Objective meat quality, composition and sensory profiling of New Zealand lamb from different production systems

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Abstract

Lamb production systems in New Zealand can vary widely in animal characteristics such as breed, sex, diet and age at slaughter. In order to move towards production systems that are more consumer-focused, there is a need to understand meat quality characteristics and cross-cultural consumer preference of loins from lambs reared under a wide range of commercial production systems. This thesis conducted a comprehensive assessment of the loins (*M. longissimus thoracis*) of 150 lambs from 10 forage production systems (n=15) and assessed meat quality, fatty acid composition, volatile and proteomics profiles, and consumer sensory evaluations.

Instrumental measurement of meat quality (shear force, pH, colour, and water holding capacity) showed that animal age at slaughter and diet had a greater effect on meat quality than the sex of the lamb. A chicory diet increased carcass weight (CW) of lambs resulting in a greater loin intramuscular fat (IMF) percentage compared to a perennial ryegrass diet (18.1±0.1kg vs. 16.9±0.1kg CW and 2.0-2.6% vs. 1.3-1.6% IMF, respectively). Finishing lambs in New Zealand forage systems for 12 months resulted in lower proportions of n-3 fatty acids in meat as well as a lower PUFA:SFA ratio compared to lambs slaughtered at 4- or 8-month-old (2.93-3.41% vs. 4.79-5.86% n-3 fatty acids and 0.17-0.19 vs. 0.27-0.35 PUFA:SFA, respectively). Of the 286 proteins identified among the raw lamb loins, only 17 showed significant differences in abundance between production systems which indicated that the expression of proteins was rarely affected by the production factors. For both New Zealand and Chinese consumers, the average liking scores differed among the different types of commercial lambs. Consumer clusters showed that the fatty acid profile and the volatile compounds derived from lipid oxidation seem to be a stronger driver of consumer liking of lamb for some consumers than other.

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List of abbreviations

Abbreviation	Explanation
CW	Carcass weight
IMF	Intramuscular fat
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
BCFA	Branched chain fatty acids
LC n-3 PUFA	n-3 long chain polyunsaturated fatty acids
CLA	Conjugated linoleic acids
HS-SPME	Headspace-Solid phase microextraction
DVB/CAR/PDMS	Divinylbenzene-Carboxen-Polydimethylsiloxane
SEM	Standard error of the mean
°C	degree Celsius
FSANZ	Food standards of Australia and New Zealand
VSGR	VIAScan® estimated GR soft tissue depth
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
FID	flame ionization detector
GC	Gas chromatography
FAME	Fatty acid methyl esters
AOSC	American oil chemists' society
h/H	hypocholesterolaemic/Hyperdolesterolaemic
PCA	Principal component analysis
ANOVA	Analysis of variance
RI	Retention index
Dim	Dimension
SDC	Sodium deoxycholate
LC-MS	Liquid chromatography-mass spectrometry
Q-TOF	Quadrupole-time-of-flight
FDR	False discovery rate
CID	Collision-induced dissociation
LFQ	Label-free quantification
PTMs	Post-translational modifications
PLS-DA	Supervised partial least square discriminant analysis
WEAN-W	4-month-old wether lambs of a composite breed at weaning
REDC-W	6- to 8-month-old wether lambs of a composite breed that had been grazing red clover
REDC-E	6- to 8-month-old ewe lambs of a composite breed that had been grazing red clover
GRASS-W	6- to 8-month-old wether lambs of a composite breed that had been grazing perennial ryegrass-based pasture
GRASS-E	6- to 8-month-old ewe lambs of a composite breed that had been grazing perennial ryegrass-based pasture

CHIC-W	6- to 8-month-old wether lambs of a composite breed that had been grazing chicory
CHIC-E	6- to 8-month-old ewe lambs of a composite breed that had been grazing chicory
MIX-W	6- to 8-month-old wether lambs of a composite breed that had been grazing mixed pasture
MXME-W	12-month-old wether Merino lambs that had been grazing a mixed pasture
MXME-C	12-month-old cryptorchid Merino lambs that had been grazing a mixed pasture

Introduction

Producing lamb meat with desirable eating quality is a key goal of the New Zealand lamb industry. The diet (Bailey et al., 1994; Chevance and Farmer, 1999; De Brito et al., 2016; Priolo et al., 2004; Santos-Silva et al., 2002; Watkins et al., 2013), sex (Diaz et al., 2003; Horcada et al., 1998; Johnson et al., 2005), age (Hopkins et al., 2006; Mashele et al., 2017; Mcphee et al., 2008; Wood et al., 2008) and breed (Hopkins and Mortimer, 2014) of the lamb are all factors that have been associated with differences in carcass and meat quality, fatty acid and volatile composition and eating quality.

The New Zealand climate favours pasture growth throughout the year and hence, a wide range of commercial pasture lamb production systems are used. Typically, lambs are born in late winter or early spring with minor differences between regions in New Zealand (Geenty, 2010). A proportion of pre-weaning lambs can be sold directly to slaughter when target weight is reached (32-38kg live weight or 14-16kg carcass weight, Geenty, 2010), while the other lambs are kept for finishing during summer and autumn, and will be regular weighed and sold to slaughter when target slaughter weights are reached. Lamb production systems in New Zealand have traditionally relied on perennial ryegrasses and white clover pastures. There has, however, been an increase in the use of alternative forage types such as plantain, chicory, and red clover in order to offer a diet with a higher nutritive value than traditional pasture.

Consumers as the ultimate arbiters require nutritious meat with good eating quality. Red meat is an important source of protein, fatty acids and vitamins in the human diet, which are essential for human health (Pereira and Vicente, 2013). A high intake of n-3 fatty acids has been reported to decreased the risk of coronary heart disease (Hu et al., 2002). Understanding the effect of diverse

forage production systems on the fatty acid composition of lamb meat, in particular n-3 fatty acids, could provide an opportunity to differentiate lamb products for those discerning consumers that are willing to pay a premium for perceived healthiness attributes.

To be able to understand the drivers and determinants of tenderness of meat, research has utilised proteomic approches and identified several myofibrillar proteins, such as actin and myosin regulatory light chain 2 as biomarkers for tenderness (Picard and Gagaoua, 2020). Currently, however, there is still a knowledge gap on how production systems influence the protein profile of lamb meat.

The liking for sheep meat flavour is not universal. Volatile profiles of lamb have been scrutinized and identified aldehydes, alcohols and ketones as possible drivers of sheep meat flavour (Resconi et al., 2013). Prescott et al., (2001) indicated the presence of volatile branched-chain fatty acids and skatole as one cause of rejection of lamb for Japanese women, while Frank et al., (2016) suggested that up to a certain threshold concentration, the total branched-chain fatty acids play a positive role in defining lamb flavour and acceptance for Chinese and non-Chinese Australian consumers. Most considerations of volatiles utilise cooked meat samples, however, being able to differentiate lambs on their raw meat volatiles would enable scope for positive or negative attributes associated with volatiles to be identified before meat is sent to market. Although laboratory testing can help identify drivers of lamb meat quality, the consumer's perspective of the of lamb meat quality is important to consider as it incorporates the complexities associated with an individual's sensitivity, preference and background experience.

Hypothesis and Research Objectives

Value creation for New Zealand lamb is dependent on understanding and managing the underpinning the key quality attributes of the primary meat components. The farm system is the first stage of the meat production value chain that could be manipulated to have an impact on the meat product. Commercial forage-based production systems for lamb can differ in the types of forage used and will incorporate different slaughter ages. Some lambs will be slaughtered at weaning, but most lambs in New Zealand forage-based systems will require some months after weaning to achieve a suitable level of finish. Both male and female lambs can be raised for meat production with males being entire or, either fully castrated or made into a cryptorchid. A review of the literature (Chapter 1) highlights that on-farm production factors such as diet, sex, and age at slaughter can influence carcass and meat quality characteristics.

It is hypothesised that differences in diet, age at slaughter and sex in difference production systems will contribute to differences in meat quality and meat characteristics of commercially reared lambs in New Zealand. A full characterisation of lamb meat sourced from difference commercial production systems has not been performed, and so considering differences in objective meat quality alongside fatty acid composition, volatile and proteomic analysis as well as consumer sensory testing will help to identify aspects of the production system that could be controlled to obtain a lamb meat product with specific characteristics for any particular market.

Therefore, this thesis set about to integrate information obtained on the intrinsic determinants of meat quality identified through objective meat quality tests, composition, fatty acid and volatile profiling and proteomics with sensory testing of the meat to understand the effect of production systems on the meat characteristics.

The objectives of the work in this thesis were to:

1. Evaluate the carcass and meat quality traits of lambs from 3 farms with diverse forage-based diets, sex, castration status and age at slaughter which are representative of the lambs commercially processed in New Zealand (Chapter 2).
2. Evaluate the meat fatty acid profiles of lambs from diverse forage-based production systems and to compare the omega-3 fatty acid levels in meat with current nutrition guidelines (Chapter 3).
3. Evaluate if there was a difference in the raw meat volatile profiles of lambs from different forage-based production systems in New Zealand (Chapter 4).
4. Compare protein profiles of meat from six types of typical commercial New Zealand forage lamb production systems (Chapter 5).
5. Investigate the eating quality, as assessed by both New Zealand and New Zealand Chinese consumers, of meat from lambs finished in different commercial production systems and evaluate the role of fatty acid and volatile composition on the sensory scores (Chapter 6).

Chapter 1

Literature review

1.1. The New Zealand lamb industry

1.1.1 New Zealand commercial production systems for sheep meat

Sheep and beef cattle production utilises 66% or 9.7 million ha of New Zealand's agriculture land (Ministry for Primary Industries, 2012). The majority of New Zealand sheep meat is shipped overseas, leading to a highly developed and export focussed sector (Prescott et al., 2001). In 2018, lamb meat to the value of $2.5 billion was exported to over 120 countries making New Zealand the largest lamb exporter in the world (Beef and Lamb New Zealand, 2018).

Forage systems are used globally for sheep meat production (Bouwman et al., 2005) and a study by Herrero et al. (2013) estimated that pasture (including grasses, clover and other pasture species) comprise 48% (2.3 billion tons) of the overall biomass used by livestock. The climate in New Zealand benefits pasture growth year-around and hence grazed pasture and forage crops supply in excess of 95% of the diet for sheep and beef production (Hodgson et al., 2005). The forage production systems are efficient, sustainable, and relatively low cost compared to grain and concentrates feeding systems that allows New Zealand sheep sector to compete globally.

Sheep numbers in New Zealand peaked of approximately 70 million in the early 1980s and have been recorded at 27 million in 2017 (Beef and Lamb New Zealand, 2018). However, the total production of sheep meat has been maintained due to improved production efficiency (Morris and Kenyon, 2014). From 1990 to 2017, the average weight of lamb carcass has increased from 13.9 kg to 18.6 kg (Beef and Lamb New Zealand, 2018). Faster growth rates result in lambs achieving a target slaughter weight at a younger age, which is considered beneficial for achieving superior market prices and minimizes maintenance feeding costs (Brito et al., 2017).

Most sheep breeds farmed in New Zealand were Romney based producing lambs for meat as well as coarse wool (Beef and Lamb New Zealand, 2016). Fewer than 10% of sheep are Merino

or Merino-based breeds, which are farmed to produce fine wools. Recent surveys suggested that there is an increasing trend of using composite breeds with Finnish Landrace and Texel genes being incorporated into an existing Romney, Coopworth and Perendale flocks (Corner-Thomas et al., 2013; Beef and Lamb New Zealand, 2016). These composite breeds displayed higher reproductive rates and greater lamb growth rates than traditional Romney, Coopworth and Perendale breeds.

Typically, lambs are born in late winter or early spring. Live weight gains of 300 g per day or more in young lambs can be achieved if the ewe is provided with a high allowance of a good quality pasture (Scales, 1993). On average, lambs are weaned at 10-14 week of age with average weaning live weight of 28-30 kg (Geenty, 2010). Under ideal management conditions, a high proportion (Figure 1.2) of the lambs are sold directly to slaughter as prime lamb when target weight is reached (32-38kg live weight or 14-16kg carcass weight) with the majority of lambs in New Zealand achieving this in December through to March (Figure 1.1). Lambs that do not reach the target live weight are kept for finishing during autumn.

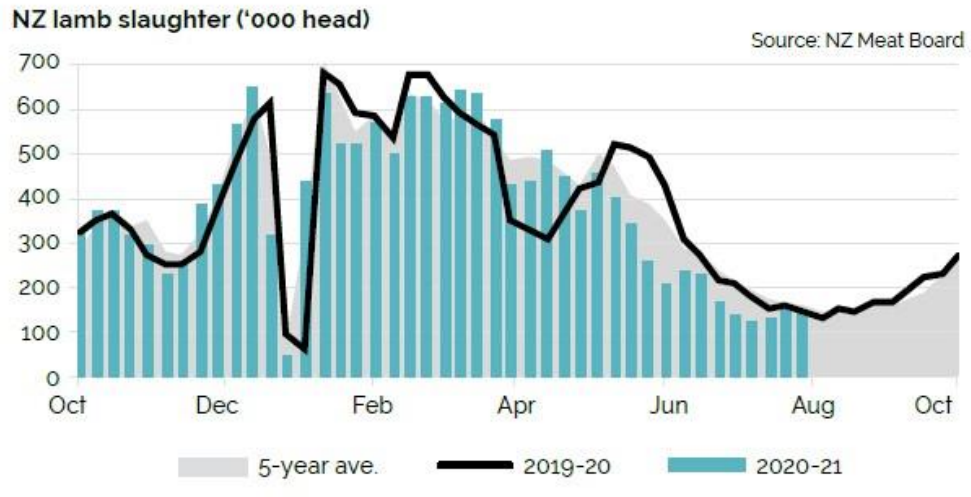

Figure 1.3 New Zealand lamb slaughter pattern 2019-2021, adapted from nzfarmlife

Small ruminants are efficient transformers of low quality forage into high quality animal products (Zervas and Tsiplakou, 2011). Sheep can generally graze pasture to a shorter residual height than cattle (Morris and Kenyon, 2014) and hence to be more suitable to graze in hill regions. As a result of the recent increase in the dairy cattle industry in New Zealand, a greater proportion of sheep farms are now located on hill country which often have low soil fertility (Beef and Lamb New Zealand, 2018). Many pastures are permanent in New Zealand especially those in less cultivatable hill country, which is the major proportion of the total area farmed for sheep and beef, Traditionally, pasture systems are based on perennial ryegrass (~80%) and white clover (~20%) in New Zealand (Kim et al., 2013). More recently, there has been increasing use of alternative forage types such as plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), and red clover (*Trifolium pratense*) to offer a diet of higher nutritive value to improve lamb weight gains (Corner-Thomas et al., 2014; Somasiri et al, 2015). Kemp, Kenyon, and Morris (2010) calculated that more than 720 kg of lamb carcass weight per ha/year could be produced on plantain, red and white clover mixed pastures compared with 400 kg/ha on traditional ryegrass and white clover pastures in farmlet experiment.

1.1.2. Oversea markets of New Zealand lamb industry

With 27 million sheep and 5 million population, New Zealand does not have a predominant domestic market for lamb meat. Approximately 95% of sheep meat was exported (Beef and Lamb New Zealand, 2018) and acceptance of sheep meat in the markets is an important issue for New Zealand meat industry. The New Zealand meat export markets are dominated by North Asia, European and North American market (Table 1.1). In 2018, China and European Union both accounted for approximately one-third of New Zealand's lamb exports. North American accounted half of New Zealand's beef exports.

Table 1.1Total global meat shipment and breakdown of meat shipments by destination (tonnes of product weight) for New Zealand meat industry for the 2017/2018 season (beef and lamb New Zealand, 2018)

	Lamb	Mutton	Beef
Total Global shipment	302,893	94,013	404,128
Shipment destination			
China	106,962	62,701	93,306
Rest of Asia	13,579	8,685	45,631
North America	31,735	6,031	206,551
UK	49,403	2,454	687
Rest of EU	61,679	5,583	6,010
Middle East	20,588	1,249	6.554

In the 1970s, most of New Zealand sheep meat was exported as whole frozen carcass with a small proportion of frozen cut. Since then, there has been a shift towards exporting more value-added products (Carson and East, 2018). More specific frozen cuts and boneless frozen cuts are developed to suit consumer demand as well as sell the cuts to the market that willing to pay the most for that cut. For example, rack was preferred in US market; striploin and rump were preferred by EU market; shoulder was preferred by middle east market (Beef and Lamb New Zealand, 2018). Meanwhile, the improvement in storage/transportation techniques make it possible to export more chilled meat (approximately 5% of beef and 32% of lamb in 2017; Carson and East, 2018) and the chilled products achieve premium prices over frozen products.

The export value of New Zealand's red meat exports has substantially increased over the recent years (Beef and Lamb New Zealand, 2018). China is the most notable market, having gone from just $260,000 of red meat exports in 1992-93 to $1.7 billion in 2017-18 which account for one third of total red meat export of New Zealand. As per capita income is projected to continue to rise in China, a model-based projection exercise indicates that under plausible assumptions, China's meat imports may rise sharply to 31.7% of its total demand, which account for nearly one-third of the world market by 2030 (Wusheng and Lijuan, 2015). New Zealand as a meat exporting country

is likely to adapt its product or implement selective trading procedures to provide consumer-acceptable products for its markets including the Chinese market.

1.2. Carcass characteristics of lamb

Carcass traits provide signals to farmers about the value of carcass, understanding carcass characteristics and finding techniques for altering the characteristics therefore, are useful in production systems to maximising returns to the producer (Ngo et al., 2016). Carcass characteristics of sheep include dressing-out percentage, carcass composition i.e., fat content and also distribution of fat. (Knight et al., 2014; Mortimer et al., 2010).

1.2.1. Carcass weight and dressing-out percentage

Carcass weight refers to the weight of an animal after removing all the internal organs, head as well as inedible portions of the tail and legs. Dressing-out percentage is expressed as the weight of the carcass as a percentage of live weight. Understanding the dressing-out percentage for sheep and the factors that affect it is important when aiming to get carcass into a particular weight range associated with a higher schedule payment (Litherland et al., 2010). Carcass classification for lamb is based on fat classes (GR; soft tissue depth over 12^{th} rib 11cm from midline) and carcass weight classes in New Zealand. The weight classes include: A = less than 9.0 kg, L = 9.0 to 12.5 kg, M = 12.0 to 16.0 kg, X = 16.5 to 20 kg, H = 20.5 kg and over. Similarly, the fat classes can be divided to: A = almost devoid of external fat, Y = low fat content, P = medium fat content, T = high fat content (cut and trim of excessive fat needed before export), F = excessive fat (must be cut and trim of excessive fat before export), and C = extreme high fat content that not suitable for export (Beef and Lamb New Zealand Reference Guide 3rd Edition, 2010).

Several on farm factors could affect the dressing-out percentage of lamb carcass includes:

a) Age/weight: dressing-out percentage increase as sheep get older and their live weight increases (Schreurs and Kenyon 2017).
b) Breed/genetic line: at same weight, a higher dressing-out percentage can be expected when the breed or genetic line has a higher level of fatness, muscularity or muscle to bone weight ratio; a lower weight of non-carcass components such as head, fleece and pelt (Schreurs and Kenyon 2017).
c) Sex/castration: at same weight, the dressing-out percentage of rams is normally approximately 1%-point lower than wethers; and 2% points lower than ewes due to the presence of testes as non-carcass components, and lower level of fat in the carcass. In young lambs, however, when the testes are not yet developed, it is often observed that ram lambs have greater dressing-out percentage compared to ewe lambs due to greater lean in the carcass (Hegarty et al., 2006).
d) Nutrition/health: the amount of gut-fill for the period leading up to slaughter can influence dressing-out percentage. The dressing-out percentage of milk-fed lambs is normally 2-4% higher than weaned lambs and lambs feed by low quality/poor digestibility pasture (Litherland et al., 2010).

1.2.2. Fat: GR index and intramuscular fat

Subcutaneous fat is easy to be trimmed off to give lean cuts (Muchenje et al., 2009). While intramuscular fat or marbling is a fine network of fat sometimes visible throughout the meat. Marbling develops with the maturity of animals and marbled meat is usually obtained from carcasses with a large amount of subcutaneous fat (Hocquette et al., 2010; Camacho et al., 2017). Measuring carcass composition either by dissection or by chemical analysis is expensive and time consuming, alternatively, indirect estimation such as fat depth over the rib (e.g. GR, soft tissue depth over the 12th rib at a point 11mm off the middle line) has been developed for lamb (Hopkins

et al., 2008). The GR measurement is moderately well related to the total carcass fatness and is the measure of fatness used in the New Zealand commercial classification system.

On-farm factors affecting fat accumulation in the sheep carcass includes:

a) Age: percentage of both subcutaneous fat and intramuscular fat in sheep carcass increase markedly as the animal gets heavier and approaches maturity (Hocquette et al., 2010; Camacho et al., 2017).
b) Breed/genetic line: at the same weight, lambs from breeds with higher mature weights will tend to be leaner because they are actually at an earlier maturity stage. When adjusting to a common muscle or meat yield between breeds, lambs from Finn and Texel sires had less fat and therefore associated with higher lean meat yield than lambs from a Romney sire (Purchas et al., 2002).
c) Sex: greater intramuscular and carcass fat in ewe lambs compared to wether and rams lambs has been reported in various studies (Anderson et al., 2015; Craigie et al., 2012).
d) Nutrition/health: greater nutrition will result in lambs that are fatter due to nutrient overflow. This is based on the theory that when the capacity to grow non-fat tissue such as muscle and bone is reached, any additional nutrients from diet intake will be used for fat growth (Hegarty et al., 2006).

1.2.3. Lean meat yield

The lamb carcass is mainly made up of muscle, fat, and bone. The measurement of carcass composition in the New Zealand sheep industry is generally focused on maximising the muscle proportion or yield of meat from a carcass. Meat yield may be represented as lean meat yield or the retail meat yield. The lean meat yield is the approximate percentage of the muscle over total carcass weight.

