.0 ± 4.3 ^{a}	63.7 ± 4.3 ^{ab}	62.5 ± 4.3 ^{ab}	67.5 ± 4.3 ^{a}	64.2 ± 4.3 ^{ab}	71.6 ± 4.3 ^{a}
n-6/*n*-3	2.5 ± 0.3	2.1 ± 0.3	2.2 ± 0.3	2.2 ± 0.3	2.5 ± 0.3	2.7 ± 0.3	2.0 ± 0.3	2.2 ± 0.3

* Values within the same row bearing different superscripts differ ($p < 0.05$); all other abbreviations are as defined in Tables 6.1–6.3.

6.4.3. Effect of pasture types on the fatty acid contents in muscle, liver, heart, and kidney

Table 6.7 shows the FA contents of the different internal organs of prime lambs as affected by the two different types of pastures. There was no significant difference in the FA content in liver of lambs grazing on different pasture types. The ALA, EPA, PUFA, n-3 PUFA, and n-6 PUFA contents in *longissimus dorsi* muscle of lucerne grazing lambs were higher than that of cocksfoot cv. porto grazing lambs. There was no difference in the n-3 LC-PUFA and EPA + DHA + DPA contents in *longissimus dorsi* muscle, liver, heart, and kidney of lambs grazing on cocksfoot cv. porto and lucerne pastures (Figure 6.2). The PUFA content in heart of lucerne grazing lambs (676.5 mg/100 g) was greater than that of cocksfoot cv. porto grazing lambs (640.6 mg/100 g). Lucerne grazing lambs had higher 20:3n-6 content in kidney than the cocksfoot cv. porto grazing lambs.

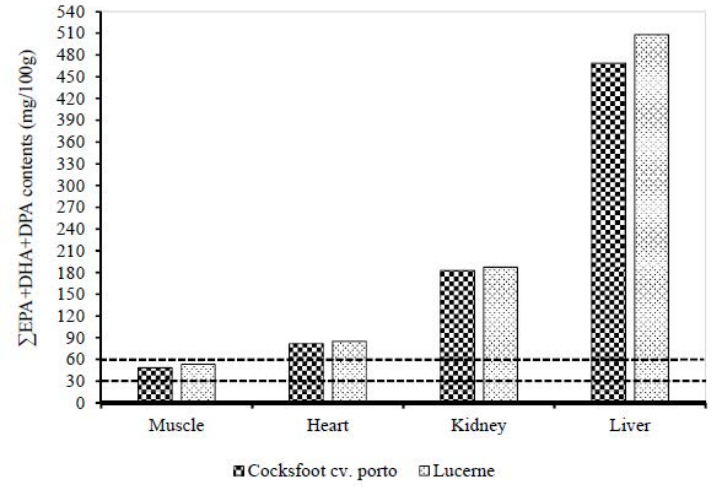

Figure 6. 2. Effect of two different pasture types (cocksfoot cv. porto and lucerne) on the contents of ∑EPA + DPA + DHA in *longissimus dorsi* muscle, heart, kidney, and liver.

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid. The lines at 30 and 60 mg/100 g represent "source" and "good source" of omega-3 FA respectively.

Table 6.7. Effect of different pasture types on total lipid percentage (g fat/100 g) and fatty acid contents (mg/100 g) in liver, kidney, heart and *longissimus dorsi* muscle of prime lambs (LSM ± SE) *.