The lean meat yield from a carcass is determined from the fat% and muscle to bone ratio (M:B) of the carcass according to the following equation:

$$\text{LMY\%} = (100\% - \text{Fat\%}) \times \frac{(\text{M:B})}{(\text{M:B} + 1)}$$

The equations indicates that increasing in LMY% may be achieved by increasing of M:B, decreasing of fat% or a combination of both. A high value of M:B is commonly associated with superior muscularity (thicker, heavier muscle) except if it is achieved by only decreasing the weight per unit length of bone (i.e. long thin bones). Differences in fatness among breeds are likely to be the strongest driver of differences in lean meat yield since the differences in M:B are relatively small between breeds (Purchas et al., 2002).

1.3. Production factors that affect meat quality of lamb

In addition to production volume, meat quality traits are becoming increasingly important to consumers over time alongside with sensory requirements and health and safety concerns (Hung et al., 2016). Meat quality characteristics tenderness and flavour are affected by the whole production process. Various single factors such as the lamb's diet (Kitessa et al., 2009; Brito et al., 2016), sex (Johnson et al., 2005), age (Mashele et al., 2017; Mcphee et al., 2008), breed (Hopkins et al., 2005) as well pre-slaughter, processing, storage factors are reported to have effect on meat quality (Table 1.2). As evidenced by the previous studies, however, the influence of these factors are sometimes mixed or inconsistent between some reports (Hopkins and Mortimer, 2014). In addition, the meat properties reported in different countries should be distinguished based on the forage and grain offered, as diet and breed can be very different between regions.

Table Table 1.2 A summary of the effect of both on- and off- farm factors that influence the appearance and palatability characteristics of sheep meat. The effect is classified as low, moderate, or high. Adapted from; Schreurs and Kenyon, (2017).

	Meat colour	Fat colour	Tenderness	Juiciness	Flavour
Animal factor					
Breed	Low	Moderate	Moderate	Moderate	Low
Genetics	Low	Low	Moderate	Moderate	Low
Sex	Low	Low	Moderate	Moderate	Low
Age	Moderate	Low	**High**	Moderate	Moderate
Pre-slaughter management					
Nutrition	Moderate	**High**	Moderate	Moderate	**High**
Stress	**High**	Low	Moderate	**High**	Moderate
Processing					
Chilling	Moderate	Low	**High**	Moderate	Low
Electrical stimulation	Low	Low	**High**	Moderate	Low
Storage of meat					
Aging	Low	Low	**High**	Moderate	Low
Packaging	**High**	Low	Low	Moderate	Moderate
Cooking	**High**	Low	**High**	**High**	Moderate

1.3.1. Tenderness of lamb meat

Tenderness can be evaluated by objectively measure of shear force required to cut through the meat (Hopkins et al., 2010) or alternatively, a trained or untrained (consumer) panel (Safari et al., 2001). No differences in objectively measured tenderness between breeds (Dransfield et al., 1979; Hopkins et al 2007; Hopkins et al., 2005) or inconsistent differences (Purchas et al., 2002) are reported. It should be noticed that post slaughter processing and storage conditions that applied before shear force measurements may vary between different experiments.

An effect of sex or castration on ultimate pH or tenderness of sheep meat is not commonly observed, the tenderness of loin meat from ram, cryptorchids, or wethers lambs up to the age of 13

months did not differ (Kerslake et al., 2012; Schreurs, 2013). A quantitative review across 55 studies by Sales (2014) concluded that sheep meat from wethers is more tender than sheep meat from rams, which mainly due to a lower total collagen content and higher soluble collagen percentage as a result of absence of testosterone.

Greater carcass weight and muscle fat accumulation of lambs grazed on chicory and plantain compared to lambs grazed on perennial ryegrass were observed (Schreurs, 2013). The greater fat accumulation were considered to be responsible for the lower shear force in the meat from chicory and plantain fed lambs (Schreurs, 2013). When the intramuscular fat was similar between meat from lambs fed on serval forage diets, similar shear force value was found (De Brito et al., 2016). For every 50g increase in the live weight of lamb, there was a 0.4 kgF increase in shear force of meat; and for every millimetre increase in GR depth, there was a 0.3 kgF decrease in shear force of meat (Campbell et al., 2011). Hence, Campbell et al., (2011) suggested the ability of the diet to deposit fat is a key driver of tenderness.

The amount and type of connective tissue in a cut of meat can also affects the tenderness of the meat. A higher concentration of collagen or less soluble collagen is associated with tougher meat. Meat tenderness declines as the animal age at slaughter and total collagen increases.

Early work from Jeremiah et al. (1971) reported that sheep ranging in age from 74 to 665 days shown a positive correlation between animal age and decreasing tenderness based on five muscles from the hindleg. Various more recent studies (Hopkins et al., 2007a; Zhang et al., 2018) suggested similar findings based on *longissimus* and *semitendinosus* muscles. While the shear force of *longissimus* muscle was higher for lambs slaughtered at 8-month-old compared to lambs slaughtered at 5-month-old or 14-month-old according to Mashele et al. (2017).

Aging is a process of proteolytic enzyme hydrolysis. Proteolytic enzymes are present within the muscle fibre and are related with protein turnover and cell renewal. After an animal is slaughtered, the proteolytic enzymes can remain active if muscle temperature is maintained above 0°C. Proteolytic activity results in a breaking down of the structural proteins within the muscle fibre, which has a tendering effect (Ma and Kim, 2020). Cold shortening is associated with tougher meat and occurs due to rapid cooling (below 10°C) of carcass before rigor mortis completed. Calcium ions will be resealed into the muscle fibre, causing sustained contraction, which is measured as a shortening of sarcomere length (Warner et al., 2010). In addition, higher levels of fatness in lamb carcass can improve tenderness of meat. Firstly, a greater subcutaneous fat cover could insulate the carcass of lamb, which makes it cool slowly and less likely to let cold shortening happen. Secondly, intramuscular fat improves meat tenderness by diluting the tougher connective tissue and muscle fibre. Thirdly, fat has a lubricating effect during chewing and stimulates the secretion of saliva to improve the sensation of tenderness during eating (Warner et al., 2010).

1.3.2. Colour of lamb lean meat and subcutaneous fat

The colour of meat is strongly affected by post-slaughter procedures and can be assessed by colour meter or a sensory panel. Discoloration is one of important indicator of freshness and wholesomeness of meat used by customers. As a result, a large amount of retail red meat (nearly 15% of beef) is discounted in price due to surface discoloration, which corresponds to a great percentage of annual revenue losses (Mancini and Hunt, 2005).

Fresh meat colour is mediated through the ultimate pH that is achieved in rigor (Purchas and Aungsupakorn, 1993). As pH increases, meat becomes darker (Fogarty et al., 2000). Abnormal colour (darker or lighter) was reported to negatively correlated with consumer liking (Miller, 2020). Meat from Merinos is reported to be more susceptible to high pH than meat from other breeds,

however, no phenotypic correlations between pH and L* values is observed, and there is little evidence that Merino lambs produce darker fresh meat than other genetic types (Fogarty et al., 2000; Hopkins et al., 2005; Hopkins et al., 2007a).

Cryptorchids had a higher pH in the *M. longissimus* than wether or ewe carcases, with 19% had a pH above the critical 5.8, but this did not translate into an effect on objective colour measured (Hopkins et al., 2001). Hopkins et al., (2007b) reported it is unlikely that consumer panel would be able to detect the slight differences between meat from different genders.

The redness of meat was reported to increase with animal age (Dawson et al., 2002). As sheep approach 12– to 13-month of age, the meat colour on average became darker, redder and less acceptable to consumers (Hopkins et al., 2007a). The preferred meat colour from suckler lambs was in part a reflection of lower myoglobin levels (Gardner et al., 2007) because a clear increase in myoglobin in meat with animals age has been observed. This also reflects the increase in muscle oxidative capacity as animals become older (Greenwood et al., 2007).

The colour of subcutaneous adipose tissue depends on animal age, gender and breed (Dunne et al., 2009). Grazing forages and pastures have been associated with increased yellow colour of fat due to the deposition of carotenoid pigments from the plants into the fat (Priolo et al., 2001). The colour changes of fat also depend on the duration of finishing, the amount of fat accumulated during finishing and the rate of utilisation of carotene from body fat (Dunne et al., 2009). Although yellow carcass fat is undesirable in many countries (Yang et al., 1992), it may be associated with a healthier fatty acid profile and greater antioxidant content from pasture feeding.

1.3.3. Water holding capacity of lamb meat

Poor water holding capacity results in abnormal loss of water and soluble proteins from the carcass and muscle cuts, which can lead to a reduction of 2% to 12% post-slaughter yield (Luca et

al., 2013). These reductions represent significant financial losses for the meat industry and decreases the palatability and processability of meat.

Safari et al., (2001) reported that genotype could affected cooking loss significantly. Loin muscle from Border Leicester x Merino lambs had lower cooking loss than all the other genotypes. High pH (above 6.10) meat was found to have a higher sarcoplasmic protein solubility and water holding capacity than normal pH (5.40-5.79) meat (Zhang at al., 2005). The ultimate pH of meat is dependent on the rate and extend of glycolysis in the post mortem muscle, which is influenced by the quantity glycogen present in the muscle at the time of slaughter and by the activity of glycolytic enzymes (Hopkins and Mortimer, 2014; Savell et al., 2005). Therefore, any initiative that reduces pre-slaughter stress and thereby reduces pH fall will improve the water holding capacity of meat products (Cheng and Sun, 2008).

1.4. Fatty acid profiles of lamb meat

1.4.1. Human nutrition guidelines related to fatty acids

Red meat is a popular source of high-quality protein and provides a variety of essential polyunsaturated fatty acids (Pighin et al., 2016). Fatty acids composition of meat not only directly affects the eating quality, but also influences purchasing decision of consumers. Although consumers generally consider the nutritional composition of food is more important than the validated claims, the use of nutritional composition claims, especially those consumers are familiar with, generates positive preference (Miklavec et al., 2015).

A controversial topic about red meat is the health concerns of its high saturated fatty acids (SFA) content, and health professionals generally recommend moderate red meat consumption (350–500g cooked weight per week, World cancer research fund, 2018). A high intake of SFA and trans-fatty acids adversely affects glucose metabolism and is associated to a number of health

concerns such as type II diabetes (Hu et al., 2001). The US Departments of Agriculture and Human Health Services (2010) have defined cholesterol-raising fatty acids as SFA from C12:0 to C16:0, including the trans-fatty acids, except for those found in products from ruminants. Not all saturated fatty acids, however, have the same level cholesterol-raising potential (Hunter et al., 2010). Bonanome and Grundy (1988) reported that a higher content of 18:0 in the diet did not elevate plasma low density lipoprotein-cholesterol because it is poorly digested and can be easily desaturated to c9-18:1. Therefore, the Atherogenic index (AI) and Thrombogenic index (TI) has been introduced to take individual fatty acids into account, and low values of AI and TI are recommended for a healthy diet (Ulbricht and Southgate, 1991; Vacca et al., 2008). The equations for the Atherogenic and Thrombogenic indices are:

$$\text{Atherogenic index (IA)} = \frac{(4 * 14{:}0) + 16{:}0 + 18{:}0}{\sum \text{PUFA} + \sum \text{MUFA}}$$

$$\text{Thrombogenic index (IT)} = \frac{14{:}0 + 16{:}0 + 18{:}0}{0.5 * (\sum \text{MUFA} + \sum \text{n6}) + (3 * \sum \text{n3}) + (\text{n3}/\text{n6})}$$

The improvement of public health awareness of SFA, atherogenic and thrombogenic risks has had a negative impact on the red meat sector. This could be one of reasons that the red meat consumption declined in Australia and New Zealand over the past 3 decades (Howes et al., 2015) despite the known nutritional benefits of meat as it provides high-quality protein and important minerals including iron and zinc (Purchas et al., 2014).

Excessive intakes of n-6 PUFA and high n-6:n-3 ratios are commonly found in modern Western diets and associated with pathogenesis of many diseases, including cardiovascular disease, inflammatory and autoimmune diseases (Simopoulos, 2008). Increasing the intake of long chain n-3 PUFA, without increasing n-6 PUFA intake can decrease the risk of these diseases (Ruxton et al., 2004). Red meat is an important source of long chain n-3 PUFA (Scollan et al., 2006). World

Health Organization, (2008) recommended that the total PUFA, n-6, and n-3 fatty acids should contribute to 6-11%, 2.5-9%, 0.5-2% of total energy intake for adults, respectively (Table 1.3). Children are recommended to have a greater intake of PUFA than adults (11-15% of total energy intake). Lack of long chain n-3 PUFA intake was linked to behavioural and learning disorders such as attention deficit hyperactivity disorder (Richardson and Ross, 2000). The World Health Organization, (2008) also recommended a combined eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) intake of 250 mg per day for adults (Table 1.3). If lamb meat with an average content of 50 mg EPA plus DHA per 100g raw meat is considered the only resource of EPA and DHA, 500g of lamb consumption per day is needed in order to reach the nutrition requirement. For pregnant and lactating females, the minimum optimal intake of EPA plus DHA is 300 mg per day, of which at least 200 mg per day should be DHA (World Health Organization, 2008).

In Australia and New Zealand, heath claims can be made with food >22 and 44 mg EPA + DHA/100 g serve for a 'source' and 'good source' of EPA + DHA, respectively (Food Standards Australia New Zealand, 2012); while the European market requires >40 mg and 80 mg EPA+DHA/100 g serve for a 'source' and 'good source', respectively (Commission Regulation of European Union, 2010). Conjugated linoleic acid (CLA) which has anticarcinogenic, antiatherogenic, antidiabetic, and antiadipogenic properties for humans is commonly found in red meat (Geay et al., 2001). The mechanism of its benefits, however, is still unclear (Ochoa et al., 2004) and the nutritional recommendations for CLA are not part of any current health guidelines.

Table 1.3 Summary of current health guidelines of fatty acids from different organizations.

Indicator	Recommended Value	Reference
Claims		
EPA+DHA, source	>22 mg/100 g product	(Food Standards Australia New Zealand, 2012)
EPA+DHA, good source	>44 mg/100 g product	(Food Standards Australia New Zealand, 2012)
EPA+DHA, source	>40 mg/100 g product	(Commission Regulation of European Union, 2010)
EPA+DHA, good source	>80 mg/100 g product	(Commission Regulation of European Union, 2010)
alfa-linolenic acid	>0.3 g/100g product	(Commission Regulation of European Union, 2010)
Intake/Day	adequate intake range	
Total Fat (Adult)	20-35% of total energy intake	(World Health Organization & Food and Agriculture Organization, 2008), (European Food Safety Authority, 2017)
Total PUFA (Adult)	6-11% of total energy intake	(World Health Organization & Food and Agriculture Organization, 2008)
Total n-6 (Adult)	2.5-9% of total energy intake	(World Health Organization & Food and Agriculture Organization, 2008)
Total n-3 (Adult)	0.5-2% of total energy intake	(World Health Organization & Food and Agriculture Organization, 2008)
Linoleic acid	4% of total energy intake	(European Food Safety Authority, 2017)
Linoleic acid	2% of total energy intake	(International Society for the Study of Fatty Acids and Lipids, 2004)
Alfa-linolenic acid	>0.5% of total energy intake	(European Food Safety Authority, 2017)
Alfa-linolenic acid	>0.7% of total energy intake	(International Society for the Study of Fatty Acids and Lipids, 2004)
EPA + DHA (children 0.5-2 years)	Adequate Intake of 0.1 g DHA	(European Food Safety Authority, 2017)
EPA + DHA (children 2-4 years)	Adequate Intake 0.1-0.15 g	(World Health Organization & Food and Agriculture Organization, 2008)
EPA + DHA (children 4-6 years)	Adequate Intake 0.15-0.2g	(World Health Organization & Food and Agriculture Organization, 2008)
EPA + DHA (children 6-10 years)	Adequate Intake 0.2g-0.25g	(World Health Organization & Food and Agriculture Organization, 2008)
EPA + DHA (Adult)	>0.5g	(International Society for the Study of Fatty Acids and Lipids, 2004)
EPA + DHA (Adult)	>0.25 g	(World Health Organization & Food and Agriculture Organization, 2008), (European Food Safety Authority, 2017)
EPA + DHA (pregnant & lactating females)	Adequate Intake 0.3 g and least 0.2 g should be DHA	(World Health Organization & Food and Agriculture Organization, 2008)
EPA + DHA (pregnant & lactating females)	0.1-0.2 g DHA additional during pregnancy and lactation	(European Food Safety Authority, 2017)
n-6: n-3	<4	(Department of Health United Kingdom, 1994)
n-6: n-3	Insufficient data on clinical and biochemical endpoints. No dietary Reference Value	(European Food Safety Authority, 2017)
PUFA: SFA	0.4	(Department of Health United Kingdom, 1994)
Conjugated linoleic acids (CLA)	No convincing evidence prevention or promotion of diet-related diseases for. No dietary Reference Value	(European Food Safety Authority, 2017)

1.4.2. Fatty acids in lamb meat

The major lipid class in lamb adipose tissue (>90%) is triacylglycerol or neutral lipid. The rest of proportions are mainly phospholipid and cholesterol, which have a much higher PUFA percentage in order to perform its function as a constituent of cellular membranes (Wood et al., 2008). Oleic acid (c9-18:1) is the most dominant fatty acid in neutral lipid. This fatty acid is formed from stearic acid (18:0) by the enzyme stearoyl Co-A desaturase, a major lipogenic enzyme. The DHA and EPA are synthesized from the n-3 precursor alpha-linolenic acid (18:3), whereas long chain n-6 PUFA such as arachidonic acid is synthesized from the n-6 precursor linoleic acid (18:2). The long chain n-3 and n-6 PUFA are mainly found in phospholipid, but also detected in neutral lipid and adipose tissue (Cooper et al., 2004). A major trans fatty acid is conjugated linoleic acid (CLA, c9t11-18:2) formed by the bacterium *Butyrivibrio fibrosolvens* in the rumen (Mir et al., 2000). Human dietary sources of CLA are almost exclusively red meat and dairy products.

Fatty acids play an important role in the overall flavour of lamb meat. Branched-chain fatty acids, mainly 4-methyloctanoic acid, 4-ethyloctanoic acid and 4-methylnonanoic acid, are the main compounds responsible for the characteristic goaty and sheepy flavour of lamb meat (Prescott et al., 2001). The level of branched-chain fatty acids is in general, negatively associated with sensory scores evaluated by Asian consumers from early studies (Prescott et al., 2001). Alternatively, the total branched-chain fatty acids can play a positive role in defining lamb flavour and acceptance for some consumers up to a certain threshold concentration (Frank et al., 2016).

Fatty acids composition of lamb also effects the mouthfeel of meat. Fat tissue with more saturated fatty acid is firmer and the melting point of fat increases as saturation increases

(Wood et al., 2004). In the C18 fatty acid series, for example, stearic acid (18:0) melts at 69.6°C, oleic acid (c9-18:1) melts at 13.4°C, n-6 linoleic acid (18:2) melts at 5°C, and alpha-linolenic acid (18:3) melts at 11°C. Therefore, when the meat is served, with internal temperature cooling down gradually, the intramuscular fat with less saturated profile is more likely to remain molten and provide a smoother mouthfeel. Angood et al., (2008) reported positive correlations between the total fatty acid content of *longissimus* (marbling level) and eating quality scores given by a trained sensory panel.

1.4.3. Effect of on farm production factors on fatty acid compositions of lamb

The production system for lamb represents the combined effects of animal-type including the age, breed and sex interacting with the farm managements. Given that beneficial fatty acids in meat are associated with purchasing and value of meat products, the influence that the production system has on the composition of fatty acids in the meat is of research interest.

1.4.3.1. ANIMAL TYPE (AGE, SEX/CASTRATION AND BREED)

Several animal-type factors like age, sex and breed have affected the fatness (subcutaneous fat depth and marbling level) of the animal as well as the composition of fatty acids (Beriain et al., 2000; Díaz et al., 2005; Ponnampalam et al., 2014). However, the effect of these factors can be confounded, and the results may not consistent between studies.

Suckling lambs essentially have a monogastric stomach and the lipid profile of their adipose tissues mainly reflects the composition of fat ingested in the milk, which contains a high content of short and medium claim SFA (Beriain et al., 2000; Bas and Morand-Fehr, 2000). The sucking lambs slaughtered at a young age (14 kg live weight) were reported to have higher medium chain SFA such as 10:0, 12:0 than post weaning lambs finished on concentrate grain diet or pasture diet (Velasco et al. 2001). Fatty acids profiles become more saturated as

animals get fatter and older (Young et al., 2006). This is because neutral lipids increased more rapidly than the phospholipids as the total fat content of the body increases (Warren et al., 2008, Jerónimo et al., 2011). Phospholipid is an essential component of cell membranes which has a higher proportion of PUFA (20-50% of total FA) than neutral lipids. The proportion of c9-18:1 tends to increase in the fat of lambs as they became older, due to an increase in the activity of stearoyl CoA desaturase on c9-18:1 (Wood et al., 2008). Similarly, Velasco et al. (2001) reported that the proportion of c9-18:1 was lowest in the meat from suckling lambs (14-28 kg live weight), and increased along with the state of fatness. A greater c9-18:1 proportion was attributed to a fatter and heavier carcass of 12-month-old compared to 4-month-old pasture reared lambs (Díaz et al., 2005). A negative association between 18:3n-3 and total lipid content has been reported in various bovine and ovine studies (Kazala et al., 1999, Salvatori et al., 2004). Trans-MUFA such as t9-18:1 and t11-18:1 are biodegradation products of 18:2n-6, the percentage of these fatty acids also increase during fattening of lambs (Jerónimo et al., 2011).