Items	Liver		*p*	Kidney		*p*	Heart		*p*	Muscle		*p*
	CFP	Lucerne		CFP	Lucerne		CFP	Lucerne		CFP	Lucerne	
Lipid percentage	6.6 ± 0.2	6.8 ± 0.2	0.460	2.9 ± 0.1	3.0 ± 0.1	0.055	2.4 ± 0.1	2.3 ± 0.1	0.865	2.9 ± 0.3	3.1 ± 0.3	0.697
14:0	28.3 ± 2.5	22.0 ± 2.5	0.089	6.1 ± 0.7[a]	4.2 ± 0.7[b]	0.047	6.8 ± 1.1	6.0 ± 1.1	0.608	48.8 ± 6.9	52.3 ± 6.9	0.727
15:0	15.1 ± 0.6	13.2 ± 0.6	0.029	4.6 ± 0.2	4.1 ± 0.2	0.097	2.9 ± 0.2	2.9 ± 0.2	0.971	6.5 ± 0.8	7.5 ± 0.8	0.381
16:1n-9c	24.8 ± 2.0	21.4 ± 2.0	0.245	4.5 ± 0.4	3.5 ± 0.4	0.088	2.9 ± 0.2	2.6 ± 0.2	0.374	6.0 ± 0.7	6.8 ± 0.7	0.361
16:1n-7c	39.6 ± 5.2	30.2 ± 5.2	0.209	5.9 ± 0.4 [a]	4.7 ± 0.4 [b]	0.049	5.6 ± 0.4	4.9 ± 0.4	0.260	29.6 ± 3.8	34.9 ± 3.8	0.347
16:0	762.5 ± 41.7	722.8 ± 41.7	0.508	303.8 ± 7.3	295.2 ± 7.3	0.413	187.2 ± 6.7	194.2 ± 6.7	0.470	527.2 ± 56.9	637.6 ± 56.9	0.183
17:0	53.1 ± 1.9	58.3 ± 1.9	0.063	18.1 ± 0.4 [b]	19.4 ± 0.4 [a]	0.031	14.0 ± 0.8	15.1 ± 0.8	0.341	22.4 ± 2.8	29.9 ± 2.8	0.064
18:2n-6 LA	377.7 ± 24.2	398.9 ± 24.2	0.542	229.7 ± 7.1	235.9 ± 7.1	0.540	377.8 ± 12.2	408.5 ± 12.2	0.088	118.3 ± 4.4 [b]	145.3 ± 4.4 [a]	0.000
18:3n-3 ALA	139.6 ± 11.4	155.8 ± 11.4	0.324	28.4 ± 2.3	28.0 ± 2.3	0.912	43.7 ± 5.1	49.1 ± 5.1	0.460	43.1 ± 2.8 [b]	54.1 ± 2.8 [a]	0.009
18:1n-9c	985.9 ± 74.2	852.3 ± 74.2	0.215	231.6 ± 7.7	211.3 ± 7.7	0.076	226.5 ± 17.3	195.0 ± 17.3	0.211	905.4 ± 98.5	1108.3 ± 98.5	0.158
18:1n-7c	77.0 ± 7.5	69.7 ± 7.5	0.495	29.8 ± 1.3	28.9 ± 1.3	0.632	36.8 ± 1.7	36.4 ± 1.7	0.864	39.3 ± 3.8	48.3 ± 3.8	0.103
18:1n-7t	237.9 ± 21.7	227.2 ± 21.7	0.732	36.9 ± 3.3	36.3 ± 3.3	0.906	45.4 ± 3.1	46.5 ± 3.1	0.800	66.1 ± 9.2	91.2 ± 9.2	0.064
18:0	876.0 ± 32.9	890.1 ± 32.9	0.764	315.6 ± 8.0	312.0 ± 8.0	0.752	291.0 ± 13.1	274.3 ± 13.1	0.376	397.6 ± 37.7	453.9 ± 37.7	0.302
20:4n-6 ARA	228.2 ± 11.6	223.9 ± 11.6	0.794	216.2 ± 8.4	210.7 ± 8.4	0.650	105.3 ± 4.0	102.7 ± 4.0	0.646	35.3 ± 1.5	36.4 ± 1.5	0.582
20:5n-3 EPA	90.8 ± 7.2	106.5 ± 7.2	0.139	63.2 ± 5.8	66.3 ± 5.8	0.709	32.5 ± 2.2	35.2 ± 2.2	0.409	21.7 ± 1.0	23.8 ± 1.0	0.140
20:3n-6	32.7 ± 1.7	38.7 ± 1.7	0.019	15.3 ± 0.7 [b]	17.7 ± 0.7 [a]	0.024	10.7 ± 0.3	11.5 ± 0.3	0.055	6.2 ± 0.2 [b]	7.4 ± 0.2 [a]	0.001
20:4n-3	10.0 ± 0.7	11.5 ± 0.7	0.112	3.3 ± 0.3	3.3 ± 0.3	0.968	2.1 ± 0.2	1.9 ± 0.2	0.400	1.9 ± 0.1	2.0 ± 0.1	0.802
20:2n-6	5.9 ± 0.4	6.3 ± 0.4	0.513	4.4 ± 0.3 [b]	5.4 ± 0.3 [a]	0.043	1.9 ± 0.1	2.1 ± 0.1	0.170	1.5 ± 0.1	1.8 ± 0.1	0.073
20:0	6.1 ± 0.4	5.3 ± 0.4	0.143	5.3 ± 0.2	5.5 ± 0.2	0.500	3.6 ± 0.1	3.3 ± 0.1	0.114	3.6 ± 0.3	3.7 ± 0.3	0.713
22:5n-6 DPA-6	8.3 ± 1.1	5.5 ± 1.1	0.081	1.3 ± 0.1 [a]	1.0 ± 0.1 [b]	0.003	1.0 ± 0.1	1.0 ± 0.1	0.922	1.3 ± 0.1	1.2 ± 0.1	0.454
22:6n-3 DHA	169.7 ± 13.1	165.8 ± 13.1	0.833	51.8 ± 2.7	47.6 ± 2.7	0.279	16.6 ± 1.0	15.9 ± 1.0	0.607	6.2 ± 0.4	6.7 ± 0.4	0.363
22:5n-3 DPA-3	208.2 ± 10.1	235.9 ± 10.1	0.064	67.4 ± 2.9	73.3 ± 2.9	0.166	32.7 ± 1.2	33.8 ± 1.2	0.496	20.6 ± 0.5 [b]	22.6 ± 0.5 [a]	0.015
22:0	8.6 ± 0.2	7.9 ± 0.2	0.057	29.2 ± 0.9	29.6 ± 0.9	0.775	5.6 ± 0.1	5.7 ± 0.1	0.863	1.4 ± 0.1	1.5 ± 0.1	0.208
23:0	18.8 ± 1.1	21.0 ± 1.1	0.181	9.2 ± 0.3	9.8 ± 0.3	0.203	8.4 ± 0.4	9.2 ± 0.4	0.138	1.6 ± 0.1 [b]	1.8 ± 0.1 [a]	0.022
24:0	16.1 ± 0.5	15.4 ± 0.5	0.288	31.9 ± 1.3	32.2 ± 1.3	0.867	5.7 ± 0.1	6.1 ± 0.1	0.067	2.0 ± 0.1	2.1 ± 0.1	0.089
Total FA	4829.7 ± 223.0	4680.3 ± 223.0	0.640	1880.6 ± 37.9	1854.5 ± 37.9	0.631	1735.4 ± 43.8	1737.3 ± 43.8	0.976	2505.3 ± 233.9	2987.9 ± 233.9	0.158
∑SFA	1784.5 ± 71.5	1756.0 ± 71.5	0.781	723.9 ± 16.1	712.1 ± 16.1	0.610	525.1 ± 21.5	516.6 ± 21.5	0.782	1010.9 ± 104.0	1190.4 ± 104.0	0.234
∑MUFA	1582.7 ± 115.5	1405.7 ± 115.5	0.290	394.4 ± 12.6	372.2 ± 12.6	0.223	403.9 ± 20.7	369.7 ± 20.7	0.254	1140.3 ± 121.4	1401.9 ± 121.4	0.141
∑PUFA	1333.6 ± 48.3	1404.9 ± 48.3	0.306	698.1 ± 13.2	706.1 ± 13.2	0.673	640.6 ± 11.2 [b]	676.5 ± 11.2 [a]	0.033	266.9 ± 8.6 [b]	312.4 ± 8.6 [a]	0.001
∑*n*-3 LC-PUFA	478.8 ± 24.8	519.7 ± 24.8	0.255	185.7 ± 9.8	190.5 ± 9.8	0.729	83.9 ± 3.9	86.8 ± 3.9	0.603	50.4 ± 1.8	55.1 ± 1.8	0.074
∑*n*-3 PUFA	626.4 ± 34.1	681.3 ± 34.1	0.266	214.1 ± 11.6	218.6 ± 11.6	0.788	127.9 ± 8.5	136.2 ± 8.5	0.500	94.0 ± 3.8 [b]	110.1 ± 3.8 [a]	0.007
∑*n*-6 PUFA	683.3 ± 38.0	702.6 ± 38.0	0.722	473.5 ± 13.4	478.0 ± 13.4	0.816	501.5 ± 14.6	530.8 ± 14.6	0.170	166.1 ± 5.4 [b]	196.2 ± 5.4 [a]	0.001
∑other FA	127.6 ± 8.1	113.1 ± 8.1	0.220	64.1 ± 2.1	64.2 ± 2.1	0.979	165.7 ± 9.2	174.4 ± 9.2	0.510	86.1 ± 6.4	82.2 ± 6.4	0.674
n-6/*n*-3	1.1 ± 0.1	1.1 ± 0.1	0.957	2.3 ± 0.1	2.3 ± 0.1	0.928	4.2 ± 0.3	4.2 ± 0.3	0.929	1.8 ± 0.0	1.9 ± 0.0	0.788