It is generally accepted that the influence of sex on fatty acid composition of meat is small and mainly expressed by fatness, with females display higher values than males for subcutaneous, intermuscular, and intramuscular fat measurements (Diaz et al., 2003; Horcada et al., 1998). This is ascribed to the fact that females display a greater tendency to accumulate fat, especially from an early age. Thus, sex differences were greater for suckling lambs than for heavier lambs (Horcada et al., 1998). In addition, the wether lambs were reported to have higher levels of as 4-alkyl-branched-chain fatty acids than the ewes at 8 months of age (Salvatore et al., 2007).

Relatively lean breeds or genetic types of lamb were reported to have higher proportions of PUFA in total lipid, because phospholipid contribute to a higher proportion of the total fat

(Warren et al., 2008, Jerónimo et al., 2011). When Welsh Mountain and Soay sheep were reared on pasture diet and slaughtered at the same body weight. Soays had much leaner carcasses and less lipid in the muscle, as well as lower proportions of c9-18:1 and higher proportions of all PUFA in muscle than Welsh Mountain sheep (Fisher et al., 2000). Sañudo et al., (2000) found some fatty acid differences between 4 sheep breeds: Spanish Rasa Aragonesa, Merino, British Welsh Mountain breed, and typical British early lambs. The British early lambs had higher percentages of 18:0, n-3 18:3, long chain n-3 PUFA and lower percentages of n-6 18:2, long chain polyunsaturated n-6 FA than Spanish lambs. However, the authors suggested this is likely a reflection of the level of difference in origin and diet between sheep breeds. The effects of breed are often confounded with other factors that affect the amount of fat in carcass (Wood et al., 2008).

1.4.3.2. EFFECT OF LAMB DIET ON FATTY ACID PROFILES OF MEAT

There are some clear fatty acid profile differences between concentrate fed and forage fed lambs (Santos-Silva et al., 2002; Sañudo et al., 2000; Ponnampalam et al., 2014). Some of the key findings include:

- Faster growth and fat deposition of concentrate diet lamb was frequently reported due to higher energy density intensity.
- PUFA: SFA ratio are lower in the animals on a forage diet since they were frequently reported to be leaner than concentrate fed animals.
- There is a higher n-6/n-3 ratio and n-6 PUFA content were observed in the meat of concentrate fed lambs than forage fed lambs because n-6 is the dominate PUFA from grains.
- Lambs fed high energy concentrate diets yield higher concentrations of branched-chain fatty acids in fat than pasture diets (Young and Braggins, 1998).

- The level of CLA was found higher for lambs grazed on pasture compared to concentrate.

In contrast, differences between lamb meat from different forage diets are more subtle and less reported. When the forage diets are applied post weaning, the active rumen manipulates fatty acid composition. A high proportion (>90%) of dietary PUFA is hydrogenated in rumen leading to a high concentration of saturated fatty acids in the meat (Bessa et al., 2015). Fraser et al., (2004) suggested that grazing forage legume swards resulted in an increased concentration of unsaturated fatty acids in lamb meat compared to grazing perennial ryegrass, thereby improving the PUFA:SFA ratio of muscle.

1.5. Volatile compounds from raw and cooked lamb meat

1.5.1. Formation of volatile compounds in raw lamb meat

Volatile compounds of raw meat can originate from several pathways including lipid oxidation and microbiology activity (Elmore et al. 2005; Gravador et al. 2015; Vasta et al. 2012). Oxidation of fatty acids can generate a serious of volatile compounds. The rate of oxidation depends on the fatty acid composition, the concentration and activities of pro- and anti-oxidants, the oxygen partial pressure, the method of processing, and the conditions in which the meat is stored (Ladikos and Lougovois, 1990). The initial oxidation phase involves the removal of hydrogen from a methylene group, typically in a cis double-bond pair of an unsaturated fatty acid which forms a lipid radical that can be rearranged. The secondary phase includes the decomposition of hydroperoxides and involves a complex series of reactions (Resconi et al., 2013). Therefore, polyunsaturated fatty acids that have two or more double bonds, are more susceptible to oxidation than are the fatty acids that have one or no double bonds because in the first step the hydrogen can be more easily removed due to the formation of a stable allylic radical in which the electrons are delocalized over three carbon atoms (Resconi et al., 2013).

Secondly, it is inevitable that meat will carry some level of microbial contamination during production, and storage (Biss and Hathaway, 1998). The presence of significant populations of spoilage bacteria (notably *psychrotrophic Clostridium spp., Enterobacteriaceae, Brochothrix thermosphacta* and *Shewanella putrefaciens*) will cause loss of shelf-life through off-flavours and odours, and discolouration (Mills et al., 2014). Enterobacteriaceae (e.g., Serratia, Erwinia, Enterobacter) could produce acetoin or its oxidation (diacetyl) or reduction (2,3-butanediol) products though different pathways (Moat et al., 2003). In addition, many

linear acids such as hexanoic acid and butanoic acid are considered as result of bacterial activities (Resconi et al., 2018). According to Reis et al., (2016), 3-methyl-butanal, 3-methyl-1-butanol and 2-methyl-1-butanol were detected from commercial vacuum-packed lamb legs stored at −1.5 °C from 7 to 13 weeks, and these compounds directly related to undesirable odours. 3-Methyl-butanal is an intermediate of catabolism pathway from branch chain amino acids that produces 3-methyl-1-butanol, 2-methyl-1-butanol, 2-methyl-propanol mainly by lactic acid bacteria (Fernández and Zúñiga, 2008). Acetic acid, ethanol, 3-hydroxy-2-butanone (acetoin), 2,3-butanediol, which are products from fermentation of glucose by lactic acid bacteria (Liu, 2003) were reported to be related to odour scores.

1.5.2. Effect of cooking on volatile profiles of lamb

Raw meat is cooked to achieve palatable and safe products. Various volatiles can be formed during cooking from non-volatile precursors as well as from the release of volatiles already exist with the meat tissue (usually the fat tissue; Mottram, 1998). According to Domínguez et al., (2014), thermal treatments (microwaved, fried, grilled, roasted) increased the total volatile compounds in head space of cooked steaks (ranging from 563 to 949 X 10^6area units /g dry matter) compared to raw foal steaks (459 X 10^6 area units/g dry matter). The most abundant volatile compounds in raw steaks were esters, which decreased during all types of thermal treatments (Domínguez et al., 2014). Whereas aldehydes, especially hexanal increased significantly during cooking and becomes the main compound family in cooked samples (Domínguez et al., 2014). Similarly, the abundance of aldehydes and several other volatile compounds increased when the lamb *longissimus dorsi* muscle cooked to an internal temperature of 70°C (Gravador et al., 2015). The types of thermal treatments also had significant effects on volatile profiles of cooked lamb, for example, pyrazines are only isolated

in the headspace of fired foal samples by solid-phase microextraction, which are considered as Maillard reaction products when cooking temperature was above 110°C (Domínguez et al., 2014). Alkyl-pyrazines is formed from the condensation of two alpha-aminoketone molecules produced in the Strecker degradation of amino acids, which represented from 0.10% to 1.27% of total volatile compounds in fried meat (Ramírez et al., 2004).

1.5.3. Methods and factors influence volatile identification techniques

Many methods have been developed to isolate and identify the volatiles in foods, such as headspace-solid-phase microextraction (HS-SPME). Static HS-SPME is an inexpensive, fully automatic, rapid, efficient volatile identification method (Vasta, et al., 2012), however, one single extraction method is hard to produce a complete flavour profile of meat. This is because almost all common extraction techniques applied in meat studies have advantages and disadvantages, which could contribute to the differences observed between studies.

Moreover, with sampling, absorption fibre and equilibrium condition could also influence the result of volatile studies. Firstly, with sampling, the marbling content differences between meat samples might create concentration variations of volatile compounds. This is because volatile compounds are more concentrated in adipose tissue than in lean muscle and can be released into the headspace more easily (Sutherland and Ames, 1995). Branched chain volatile fatty acids including 4-methyloctanoic, 4-ethyloctanoic, and 4-methylnonanoic that are known to be responsible for mutton flavour are often below the detection threshold of HS-SPME method in lean meat (Brennand and Lindsay, 1992). Secondly, selection of headspace absorption fibre and equilibrium condition may determine the number and type of compounds identified (Reis et al., 2016). Thin fibre coatings ensure a fast diffusion and release of semi-volatile compounds, while thicker fibre coatings retain highly volatile compounds better.

According to Reis et al., (2016), the CAR/PDMS fibre captures larger number of ketone and aldehyde compounds in the headspace of vacuum-packed raw lamb meat than CAR/PDMS/DVB fibre. In contrast, the CAR/PDMS/DVB fibre have better performance for long chain fatty acids and some alcohols than CAR/PDMS fibre. Polar compounds (e.g., carboxylic acids, alcohols, lactones) are less likely to be retained in a trap when the humidity is high in the headspace of sample (Canac-Arteaga et al., 2000), indicating that meat samples with high water content may result in a mistakenly low volatiles abundance.

1.5.4. The effect of production factors on volatile compounds of lamb meat

Most of the meat volatile compounds are considered to be of "metabolic origin" (Coppa et al., 2011) which can be found in all types of sheep meat. Nevertheless, on farm factors including animal age and the type of feed offered to the animal could influence the level of volatiles (both raw and cooked) and flavour of meat.

There is a general consensus that as sheep becomes older, the meat becomes more strongly flavoured (Watkins et al., 2014) and the consumer scores decline (Hopkins et al., 2006). At an early age, however, Gkarane et al., (2018) reported that no consistent age effect on the volatiles of meat between lambs slaughtered at age from 4 months to 12 months. Changes in animal metabolism and changes in muscle composition are likely to be involved with meat flavour differences in animals at different ages. As sheep age the fatty acid composition of fat depots changes and generally the fats become more saturated. It has also been suggested that the content of volatile branched-chain fatty acids in adipose tissue as well as total intramuscular fat content increase with age (Young et al., 1997). The odour of livestock was clearly linked to 3-methylindole, a rumen breakdown product of tryptophan. This compound was also

responsible for rancid odour, rather than hexanal and its analogues according to Young et al., (1997).

In addition, plant-derived compounds such as alpha- and beta-pinene could be transferred into the meat, which accumulate within the muscle tissue as animal age (Chevance and Farmer, 1999; Priolo et al., 2004). o-Xylene, which has been considered as an environmental pollutant and been found at greater amounts in the meat of grass-fed animals compared to indoor concentrate-fed ones (Vasta et al., 2012), is also likely to increase with the animal age.

Diet appears to have strong influence in lamb meat flavour in both direct and indirect effects (Duckett and Kuber, 2016). Direct effects of diets result in accumulation of specific components in meat. Terpenes such as alfa pinene and the p-cymene were higher in meat of pasture feed lambs because the terpenes are synthesized exclusively by plants, and they have been shown to be transferred from green herbage to animal tissue (Vasta et al., 2012; Vasta and Priolo, 2006). Perennial ryegrass/white clover pasture diets are relatively rich in soluble protein and rumen microbes breakdown approximately 70% of that protein to yield amino acids (Waghorn and Barry, 1987). An excess of amino acids in the rumen when animals consume pasture are broken down to products that contribute to "pasture" flavour (Schreurs et al., 2007).

Indirect effects of diets can be expressed via effects on fatty acid profiles of meat. Elmore et al., (2005) reported that dietary supplements (linseed oil, fish oil, protected lipid supplement, and marine algae) could alter fatty acid and volatile profile of cooked lamb loin meat with intense feeding (live weight gain from 29kg to 40kg). In the 111 identified volatile compounds, 78 were significantly affected by dietary treatment and almost all these compounds were formed from lipid oxidation instead of the Maillard reaction (Elmore et al., 2005). Volatile compounds derived from n-3 fatty acids were highest in the meat from the lambs fed the fish

oil/algae diet (Elmore et al., 2005). The amount of supplements/diet change and period of feeding is important for whether effect of diets can be found. This is because most of the odour active volatile compounds are lipophilic, their accumulation in animal muscle is also linked to the level of intramuscular fat deposition (Vasta et al., 2012).

Studies looking at the flavour differences between sexes have been inconsistent and it is probable that these results are often confounded by factors such as weight or fat content. From 63 volatile compounds determined in grilled lamb loin, 29 compounds were affected by gender (Mottram, 1998). Rams had higher relative abundance of lipid oxidation products, which, in many cases, leads to deterioration in the quality of meat and unacceptable flavour changes for consumers (Mottram, 1998). A recent study suggest that undesirable flavour in rams may be related to differences in hormone-dependent fatty acid metabolism (Gkarane et al., 2018a). Meanwhile, castrates had higher relative abundance in pyrazines and benzenoid compounds, which associated with a roast meat aroma, may be due to differences in amino acid composition that promote their formation (Gkarane et al., 2018a). Young et al., (2003) indicated that castration did not affect the level of branch chain fatty acids concentration and meat flavour at young age. Boar taint is a common complaint from the consumption of pork result from the production of a pheromonal testicular steroid called androstenone. Comparable meat flavour problems have not been found in rams (Purchas and Aungsupakorn, 1993).

Sheep meat flavour in the lean was stronger in Coopworth than Merino, and foreign flavour was stronger in Merino than Coopworth, however, there was confounding effects of pH(Young et al., 1993). Many common volatile compounds are reported in grilled sheep meat from various breeds (Frank et al., 2017) including Coopworth composite and first cross Border Leicester Merino. When the age and level of fatness are constant, there are no flavour

differences among breeds (Frank et al., 2017). In contrast, Elmore et al., (2000) found that aroma composition was affected by breed with the concentration of 54 compounds identified as being significantly different in meat samples obtained from Suffolk compared to Soay breeds. An early study indicated some fine-wool breeds of sheep produce meat with more intense mutton flavour than coarse wool breeds (Cramer et al., 1970).

1.6. Protein profile of lamb meat

1.6.1. Proteins in lamb skeletal muscle

Meat commonly refers to skeletal muscle that has undergone a series of complicated biochemical and structural changes during its post-mortem storage to reach a state more acceptable to consumers. Protein is a key component in meat and contributes significantly to its nutritional and sensory qualities. Meat (skeletal muscle) proteins consists approximately 20% to total weight and can be generally classified into three main groups based on their solubility (Table 1.4). The protein from lamb is highly digestible, around 94% compared with the protein digestibility of 78% in beans and 86% in whole wheat (Bhutta, 1999). Protein digestibility corrected amino acid score is a method of evaluating the protein quality, which has a maximum score of 1.0. Lamb meat have a score of approximately 0.9, compared with values of 0.5–0.7 for most plant foods (Schaafsma, 2000).

Table 1.4 The three classes of skeletal muscle proteins based on their solubility and location. The information in this table summarised from Goll, et al., (2007) and Xiong, (2017)

Classes	Location	Abundance in meat by weight	Solubility

Sarcoplasmic	Cytoplasm	~30 to 35% of total protein	Soluble at low (<0.05 M) ionic strength
Myofibrillar	Myofibril	~55 to 60% of total protein	Soluble at relatively high (>0.2 M) ionic strength
Stromal	Principally extracellular, including collagen, reticulin and elastin; some membrane proteins that are detergent-soluble	~10 to 15% of total protein	Insoluble in neutral aqueous solvents

Skeletal muscle consists of approximately 90% muscle fibres and 10% of connective and fat tissues (Listrat et al., 2016). Within the fibres, the myofibrils occupy nearly the entire intracellular volume (75–90%) and the myofibers size (diameter ranges from 10 to 100μm) increases with lamb age which is an important parameter of muscle growth (Lefaucheur, 2010; Listrat et al., 2016). Connective tissue is the protein structure which holds muscles together (Listrat et al., 2016). It is found in two places: between individual muscle fibres and holding bundles of fibres together or between whole muscles anchoring muscles to bone.

The sarcoplasm is the cytoplasm of a myocyte. Sarcoplasmic proteins in meat contain a widely divergent group of proteins that control different cellular functions including muscle contraction, metabolism, heat stress, oxidation, proteolysis, and apoptosis (Huang et al., 2020). According to an in-depth characterisation study by Yu et al., (2015), the 207 proteins found in the sarcoplasmic fraction of raw lamb *longissimus lumborum* muscle were dominated by glycolytic enzymes and mitochondrial proteins.

Certain muscle sarcoplasmic proteins, such as glyceraldehyde-3-phosphate dehydrogenase can be denatured on account of post-mortem pH decline (Warriss, 2010). The alteration of the sarcoplasmic fraction is not directly involved in the muscle tenderness, because the sarcoplasmic proteins have non-structural functions and they are soluble in situ (Ohlendieck, 2010). However, cathepsins and calpains are two important sarcoplasmic protein

that involved in tenderization process during aging. Cathepsins that occur in the lysosomes have maximum activity in mildly acid conditions. While calpains are activated by calcium ions and have maximum activity in neutral to alkaline conditions. Cathepsins and calpains could degrade troponin T, some collagen cross-links and mucopolysaccharides of the connective tissue ground substance. They only appear to degrade actin and myosin below a pH of 5, so this is unlikely to occur under normal conditions in meat (Warriss, 2010).

Joo et al. (1999) reported that the denaturation of sarcoplasmic proteins has an impact on colour and water holding capacity of porcine *longissimus* muscle. Light pork colour was associated with precipitation of sarcoplasmic proteins onto the myofibrillar proteins and that the major denatured sarcoplasmic proteins are phosphorylase, creatine kinase, triosephosphate isomerase and myokinase (Joo et al., 1999).

The myofibrillar proteins make up the structures responsible for muscle contraction and therefore directly related to tenderness of meat. During contraction, actin and myosin filaments slide together to form a more complex protein known as actomyosin. Myofibrillar proteins can be classified as three subgroups based on their physiological and structural roles (Xiong, 2017):

i. The major contractile proteins, including myosin and actin. Myosin (223kDa) is the major structural proteins accounting for approximate 45% of the total myofibrillar proteins in muscle (Lametsch et al., 2003). Myosin is a hexamer and is best known for its roles in muscle contraction and in a wide range of other motility processes. The mass of myosin ranged from 470kDa to 510KDa and can be further hydrolysed to myosin heavy chain and myosin light chain. Actin has the mass of roughly 42 kDa and participates in many important cellular processes, including muscle contraction, cell motility, cell division and cytokinesis.

ii. Regulatory proteins that are involved in the initiation and control of contraction like tropomyosin. Tropomyosin is a two-stranded alpha-helical coiled coil protein present in muscle cells with a molecular weight around 34kDa to 38kDa.

iii. The primary function of cytoskeletal proteins is to give the cell its shape and resistance to deformation. This subgroup includes titin (Linke and Krüger, 2010) and nebulin (Ottenheijm and Granzier, 2010).

The stromal fraction contains mainly the connective tissue proteins, such as collagens, elastin and reticulin, which are part of the supporting framework for muscle cells (endomysium) and that for muscle bundles (perimysium). Collagen is specialized protein which serves a variety of functions. The primary functions of collagen are to provide strength and support and to help form an impervious membrane. In meat, collagen which does not broken down easily in cooking is one of major factors influencing the tenderness of the meat. Collagen is colourless, thin and transparent. Microscopically, it appears in a coiled formation which softens and contracts to a short, thick mass when it is heated, and helping give cooked meat a plump appearance. Collagen itself is tough, insoluble in water and would shrinks when heated, however, heating to the appropriate temperature eventually converts collagen to gelatin which is viscoelastic rather than rigid.. Elastin is also insoluble in water, but unlike collagen and reticulin, it cannot be hydrolysed after heating, hence its nutrition value is lower. Elastin is found in the walls of the circulatory system as well as in connective tissues throughout the animal body and provide elasticity to those tissues. Muscles from young animals contains relatively little elastin. Reticulin is present in much smaller amounts than either collagen or elastin. It is speculated that reticulin may be a precursor to either collagen and/or elastin as it is more prevalent in younger animals.

1.6.2. Quantification of proteins in meat

There are two major mass-spectrometry-based quantification methods (Bantscheff and Schirle, 2007). The first type is bottom-up approaches, the extracted, purified proteins are subjected to proteolytic cleavage (enzymatically or non-enzymatically), and the peptide products are analysed by mass spectrometry; The second type is top-down approaches, intact protein ions or large protein fragments are subjected to gas-phase fragmentation for mass spectrometry analysis directly.

Label-free quantification is a bottom-up method that aims to determine the relative amount of proteins between two or more biological samples. Over the years, protein digestion techniques have been improved using novel approaches for better protein identification and reproducibility of results (Ferreira et al., 2017). Commonly applied methods for protein digestion involve the use of enzymes. Trypsin is the most widely applied enzyme because it is highly specific, only cleaving the peptide bonds C-terminal to the basic residues lysine and arginine, except when followed by proline (Bantscheff and Schirle, 2007). Protein solubility is a pre-requisite as certain classes of proteins from meat are very difficult to solubilise and digest, which complicates their analysis with bottom-up proteomics approach. The solubilisation of such proteins can be improved using detergents, and most detergents (such as sodium deoxycholate) are incompatible with mass spectrometry analysis and must be removed before LC-MS/MS analysis (Ong and Mann, 2013). Unlike quantitative method, label-free quantification does not use a stable isotope containing compound to chemically bind to and label the protein. Therefore, analysis error can occur during protein purification stage, protein digestion or mass spectrometry data analysis (Bantscheff and Schirle, 2007; Ong and Mann, 2013). Label-free approaches are less accurate compared to labelled techniques when

considering the overall experimental process because all the systematic and non-systematic variations between experiments are reflected in the obtained data. However, label-free protein quantification is worth considering for several reasons concluded by Bantscheff and Schirle: (2007).

- Time consuming and expensive steps of introducing a stable isotope label into proteins or peptides can be omitted.
- No principal limit to the number of treatments that can be compared, while stable isotope labelling techniques that are typically limited to 2–8 treatments that can be directly compared.
- Mass spectral complexity (in terms of detected peptide species within a particular chromatographic time window) is not increased which might provide more analytical depth (i.e., number of detected peptides/ proteins in an experiment) because the mass spectrometer is not occupied with fragmenting all forms of the labelled peptide.
- Provide higher dynamic range of quantification than stable isotope labelling and therefore may be advantageous when large and global protein changes between treatments are observed.

1.6.3. Relationships between protein profile and tenderness, water-holding capacity, and meat colour

The tenderness of aged meat is determined by the rate and extent of proteolysis of the key myofibrillar proteins in the post-mortem muscle. Both regulatory and cytoskeletal proteins of myofibrillar protein have found to be degraded during post mortem tenderisation include actin, myosin, titin, nebulin, desmin, vinculin, dystrophin and troponin-T (Pearce et al., 2011). Some commonly reported meat tenderness biomarkers are:

1. Metabolic enzymes e.g.., glyceraldehyde-3-phosphate dehydrogenase (GAPDH), triosephosphate isomerase (TPI).

2. Cell death and signalling proteins e.g.., four and a half LIM domains 1(FHL1) and tripartite motif protein 72 (TRIM72).

3. proteolysis enzymes e.g.., μ-calpain (CAPN1).

4. oxidative proteins e.g.., protein deglycase DJ-1 (PARK7).

5. structural proteins e.g.., α-actin (ACTA1), titin, myosin heavy chain-I, myosin light chain 3 and myosin light chain 1 (Huang et al., 2020).

The mechanism that each of these biomarkers affected tenderness is different. GAPHD is a metabolic enzyme about 37kDa that catalyses the sixth step of glycolysis. Previous studies reported that GAPDH levels are negatively correlated with shear force, and positively correlated with the sarcomere length (Sierra et al., 2012). It was reported that FHL1 is positively correlated with the meat tenderness due to its role in regulating gene transcription, cell proliferation, metabolism and apoptosis (Gagaoua et al., 2018a). FHL1 is linked to the release of intact α-actinin from bovine myofibrils and contributes to the weakening of the Z-line during meat tenderizing (Gagaoua et al., 2021a). μ-Calpain was found to interact with several proteins belonging to numerous biological pathways in post-mortem, such as Ca2+ homeostasis, protein structure, glucose metabolism, heat stress, mitochondria and apoptosis (Huang et al., 2020). Guillemin et al., (2011) showed positively correlation between μ-calpain and tenderness of bovine semitendinosus muscle. It was hypothesized that μ-calpain catalyses the proteolysis of myofibril and increased the release of myosin light chain 1 into the sarcoplasmic fraction (Anderson et al., 2012). The PARK7 levels were positively correlated with meat tenderness by altering the degradation of myofibrillar proteins by Gagaoua et al., (2015), but were negatively contributes to the meat tenderness as a quencher for reactive oxygen species (Guillemin et al., 2011). This implicated the limited accuracy and reliability

for scientific research and industrial applications. There are many potential protein biomarkers related to meat quality, but these protein biomarkers are affected by breed, age, sex, feeding patterns, muscle type, and other factors. Therefore, protein biomarkers cannot yet be used accurately to consistently evaluate and predict meat quality.

Water holding capacity is defined as the ability of fresh meat to retain moisture that affected by many factors such as pH, hydrolysis and oxidation of cytoskeletal proteins, and the permeability of the cell membrane (Zhang et al., 2019). Many meat tenderness biomarker such as GAPDH, TPI1, PARK7 and ACTA1 can simultaneously monitor tenderness and water holding capacity of meat (Huang et al., 2020). The abundance of metabolic enzymes GAPDH and ATP synthase subunit were higher in the meat from high drip goose group than low drip goose group (Zhang et al., 2019). The formation of drip is influenced by structural changes such as the oxidation and proteolytic degradation of structural proteins and shrinkage of myofibrils during rigor development (Huff-Lonergan and Lonergan, 2007). The structural proteins myosin regulatory light chain 2 was reported to be more abundant in high drip groups than low drip groups (Zhang et al., 2019).

Heat shock cognate 70 was reported to be more abundant in low-drip group pork meat compared to high-drip pork (Luca et al., 2013). Heat shock proteins are important contributor to the cellular defence mechanism against oxidative stress, and they could prevent cell and tissue damage caused by reactive oxygen species (Guillemin et al., 2011). Besides, heat shock proteins also have the ability to bind with cell membranes and maintain the integrity and stability of membranes (Zhang et al., 2019).

Before slaughter, myoglobin and hemoglobin are the main pigment proteins, but after slaughter, most of the hemoglobin is lost along with blood, and the myoglobin within muscle

is the key substance that determines meat colour (Suman and Joseph, 2013). Wu et al., (2015) reported that the redness of beef M. semitendinosus decreased gradually during 15 days of post-mortem storage. This colour changing was highly correlated to some metabolism proteins (fructose-bisphosphate aldolase A isoform, triosephosphate isomerase, L-lactate dehydrogenase A chain isoform, pyruvate kinase isozymes M1/M2 isoform), and antioxidant proteins (peroxiredoxin-6) in sarcoplasmic. These proteins are several representatives of the glycolytic metabolism and the oxidative metabolic pathways. For example, fructose bisphosphate aldolase can catalyse the reversible aldol cleavage reaction between glyceraldehyde 3-phosphate and dihydroxyacetone phosphate during glycolysis. The up-regulated expression of this enzyme during storage implies the conversion toward an anaerobic metabolism due to depleted phosphocreatine in meat, and the various substrates from anaerobic metabolism can be further converted into lactic acid, which indirectly affects the meat colour (Mancini and Hunt, 2005; Wu et al., 2015).

1.7. Consumer requirements for sensory properties of meat

Consistent and high-quality meat production that satisfies consumer expectations is of most importance to the meat industry to maintain and expand markets. The main sensory attributes of meat from a consumer perspective, are tenderness, juiciness and flavour liking which are influenced by several pre- and post-harvest variables (Pannier et al., 2018). Although consumers consider a range of attributes in their meat purchasing decisions, with factors such as animal welfare, sustainability and authenticity are becoming increasingly important, eating quality is still a major determinant of satisfaction, re-purchase and willingness to pay for lamb (Realini et al., 2021).

1.7.1. Tenderness of meat

Low tenderness will lead to a decline in overall liking score but the threshold of tenderness satisfaction is a topic of debat. Based on sensory tests with Australian consumers, Hopkins et al. (2006) imply that for sheep meat to be classified as good every day quality must have a shear force value less than 40 N in a dimensionally defined cooked meat sample. As for beef, similar findings were reported that consumers considered meat to be 100% acceptable for tenderness when the shear force was about 31 N (Miller et al., 1998).

1.7.2. Flavour of lamb meat

Flavour is an important aspect for the overall acceptability of meat products, which is frequently considered as the most important palatability characteristic of cooked sheep meat (Hopkins et al., 2005; Phelps et al., 2018; Thompson et al., 2005; Pavan et al., 2021). Although the perception of flavour has been defined as integrated sensations of smell, taste and touch (Noble, 1996), the cooked meat flavour is mainly influenced by volatile compounds that reach the receptors in the nasal epithelium through the nose or through the posterior nares at the back of the nose when food is chewed (Farmer, 1994; Mottram, 1998).

Just as significant industry efforts have been dedicated to improve meat tenderness, there are studies that report that flavour is becoming a more important driver of overall palatability (O'Quinn et al., 2018). A shift from traditional commodity to value-based marketing requires optimization of production and processing to guarantee products with favourable meat flavour.

Over 1000 volatile compounds have been associated with meat flavour (Mottram, 1998), however, whether a compound has an impact on flavour or odour depend on its concentration and detection threshold (Farmer, 1994). However, only a few compounds actually contribute to the baseline aroma of lamb meat according to Watkins et al., (2013) and these compounds

(listed in decreasing rank) are: 4-ethyloctanoic acid (mutton-like), 1-octen-3- one (mushroom, earth), (E,E)-2,4-decadienal (fatty, fried), (Z)-2-nonenal (plastic, chlorine), 2-acetyl-1-pyrroline (popcorn, roasted), furaneol (caramel), (E)-2-heptenal (fish, fried), methional (cooked vegetables, potato), 2,3-diethyl-5-methylpyr-azine (nutty, roasted), dimethyl trisulfide (sulphur), (E)-2-nonenal (cardboard, wood), decanal/2,4-(E,E)-heptadienal (roast meat, potato), 4-methylphenol (stable, animal), octanal (lemon, floral), and (E)-2-octenal (grass). The overall meat flavour is a balance of various compounds (Basant et al., 1975). Some volatile compounds have a significant effect on flavour may present at very low concentrations. For example, 4-ethyloctanoic acid makes the major contribution of the characteristic sheepy flavour has very low flavour threshold (0.00043–0.0018 μg/g) compared to 4-methyloctanoic acid (0.02–0.6 μg/g) (Brennand et al., 1989; Teng et al., 2018).

Lastly, detection thresholds vary from person to person resulting in different sensitiveness to particular flavour compounds (Prescott et al., 2001). The taste detection thresholds increased with age in general across all taste modalities via a systematic review (Methven et al., 2012). The 'taster' group could be divided into 'supertaster', 'medium tasters' and 'non-tasters' depending on the perceived intensity of propylthiouracil or perception of sweetness and bitterness, making up 20%, 50% and 30% of the population, respectively (Bartoshuk et al., 1992; Feeney et al., 2011).

In addition, meat flavour is also affected by consumers' previous experience and cultural background (Font-i-Furnols and Guerrero, 2014). Country of origin of consumers has been reported to exert a significant effect on their preferences for lamb-meat, with consumers preferring forage- or concentrate-fed lamb depending on their nationality (Sañudo et al., 2007). Consumers from Japan are reported to be sensitive to "mutton" flavour caused by branched-

chain fatty acids which may be a barrier to acceptance of sheep meat (Prescott et al., 2001; Watkins et al., 2010). A study from Australia (Frank et al., 2016a) investigated eating quality of lambs from low and high intramuscular fat sires allocated to one of four finishing feeds: perennial ryegrass (*Lolium perenne*), lucerne (*alfalfa; Medicago sativa*), and two brassica (*Brassica napus*) forages. Unlike previous report, Frank et al. (2016) suggested that up to a certain threshold concentration, the total BCFAs play a positive role in defining lamb flavour and acceptance, and Chinese background consumers were not inherently more sensitive to the presence of branch chain fatty acids than Australian consumers.

1.7.3. Juiciness of meat

Many studies have reported that consumers prefer juicier meat in pork, beef and lamb (Font-i-Furnols and Guerrero, 2014). Raw meat contains about 75% of water, and the juiciness of meat depends on both raw meat quality and on the cooking procedure. Lower cooking loss was reported from grilled meat (19.1%) compared to fired, roasted, or microwaved (29.9%, Domínguez et al., 2014). Protein denaturation during cooking is the most probable reason for water loss during cooking, which causes less water to be entrapped within the protein structures held by capillary forces (Aaslyng et al., 2003).

1.8. Summary of literature

- The meat industry is a major export industry for New Zealand with approximately 95% of sheep meat been exported. New Zealand exported 386,250 tonnes of lamb and mutton (equivalent to an export value of $3.54 billion) in 2019/20 season, and 54% of New Zealand's total sheep meat exports by volume during the year was exported to China (Meat Industry Association, 2020).

- Many studies have been done to understand individual effect of on lamb age, breed, sex, and diet on various meat quality characteristics. However, these production factors often varied between different regions, and therefore both strong, weak, or no effect of lamb age, breed, sex, and diet on meat quality has been reported. Clear correlation between these on farm factors and physicochemical properties of meat products has not been fully understood.
- There is a raising health concern about red meat for its high saturated fatty acids, and meat with high proportions of long chain unsaturated fatty acids have an chance to be considered as a 'source' or 'good source' of EPA + DHA (Food Standards Australia New Zealand, 2012). Nevertheless, a consumer will not compromise meat eating quality for healthiness attributes (Verbeke, 2006).
- Protein is a key component in meat and contributes significantly to its nutritional and sensory qualities. The myofibrillar and stromal make up the structures responsible for muscle contraction, or are part of the supporting framework for muscle cells, and therefore directly related to tenderness of meat. While sarcoplasmic proteins involved different cellular functions including muscle contraction, metabolism, heat stress, oxidation, proteolysis (Huang et al., 2020) and indirectly related to many meat quality characteristics.
- Eating quality of cooked meat is determined by the combined acceptance of flavour, tenderness and juiciness (Miller, 2020). Flavour was considered as the most important attribute driving consumer liking for lamb, especially when acceptable tenderness (<40N) was reached (Hopkins et al., 2006; Miller, 2020).
- The effect of production systems on the meat quality, fatty acid, volatile and protein profile of lamb meat has not been considered in-depth for commercial lambs in a forage-based

production system. Therefore, studies were conducted in this thesis to look at production system effects of diet, sex, and age at slaughter.

Chapter 2

Carcass characteristics and meat quality of commercial lambs reared on different forage systems

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Abstract

The objective of this study was to evaluate the effect of production factors including age, forage types, sex or castration on carcass and meat quality traits in New Zealand. The carcass attributes and meat quality characteristics of the *Longissimus thoracis* were evaluated for 150 lambs from 10 commercial forage production systems (n=15) on 3 commercial Farms A, B and C. Lambs that were processed at weaning (at 4-month-old) had heavier carcasses, and their meat had greater intramuscular fat and shear force than lambs slaughtered after further finishing on pasture or chicory ($P<0.05$). Chicory-fed lambs had heavier carcass weights and a greater intramuscular fat content in their meat compared to pasture-fed lambs ($P<0.05$), but similar eye muscle area and fat depth over the loin ($P>0.05$). The effects of the sex of the lamb, castration status, forage diet and age relative to weaning on meat quality was subtle.

2.1. Introduction

Producing lamb meat with desirable eating quality is a key goal of the New Zealand lamb industry. Meat quality includes many factors including water-holding capacity, colour, and nutritional value (Hopkins and Mortimer, 2014). The quality of meat is important for consumer satisfaction and to entice repurchase (Jiang et al., 2015). Globally, lamb production occurs under different production and management systems. Factors such as diet (De Brito et al., 2016), sex (Johnson et al., 2005), age (McPhee et al., 2008; Mashele et al., 2017) and breed (Hopkins and Mortimer, 2014) of the lamb have been associated with differences in carcass and meat quality. The impact of animal age on meat quality traits is of particular importance because it can help to make marketing decisions, but determining age effects is not straight forward as it can be confounded by many other factors (Purchas, 2007). Older animals are often heavier and have more subcutaneous and intermuscular fat than the younger animals which can slow cooling rates resulting in a decline in pH and subsequent traits such as tenderness and colour. The effect of the sex of the lamb or castration status of male lambs on ultimate pH or tenderness of their meat is not commonly observed (Schreurs, 2013; Kerslake et al., 2012). Hopkins et al. (2007), however, reported that meat from castrated male lambs was tougher than ewe lambs (Hopkins et al., 2007). Feeding plantain has been reported to increase lamb growth rates from birth to weaning compared with feeding perennial ryegrass (Campbell et al., (2011), due to differences in the ability of the lamb to deposit fat on the carcass and within the muscle which are key drivers of meat quality traits such as tenderness.

Around the world forage systems are used for the production of sheep meat (Bouwman et al., 2005). Herrero et al. (2013) estimated that grazed pasture comprised 48% (2.3 billion tons) of the overall biomass used by livestock. The New Zealand climate favours pasture growth

throughout the year and as a result grazed pastures and forage crops supply approximately 95% of the diet for sheep and beef production (Hodgson et al., 2005). Sheep and beef cattle production utilises 9.7 million ha (66%) of New Zealand's agriculture land (Ministry for Primary Industries, 2012) and played an important role in the New Zealand economy, contributing to 3.2% of New Zealand's Gross Domestic Product. In 2018, New Zealand exported over 90% of its total lamb produced, which value $2.5B to over 120 countries, making New Zealand the largest lamb exporter in the world (Beef and Lamb New Zealand, 2018). Understanding the impact of current New Zealand production systems on carcass and meat quality is required to ensure that meat with attributes that meet or exceed consumer expectations in varied and discerning markets is delivered.

Lamb production systems in New Zealand have traditionally relied on perennial ryegrasses and white clover pastures (Beef and Lamb New Zealand, 2018). There has, however, been an increase in the use of alternative forage types such as plantain, chicory, and red clover in order to offer a diet with a higher nutritive value than traditional pasture (Kim et al., 2013). The greater nutritive value of the alternative forages allows fast lamb liveweight gains (Somasiri et al., 2015). Kemp et al. (2010) calculated that more than 720 kg of lamb carcass per ha per year could be produced on plantain, red and white clover mixed pastures compared with 400 kg/ha on perennial ryegrass/white clover pastures.

Sheep production in New Zealand has traditionally been based on a maternal Romney breed or Romney derived breeds (Coopworth, Perendale) and fewer than 10% of sheep are Merino or Merino-based breeds primarily farmed to produce fine wool (Beef and Lamb New Zealand, 2018). A survey in 2012 identified that approximately 40% of the New Zealand sheep flock were of a composite breed whereby Finnish Landrace and Texel genetics have been

incorporated into Romney, Coopworth and Perendale flocks in order to increase the number of lambs born and carcass meat yield (Corner-Thomas et al., 2013). Increased growth rates have also been achieved through genetic selection within existing breeds and through improved feeding and management (ref needed). Between 1990 to 2017, although the number of sheep in New Zealand has decreased from 57 million to 27 million, the average carcass weight of lambs increased from 13.9 kg to 18.6 kg to maintain the production volume (Beef and Lamb New Zealand, 2018). There are a number of advantages in achieving faster lamb growth rates as it results in lambs achieving target slaughter weights at a younger age, which can result in superior market prices while minimizing maintenance feeding costs (Brito et al., 2017).

As a result of the gains in production efficiency of lamb growth that have been achieved there is a concern that fast growing, lean lambs may be associated with meat with a low intramuscular fat (IMF) content and negative eating quality attributes (Hopkins et al., 2006; Frank et al., 2017). It is generally considered that as an animal ages the meat becomes more strongly flavoured (Watkins et al., 2014) which could have both positive or negative influences on consumer sensory scores (ref needed). In addition, a higher concentration of collagen or less soluble collagen has been associated with tougher meat as tenderness declines as animals age and total collagen increases (Hopkins et al., 2007). Hopkins et al., (2007) reported a clear advantage in the ability of suckler lambs (4 months of age and still on their mothers) to withstand stress or reduce the preslaughter depletion of glycogen as seen in the semitendinosus muscle through pH measures than older weaned lambs. High growth rates, however, have also been associated with changes in muscle structure and metabolism that can have a negative effect on meat quality of lamb (Brito et al., 2017).

There are a broad range of commercial production systems utilized in New Zealand including different combinations of animal genetics, sex, diet and age at slaughter which provide the potential for variation in carcass and meat quality characteristics of lambs. The objective of this study was to evaluate the carcass and meat quality traits of lambs from 3 farms with diverse forage-based diets, sex, castration status and age at slaughter which are representative of the lambs commercially processed in New Zealand.

2.2. Materials and methods

2.2.1. Animals

Lambs were finished on one of three commercial sheep farms north of Invercargill, New Zealand which implemented different production approaches. Lambs from Farm A and C were born in August 2017, and lambs from Farm B were born in December 2016 (Table 2.1). Ten production systems representing forage fed lambs typically processed in New Zealand were selected. These included 6 to 8-month-old wether and ewe lambs of a composite breed that had been grazing red clover, 6 to 8-month-old ewe lambs of a composite breed that had been grazing perennial ryegrass, red clover and white clover mix pasture (Farm A); 12-month-old, wether and cryptochid Merino lambs that had been grazing a mixed pasture (perennial ryegrass and white clover mix followed by fescue, red and white clover and plantain mix in the previous 2 weeks; Farm B); 6 to 8-month-old wether and ewe lambs of a composite breed that had been grazing a predominantly Italian and perennial ryegrass, and red and white clover mix pasture; 6 to 8 month old wether and ewe lambs of a composite breed that had been grazing chicory; 4-month-old wether lambs of a composite breed at weaning that had been grazed mixed pasture (Farm C). Decisions regarding the animals that were sent for slaughter were made by the farmer based on criteria to obtain a target carcass weight of 17-21 kg. Fifteen lambs from each

production system were randomly selected and identified from the slaughter chain from a larger group of lambs that were sent for slaughter from each of the three farms.

Table 2.1 Production systems of animals (n=15) selected according to the pre-slaughter factors (age, sex, diet, breed) included in the study.

Production systems	Approximate age at slaughter (months)	Sex	Finishing diet[1]	Breed[2]	Farm	Distance to meat plant (km)
REDC-W	6-8	Wethers	Red Clover	Perendale × LambSupreme	A	80
REDC-E	6-8	Ewes	Red Clover	Perendale × LambSupreme	A	80
MIX-W	6-8	Wethers	Pasture	Perendale × Romney	A	80
MXME-W	12	Wethers	Pasture	Merino	B	250
MXME-C	12	Cryptochids	Pasture	Merino	B	250
WEAN-W	4	Wethers	Pre-weaning	Composite	C	100
GRASS-W	6-8	Wethers	Pasture	Composite	C	100
GRASS-E	6-8	Ewes	Pasture	Composite	C	100
CHIC-W	6-8	Wethers	Chicory	Composite	C	100
CHIC-E	6-8	Ewes	Chicory	Composite	C	100

[1] Animal diet: Pre-weaning, suckled and grazing mothers' diet of a chicory (Cichorium intybus) and red clover (Trifolium pratense) mix;
Farm A-Pasture, predominantly Italian and perennial ryegrass (Lolium perenne) and red and white (Trifolium repens) clover mix;
Farm B-Pasture, permanent pasture, perennial ryegrass, red clover and white clover mix;
Farm C-Pasture, ryegrass and white clover mix followed by fescue (Lolium arundinaceum), red and white clover and plantain (Plantago lanceolata) mix during the last 2 weeks.
[2] Composite: Perendale, Texel, Finnish Landrace and Romney genetics; LambSupreme: lean-selected Poll Dorset, Wiltshire, Romney x Dorset, Coopworth, Texel, and high-growth Romney.