* Values within the same row bearing different superscripts differ ($p < 0.05$); all other abbreviations are as defined in Tables 6.1–6.3.

6.5. Discussion

6.5.1. FA of pastures and supplementary feeds

The cocksfoot cv. porto and lucerne pastures in this study were abundant in ALA and total n-3 PUFA. Casey et al. (1988) reported that cocksfoot pasture had 39.1% of ALA which is considerably lower than the result obtained in this study (57.6%). This could be attributed to the fact that the FA composition of pastures depend on many factors such as cultivar, cutting age, and season. Meľuchová et al. (2008) found that ALA concentration of pasture plants (mainly lucerne, grass, and herbs) decreased from 62% to 39% (of total FA) from May to August. Garcia et al. (2016) also found that cultivar, cutting date, and season significantly influenced the FA composition, the ALA/LA ratio and PUFA. The relative level of ALA of lucerne pasture in this study was 51.9% which was similar to the finding of Wiking et al. (2010) (53.5%) and doubled the ALA proportion in lucerne hay (22.1%) as reported by Nguyen et al. (2017b) in the same region. Glasser et al. (2013) also found that the ALA proportion of fresh alfalfa was double that of alfalfa hay. The supplementary feeds used in the current study were rich in LA and total n-6 PUFA. Nguyen et al. (2017c) also found that 5% canola oil pellet contained high relative levels of LA and n-6 PUFA (26.7% and 27.4%, respectively).

6.5.2. Effect of supplements on the fatty acid contents in longissimus dorsi muscle, liver, heart and kidney

FA of *longissimus dorsi* muscle: Supplementation of omega-3 rich feed to lambs in indoor systems can increase the content of health benefit claimable FA in muscle (Nguyen et al., 2018a). However, unlike an indoor system, the response of FA content in muscle of grazing ruminants to supplements is not stable, and depends on the quality and quantity of pastures and supplements. Boughalmi and Araba (2016) conducted a trial on the Timahdite lamb breed that

revealed that lambs raised under pasture only had higher percentages of ALA and n-3 PUFA in the semimembranosus muscle than lambs did under the pasture and concentrate diet. Turner et al. (2014a) revealed that supplementation with whole cottonseed increased LA and the n-6/n-3 ratio and decreased ALA and n-3 PUFA in *longissimus dorsi* muscle of Suffolk lambs and Katahdin lambs grazing on a grass–legume pasture. Ponnampalam et al. (2012) found that adding oat grain at 245 g or at 175 g with flaxseed or 175 g with flaxmeal per day in the diet of grazing lambs increased the LA content and the n-6/n-3 ratio and did not affect n-3 PUFA and n-3 LC-PUFA content in the *longissimus lumborum*, compared with lambs grazing pasture only. In addition, Fruet et al. (2018) reported that beef cattle grazing on legume-grass pasture had higher concentrations of ALA in *longissimus thoracis* muscle than those grazing on legume-grass pasture supplemented with whole corn grain at 1.4% of body weight. The results of the current study were in line with previous findings (Boughalmi and Araba, 2016; Turner et al., 2014a) that reported supplementation of pellets with or without oil infusion to grazing lambs led to a decrease in ALA and n-3 PUFA contents and increased the n-6/n-3 ratio in *longissimus dorsi* muscle. The increase of the LA content in *longissimus dorsi* muscle of lucerne grazing lambs supplemented with NOP and RBO pellets could be due to the high n-6 concentration of supplementary diets leading to more n-6 FA being digested, absorbed and finally incorporated in *longissimus dorsi* muscle. The decrease of the 18:0 content in *longissimus dorsi* muscle of cocksfoot cv. porto grazing lamb with pellet supplementation in this study was in agreement with the findings of Fruet et al. (2018) , in that grass-fed beef had higher concentration of 18:0 when compared to grain-fed animals. The conversion of LA and ALA to their long-chain FA products share several of the elongation and desaturation enzymes and there was competition for incorporation into phospholipids between n-6 and n-3 FA (Raes et al., 2004). Thus, the reduction of n-3 LC-PUFA and EPA + DHA + DPA contents in the *longissimus dorsi* muscle of lucerne grazing lambs might be due to significant increases in LA

content, and therefore, the competition for incorporation of n-6 and n-3 FAs into phospholipids. The lambs grazing cocksfoot cv. porto only had high contents of n-3 LC-PUFA (55.2 mg/100 g) in the *longissimus dorsi* muscle, which was similar to that of only lucerne grazing lambs (60.4 mg/100 g) ($p > 0.3150$). The high content of n-3 LC-PUFA in lamb would be beneficial for meat consumers. According to Nichols et al. (2010), the daily requirement per person was 500 mg of the LC omega-3 and a standard serve of red meat was 135 g under Australia and New Zealand regulation (Ponnampalam et al., 2014b). Therefore, consumers having two serves of cocksfoot cv. porto and lucerne grazing lamb meat (= 270 g) each day can meet about 30% of LC omega-3 daily requirement, which could result in a significant increase in LC omega-3 intake to Australians.

FA of liver, heart and kidney: The FA contents of organs (liver, heart, and kidney) can be affected by breeds and nutritional manipulation. Malau-Aduli et al. (2016) reported that there were significant sire-breed variations in the FA content of kidney and muscle. (Kashani et al., 2015) found that Spirulina supplementation to lambs grazing on ryegrass pasture significantly increased the n-3 and n-6 PUFA composition in all organs (liver, heart, and kidney). The results of Nguyen et al., (2017c) demonstrated that there was no significant difference between liver FA profiles of 5% canola oil pellet-fed and control lambs in an indoor feeding system. This current study clearly demonstrated that supplementation with NOP and CO tended to increase the ALA content in the liver of cocksfoot cv. porto grazing lambs. The provision of NOP and CO supplements to grazing lambs resulted in adding more ALA to the lamb diet, which in turn, could explain the increased ALA content in the liver of cocksfoot cv. Porto grazing lambs. In addition, among the supplemented treatments, the cocksfoot cv. porto grazing lambs with RBO supplementation had lower EPA, DPA, n-3 LC-PUFA, PUFA, and EPA + DHA + DPA contents of liver than those lambs in the CO treatment. This is likely due to the large difference

in the ALA proportions of the CO (5.7%) and RBO treatments (2.7%). The competition for incorporation of n-6 and n-3 FAs into the phospholipids is a contributing factor, as previously discussed. The competition of incorporation of n-6 and n-3 FAs into the phospholipids also occurred in heart tissue, and could be the reason for the observed lowering of both ARA and DPA contents in heart of cocksfoot cv. porto grazing lambs with RBO and CO supplementation. The increase of the n-6 PUFA, PUFA, and total FA contents in kidney tissues of lucerne grazing lambs with NOP supplementation could also result from the high n-6 proportion (50.3%) of the NOP supplement. The kidney and liver of all lambs in this study contained high n-3 LC-PUFA contents (ranging from 163.2 mg/100 g to 572.6 mg/100 g), equal to and for many species over the n-3 LC-PUFA contents of wild Australian seafood such as fish, shellfish and lobster (Nichols et al., 2010). In addition, the n-6/n-3 ratio of liver and kidney (from 1.0 to 2.6) were well below the desirable ratio (Simopoulos, 2008). Therefore, the liver and kidney of grazing lambs could be considered as good sources of omega-3 (Zealand, 2012).