2.2.2. Slaughter

Lambs were transported by a commercial trucking firm from the respective farms of origin which were a distance of 80-250 km from the Alliance Group Ltd, Lorneville plant near Invercargill (Table 2.1). Lambs were slaughtered within 24 h after leaving the farm following standard practice for commercial processing. Lambs had ad libitum access to water while in lairage. Lambs were electrically stunned (50Hz, 2 secs, 1A), exsanguinated, and dressed according to standard commercial procedures. Electrical stimulation (230 voltages for 50 seconds) was applied to the carcass of all lambs. The 12-month-old lambs from Farm B and the 4-month-old lambs from Farm C were slaughtered on the 7th December 2017 while the 6 to 8-month-old lambs from Farms A and C were slaughtered on the 1st March 2018. Carcasses were chilled at 0°C for 24 h before being boned out.

2.2.3. Carcass characteristics and sampling

Hot carcass weight and the VIAScan® estimated meat yield from the loin, leg, and shoulder as well as the VIAScan® estimated GR soft tissue depth (VSGR) were collected for each lamb. VIAScan® has been accredited by relevant authorities in New Zealand to predict fatness in commercial abattoirs using a broad classification based on GR (the depth of muscle and fat tissue from the surface of the carcass to the lateral surface of the twelfth rib, at a point 110 mm from the midline) ranges. VIAScan® uses an image of the dorsal view of each carcass obtained with a camera perpendicular to the long axis of carcass. Reference points were used to calculate the dimensions of the carcass (Hopkins et al., 2004) and meat yield of total carcass weight. The *Longissimus thoracis et lumborum* muscle (loin) was removed with the fat-cap from the left side of each carcass and a section (approximately 10 cm in length) was used for meat quality assessment.

All samples were then vacuum packed and chilled at -1.5°C for 21 days followed by storage at -20°C until further analysis.

Eye muscle area was measured on a transverse cut at the thoracic region of the loin by tracing the area and subsequently measuring the area using a planimeter (Placom KP-90N, Japan). At the same time as the eye muscle area was measured, the fat depth over the loin was measured using a ruler at a point where the muscle was deepest. Intramuscular fat (IMF) was measured using Soxhlet extraction method(AOAC 991.36). The equations for generating the response and conversion factors for calculating total fat content were obtained from American Oil Chemists' Society (6th edition, Ce 1f-96).

2.2.4. Meat quality

2.2.4.1. PH AND SARCOMERE LENGTH

The loin sections were thawed at 2°C for 24 h prior to objective meat quality analysis at the Massey University meat laboratory. The pH was measured on two occasions: firstly, 24 h post-mortem and then at the time of objective meat quality testing. Measurements were made in triplicate on an internal cut of the loin using a pH spear calibrated with 4.01, 7.00, and 10.01 pH standard buffers at 10°C (Eutech Instruments, Singapore). Sarcomere length was measured by laser diffraction (helium-neon, 632.8 nm, Melles Griot, Carlsbad, CA, USA) using muscle fibre samples held between glass microscope slides (Purchas and Barton, 1976).

2.2.4.2. WATER HOLDING CAPACITY

A 25 mm thick subsample cut from the loin was weighed, placed into a plastic bag and suspended in a water bath at 70°C to cook for 90 min (Contherm®, Model 370HL, Australia). After

cooking, the samples were cooled to room temperature and then chilled at 1°C for 4 h. The cooked sections were pat dried with tissue paper and weighed. Cooking loss was calculated as the percentage of weight lost from the 25 mm loin section after cooking. To further assess water-holding capacity, a 30 mm cube of raw lean meat with was cut from the loin. The 30 mm cube was weighed and then suspended on metal hook in a plastic bag free from contact with the bag at 1°C. The suspended cube was then blotted dry using tissue and reweighed at 24 and 48 h. Drip loss was calculated as percentage of weight loss after 24 or 48 h.

2.2.4.3. COLOUR

Lean meat colour was assessed at 24 h post-mortem at the meat plant directly after boning out (Colour Minolta CR-400, USA) and at the time of objective meat quality testing (Colour Minolta CR-200, USA). Lean meat colour was measured on a transverse surface of a fresh cut after 30 min of exposure to air. At both time points, the CIE L* (lightness), a* (redness) and b* (yellowness) values were measured (Illuminant D65, 8 mm aperture, 0° standard observer).

Fat colour was assessed after the subcutaneous fat was scraped with the edge of a knife to remove surface pigments, and using a Minolta CR-200 Colour Meter using CIELAB at the time of objective meat quality testing. For both the fat and lean meat colour, readings were taken at three points across the sample and these values were averaged to get a representative reading of colour. The colour saturation (Chroma (C*)) and the proportion of redness to yellowness (hue angle (H°)) were calculated as:

$$\text{Chroma}(C^*) = \sqrt{(a^*)^2 + (b^*)^2}$$

$$\text{Hue angle}(H°) = \text{arctangent}(\frac{b^*}{a^*})$$

2.2.4.4. SHEAR FORCE

From the 25 mm section of cooked loin, 4 to 6 cores of 13 mm × 13 mm were cut along the direction of the muscle fibres. Shear force measurements were performed by shearing perpendicular to the muscle fibre direction (V-shaped blade, 20 mm/s TMS-Pilot Texture Analyzer, USA). The peak force required to shear through the meat sample was recorded by TL-Touch texture software (version 1.18-408) and expressed as shear force in Newtons.

2.2.5. Statistical analysis

Data were analysed using the GLIMMIX procedure of SAS (SAS® University Edition 3.8; SAS Institute Inc., Cary NC, USA). Sex (wether vs. ewe) and castration status (wether vs. cryptorchid) were included in the model as fixed effects for Farm A and B, respectively. Diet (red clover vs pasture) was included in the model as fixed effect for Farm A. For Farm C, pre-planned non-orthogonal contrasts were used to compare means from 6 to8 month old lambs fed pasture vs. chicory (diet) and wether vs. ewe (sex) and their interaction. In addition, animal age at slaughter for wether lambs (4 vs. 6- to 8-month-old) was contrasted. Hot carcass weight was included in the model as a covariate for other carcass variables. Muscle pH-24 h was included in the model as a covariate for the estimated lean meat colour at 24 h post-mortem, and muscle pH-after aging was included in the model as a covariate for the estimated meat quality measurements determined post-aging. Differences among least square means were considered significant at a probability level of <0.05.

2.3. Results

2.3.1. Carcass characteristics

There were no differences (P>0.05, Table 2.2) in any of the carcass parameters measured between wether and ewe lambs grazed on Farm A. The IMF percentage in the loin of lambs fed pasture was greater than those fed red clover (P<0.05, Table 2.2), and the shoulder yield of carcass was greater from lambs fed red clover than pasture (P<0.05, Table 2.2) in Farm A. On Farm B eye muscle area and the IMF percentage in the loins of Merino wether lambs was greater than Merino cryptorchid lambs (P<0.05), while there were no differences (P>0.05) were observed in the other carcass parameters.

On Farm C, wether lambs had a heavier hot carcass, greater shoulder lean meat yield and greater loin IMF percentages compared to ewe lambs (P<0.05, Table 2.3). Lambs grazing on chicory had heavier carcasses, greater shoulder yields and a greater IMF percentage in the loin muscle compared to lambs grazing the pasture diet (P<0.05). The influence that the diet had on the meat, loin and leg yields was dependent on the sex of the lamb. Ewe lambs on chicory had a greater meat and loin yield than ewes on pasture and greater leg yields than wether lambs on chicory (P<0.05). Lambs that were slaughtered at weaning had heavier carcasses, greater estimated VSGR and greater IMF percentages compared to lambs that had a finishing period grazing on forages after weaning (P<0.05).

Table 2.2 Effect of sex (wethers vs. ewes), castration condition (wethers vs. cryptorchids) and diet (red clover wethers vs pasture wethers) on carcass characteristics of lambs from Farms A and B, respectively.

	Farm A					Farm B		
Diet	Red clover	Red clover	Pasture	P-value		Pasture		P-value
Sex/Castration	Wether	Ewe	Wether	Sex	Diet	Wether	Cryptorchid	Castration
Hot carcass weight (kg)	17.9 ± 0.2	17.7 ± 0.2	18.7 ± 0.3	0.541	0.012	19.0 ± 0.2	19.5 ± 0.3	0.191
Meat yield (% of carcass)[1]	52.1 ± 0.6	53.0 ± 0.6	51.4 ± 0.4	0.328*	0.188	53.6 ± 0.7	52.8 ± 0.6	0.371
Loin yield (% of carcass)[1]	14.0 ± 0.2	14.4 ± 0.2	14.1 ± 0.1	0.113*	0.855	14.6 ± 0.2	14.4 ± 0.2	0.409
Leg yield (% of carcass)[1]	22.2 ± 0.4	22.5 ± 0.3	22.2 ± 0.1	0.531	0.920	21.7 ± 0.3	21.8 ± 0.3	0.143
Shoulder yield (% of carcass)[1]	15.9 ± 0.2	16.1 ± 0.2	15.1 ± 0.2	0.577*	0.002*	16.5 ± 0.2	16.6 ± 0.3	0.881
VSGR (mm)[1]	8.0 ± 0.6	9.0 ± 0.4	8.9 ± 0.5	0.163	0.541	3.0 ± 0.5	3.0 ± 0.5	0.896
Fat depth (mm)	3.4 ± 0.7	3.7 ± 0.5	4.7 ± 0.6	0.751	0.358	4.4 ± 0.6	4.2 ± 0.7	0.782
Intramuscular fat (% of loin)	2.2 ± 0.1	2.8 ± 0.2	3.0 ± 0.2	0.100	<0.001	4.4 ± 0.2	3.2 ± 0.2	0.025
Eye muscle area (cm^2)	11.1 ± 0.8	10.7 ± 0.6	9.7 ± 0.4	0.709	0.197	10.3 ± 0.3	9.1 ± 0.5	0.025

[1] Meat, loin, leg and shoulder yields and VSGR were estimated by VIAScan®.
*Hot carcass weight covariate ($P<0.05$).

Table 2.3 Effect of diet, sex, and their interaction, and effect of age at slaughter (at weaning with 4-month-old vs. 6- to 8-month-old) on carcass characteristics of lambs from Farm C.

Diet:	Pasture		Chicory		Wean	Non-orthogonal contrast[2], P-value			
Sex:	Wether	Ewe	Wether	Ewe	Wether	Diet	Sex	Diet x Sex	Age
Hot carcass weight (kg)	17.1 ± 0.1	16.7 ± 0.1	18.1 ± 0.1	18.0 ± 0.1	18.8 ± 0.1	<0.001	0.044	0.275	<0.001
Meat yield (% of carcass)[1]	52.9 ± 0.6	51.5 ± 0.7	53.1 ± 0.5	54.2 ± 0.5	52.6 ± 0.7	0.055	0.740	0.016	0.606
Loin yield (% of carcass)[1]	14.3 ± 0.2	13.8 ± 0.2	14.7 ± 0.2	15.0 ± 0.2	14.3 ± 0.2	0.002	0.598	0.011	0.414
Leg yield (% of carcass)[1]	22.2 ± 0.2	21.9 ± 0.2	21.5 ± 0.2	22.4 ± 0.2	21.5 ± 0.2	0.749	0.199	0.009	0.267
Shoulder yield (% of carcass)[1]	16.4 ± 0.2	15.7± 0.3	17.0 ± 0.2	16.7 ± 0.2	16.9 ± 0.3	0.006	0.043	0.384	0.463
VSGR (mm)[1]	7.2 ± 0.5	8.1 ± 0.6	7.8 ± 0.5	7.8 ± 0.5	8.9 ± 0.6	0.781	0.330	0.288	0.054*
Fat depth (mm)	3.6 ± 0.6	5.0 ± 0.7	4.5 ± 0.6	5.0 ± 0.6	4.8 ± 0.7	0.586	0.091	0.439	0.420
IMF (%)	1.6 ± 0.2	1.3 ± 0.3	2.6 ± 0.2	2.0 ± 0.2	3.2 ± 0.3	0.004	0.040	0.572	0.001
Eye muscle area (cm^2)	11.5 ± 0.7	10.3 ± 0.8	11.1 ± 0.6	10.6 ± 0.6	12.2 ± 0.8	0.919	0.180	0.549	0.370

[1] Meat, loin, leg and shoulder yields and VSGR were estimated by VIAScan®.
[2] Diet: Pasture-Ewe & Pasture-Wethers vs. Chicory-Ewes & Chicory-Wethers; Sex: Pasture-Ewes & Chicory-Ewes vs. Pasture-Wethers & Chicory-Wethers; Diet x Sex: Pasture-Ewes & Chicory-Wethers vs. Pasture-Wethers & Chicory-Ewes); Age: Wean-Wethers vs. Pasture-Wethers & Chicory-Wethers.
*Hot carcass weight covariate (P<0.05).

2.3.2. Meat quality

Meat quality parameters did not differ (P>0.05) for wether and ewe lambs from Farm A, except for lean meat colour measured at 24 h post-mortem (P<0.05). Loins from wether lambs tended to have higher L* (P<0.10), and had higher a*, b*, Hue and Chroma values (P<0.05, Table 2.4) than loins from ewe lambs. Similarly, there were no differences (P>0.05) in meat quality parameters between lambs fed red clover and pasture on Farm A except loins from red clover fed lambs had a higher L*, a*, b* and Chroma values (P<0.05, Table 2.4) than loins from pasture fed lambs at 24 h post-mortem. From Farm B, the loins from wether lambs had similar (P>0.05) meat quality characteristics to cryptorchid lambs except for pH measured after aging which was slightly higher (P<0.05) in loins of wether lambs.

There were no interactions (P>0.05, Table 2.5) between animal diet and sex for the meat quality parameters of lamb loins from Farm C. The diet of lambs from Farm C influenced some meat colour parameters measured at 24 h post-mortem and subcutaneous fat colour determined after aging. Loins from lambs fed pasture showed higher a* and Chroma values (P<0.05) than loins from chicory fed lambs. The subcutaneous fat over the loin from lambs fed on pasture had higher a* (P<0.05) and L* (P<0.10) values than those from lambs fed on chicory. The sex of the lamb had an effect (P<0.05) on the water holding capacity of meat, while other loin meat quality characteristics were similar (P<0.05) between wethers and ewes. Loins from wethers had a greater (P<0.05) cooking loss and drip loss at 24 and 48 h than loins from ewe lambs. The age at slaughter had an effect (P<0.05) on pH after aging, shear force, sarcomere length, cook loss and some lean colour parameters at 24 h post-mortem. Loin meat from wether lambs slaughtered at weaning had a higher (P<0.05) pH, shear force and meat yellowness and Hue angle, and shorter (P<0.05) sarcomere length and less (P<0.05) cooking loss compared with wethers slaughtered after a finishing period at 6- to 8-month-old.

Table 2.4 Effect of sex (wethers vs. ewes), castration condition (wethers vs. cryptorchids) and diet (red clover wethers vs pasture wethers) on meat quality characteristics of lamb loins (*Longissimus thoracis et lumborum*) from Farms A and B, respectively.

	Farm A					Farm B		
Diet	**Red clover**	**Red clover**	**Pasture**	**P-value**		**Pasture**	**Pasture**	**P-value**
Sex/Castration	**Wether**	**Ewe**	**Wether**	**Sex**	**Diet**	**Wether**	**Cryptochid**	Castration
pH (24 h)	5.58 ± 0.01	5.58 ± 0.01	5.58 ± 0.01	0.681	0.882	5.87 ± 0.05	5.78 ± 0.03	0.142
pH (after aging)	5.43 ± 0.02	5.39 ± 0.02	5.41 ± 0.01	0.197	0.352	5.78 ± 0.05	5.66 ± 0.03	0.038
Shear force (N)	25.1 ± 0.8	24.9 ± 1.3	24.9 ± 0.8	0.903	0.859	27.9 ± 1.8	29.3 ± 1.2	0.471*
Sarcomere length (μm)	1.69 ± 0.01	1.70 ± 0.02	1.68 ± 0.02	0.862	0.639	1.57 ± 0.02	1.58 ± 0.03	0.708
Water holding measurements								
Cooking loss (%)	27.9 ± 0.9	26.2 ± 1.0	26.3 ± 0.8	0.209	0.470	28.2 ± 1.0	26.9 ± 0.9	0.371
Drip loss after 24 h (%)	1.8 ± 0.1	2.0 ± 0.2	1.7 ± 0.1	0.277	0.861	2.2 ± 0.1	1.9 ± 0.1	0.555
Drip loss after 48 h (%)	2.7 ± 0.1	2.9 ± 0.2	2.9 ± 0.2	0.351	0.417	2.8 ± 0.1	2.9 ± 0.1	0.904
Subcutaneous fat colour								
L*	68.5 ± 1.6	70.0 ± 1.4	68.5 ± 1.3	0.493	0.866	70.6 ± 1.1	69.1 ± 1.2	0.379
a*	5.6 ± 0.6	5.0 ± 0.6	5.3 ± 0.4	0.425	0.688	4.0 ± 0.4	4.6 ± 0.5	0.344
b*	6.1 ± 0.4	5.9 ± 0.3	6.5 ± 0.4	0.731	0.398	7.6 ± 0.5	7.8 ± 0.5	0.762
Lean meat colour 24 h post-mortem								
L*	40.0 ± 0.8	38.2 ± 0.5	37.9 ± 0.4	0.077	0.042	37.2 ± 0.9	38.4 ± 1.1	0.414
a*	21.7 ± 0.3	19.6 ± 0.3	20.2 ± 0.4	<0.001	0.009	18.6 ± 0.5	18.7 ± 0.5	0.892
b*	9.3 ± 0.3	7.3 ± 0.1	8.1 ± 0.2	<0.001	0.006	9.2 ± 0.4	9.2 ± 0.4	0.959
Hue	23.2 ± 0.5	20.5 ± 0.2	22.0 ± 0.4	<0.001	0.062	26.2 ± 0.9	26.1 ± 0.9	0.915
Chroma	23.6 ± 0.4	20.9 ± 0.3	21.8 ± 0.4	<0.001	0.005	20.8 ± 0.5	20.9 ± 0.6	0.930
Lean meat colour after aging								
L*	42.7 ± 0.7	41.8 ± 0.5	41.5 ± 0.6	0.308	0.314	40.2 ± 1.0	39.5 ± 1.1	0.644
a*	14.6 ± 0.2	14.1 ± 0.3	14.4 ± 0.3	0.209	0.826	13.2 ± 0.4	12.9 ± 0.3	0.583*
b*	4.1 ± 0.2	3.5 ± 0.2	3.9 ± 0.3	0.103	0.853	2.6 ± 0.3	2.8 ± 0.4	0.691
Hue	15.4 ± 0.7	13.9 ± 0.7	14.7 ± 0.8	0.150	0.881	11.0 ± 0.9	11.9 ± 1.3	0.604
Chroma	15.2 ± 0.3	14.6 ± 0.3	15.0 ± 0.3	0.159	0.809	13.2 ± 0.4	13.4 ± 0.4	0.699*

*pH (24h or after aging) covariate ($P<0.05$).

Table 2.5 Effect of diet, sex, and their interaction, and effect of age at slaughter (at weaning with 4-month-old vs. 6-to 8-month-old) on meat quality characteristics of lamb loins (*Longissimus thoracis et lumborum*) from Farm C.

	Farm C								
Diet	Pasture		Chicory		Wean	Non-orthogonal contrasts[1], P-value			
Sex	Wether	Ewe	Wether	Ewe	Wether	Diet	Sex	Diet x Sex	Age
pH (24 h)	5.60 ± 0.01	5.61 ± 0.01	5.61 ± 0.01	5.60 ± 0.01	5.59 ± 0.01	0.873	0.873	0.750	0.290
pH (after aging)	5.43 ± 0.03	5.47 ± 0.03	5.41 ± 0.03	5.40 ± 0.03	5.53 ± 0.03	0.105	0.502	0.367	0.038
Shear force (N)	26.0 ± 2.0	24.5 ± 1.2	26.4 ± 2.0	26.4 ± 2.0	33.2 ± 2.1	0.580	0.717	0.694	0.011*
Sarcomere length (μm)	1.68 ± 0.02	1.67 ± 0.02	1.69 ± 0.02	1.72 ± 0.02	1.61 ± 0.02	0.285	0.763	0.363	0.011
Water-holding capacity measurements									
Cooking loss (%)	29.3 ± 1.0	27.0 ± 1.0	29.3 ± 1.0	26.8 ± 1.0	26.7 ± 1.0	0.943	0.015	0.901	0.049
Drip loss after 24 h (%)	2.1 ± 0.1	1.7 ± 0.1	2.2 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	0.474	0.013	0.670	0.792
Drip loss after 48 h (%)	3.1 ± 0.2	2.6 ± 0.2	3.1 ± 0.2	2.8 ± 0.2	3.2 ± 0.2	0.314	0.009	0.479	0.630
Subcutaneous fat colour									
L*	69.0 ± 1.0	68.1 ± 1.0	70.7 ± 1.0	70.1 ± 1.0	69.3 ± 1.0	0.083	0.510	0.895	0.700
a*	5.5 ± 0.3	5.3 ± 0.3	4.7 ± 0.3	4.7 ± 0.3	5.6 ± 0.3	0.048	0.722	0.781	0.234
b*	6.5 ± 0.4	6.3 ± 0.4	6.4 ± 0.4	6.4 ± 0.4	5.8 ± 0.4	0.974	0.855	0.733	0.172
Lean meat colour-24 h postmortem									
L*	39.7 ± 0.6	38.1 ± 0.6	38.5 ± 0.6	38.6 ± 0.6	38.7 ± 0.6	0.618	0.191	0.145	0.605
a*	19.0 ± 0.4	18.9 ± 0.4	17.6 ± 0.4	17.9 ± 0.4	17.5 ± 0.4	0.007	0.910	0.631	0.138
b*	7.3 ± 0.4	7.3 ± 0.4	6.8 ± 0.4	7.0 ± 0.4	8.1 ± 0.4	0.121	0.524	0.567	<0.001
Hue	20.9 ± 0.7	21.2 ± 0.7	21.0 ± 0.7	21.5 ± 0.7	24.7 ± 0.7	0.726	0.430	0.774	<0.001
Chroma	20.3 ± 0.5	20.2 ± 0.5	18.9 ± 0.5	19.2 ± 0.5	19.3 ± 0.5	0.008	0.822	0.595	0.612
Lean meat colour after aging									
L*	42.1 ± 0.5	41.6 ± 0.6	42.1 ± 0.5	41.2 ± 0.5	41.4 ± 0.5	0.671	0.189	0.783	0.349
a*	13.3 ± 0.3	13.5 ± 0.3	13.4 ± 0.3	14.0 ± 0.3	13.6 ± 0.3	0.324	0.125	0.550	0.506*
b*	3.8 ± 0.4	3.1 ± 0.4	3.0 ± 0.4	3.2 ± 0.4	3.5 ± 0.4	0.384	0.540	0.261	0.926*
Hue	15.5 ± 0.5	12.9 ± 0.5	12.6 ± 0.5	13.0 ± 0.5	14.4 ± 0.6	0.347	0.453	0.330	0.878*
Chroma	14.1 ± 0.3	13.9 ± 0.3	13.7 ± 0.3	14.4 ± 0.3	14.1 ± 0.3	0.973	0.490	0.171	0.703*

[1]Diet: Pasture-Ewe & Pasture-Wethers vs. Chicory-Ewes & Chicory-Wethers; Sex: Pasture-Ewes & Chicory-Ewes vs. Pasture-Wethers & Chicory-Wethers; Diet x Sex: Pasture-Ewes & Chicory-Wethers vs. Pasture-Wethers & Chicory-Ewes); Age: Wean-Wethers vs. Pasture-Wethers & Chicory-Wethers.
*pH (24h or after aging) covariate (P<0.05).