6.5.3. Effect of pasture types on the fatty acid contents in muscle, liver, heart, and kidney of lambs

Pasture type did not affect the FA contents in liver of grazing lambs. However, pasture type impacted the FA contents in the muscle, heart, and kidney tissues. Lambs grazing on lucerne pasture had higher contents of ALA, 20:3n-6, DPA, PUFA, n-3 PUFA, and n-6 PUFA in *longissimus dorsi* muscle compared with lambs grazing on the cocksfoot cv. porto pasture. In the present study, the fatty acid composition of cocksfoot cv. porto and lucerne pasture was similar, therefore, the difference in FA content in the *longissimus dorsi* muscle of lambs grazing on these two types of pasture could be attributed to the distinctive characteristics of grass and legume pastures in terms of feed intake and the activity of stearoyl CoA desaturase enzyme. The findings of a meta-analysis conducted by Johansen et al. (2017) revealed that

cows grazing on legume species had 1.3 kg dry matter intake higher than cows grazing on grass species. Wiking et al. (2010) found that transcription of stearoyl CoA desaturase in mammary tissue of cows grazing on high proportions of legume (white clover, red clover, and lucerne pasture) was significantly increased in comparison to cows fed maize/grass silage. Fraser et al. (2004) also found that lambs finished on legume swards (red clover and lucerne) had significantly higher proportions of ALA in *longissimus dorsi* muscle than lambs finished on perennial ryegrass sward. The n-3 LC-PUFA content in *longissimus dorsi* muscle of lambs grazing on the lucerne and cocksfoot pastures (50.4 and 55.1 mg/100 g, respectively) were similar and well above the 30 mg cut-off point for "omega-3 source" claim under Australian guidelines (Zealand, 2012). This result could be due to the fact that n-3 LC-PUFA content in *longissimus dorsi* muscle of grazing lambs was mainly synthesised from the ALA precursor (Kitessa et al., 2010). It could also be due to the low elongation and desaturation of ALA into n-3 LC-PUFA, and the limited capacity of muscle lipids to incorporate n-3 LC-PUFA as occurs in ruminants (Bessa et al., 2015). Furthermore, cocksfoot cv. porto and lucerne pastures had similar proportions of ALA (57.6% vs. 51.9%, respectively). Ponnampalam et al. (2012) performed a trial with lambs grazing on perennial lucerne and annual phalaris pasture, in which they also found no difference in the n-3 LC-PUFA content in muscle tissue of lambs grazing these two pasture types.

6.6. Conclusion

Lambs grazing on lucerne pasture showed higher contents of ALA, 20:3n-6, EPA, PUFA, n-3 PUFA, and n-6 PUFA in *longissimus dorsi* muscle in comparison with lambs grazing on the cocksfoot cv. porto pasture. All grazing lambs with or without supplements had high n-3 LC-PUFA content in *longissimus dorsi* muscle (50.4 mg/100 g and 55.1 mg/100 g, respectively), which was well over the 30 mg cut-off point for labelling as a source of omega-3. A larger

serve size, e.g., 135 or 150 g, as has been used in other studies, would see a good source of omega-3 (60 mg per serve) achieved. Lambs grazing on cocksfoot cv. porto pasture only also achieved high contents of ALA and n-3 LC-PUFA contents (67.1 mg/100 g and 55.2 mg/100 g, respectively), which was the same as those contents of only lucerne grazing lambs, with cocksfoot cv. porto clearly demonstrated to produce premium quality, healthy lamb meat, based on omega-3 PUFA content. Supplementation using pellets with or without oil infusion to grazing lambs generally decreased the ALA and n-3 PUFA contents and increased the n-6/n-3 ratio in *longissimus dorsi* muscle. The addition of pellets to grazing lambs decreased the 18:0 content in *longissimus dorsi* muscle of cocksfoot cv. porto grazing lambs. NOP and RBO supplementation increased the LA content in *longissimus dorsi* muscle of lucerne grazing lambs. Pellet supplementation tended to reduce the EPA + DHA + DPA content in *longissimus dorsi* muscle of lucerne grazing lambs. The fatty acid contents of internal organs of grazing lambs were affected by pellet supplementation. The n-3 LC-PUFA contents in the liver and kidney of grazing lambs were equal to the n-3 LC-PUFA contents of wild Australian seafood such as fish, shellfish, and lobster and can be considered and used as a good source of omega-3.

Chapter 7: General discussion, conclusions and implications

The influence of supplementing weaner lambs with oil-infused pellets rich in polyunsaturated fatty acids on growth, carcass traits and n-3 LC-PUFA composition of the *longissimus dorsi* muscle, heart, liver and kidney was investigated in both in-door lot-fed and out-door grazing systems. The primary objectives were to ascertain if there were health beneficial improvements in n-3 LC-PUFA content attributable to canola, rice bran, flaxseed, safflower and rumen protected oil-infused pellets and variations in cost of production relative to liveweight gain.