2.4. Discussion

To increase efficiency of lamb production there has been a change in the variety of forage species used for finishing lambs (e.g. *chicory*) in New Zealand in order to improve lamb growth rate and finishing weights (Hopkins and Mortimer, 2014; Somasiri et al., 2015; Young et al., 2006; Hopkins et al., 2007). Genetic selection and on-farm management of sheep for meat production has been orientated towards maximizing retail yield of meat through greater carcass weights and the improvement in growth rate of lambs in pasture-based production systems (Corner-Thomas et al., 2013). As well as achieving desirable carcass characteristics to maximise the return for the farmer, it is important to consider the influence of the production system on meat quality to meet the demands of discerning consumers and maximise the value of the product (Hopkins and Mortimer, 2014). The objective of this study was to evaluate lambs from three farms for the effect that diet, sex, castration status as well as the choice of slaughtering at weaning or after a finishing period on carcass attributes and meat quality characteristics.

2.4.1. Carcass characteristics

Similar carcass weight, meat yield of carcass, VSGR (a measure of soft tissue depth over the ribs), and fat depth over the loin were found between wether and ewe lambs from Farm A, and wether and cryptorchid lambs from Farm B. These results are in agreement with Hopkins et al. (2001) and Kaić et al. (2016) who reported that lambs slaughtered at a similar age and reared under similar conditions had similar carcass traits regardless of their gender. Previous studies suggest that sex and castration status have little effect on IMF deposition in young lambs (Hopkins and Mortimer, 2014; Tejeda et al., 2008), however, in the current study Merino wether lambs from Farm B had greater intramuscular fat and eye muscle area compared to the Merino cryptorchid

lambs from the same farm. Similarly, wether lambs from Farm C had greater carcass weight, shoulder yield and IMF content in the loin compared to ewe lambs. The removal of androgenic hormones by castration has been associated with increased fat and lean deposition in older lambs (McPhee et al., 2008; Mashele et al., 2017). ,In addition, lambs that are heavier at the time of slaughter generally have greater IMF concentrations in their meat (McPhee et al., 2008; Mashele et al., 2017). The growth of the eye muscle and intramuscular fat is more evident in older, heavier lambs (Mashele et al., 2017) so it is likely that the older slaughter age of the lambs from Farm B and the heavier weight of carcasses for wether lambs from Farm C allowed for the castration effect on lean and fat deposition to be expressed.

The chicory-fed lambs from Farm C had heavier carcasses, greater IMF deposition in the loin and increased shoulder yield compared with pasture-fed lambs. In addition, ewe lambs fed chicory had higher meat and loin yields than ewe lambs fed pasture. These results indicate that feeding chicory to lambs provides a diet with a high feeding value that encourages faster growth and the deposition of both lean and fat. Farmlet studies have shown lambs grazing herbs and alternative forages have a greater ability than lambs grazing ryegrass pasture to deposit fat and thus achieve heavier carcass weights and greater subcutaneous fat depth (Somasiri et al., 2015). Greater lamb meat yields can be a consequence of either increased muscle deposition or the deposition of fat that is not trimmed resulting in increased conformation scores. Alternatively, a lamb that has finer, lighter bones will have a greater muscle to bone ratio which is also associated with greater meat yields (McCutcheon et al., 1993). Ewe lambs have been reported to have greater fat deposition and smaller, lighter bones, although their muscle weight was similar to rams, resulting in greater muscle to bone ratio and meat yields for ewe-lambs (Johnson et al., 2005). The greater meat yields

of ewe lambs fed chicory from Farm C compared to those fed pasture suggests that chicory allows for greater muscle to bone ratio and conformation to be expressed relative to the pasture diet.

Heavier carcass weight and greater IMF% of lambs slaughtered directly after weaning compared to those slaughtered after an additional finishing period on pasture or chicory was observed in Farm C. This was likely due to the faster growth from having access to milk resulting in greater muscle development and fat deposition due to a higher plane of nutrition (McPhee et al., 2008). Average lamb growth rates from birth to 15 weeks of age were 343 g/d and 292 g/d for single and twin lambs, respectively indicating that the greater milk supply to the single lamb had an effect on early growth (Muir et al., 2000). In comparison to muscle, fat is considered to be a later developing tissue (Ponnampalam et al., 2008), therefore, greater subcutaneous fat depths and IMF concentrations are usually seen with animals that are older at slaughter compared to younger animals (Pannier et al., 2014; 2014; Mashele et al., 2017). For lambs from Farm C that were slaughtered after weaning, the greater IMF% and numerically greater VSGR contradicts this, but the literature also indicates that heavier live weights and carcass weights are associated with increased carcass fat and IMF (Anderson et al., 2015). The greater IMF content in the loin of lambs slaughtered directly after weaning and processed at a younger age, is therefore, either a consequence of a diet that included milk prior to slaughter or being heavier at slaughter rather than a direct age effect. It should be noted that the IMF level of lamb loins in the current study was comparable to those previously reported for lambs grown in New Zealand forage-based systems (2.69%; Craigie et al., 2017). IMF is a carcass characteristic that can greatly influence the eating quality of meat, as IMF has been reported to account for 11% of the variability in the overall liking of lamb as assessed by a trained sensory panel (Lambe et al., 2017). The melted fats, in combination with water, are released upon chewing, which helps stimulate the flow of saliva,

creating an even greater sense of eating pleasure also known as ‘mouth feel’ (Wood et al., 2004). The developed adipose tissue weakened structures of the intramuscular connective tissue and contributed to tenderization of meat (Nishimura et al., 1999). The importance of the variation in IMF concentration obtained from the different production factors such as sex, castration status, diet, and age of lambs warrants further investigation due to the influence it can have on eating quality (Hopkins et al., 2006).

2.4.2. Meat quality

The colour of loin meat from wether lambs from Farm A was lighter, redder and had greater colour intensity compared to ewe lambs from the same farm, however, lambs from Farm B and C did not differ. The differences of lambs from Farm A are difficult to explain as generally lambs of different sexes or castration status slaughtered at the same age grazing the same diet have no difference for meat colour (Hopkins et al., 2007; Tejeda et al. 2008; Schreurs and Kenyon, 2017). Meat colour between animals can vary due to differences in ultimate pH (Savell et al., 2005; Hopkins and Mortimer, 2014; Schreurs and Kenyon, 2017), however there was no difference in pH either at 24 h post mortem or after aging, indicating that pH did not explain the differences in meat colour on Farm A. When considering the absolute difference in colour values between the wether and ewe lambs from Farm A, the difference is small. Although statistically different, the difference in colour values is unlikely to be visually observed (Priolo et al., 2001).

When pH was measured in loins after aging, wether Merino lambs from Farm B had a higher pH compared to cryptorchid lambs. Wethers slaughtered at weaning from Farm C also had a higher pH after aging compared to wether lambs that were slaughtered at 6- to 8-month-old. The pH of meat is an important determinant of meat quality as it influences colour, water holding capacity

and tenderness (Hopkins and Mortimer, 2014; Savell et al., 2005). The pH values for lambs from Farm B and C, however, were below 5.8 and the differences in pH observed were small and similar to differences observed by Hopkins et al. (2011) in a study where the pH had no effect on meat quality of lamb. The reason for the differences in pH after aging for lambs from Farm B and C are difficult to reconcile. The variability in pH from different studies that examined meat from lambs of different castration status or age (Hopkins and Mortimer, 2014) suggested that no distinct effect could be attributed to these factors. Other factors are likely to be more important such as exposure to stressors or excessive activity depleting glycogen in the muscle (Devine et al., 1993; Pethick and Rowe, 1996; Johnson et al. 2005; Johnson et al., 2017).

Water holding capacity measurements indicate whether muscles lose water easily during refrigeration, storage, in retail packaging and during cooking (Kadim et al., 2013). Ewe lambs from Farm C showed a greater water holding capacity than wethers as indicated by wethers having a greater loss of moisture during cooking and drip loss when stored in the chiller. This sex effect on meat water holding capacity was not identified in lambs from Farms A or B. Wethers from Farm C had a lower water holding capacity than the ewes, which could be attributed to wether lambs being heavier at slaughter (Vergara et al., 1999). Post-slaughter treatments such as chilling and aging can have a major impact on water holding capacity (Cheng and Sun, 2008). The subtle differences seen between the wether and ewe lamb carcasses, in response to chilling and aging procedures, may have caused the differences in water holding capacity seen in the current study.

Grazing forages and pastures has been associated with increased yellow colour of fat due to the deposition of carotenoid pigments from the plants into the fat (Priolo et al., 2001). The forage diets fed to lambs on Farm C did not influence the subcutaneous fat yellowness (b*) values although there were some statistically significant effects on lightness (L*) and redness (a*). The

subcutaneous fat from chicory fed lambs was lighter in colour and less red compared to pasture fed lambs but the difference in the absolute values was small and would not be visually detected by consumers (Carrasco et al., 2009). The chicory fed lambs from Farm C had meat that was less red and had less intensity of redness at 24 h post-mortem compared to the pasture fed lambs. Chicory fed lambs also had a greater IMF% which has been associated with lower meat redness when measured objectively, and this ascribed to a dilution of the muscle fibres (Franco et al., 2009; Mashele et al., 2017). This finding was in agreement with the lower redness and saturation of meat colour in lambs grazing pasture compared to offered red clover on Farm A. IMF level was also greater in pasture fed lambs. It should be noted that the breed of lambs grazing pasture and red clover differed. In a large study across 6 countries it was suggested that most of the breed effects were due to differences in sarcomere length, final pH of lambs raised under different production systems and slaughtered in different countries over a wide range in carcass weights (5.4–30.5 kg)(Berge et al., 2003), .

Wether lambs slaughtered at weaning had meat with a shorter sarcomere length, higher shear force (when pH was included as a covariate in the analysis), and meat colour that was more yellow and with a greater hue angle compared to wether lambs that had an additional finishing period on pasture or chicory. Although statistically significant, the difference in sarcomere length was 0.08 µm. This difference was unlikely to be of consequence to meat quality and unlikely to be a result of cold shortening. All lambs investigated in the current study had a sarcomere length within the range expected for lamb of 1.57-1.72 µm (Davey and Garnett, 1980; Savell et al., 2005; Hopkins et al., 2007). The higher feeding value of milk (Muir et al., 2000) would likely ave allowed for greater muscle fibre hypertrophy increasing the muscle fibre diameter (Greenwood et al., 2006) which has been associated with small decreases in sarcomere length (Lewis et al., 1977; Lieber et

al., 1984). Weaned lambs had numerically greater eye muscle areas than wether lambs fed pasture or chicory. Given that the shear force values were compared at an equal pH, the increased muscle fibre size was also likely to have contributed to higher shear force values of the weaned lambs compared to the older lambs. An increased muscle fibre diameter, as well as more collagen and stronger collagen networks associated with larger muscle fibres, will increase shear force values of lamb (Young et al., 1993; Schreurs and Kenyon 2017). Nevertheless, the shear force values were below 40 N which is considered the threshold for consumer acceptance of tenderness of lamb (Hopkins et al., 2006).

The greater hue angle and b* values from lambs that were processed at weaning indicates that the meat was browner in colour compared to the meat from lambs processed after finishing on forages. Brown colours in meat are associated with a higher concentration of metmyoglobin which can occur when a meat is less oxidatively stable (Priolo et al., 2001). Secondary compounds in plant diets of lambs can produce meat that has a greater oxidative stability (Luciano et al., 2009a, b).

2.5. Conclusions

This study indicated that castration of male lambs and a diet of chicory can increase intramuscular fat in lambs. Sending lambs with suitable live weights for processing at weaning will produce a heavier carcass and meat that has a greater intramuscular fat content than finishing lambs for an addition period post-weaning. Greater carcass weight and meat yield was observed in lambs grazing chicory than pasture. The differences in meat yield of lambs finished on chicory was also dependent on the sex of the lamb. The effect that sex, castration status, forage diet and the processing of lambs after weaning compared to after a finishing period on forages on meat

quality was subtle. There were some differences in meat colour that could be attributed to either a direct effect of diet, and castration status or age at processing on pH and shear force that are likely to be working through an indirect effect of growth rate and carcass characteristics. To further elucidate the effect of New Zealand production systems on the meat produced, chemical and sensory properties of the meat will be investigated in following Chapters.

Chapter 3

Fatty acid composition of *Longissimus thoracis* from commercial lambs reared in different forage-based production systems

This chapter formed part of the following publication:

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Abstract

On farm animal production factors can affect the fatty acid profile of lamb meat. The fatty acid composition of the *Longissimus thoracis* was evaluated from 150 lambs from 10 groups of commercial lambs that different in age, sex, diet and breed on three farms. These 10 groups represent typical forage lamb production systems in New Zealand. The meat from 4-month-old lambs that was slaughtered at weaning had greater proportion of saturated fatty acids and lower proportion of monounsaturated fatty acids compared to 6- to 8-month-old composite lambs as a reflection of fatty acid profile of milk. The polyunsaturated to saturated fatty acid ratio was similar between these lambs and all ratios were lower than 0.4. All lamb production systems produced meat with an omega-6 to omega-3 ratio below 1.5. Chicory diet increased the proportion of poly unsaturated fatty acids and decreased the proportion of saturated fatty acids and branch chain fatty acids in meat compared to perennial ryegrass diet. Meat from lambs processed at weaning contained the greatest content of eicosapentaenoic and docosahexaenoic acids, and meat from Merino lambs contained the least content of eicosapentaenoic and docosahexaenoic acids. The combined eicosapentaenoic acid plus docosahexaenoic acid content in muscle and the proportion of lambs that can be considered a ‘source’ or ‘good source’ of these target fatty acids differed significantly among the ten evaluated forage-based production systems. However, the potential to aim for nutritional claims on eicosapentaenoic acid plus docosahexaenoic acid is limited by total saturated fatty acids plus trans fatty acids and would depend on the nutritional guidelines and regulations of the export markets.

3.1. Introduction

Red meat is an important source of protein, fatty acids and vitamins in the human diet, which are essential for human health (Pereira and Vicente, 2013). Dietary fat from meat plays an important role in health maintenance and disease prevention, delivers nutrients such as vitamins A, D, E, carotenoids (Scollan et al., 2006), long chain omega-3 polyunsaturated fatty acids (LC-n-3-PUFA) and conjugated linoleic acid (CLA). CLA is almost exclusively sourced from red meat and dairy products (McGuire and McGuire, 2000). A high intake of n-3 fatty acids has been reported to decreased the risk of coronary heart disease (Hu et al., 2002), while a lack of dietary n-3 fatty acids in childhood is linked to behavioural and learning disorders such as attention deficit hyperactivity disorder (Richardson and Ross, 2000). A high intake of saturated fatty acids (SFA) and trans-fatty acids has been found to adversely affect glucose metabolism and insulin resistance, which have been associated with Type II diabetes and other health concerns such as cardiovascular disease (Hu et al., 2001). In addition, the U.S. Department of Agriculture and Human Health Services (2010) has verified that the cholesterol-raising fatty acids include SFA from C12:0 to C16:0, which are commonly found in meat from ruminant animals (Díaz et al., 2005).

A recent systematic review suggested that the certainty of evidence between cardiovascular mortality, Type II diabetes and overall cancer mortality to high meat consumption is very low (Johnston et al., 2019). However, various epidemiological studies have correlated SFA and meat intake with cardiovascular disease since the 1980s (Johnston et al., 2019). The average European and North American adult consumes nearly 80 kilograms and more than 110 kilograms meat per year, respectively (Ritchie, 2017). In high-income countries, changes in meat consumption have stagnated or even decreased over the last 50 years (Ritchie, 2017). There is an increasing awareness by consumers about how food influences their health (Fowler et al., 2019), which poses challenges

for lamb producers and processors. Consumers are selecting food items with a higher concentration of n-3 fatty acids and lower concentrations of SFA (Font-i-Furnols and Guerrero, 2014). In Australia and New Zealand, food containing over 22 and 44 mg per 100 g serve of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) could be considered as a 'source' and a 'good source' of them respectively (Food Standards Australia New Zealand, 2012). While in the European Union, over 40 and 80 mg per 100 g serve of EPA plus DHA are needed for a food to be considered as a 'source' and a 'good source', respectively (Commission Regulation of European Union, 2010).

Understanding the effect of diverse forage production systems on the fatty acid composition of lamb meat, in particular n-3 fatty acids, could provide an opportunity to differentiate lamb products for discerning markets. Production systems in New Zealand can vary depending on the animal characteristics such as breed, sex and age at slaughter, and management factors such as the animal's diet. These factors that have been reported to contribute to variations in the fatty acid composition of meat (Díaz et al., 2005). For instance, the fatty acid profile of lamb becomes more saturated as the animal ages (Wood et al., 2008). Similarly, female lambs have a tendency to deposit more fat and have a greater saturated fatty acid profile than male lambs at a given weight (Diaz et al., 2003; Horcada et al., 1998). Branched-chain fatty acids, in particular 4-methyloctanoic and 4-methylnonanoic acids that have been identified in sheep meat in sufficient quantities to provide the characteristic lamb odour (Mottram, 1998). There is potential for the diversity in lamb production systems between countries and farms within countries to contribute to differences in the fatty acid composition of meat.

Forage and concentrate feeds have been reported to influence fatty acids composition of lamb meat. Forage-based animal diets can enhance the proportion of n-3-PUFA in meat, and often enrich

the meat with antioxidants in comparison with meat from animals fed concentrate-based diets (Howes et al., 2015). The meat of lambs fed lucerne, clover, lotus or ryegrass had higher concentration of medium chain fatty acids containing 6–12 carbon atoms than lambs fed corn (Bailey et al., 1994). The PUFA to SFA ratios are lower in the meat of forage fed animals as they are frequently reported to be leaner than concentrate diet animals due to lower energy density (Santos-Silva et al., 2002). There is, however, limited information regarding the fatty acid composition of meat from lambs finished in different grazing systems, which was considered to be more subtle in general.

Feeding concentrates is impractical and uneconomic for most New Zealand production systems that rely on the grazing of pasture, but the broad range of commercial production systems utilized in New Zealand suggests that there is potential for variation in fatty acid profiles in lamb meat. The objective of this study was to evaluate the meat fatty acid profiles of lambs from diverse forage-based production systems in New Zealand and to compare the omega-3 fatty acid levels in meat with current nutrition guidelines.

3.2. Materials and methods

3.2.1. Animals and management

To encompass a range of forage-based production systems, lambs were sourced from three commercial farms north of Invercargill, New Zealand. Lambs from Farm A and C were born in August 2017, and lambs from Farm B were born in December 2016 (Table 3.1). These three farms provided ten production systems that represented forage fed lambs processed in New Zealand. The ten production systems included: 4-month-old wether lambs of a composite breed at weaning (WEAN-W); 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing perennial ryegrass based pasture (GRASS-W, GRASS-E, respectively); 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing chicory (CHIC-W, CHIC-E, respectively); 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing red clover (REDC-W, REDC-E, respectively); 6- to 8-month-old wether lambs of composite breed that had been grazing a mixture of perennial ryegrass, red- and white-clover mixed pasture (MIX-W); and 12-month-old wether and cryptorchid Merino lambs that had been grazing a mixed pasture (MXME-W, MXME-C, respectively). Fifteen lambs from each production system were randomly selected and identified at slaughter from a larger group of lambs.

Table 3.1 Production systems of animals (n=15) selected according to the pre-slaughter factors (age, sex, diet, breed) included in the study.