The series of studies presented in this thesis aimed to investigate the responses of confined and grazing prime lambs to dietary n-3 PUFA-rich oil supplementation with regards to animal performance, carcass characteristics, feed costs and fatty acid profiles of edible muscle, liver, kidney and heart. The primary objective was to provide lamb producers with scientific evidence for better utilization of their feed resources to produce premium healthy lamb at comparatively lower feed costs without compromising productivity and product quality. In order to achieve this overarching objective, a comprehensive literature review of the benefits of n-3 LC-PUFA for human consumption, underlying biological mechanisms and potential for improvement of the n-3 LC- PUFA content in lamb was presented in Chapter 2.

A series of hypotheses were tested in the following chapters:

Chapter 3 tested the hypothesis that supplementation of lambs with a variety of dietary oil based PUFA-enriched pellets would improve growth and carcass characteristics in comparison to control lambs fed lucerne hay only. The research question herein was: What are the responses of confined lambs to dietary oil based PUFA-enriched pellet supplementation with respect to

lamb growth and carcass characteristics? To answer this question, a 10-week feeding trial in a confined production system was conducted. Seventy-two White Suffolk x Corriedale first-cross weaner lambs were randomly allocated to the following six treatments: (1) Control: Lucerne hay only; wheat-based pellets infused with 50 ml/kg (on DM basis) of oil from (2) rice bran (RBO); (3) canola (CO); (4) rumen-protected (RPO), (5) flaxseed (FSO) and (6) safflower (SO) in a completely randomized experimental design.

The findings revealed that supplementation of lambs on a lucerne basal diet with wheat-based pellets enriched with different PUFA-containing oils increased total dry matter feed intake, final LWT, ADG, body conformation traits and carcass characteristics. The use of RBO and CO in fattening prime lambs reduced about 65% of feed costs compared with control lambs, and did not negatively impact live animal performance, carcass characteristics and OTH trade income in comparison to other sources of PUFA. The low feed cost of RBO and CO supplementation could be attributed to the lower price of RBO and CO compared to other sources of PUFA. Furthermore, the percentage of all oils using in this study being lower than 6%, which reported to be unlikely to have negative effects on feed intake, growth and carcass trait of lambs (Nguyen et al., 2018a), could explain no different in live animal performance, carcass characteristics and OTH trade income of lambs fed different sources of PUFA.

Previous studies showed improvement in animal performance and carcass characteristics (Beigh et al., 2017; Safari et al., 2009) when confined lambs on a basal diet of low quality hay were supplemented with concentrate or pellets. However, in the current study, it was demonstrated that supplementation of confined lambs on a high quality basal hay (lucerne hay)

containing high CP content with PUFA-rich oil pellets, improved animal performance and carcass traits in addition to reducing feed costs.

While Chapter Three demonstrated good live animal performance, carcass characteristics and OTH income at comparatively lower feed cost, the following research questions remained unanswered:

1. Will good animal performance, carcass characteristics and OTH income on low cost feed lead to the attainment of 'source' or 'good source' levels of n-3 LC-PUFA in the meat of supplemented lambs?

2. Will there be significant variation in n-3 LC-PUFA content between the *longissimus dorsi* muscle, heart, kidney and liver of supplemented lambs?

3. Can edible organs provide 'source' and 'good source' EPA+DHA contents considered beneficial for human health?

To provide answers to these questions, the experiment described in Chapter Four was conducted.

Chapter 4 analysed the fatty acid profiles of confined lambs fed a lucerne hay basal diet plus n-3 PUFA-rich oil pellets to evaluate impact on FA content variation in the *longissimus dorsi* muscle, heart, kidney and liver tissues. Results demonstrated that lambs fed a lucerne-basal diet and supplemented with CO, FSO, SO and RPO pellets had EPA and DHA contents in the *longissimus dorsi* muscle above the 30 mg per standard serve threshold for omega-3 "source" claim under the Food Standards Australia and New Zealand (FSANZ) guidelines. There was no difference in ALA and n-3 LC-PUFA contents in the *longissimus dorsi* muscle of lambs fed different dietary oils of plant origin. This could be due to the limited bioconversion of ALA to

n-3 LC-PUFA (Shahidi and Ambigaipalan, 2018). This finding is in line with the results of Nguyen et al., (2017b) who did not observe any difference in n-3 LC-PUFA contents of the *longissimus dorsi* muscle between lambs supplemented with canola and flaxseed oil infused pellets.

The liver and kidney play crucial roles in lipid metabolism (Liu et al., 2014; Moestrup and Nielsen, 2005). Furthermore, the liver is the hub of fatty acid synthesis and lipid circulation through lipoprotein synthesis (Nguyen et al., 2008). Therefore, the liver and kidney have high contents of n-3 LC-PUFA. The variation in fatty acid contents of the different tissues and organs and high n-3 LC-PUFA content in the liver and kidney of supplemented lambs were in agreement with the findings of Malau-Aduli et al., (2016). The results of previous studies and this study demonstrated that confined lambs supplemented with PUFA-rich feeds consistently produced healthy lamb products with 'source' level n-3 LC-PUFA.