Production systems	Approximate age at slaughter (months)	Sex	Finishing diet[1]	Breed[2]	Farm	Distance to meat plant (km)
REDC-W	6-8	Wethers	Red Clover	Perendale × LambSupreme	A	80
REDC-E	6-8	Ewes	Red Clover	Perendale × LambSupreme	A	80
MIX-W	6-8	Wethers	Pasture	Perendale × Romney	A	80
MXME-W	12	Wethers	Pasture	Merino	B	250
MXME-C	12	Cryptochids	Pasture	Merino	B	250
WEAN-W	4	Wethers	Pre-weaning	Composite	C	100
GRASS-W	6-8	Wethers	Pasture	Composite	C	100
GRASS-E	6-8	Ewes	Pasture	Composite	C	100
CHIC-W	6-8	Wethers	Chicory	Composite	C	100
CHIC-E	6-8	Ewes	Chicory	Composite	C	100

[1] Animal diet: Pre-weaning, suckled and grazing mothers' diet of a chicory (Cichorium intybus) and red clover (Trifolium pratense) mix;
Farm A-Pasture, predominantly Italian and perennial ryegrass (Lolium perenne) and red and white (Trifolium repens) clover mix;
Farm B-Pasture, permanent pasture, perennial ryegrass, red clover and white clover mix;
Farm C-Pasture, ryegrass and white clover mix followed by fescue (Lolium arundinaceum), red and white clover and plantain (Plantago lanceolata) mix during the last 2 weeks.
[2] Composite: Perendale, Texel, Finnish Landrace and Romney genetics; LambSupreme: lean-selected Poll Dorset, Wiltshire, Romney x Dorset, Coopworth, Texel, and high-growth Romney.

3.2.2. Slaughter and sampling

Lambs were processed at Alliance Group Ltd, Lorneville plant, Invercargill, New Zealand. The 4- and 12-month-old lambs were slaughtered on the 7th December 2017 and the 6- to 8-month-old lambs were slaughtered on the 1st March 2018. All lambs were electrically stunned, exsanguinated, and dressed according to standard commercial procedures. The *Longissimus thoracis* (loin) muscle was removed from the carcass, vacuum packed and chilled at -1.5°C for 21 days then frozen at -20°C until further analysis. A detailed description of animal groups, sample collection and carcass and meat quality characteristics corresponding to this study were previously reported in Chapter 2.

3.2.3. Fatty acid composition

Prior to fatty acid composition analysis all samples were freeze-dried (Cuddon Freeze Drier, Blenheim, NZ) and ground into a fine powder. Fatty acid concentrations were measured using trans-methylation of the fatty acids and quantification by gas chromatography (GC) as described by Agnew et al. (2019). Freeze-dried ground meat (300 mg), 4 mL of toluene, 0.3 mL of internal standard (C11 triglyceride in toluene), and 4 mL of 5% of sulphuric acid in methanol were mixed thoroughly, by vortex and incubated at 70°C for 2 h, the sample was mixed every 30 min during the incubation time. After 25 min of equilibration at room temperature, 5 mL of saturated NaCl was added, mixed and then centrifuged at 2300 rpm for 2 min to separate solvent layers. The top layer containing the fatty acid methyl esters (FAME) was transferred into 1.5 mL GC autosampler vial (Hewlett Packard, model 6890). The GC was a Shimadzu GC-2010 plus (Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector (FID). The column was a Restek RTX 2330 column of 105 m length, 0.25 mm i.d., and 0.20μm film thickness (Restek Corporation,

Bellefonte, PA, USA). The thermal program used an initial temperature of 175°C for 17 min, which was increased to 220°C at a rate of 6°C per min and held for 10 min. The carrier gas was hydrogen with a linear velocity of 50 cm/s. The injection volume was 1 μL, with a split ratio of 80:1. The injector temperature was 260°C and the detector temperature was 300°C.

The peak areas obtained from the GC were integrated using the Shimadzu Lab-solution software (version 4.20) in a post-run analysis. Peaks were identified by comparison of their retention times with those of commercial standard mixtures (FAME mix 37 components from Supelco Inc., Bellefont, PA, USA) and quantified by using the internal standard (C11:0) and theoretical FID response factors (Agnew et al., 2019). The equations for generating the response and conversion factors to quantify individual fatty acids from FAME were obtained from American Oil Chemists' Society 6th edition (AOCS Ce1f-96, Ce 1 h-05 and Ce 1i-07). Individual fatty acid content was expressed as mg per 100 g of raw meat (m. longissmus thoracis) and as a percentage of total fatty acids. For 10% of the samples, a randomly allocated duplicate sample was analysed to verify consistency. Major fatty acid groups and PUFA:SFA ratio, n-6:n-3 ratio, thrombogenic index (Ulbricht and Southgate, 1991), atherogenic index (Ulbricht and Southgate, 1991), hypocholesterolaemic/Hyperdolesterolaemic (h/H) ratio (Santos-Silva et al., 2002) and nutritional ratio (Estévez et al., 2004) were calculated from the relevant individual fatty acids to enable comparison with human nutritional guidelines.

3.2.4. Statistical analysis

Data were analysed using Genstat 18.1.0 (VSN International Ltd, UK). Sex (wethers vs. ewes), castration status (wethers vs. cryptorchids) and diet (MIX-W vs REDC-W) were included in the model as fixed effects for Farm A and B, respectively. For Farm C, pre-planned non-orthogonal

contrasts were used to compare means from 6–8 months old lambs fed pasture vs. chicory (diet) and wethers vs. ewes (sex) and their interaction. In addition, animal age at slaughter for wether lambs (4 vs. 6–8 months old) was contrasted. Differences amongst least square means were considered significant at a probability level of <0.05.

In order to summarise the relative differences amongst samples in relation to their overall fatty acid profiles, a Principal Component Analysis (PCA) was performed using the content of identified fatty acids (mg/ 100 g raw meat) using "factoextra" package in RStudio (Version 1.2.5001).

3.3. Results

3.3.1. Effect of gender and castration

The content of 22:5n-3 and LC n-3 PUFA were greater in wethers than ewes for lambs grazed on chicory or perennial ryegrass ($P<0.05$; Table 3.4). The content of ai-15:0, ai-17:0, t9-18:1, t11-18:1, c9t11-18:2 was greater in MXME-W lambs than MXME-C lambs ($P<0.05$; Table 3.3).

For lambs grazed on red clover, wethers had greater proportion of 12:0, 15:0, ai-15:0, i-17:0 compared to ewes ($P<0.05$; Table 3.5). The proportion of 22:0, c11-18:1, 22:5n-3, 22:6n-3 were greater in cryptorchid Merino lambs compared in that of wether Merino lambs ($P<0.05$; Table 3.6). While the proportion of ai-17:0, t11-18:1, c9t11-18:2 was greater in wether Merino lambs compared in that of cryptorchid Merino lambs ($P<0.05$; Table 3.6).

Table 3.2 Fatty acid content (mg/100g raw meat, means ± standard error of the means) of *m.longissimus thoracis* from lambs reared under different New Zealand commercial forage production systems in Farm A.

Fatty acid content (mg/100g raw meat)	Red clover	Red clover	Pasture	P-value	
	Wether	Ewe	Wether	Sex	Diet
10:0	3.17±1.54	3.09±1.18	3.73±1.11	0.884	0.261
12:0	5.89±3.83	3.37±1.57	5.45±2.42	**0.026**	0.709
14:0	56.82±38.99	46.82±18.54	66.08±23.83	0.378	0.439
15:0	7.94±4.84	6.53±2.18	8.67±2.83	0.312	0.619
16:0	503.66±275.61	497.20±145.44	579.09±146.41	0.937	0.357
17:0	22.99±11.92	21.63±5.81	25.95±6.27	0.696	0.401
18:0	362.39±190.56	351.25±90.70	414.19±89.52	0.839	0.349
22:0	Bd[1]	Bd	Bd	-	-
24:0	1.39±0.70	1.43±0.31	1.35±0.21	0.841	0.835
i-14:0	Bd	Bd	Bd	-	-
i-15:0	2.17 ±2.17	1.56±0.95	2.46±1.10	0.326	0.645
ai-15:0	3.50±2.26	2.70±0.98	3.60±1.05	0.219	0.877
i-16:0	2.69±1.77	2.23±0.87	2.12±0.91	0.374	0.729
i-17:0	8.31±3.97	7.23±1.95	9.64±2.71	0.351	0.291
ai-17:0	8.11±5.66	10.85±2.77	10.85±3.28	0.554	0.306
14:1	1.51±1.43	1.17±0.85	1.91±0.82	0.443	0.346
16:1	23.85±13.78	22.85±6.71	27.99±8.09	0.804	0.324
17:1	10.93±5.44	11.95±2.72	14.15±2.99	0.518	0.053
t9-18:1	4.77±2.99	4.50±1.58	5.29±1.44	0.757	0.548
t11-18:1	69.80 ±49.58	62.51±22.50	66.83±22.18	0.608	0.834
c9-18:1	755.61 ±401.68	783.97±226.82	964.95±239.28	0.814	0.094
c11-18:1	24.58 ±8.43	24.28±4.88	27.98±5.09	0.906	0.192
18:2n-6	105.13±26.04	103.34±11.25	98.32±16.48	0.809	0.400
c9t11-18:2	26.67±19.12	23.93±10.18	30.44±12.82	0.629	0.531
20:4n-6	38.00±8.25	36.29±5.11	38.21±5.54	0.501	0.936
22:2n-6	0.45±0.86	0.13±0.52	Bd	0.228	-
18:3n-3	61.44±17.02	59.16±10.61	58.10±11.18	0.663	0.531
20:5n-3	30.87±6.31	30.44±4.20	31.89±3.89	0.829	0.596

22:5n-3	28.29 ±5.33	27.51±1.94	29.35±2.54	0.595	0.493
22:6n-3	8.47 ±1.96	8.15±1.81	8.93±1.47	0.638	0.478
BCFA[2]	24.78±15.73	24.57±7.30	28.67±8.88	0.384	0.442
SFA[3]	987.64±538.28	953.16±264.34	1133.91±275.29	0.813	0.364
MUFA[4]	891.04±478.60	911.27±255.74	1109.14±273.21	0.886	0.137
PUFA[5]	299.33±69.90	288.97±29.23	295.25±38.39	0.601	0.844
EPA+DHA[6]	39.34±7.91	38.59±5.69	40.82±5.01	0.769	0.545
n-6 PUFA sum[7]	143.13±32.05	139.64±12.93	136.54±18.55	0.696	0.494
n-3 PUFA sum[8]	129.08±26.77	125.27±13.84	128.28±14.34	0.638	0.611
LC n-3 PUFA[9]	67.75±12.88	67.75±6.92	70.17±6.81	0.564	0.455
Unreported	214.70±66.04	197.75±28.08	222.63±27.91	0.368	0.672
Total FA	2392.71±1130.01	2351.36±553.42	2761.45±599.00	0.892	0.278

1. bd: below detection threshold of 0.01 mg/100g raw meat.
2. BCFA =∑ i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
3. SFA = ∑ 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0, i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
4. MUFA = ∑ 14:1, 16:1, 17:1, t9-18:1, t11-18:1, c9-18:1, c11-18:1, 20:1, 22:1, 24:1.
5. PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n6, 18:3n-3, 20:5n3, 22:5n3, 22:6n-3, CLAc9t11.
6. EPA+DHA= ∑ 20:5n-3, 22:6n-3.
7. n-6 PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n-6.
8. n-3 PUFA = ∑ 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.
9. LC n-3 PUFA = ∑ 20:5n-3, 22:5n-3, 22:6n-3.

Table 3.3 Fatty acid content (mg/100g raw meat, means ± standard error of the means) of *m.longissimus thoracis* from wether and cryptorchid Merino lambs reared in Farm B.

Fatty acid content (mg/100g raw meat)	**Pasture**	**Pasture**	**P-value**
	Wether	**Cryptorchid**	
10:0	4.31±0.77	3.94±1.16	0.315
12:0	4.03±1.73	3.47±1.98	0.422
14:0	69.03±16.61	56.69±24.95	0.122
15:0	12.03±2.71	9.95±3.78	0.095
16:0	733.65±170.97	634.20±215.56	0.172
17:0	37.37±9.64	30.65±9.37	0.063
18:0	557.91±131.88	468.19±152.27	0.096
22:0	1.22±0.73	1.79±0.38	**0.012**
24:0	Bd[1]	Bd	-
i-14:0	0.63±0.81	0.40±0.70	0.408
i-15:0	4.38±1.04	3.71±1.55	0.179
ai-15:0	5.56±1.19	4.30±1.64	**0.023**
i-16:0	2.16±0.25	2.16±0.35	0.716
i-17:0	13.19±2.79	11.47±3.31	0.134
ai-17:0	17.73±4.22	13.75±4.69	**0.021**
14:1	1.46±0.80	1.22±0.99	0.468
16:1	34.85±8.19	31.84±9.37	0.358
17:1	2.45±0.68	3.47±4.46	0.432
t9-18:1	6.73±1.38	5.52±1.79	**0.047**
t11-18:1	78.06±18.77	54.09±20.18	**0.002**
c9-18:1	1249.19±298.79	1041.75±339.92	0.087
c11-18:1	31.39±5.81	28.35±6.42	0.185
18:2n-6	80.28±10.47	74.76±16.32	0.279
c9t11-18:2	31.43±8.14	21.35±8.83	**0.003**
20:4n-6	33.65±3.15	31.55±4.08	0.126
22:2n-6	Bd	0.17±0.45	-
18:3n-3	46.01±8.32	42.48±10.45	0.315
20:5n-3	21.32±2.42	21.54±2.27	0.799
22:5n-3	22.20±2.16	21.83±2.05	0.638

22:6n-3	6.43±1.26	6.47±0.95	0.910
BCFA[2]	43.65±9.41	35.79±11.51	0.051
SFA[3]	1463.15±332.36	1244.67±414.55	0.122
MUFA[4]	1404.15±325.50	1166.15±374.44	0.074
PUFA[5]	241.32±27.63	220.17±40.09	0.104
EPA+DHA[6]	27.77±3.34	28.01±2.77	0.832
n-6 PUFA sum[7]	113.93±12.38	106.33±19.36	0.211
n-3 PUFA sum[8]	95.97±12.74	92.31±13.32	0.338
LC n-3 PUFA[9]	51.31±5.42	50.75±4.58	0.747
Unreported	243.98±35.43	223.42±37.87	0.136
Total FA	3357.82±698.85	2857.42±845.10	0.088

1. bd: below detection threshold of 0.01 mg/100g raw meat.
2. BCFA =∑ i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
3. SFA = ∑ 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0, i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
4. MUFA = ∑ 14:1, 16:1, 17:1, t9-18:1, t11-18:1, c9-18:1, c11-18:1, 20:1, 22:1, 24:1.
5. PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n6, 18:3n-3, 20:5n3, 22:5n3, 22:6n-3, CLAc9t11.
6. EPA+DHA= ∑ 20:5n-3, 22:6n-3.
7. n-6 PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n-6.
8. n-3 PUFA = ∑ 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.
9. LC n-3 PUFA = ∑ 20:5n-3, 22:5n-3, 22:6n-3.

Table 3.4 Fatty acid content (mg/100g raw meat, means ± standard error of the means) of *m.*longissimus *thoracis* from lambs reared under different New Zealand commercial forage production systems in Farm C.

Fatty acid content (mg/100g raw meat)	**Pasture**		**Chicory**		**Wean**	**Non-orthogonal contrast[2], P-value**			
	Wether	**Ewe**	**Wether**	**Ewe**	**Wether**	**Diet**	**Sex**	**Diet x Sex**	**Age**
10:0	3.09±0.65	3.57±1.08	2.97±0.88	2.73±1.03	6.64±2.15	**0.046**	0.636	0.137	**<0.001**
12:0	6.23±2.80	6.05±2.86	4.96±2.14	7.00±3.34	12.31±5.31	0.826	0.208	0.133	**<0.001**
14:0	47.69±15.31	52.23±19.76	45.51±17.35	41.49±19.57	122.15±49.63	0.167	0.956	0.363	**<0.001**
15:0	6.95±1.91	7.11±2.09	6.08±1.47	5.31±1.89	13.08±4.47	**0.007**	0.551	0.337	**<0.001**
16:0	423.61±113.16	499.05±160.24	458.19±121.13	431.67±133.03	645.84±152.07	0.637	0.481	0.144	**<0.001**
17:0	20.55±5.50	21.28±5.73	19.17±3.81	17.50±4.95	26.55±5.47	0.050	0.726	0.363	**<0.001**
18:0	320.44±79.42	333.71±87.74	308.54±72.33	297.93±75.61	349.26±64.63	0.240	0.948	0.561	**0.132**
22:0	2.19±0.89	0.87±0.89	1.18±1.00	2.11±0.69	Bd	0.664	0.478	**<0.001**	-
24:0	Bd[1]	Bd	Bd	Bd	1.88±0.20	-	-	-	-
i-14:0	0.20±0.53	0.09±0.37	Bd	Bd	Bd	0.080	0.529	0.524	0.322

i-15:0	2.37±1.17	2.07±1.10	1.65±0.79	1.05±1.06	2.67±1.82	**0.002**	0.129	0.578	0.064
ai-15:0	3.23±1.04	3.15±1.06	2.75±0.74	2.10±1.13	5.09±1.82	**0.005**	0.189	0.272	**<0.001**
i-16:0	1.98±0.31	1.86±0.42	1.78±0.24	1.75±0.22	3.91±1.37	0.060	0.374	0.564	**<0.001**
i-17:0	7.57±1.81	7.27±2.00	6.00±1.46	5.01±1.53	9.47±2.90	**<0.001**	0.206	0.447	**<0.001**
ai-17:0	8.45±2.29	8.86±3.01	7.90±2.14	9.09±2.34	13.01±4.09	**0.019**	0.379	0.122	**<0.001**
14:1	1.00±0.90	1.14±1.34	0.81±1.02	0.75±1.02	4.07±2.02	0.295	0.895	0.712	**<0.001**
16:1	19.53±5.09	23.70±8.99	19.94±6.68	18.85±6.14	35.82±12.31	0.220	0.396	0.144	**<0.001**
17:1	3.73±3.19	2.42±0.78	3.90±2.01	2.85±0.76	13.57±3.27	0.569	0.022	0.794	**<0.001**
t9-18:1	3.77±0.98	3.73±1.31	3.87±1.21	3.33±1.48	5.41±1.56	0.650	0.374	0.449	**<0.001**
t11-18:1	42.34±12.60	48.71±19.44	54.54±21.73	45.91±19.26	73.36±23.80	0.333	0.816	0.123	**<0.001**
c9-18:1	672.16±202.04	742.11±243.18	676.69±184.39	625.41±189.58	816.06±177.73	0.293	0.862	0.260	**0.020**
c11-18:1	20.26±3.02	21.47±4.09	21.09±3.22	19.29±3.59	27.56±4.66	0.463	0.744	0.101	**<0.001**
18:2n-6	92.03±14.87	92.63±10.91	119.99±15.85	115.69±16.32	148.49±22.78	**<0.001**	0.712	0.520	**<0.001**
c9t11-18:2	18.73±6.56	20.65±10.89	22.45±9.01	18.64±9.39	35.78±13.93	0.717	0.687	0.227	**<0.001**
20:4n-6	37.05±11.16	31.46±8.40	32.29±4.44	30.12±3.68	36.85±5.09	0.139	0.051	0.399	0.385
22:2n-6	Bd	Bd	Bd	Bd	0.78±0.92	-	-	-	-
18:3n-3	46.43±8.18	49.35±6.95	61.21±7.61	58.16±11.93	84.05±16.65	**<0.001**	0.982	0.197	**<0.001**
20:5n-3	24.65±4.74	23.92±3.07	26.29±4.52	25.05±3.25	33.03±4.40	0.179	0.341	0.808	**<0.001**
22:5n-3	23.94±2.42	22.41±2.33	23.37±2.73	22.27±2.13	31.59±3.39	0.577	**0.037**	0.729	**<0.001**
22:6n-3	7.49±1.27	7.21±0.98	6.87±1.29	6.31±1.60	10.49±2.66	**0.027**	0.226	0.686	**<0.001**
BCFA[2]	23.82 ±6.24	23.33±7.17	20.09±4.91	16.24±5.76	34.14±11.12	**0.001**	0.210	0.290	**<0.001**
SFA[3]	855.57±208.51	947.21±268.02	866.63±208.80	821.99±232.32	1213.86±272.14	0.344	0.689	0.254	**<0.001**
MUFA[4]	762.79±215.22	843.23±270.32	780.85±209.32	716.39±218.14	975.88±210.20	0.361	0.893	0.227	**0.004**
PUFA[5]	250.32±26.87	247.64±16.91	292.57±33.95	276.23±39.51	381.08±59.96	**<0.001**	0.299	0.389	**<0.001**
EPA+DHA[6]	32.13±5.78	31.13±3.67	33.15±5.12	31.35±4.48	43.52±6.83	0.619	0.256	0.749	**<0.001**
n-6 PUFA sum[7]	129.09±24.14	124.07±17.49	152.38±18.45	145.81±18.49	185.35±26.47	**<0.001**	0.325	0.880	**<0.001**
n-3 PUFA sum[8]	101.54±14.58	103.73±12.11	117.74±13.52	111.77±17.69	159.18±24.96	**<0.001**	0.866	0.208	**<0.001**
LC n-3 PUFA[9]	56.60±7.38	55.02±5.81	57.76±7.40	54.66±6.44	75.11±9.41	0.191	**0.042**	0.946	**<0.001**
Unreported	187.44±23.04	190.99±21.78	177.62±21.78	174.03±25.66	223.00±41.88	**0.037**	0.992	0.557	**<0.001**
Total FA	2084.40±451.89	2241.72±557.81	2118.91±445.16	1989.71±497.95	2792.48±560.58	0.487	0.862	0.237	**<0.001**

1. bd: below detection threshold of 0.01 mg/100g raw meat.
2. BCFA =∑ i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
3. SFA = ∑ 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0, i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.