From the findings reported in Chapter Four, the following further questions needed to be answered:

1. Is there any added advantage of supplementing lambs grazing lucerne and cocksfoot pastures with CO and RBO oil-based pellets in enhancing EPA+DHA contents?
2. Are animal performance, carcass traits, feed conversion efficiency and OTH trade incomes of grazing lambs improved by additional supplementation with RBO and CO?

Therefore, the experiment reported in Chapter 5 was designed to answer these questions and test the hypothesis that the productive performance, carcass characteristics, feed conversion efficiency and OTH trade incomes of grazing prime lambs would be improved by supplementation with n-3 PUFA-rich oil pellets and vary between pasture types. In order to test this hypothesis, a 9-week grazing trial with forty-eight first-cross weaner lambs was

conducted. The lambs were randomly assigned into a split-plot experimental design with pasture type as the main plot effect and pellet supplementation as a sub-plot effect.

The findings revealed that lambs grazing CFP pasture and supplemented with CO had lower FCE and higher OTH trade income than the CFP grazing only lambs. Pellet supplementation did not affect animal performance and carcass traits of lucerne grazing lambs. Lambs grazing lucerne pasture had higher performance and carcass traits than lambs grazing CFP pasture. The results herein could be attributed to the difference in diet composition, especially protein content. For instance, supplementation of CFP grazing lambs with CO led to more protein intake, digestion and absorption, resulting in higher performance and better carcass traits. Similarly, the higher protein content of lucerne pasture in comparison to CFP could be the likely reason for higher performance and better carcass traits of lucerne grazing lambs compared with those grazing CFP. The current findings are supported by the study of De Brito et al., (2017b) who reported that lambs grazing legumes with high protein content had faster growth than lambs grazing grass.

There was still the unanswered question of any likely added advantage of supplementing lambs grazing lucerne and cocksfoot pastures with CO and RBO oil-based pellets in enhancing EPA+DHA contents. Therefore, Chapter 6 aimed to answer this question and tested the following three hypotheses:

(1) Muscle, heart, kidney and liver tissues of confined prime lambs fed different n-3 PUFA rich oil pellets would have higher PUFA contents than grazing only lambs (control);

(2) Fatty acid profiles of grazing lambs supplemented with different n-3 PUFA-rich oil pellets would vary according to tissue type (muscle, heart, kidney and liver); and

(3) Fatty acid profiles in muscle, heart, kidney and liver tissues of grazing lambs would have different responses to different pasture types and PUFA rich oil pellets.

The results from this study proved that supplementation of grazing lambs with pellets including or without oil infusion generally decreased the ALA and n-3 PUFA contents and increased the n-6/n-3 ratio in *longissimus dorsi* muscle. Many previous studies reported a reduction in ALA and n-3 PUFA contents in the muscle of supplemented grazing lambs (Boughalmi and Araba, 2016; Ponnampalam et al., 2012; Turner et al., 2014a). This result could be explained by an increase in LA content from supplementary feeds. Both ALA and LA share and compete for the same enzymes (Δ^6-desaturase, Δ^5-desaturase, and elongase) in their biosynthesis, and therefore, the increase in LA content reduces ALA accumulation, and *vice versa* (Raes et al., 2004).

The fatty acid profiles of organs and tissues of grazing lambs were also impacted by plant oil infused pellets. Lucerne grazing lambs had higher contents of ALA, 20:3n-6, EPA, total PUFA, n-3 PUFA and n-6 PUFA in the *longissimus dorsi* muscle than in lambs grazing cocksfoot pasture. Fatty acid contents in the muscle, heart and kidney of grazing lambs were affected by different types of pastures. The possible explanation for this difference could be due to the higher quality of lucerne compared with CFP resulting in faster rate of digestion better fatty acids absorption and accumulation in lambs on lucerne pasture. This finding is similar to the results of De Brito et al., (2017a). Fatty acid profiles of the liver and kidney of grazing lambs met the 'good source' of n-3 LC-PUFA criteria. CFP demonstrated the potential for producing premium quality, healthy lambs with high ALA and n-3 LC-PUFA contents.

In summary, the findings reported in this thesis provide Australian lamb producers and research scientists with scientific evidence that adds to current knowledge on the improvement of n-3 LC-PUFA content in lamb using PUFA- rich feeds in both indoor lot-fed and outdoor grazing production systems.

A) In indoor, lot-fed production system:

- RPO supplementation was the most effective in increasing n-3 LC-PUFA content in the *longissimus dorsi* muscle of confined lambs;
- RBO and CO can be used to improve the n-3 LC-PUFA content of lamb *longissimus dorsi* muscle at comparatively lower feed costs;
- Future studies could focus on whole production system costs and the effect of marketing labelled n-3 LC-PUFA 'source' and 'good source' lamb products;

B) In outdoor, grazing production system:

- CO can be utilised as a strategic nutritional tool to increase carcass weight and OTH trade of lambs with a better FCE;
- Cocksfoot pasture has the potential to produce premium quality, healthy lamb meat, based on omega-3 PUFA content;
- Future grazing studies should explore other legumes and grasses with the potential to produce premium and healthy lamb meat with high content of n-3 LC PUFA.
- Future studies on rumen biohydrogenation pathways in grazing lambs are needed to find novel ways of increasing n-3 LC-PUFA content in meat.

In conclusion, the overall results of this study offer lamb producers with scientific data for better matching their existing sheep genetics with PUFA-rich feed resources under the 'real

world' on-farm management conditions in order to achieve their targets in terms of continuous improvement of lamb productivity and quality.

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Appendix 1

SCIENTIFIC REPORTS

OPEN

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Fatty acid profiles of muscle, liver, heart and kidney of Australian prime lambs fed different polyunsaturated fatty acids enriched pellets in a feedlot system

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We investigated the effect of various dietary polyunsaturated fatty acid (PUFA) sources on the fatty acid profiles of muscle, liver, heart and kidney of Australian prime lambs. Seventy-two White Suffolk x Corriedale first-cross lambs weaned at 6 months of age were randomly allocated to the following six treatments: (1) Control: Lucerne hay only; wheat-based pellets infused with 50 ml/kg dry matter (DM) of oil from (2) rice bran (RBO); (3) canola (CO); (4) rumen-protected (RPO), (5) flaxseed (FSO) and (6) safflower (SO) sources in a completely randomized experimental design. Lambs in CO, FSO, SO and RPO treatments achieved contents of eicosapentaenoic acid (EPA, 22:5n-3) plus docosahexaenoic acid (DHA, 22:6n-3) in the *longissimus dorsi* muscle ranging from 31.1 to 57.1 mg/135 g, over and above the 30 mg per standard serve (135 g) threshold for "source" claim under the Australian guidelines. There was no difference in n-3 LC-PUFA contents in *longissimus dorsi* muscle of lambs fed dietary oils of plant origin. The highest 18:3n-3 (ALA) contents achieved with FSO diet in the muscle, liver and heart were 45.6, 128.1 and 51.3 mg/100 g, respectively. Liver and kidney contained high contents of n-3 LC-PUFA (ranging from 306.7 to 598.2 mg/100 g and 134.0 to 300.4 mg/100 g, respectively), with all values readily exceeding the 'good source' status (60 mg per serve under Australian guidelines). The liver and kidney of PUFA fed lambs can be labelled as 'good source' of n-3 LC-PUFA based on EPA and DHA contents stipulated by the Food Standards of Australia and New Zealand guidelines. Therefore, if lamb consumers consider eating the liver and kidney as their dietary protein sources, they can adequately obtain the associated health benefits of n-3 LC-PUFA.

Appendix 2

nutrients

MDPI

Article

Enhanced Omega-3 Polyunsaturated Fatty Acid Contents in Muscle and Edible Organs of Australian Prime Lambs Grazing Lucerne and Cocksfoot Pastures

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Abstract: The enhancement of health-beneficial omega-3 long–chain (≥C20) polyunsaturated fatty acid (*n*-3 LC-PUFA) contents in the muscle, liver, heart, and kidney of Australian prime lambs through pasture grazing and supplementation with oil infused pellets was investigated. Forty-eight first-cross prime lambs were randomly assigned into a split-plot design with pasture type as the main plot effect and pellet supplementation as a sub-plot effect in a feeding trial that lasted for nine weeks. The *n*-3 LC-PUFA content in *Longissimus dorsi* muscle of all lambs was well above the 30 mg threshold for "omega-3 source" nutrition claim under the Australian Food Standards and Guidelines. Pasture type impacted the fatty acid contents in muscle, heart, and kidney of prime lambs. Lambs grazing cocksfoot grass only had high 18:3n-3 (ALA) and *n*-3 LC-PUFA contents (67.1 mg/100 g and 55.2 mg/100 g, respectively) in the *Longissimus dorsi* muscle, which was not significantly different ($p > 0.8990$) from the contents of lambs grazing only lucerne. Supplementation of pellets with or without oil infusion to grazing lambs generally decreased the ALA and *n*-3 LC-PUFA contents and increased the *n*-6/*n*-3 ratio in the *Longissimus dorsi* muscle. The fatty acid content in the internal organs of grazing lambs was also affected by pellet supplementation. The liver and kidney of grazing lambs were both "good sources" (60 mg/100 g) of omega-3. The cocksfoot grass showed considerable potential for producing healthy, premium quality meat with high contents of *n*-3 and *n*-3 LC-PUFA, which may consequently enhance the omega-3 intake of Australian lamb consumers.

Keywords: lamb; *n*-3 LC-PUFA; muscle; liver; heart; kidney; rice bran; canola; cocksfoot; lucerne

Appendix 3

animals

MDPI

Article

Nutritional Supplements Fortified with Oils from Canola, Flaxseed, Safflower and Rice Bran Improve Feedlot Performance and Carcass Characteristics of Australian Prime Lambs

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Simple Summary: This study evaluated the feedlot response of Australian prime lambs to supplementation with oil based polyunsaturated fatty acid enriched pellets. The results demonstrated that live animal performance and carcass characteristics of prime lambs on a lucerne basal diet were improved after the supplementation with oil based polyunsaturated fatty acid enriched pellets. Supplementation of lambs with rice bran oil and canola oil resulted in improved live animal performance and carcass characteristics of prime lambs at comparatively lower feed costs than oils from flaxseed, safflower and rumen-protected sources. These results are very useful for prime lamb producers in increasing product quality and farm profitability without compromising animal performance and well-being.