4. MUFA = ∑ 14:1, 16:1, 17:1, t9-18:1, t11-18:1, c9-18:1, c11-18:1, 20:1, 22:1, 24:1.
5. PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n6, 18:3n-3, 20:5n3, 22:5n3, 22:6n-3, CLAc9t11.
6. EPA+DHA= ∑ 20:5n-3, 22:6n-3.
7. n-6 PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n-6.
8. n-3 PUFA = ∑ 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.
9. LC n-3 PUFA = ∑ 20:5n-3, 22:5n-3, 22:6n-3.

3.3.2. Effect of diet

Higher content of 10:0, 15:0, i-15:0, ai-15:0, i-17:0, ai-17:0, 22:6n-3, BCFA; and lower content of 18:2n-6, 18:3n-3, PUFA, n-6 and n-3 PUFA were observed in loins from lambs grazed on perennial ryegrass than chicory ($P<0.05$; Table 3.4).

In Farm A, the wether lambs grazed on mixed pasture had greater proportion of c9-18:1, MUFA than wether lambs grazed on red clover, while the proportion of 18:2n-6, PUFA, n-6 PUFA, n-3 PUFA was lower in wether lambs grazed on red clover than mixed pasture ($P<0.05$; Table 3.5). In Farm C, the proportion of 10:0, 15:0, 17:0, i-15:0, ai-15:0, i-17:0, ai-17:0 and BCFA, SFA were greater in lambs grazed on perennial ryegrass than chicory ($P<0.05$; Table 3.7). The proportion of 18:2n-6, 18:3n-3, n-3 PUFA, n-6 PUFA and PUFA were greater in lambs grazed on chicory than perennial ryegrass ($P<0.05$; Table 3.7).

3.3.3. Interaction of diet and sex

The only interaction between diet and sex was observed in 22:0. The content and proportion of 22:0 was greater in wether than ewe for lambs grazed on perennial ryegrass, while the content and proportion of 22:0 was lower in wether than ewe for lambs grazed on chicory ($P<0.05$; Table 3.7).

3.3.4. Effect of age and age at slaughter

The loins from 4-month-old wether lambs had greater content of all identified SFA, MUFA, PUFA except 18:0 and 20:4n-6 than 6- to 8-month-old wether lambs that grazed on chicory or perennial ryegrass ($P<0.05$; Table 3.4). Highest content of n-6 (185 mg/100g,) and n-3 (159 mg/100g) fatty acids were observed in WEAN-W (Table 3.4). The combined EPA (20:5n-3) plus DHA (22:6n-3) content and long chain n-3 PUFA content was greater in WEAN-W lambs compared to GRASS-W, and CHIC-W lambs ($P<0.05$, Table 3.4).

The WEAN-W lambs had significantly greater proportion of 10:0, 12:0, 14:0, 15:0, 16:0, ai-15:0, i-16:0, ai-17:0, SFA, 14:1, 16:1, 17:1, c9t11-18:2, 18:3n-3, and lower proportion of 18:0, c9-18:1, MUFA, 20:4n-6 compared to GRASS-W and CHIC-W lambs ($P<0.05$, Table 3.7). 18:2n-6 and 18:3n-3 formed the highest proportion of PUFA in all lambs, and the MXME-W lambs had the lowest proportion of 18:2n-6 (2.46%) and 18:3n-3 (1.39%) among all production systems considered. The proportion of conjugated linoleic acid isomer c9t11-18:2 was greatest in WEAN-W lambs (1.25%), and lowest in MXME-C lambs (0.74%, Table 3.7).

Table 3.5 Fatty acid composition (percentage ± standard error of the means) of *m. longissimus thoracis* as a percentage of total fatty acids from lambs reared under different New Zealand commercial production systems in Farm A.

Fatty acid composition (percentage)	**Red clover**	**Red clover**	**Pasture**	**P-value**	
	Wether	**Ewe**	**Wether**	**Sex**	**Diet**
10:0	0.13±0.02	0.13±0.03	0.13±0.02	0.682	0.898
12:0	0.24±0.15	0.14±0.06	0.19±0.06	**0.014**	0.174
14:0	2.24±0.68	1.95±0.57	2.34±0.43	0.162	0.707
15:0	0.32±0.05	0.28±0.05	0.31±0.05	**0.011**	0.397
16:0	20.59±1.40	20.89±1.41	20.82±0.97	0.779	0.970
17:0	0.95±0.06	0.92±0.07	0.94±0.09	0.071	0.373
18:0	14.99±1.21	14.89±1.00	15.01±0.85	0.557	0.639
22:0	Bd[1]	Bd	Bd	-	-
24:0	0.072±0.04	0.071±0.03	0.056±0.01	0.958	0.169
i-14:0	Bd	Bd	Bd	-	-
i-15:0	0.09±0.05	0.07±0.04	0.09±0.02	0.302	0.600
ai-15:0	0.14±0.03	0.11±0.03	0.13±0.02	**0.022**	0.237
i-16:0	0.11±0.03	0.09±0.03	0.10±0.02	0.116	0.471
i-17:0	0.35±0.04	0.31±0.05	0.35±0.05	**0.013**	0.857
ai-17:0	0.37±0.07	0.34±0.06	0.39±0.05	0.209	0.359
14:1	0.06±0.04	0.05±0.04	0.07±0.02	0.530	0.291
16:1	0.97±0.15	0.96±0.12	1.00±0.11	0.756	0.687
17:1	0.47±0.14	0.51±0.05	0.52±0.05	0.237	0.224
t9-18:1	0.19±0.04	0.19±0.03	0.19±0.02	0.554	0.660
t11-18:1	2.78±0.88	2.65±0.72	2.40±0.56	0.578	0.109
c9-18:1	31.15±2.90	33.02±2.53	34.74±1.30	0.084	**<0.001**
c11-18:1	1.06±0.13	1.04±0.10	1.02±0.12	0.568	0.274
18:2n-6	4.72±1.42	4.57±1.04	3.63±0.63	0.658	**0.004**
c9t11-18:2	1.05±0.37	1.00±0.29	1.08±0.30	0.586	0.917
20:4n-6	1.77±0.72	1.62±0.46	1.45±0.45	0.411	0.099
22:2n-6	0.014±0.02	0.005±0.02	Bd	0.292	-
18:3n-3	2.71±0.67	2.59±0.57	2.13±0.29	0.507	**0.001**
20:5n-3	1.43±0.52	1.37±0.43	1.21±0.37	0.638	0.124

22:5n-3	1.30±0.45	1.23±0.36	1.11±0.27	0.550	0.089
22:6n-3	0.39±0.13	0.37±0.14	0.34±0.12	0.674	0.235
BCFA[2]	1.04±0.19	0.92±0.20	1.06±0.14	0.063	0.912
SFA[3]	40.58±1.92	40.19±1.85	40.86±1.44	0.261	0.714
MUFA[4]	36.67±2.71	38.42±2.14	39.94±1.30	0.083	**<0.001**
PUFA[5]	13.38±3.59	12.76±2.71	10.95±1.84	0.490	**0.011**
EPA+DHA[6]	1.82±0.64	1.74±0.57	1.55±0.48	0.641	0.138
n-6 PUFA sum[7]	6.50±2.06	6.20±1.42	5.08±1.02	0.543	**0.010**
n-3 PUFA sum[8]	5.83±1.71	5.56±1.37	4.79±0.97	0.546	**0.023**
LC n-3 PUFA[9]	3.12±1.08	2.97±0.91	2.66±0.74	0.551	0.101
Unreported	9.67±1.36	8.63±1.21	8.25±1.08	0.129	**0.019**
PUFA:SFA	0.33±0.09	0.32±0.07	0.27±0.05	0.658	**0.022**
n-6:n-3	1.11±0.11	1.12±0.09	1.06±0.09	0.917	0.170
Thrombogenic index 1	0.89±0.15	0.89±0.13	0.95±0.09	0.930	0.252
Atherogenic index 2	0.61±0.09	0.58±0.08	0.61±0.06	0.337	0.946

1. bd: below detection threshold of 0.01 mg/100g raw meat.
2. BCFA =∑ i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
3. SFA = ∑ 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0, i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
4. MUFA = ∑ 14:1, 16:1, 17:1, t9-18:1, t11-18:1, c9-18:1, c11-18:1, 20:1, 22:1, 24:1.
5. PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n6, 18:3n-3, 20:5n3, 22:5n3, 22:6n-3, CLAc9t11.
6. EPA+DHA= ∑ 20:5n-3, 22:6n-3.
7. n-6 PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n-6.
8. n-3 PUFA = ∑ 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.
9. LC n-3 PUFA = ∑ 20:5n-3, 22:5n-3, 22:6n-3.

Table 3.6 Fatty acid composition (percentage ± standard error of the means) of *m. longissimus thoracis* as a percentage of total fatty acids from wether and ewe lambs reared in Farm B.

Fatty acid composition (percentage)	**Pasture**	**Pasture**	**P-value**
	Wether	**Cryptorchid**	**Castration**
10:0	0.13±0.02	0.14±0.02	0.125
12:0	0.12±0.06	0.12±0.07	0.950
14:0	2.06±0.34	1.91±0.38	0.282
15:0	0.36±0.04	0.34±0.05	0.369
16:0	21.74±1.06	21.91±1.71	0.480
17:0	1.11±0.10	1.07±0.07	0.343
18:0	16.61±1.48	16.38±1.73	0.862
22:0	0.039±0.02	0.075±0.03	**<0.001**
24:0	Bd[1]	Bd	-
i-14:0	0.018±0.02	0.012±0.02	0.429
i-15:0	0.14±0.02	0.14±0.02	0.735
ai-15:0	0.17±0.02	0.15±0.03	0.072
i-16:0	0.07±0.02	0.08±0.04	0.068
i-17:0	0.39±0.03	0.40±0.04	0.234
ai-17:0	0.53±0.04	0.48±0.04	**0.002**
14:1	0.04±0.02	0.04±0.03	0.714
16:1	1.04±0.13	1.12±0.16	0.071
17:1	0.08±0.03	0.12±0.13	0.218
t9-18:1	0.20±0.01	0.19±0.02	0.247
t11-18:1	2.33±0.39	1.89±0.44	**0.005**
c9-18:1	37.04±2.08	36.17±2.64	0.476
c11-18:1	0.94±0.07	1.01±0.12	**0.023**
18:2n-6	2.46±0.51	2.71±0.65	0.165
c9t11-18:2	0.94±0.20	0.74±0.16	**0.003**
20:4n-6	1.04±0.24	1.18±0.38	0.162
22:2n-6	Bd	0.007±0.02	-
18:3n-3	1.39±0.24	1.52±0.31	0.129
20:5n-3	0.66±0.17	0.82±0.32	0.059

22:5n-3	0.68±0.14	0.82±0.26	**0.048**
22:6n-3	0.20±0.05	0.24±0.09	**0.047**
BCFA[2]	1.30±0.08	1.25±0.13	0.325
SFA[3]	43.56±1.86	43.28±2.30	0.921
MUFA[4]	41.72±2.12	40.57±2.44	0.293
PUFA[5]	7.36±1.29	8.04±1.95	0.185
EPA+DHA[6]	0.86±0.21	1.06±0.40	0.052
n-6 PUFA sum[7]	3.50±0.72	3.90±0.99	0.142
n-3 PUFA sum[8]	2.93±0.56	3.41±0.93	0.058
LC n-3 PUFA[9]	1.54±0.34	1.88±0.67	0.060
Unreported	7.36±0.55	8.12±1.24	**0.039**
PUFA:SFA	0.19±0.03	0.02±0.05	0.216
n-6:n-3	1.15±0.09	0.06±0.08	0.158
Thrombogenic index 1	1.14±0.09	0.05±0.15	0.366
Atherogenic index 2	0.62±0.05	0.03±0.08	0.940

1. bd: below detection threshold of 0.01 mg/100g raw meat.
2. BCFA =∑ i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
3. SFA = ∑ 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0, i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
4. MUFA = ∑ 14:1, 16:1, 17:1, t9-18:1, t11-18:1, c9-18:1, c11-18:1, 20:1, 22:1, 24:1.
5. PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n6, 18:3n-3, 20:5n3, 22:5n3, 22:6n-3, CLAc9t11.
6. EPA+DHA= ∑ 20:5n-3, 22:6n-3.
7. n-6 PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n-6.
8. n-3 PUFA = ∑ 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.
9. LC n-3 PUFA = ∑ 20:5n-3, 22:5n-3, 22:6n-3.

Table 3.7 Fatty acid composition (percentage ± standard error of the means) of *m. longissimus thoracis* from lambs reared under different New Zealand commercial forage production systems in Farm C.

Fatty acid composition (percentage)	**Pasture**		**Chicory**		**Wean**	**Non-orthogonal contrast2, P-value**			
	Wether	**Ewe**	**Wether**	**Ewe**	**Wether**	**Diet**	**Sex**	**Diet x Sex**	**Age**
10:0	0.15±0.02	0.16±0.03	0.14±0.03	0.14±0.03	0.23±0.05	**0.004**	0.614	0.355	**<0.001**
12:0	0.33±0.24	0.27±0.11	0.24±0.12	0.38±0.25	0.43±0.14	0.824	0.252	0.065	**0.006**
14:0	2.31±0.61	2.32±0.68	2.14±0.66	2.02±0.59	4.26±1.08	0.101	0.581	0.896	**<0.001**
15:0	0.34±0.09	0.32±0.07	0.29±0.06	0.27±0.06	0.46±0.09	**0.001**	0.188	0.824	**<0.001**
16:0	20.42±1.32	22.05±2.08	21.39±1.39	21.43±1.56	23.01±1.29	0.765	**0.047**	0.088	**<0.001**
17:0	0.99±0.07	0.96±0.09	0.91±0.08	0.88±0.10	0.95±0.05	**<0.001**	0.129	0.597	0.685
18:0	15.55±1.13	15.00±1.37	14.56±1.22	14.99±1.48	12.61±1.45	0.085	0.947	0.090	**<0.001**
22:0	0.112±0.05	0.062±0.05	0.063±0.06	0.124±0.05	Bd	0.607	0.927	**<0.001**	-
24:0	Bd[1]	Bd	Bd	Bd	0.076±0.02	-	-	-	-
i-14:0	0.012±0.03	0.004±0.02	Bd	Bd	Bd	0.086	-	-	-
i-15:0	0.13±0.07	0.11±0.06	0.09±0.03	0.05±0.05	0.10±0.03	**0.001**	0.082	0.770	0.755
ai-15:0	0.16±0.06	0.15±0.05	0.13±0.03	0.10±0.05	0.18±0.04	**0.003**	0.086	0.728	**0.039**
i-16:0	0.10±0.03	0.09±0.04	0.09±0.03	0.09±0.03	0.14±0.03	0.575	0.797	0.310	**<0.001**
i-17:0	0.37±0.09	0.33±0.08	0.29±0.07	0.25±0.05	0.33±0.06	**<0.001**	0.067	0.679	0.951
ai-17:0	0.41±0.08	0.40±0.09	0.37±0.06	0.31±0.06	0.46±0.07	**<0.001**	**0.042**	0.323	**0.006**
14:1	0.05±0.05	0.05±0.06	0.04±0.05	0.03±0.04	0.14±0.05	0.215	0.610	1.000	**<0.001**
16:1	0.94±0.10	1.04±0.24	0.92±0.19	0.94±0.14	1.26±0.22	0.119	0.209	0.456	**<0.001**
17:1	0.19±0.15	0.11±0.05	0.19±0.12	0.15±0.05	0.48±0.04	0.431	**0.020**	0.536	**<0.001**
t9-18:1	0.18±0.03	0.16±0.03	0.18±0.02	0.16±0.05	0.19±0.02	0.615	**0.028**	0.873	0.346
t11-18:1	2.08±0.59	2.19±0.60	2.53±0.78	2.24±0.60	2.62±0.65	0.113	0.439	0.284	0.187
c9-18:1	32.26±2.95	32.70±2.52	31.55±3.10	31.06±2.62	29.21±2.38	0.057	0.978	0.636	**<0.001**
c11-18:1	1.00±0.10	0.98±0.11	1.01±0.13	0.99±0.12	1.00±0.08	0.787	0.439	0.951	0.528
18:2n-6	4.66±1.34	4.42±1.46	5.85±1.43	6.03±1.33	5.41±0.81	**<0.001**	0.832	0.459	0.858
c9t11-18:2	0.91±0.28	0.92±0.38	1.03±0.29	0.90±0.31	1.25±0.31	0.538	0.332	0.419	**0.004**
20:4n-6	1.90±0.83	1.52±0.73	1.60±0.48	1.58±0.40	1.36±0.32	0.390	0.165	0.170	**0.022**

22:2n-6	Bd	Bd	Bd	Bd	0.029±0.03	-	-	-	-
18:3n-3	2.30±0.39	2.32±0.60	2.99±0.76	3.03±0.82	3.03±0.33	**<0.001**	0.804	0.925	**0.049**
20:5n-3	1.23±0.34	1.13±0.33	1.30±0.46	1.33±0.42	1.21±0.21	0.162	0.965	0.697	0.585
22:5n-3	1.21±0.30	1.06±0.30	1.14±0.28	1.18±0.34	1.16±0.20	0.740	0.705	0.348	0.846
22:6n-3	0.38±0.11	0.34±0.10	0.34±0.15	0.33±0.09	0.38±0.09	0.353	0.439	0.874	0.632
BCFA[2]	1.17±0.31	1.07±0.28	0.95±0.18	0.81±0.18	1.20±0.20	**<0.001**	0.052	0.919	0.121
SFA[3]	41.38±1.25	42.20±1.62	40.68±1.37	41.01±2.07	43.24±1.58	**0.007**	0.172	0.973	**<0.001**
MUFA[4]	36.76±2.48	37.26±2.71	36.48±2.99	35.62±2.96	34.89±2.04	0.104	0.788	0.475	**0.008**
PUFA[5]	12.59±2.76	11.70±3.17	14.24±3.29	14.37±2.98	13.83±1.62	**0.004**	0.629	0.503	0.752
EPA+DHA[6]	1.61±0.44	1.47±0.42	1.64±0.59	1.66±0.50	1.59±0.28	0.365	0.829	0.728	0.767
n-6 PUFA sum[7]	6.56±2.11	5.94±2.14	7.44±1.87	7.62±1.64	6.80±1.08	**0.008**	0.551	0.329	0.538
n-3 PUFA sum[8]	5.12±1.06	4.85±1.30	5.77±1.60	5.86±1.58	5.78±0.69	**0.014**	0.981	0.775	0.381
LC n-3 PUFA[9]	2.82±0.73	2.53±0.71	2.78±0.87	2.83±0.83	2.75±0.45	0.965	0.437	0.343	0.545
Unreported	9.28±1.10	8.84±1.44	8.60±1.38	9.00±1.24	8.04±0.64	0.438	0.880	0.285	0.019
PUFA:SFA	0.31±0.07	0.28±0.07	0.35±0.08	0.35±0.07	0.32±0.04	**0.002**	0.540	0.552	0.682
n-6:n-3	1.30±0.36	1.23±0.26	1.30±0.12	1.32±0.12	1.17±0.11	0.442	0.388	0.265	0.060
Thrombogenic index	0.95±0.09	0.98±0.12	0.89±0.12	0.90±0.12	0.97±0.07	**0.008**	0.388	0.655	0.285
Atherogenic index	0.62±0.06	0.66±0.08	0.61±0.07	0.61±0.08	0.86±0.13	0.135	0.408	0.444	**<0.001**

1. bd: below detection threshold of 0.01 mg/100g raw meat.
2. BCFA =∑ i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
3. SFA = ∑ 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0, i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, ai-17:0.
4. MUFA = ∑ 14:1, 16:1, 17:1, t9-18:1, t11-18:1, c9-18:1, c11-18:1, 20:1, 22:1, 24:1.
5. PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n6, 18:3n-3, 20:5n3, 22:5n3, 22:6n-3, CLAc9t11.
6. EPA+DHA= ∑ 20:5n-3, 22:6n-3.
7. n-6 PUFA = ∑ 18:2n-6, 20:4n-6, 22:2n-6.
8. n-3 PUFA = ∑ 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.
9. LC n-3 PUFA = ∑ 20:5n-3, 22:5n-3, 22:6n-3.

3.3.5. Nutrition indices

A lower PUFA:SFA ratio was observed in meat from MIX-W lambs than REDC-W lambs slaughtered at 6- to 8-month-old ($P<0.05$; Table 3.5). Lambs grazed on chicory had greater lower PUFA:SFA ratio and lower thrombogenic index than lambs that grazed on perennial ryegrass. The 4-month-old per-weaning wether lambs had greater atherogenic index than 6- to 8-month-old wether lambs grazed on perennial ryegrass or chicory (Table 3.7). The greatest EPA plus DHA (43.52mg/100g raw meat) content was observed in the meat of pre-weaning lambs (Figure 3.1, Table 3.4). While the lowest EPA plus DHA (27.77mg/100g raw meat) content was observed in the meat of 12-month-old Merino lambs (Figure 3.1, Table 3.4). The average EPA+DHA values of meat from all lambs used in the current study could be considered a "source" of n-3 fatty acids by the Australia and New Zealand reference standard (Food Standards Australia New Zealand, 2013), but only the meat from lambs which were slaughtered at weaning could be considered as a "good source" of n-3 fatty acids (Food Standards Australia New Zealand, 2013, Figure 3.1). Both meat from MIX-W and WEAN-W lambs were on average, able to be considered as a "source" of n-3 fatty acids by European Union regulations (Commission Regulation of European Union, 2010).

3.3.6. Principal Component Analysis

To visualise differences in fatty acid content (mg/100 g) among all 10 lamb production groups, a PCA biplot is presented in Figure 3.2. The first dimension (Dim1) of the PCA explained 50.3% of the total variation in fatty acid and volatile composition, and the second dimension (Dim2) explained 22.0% of the total variation. Dim1 separated the animal groups based on their total fatty acid content, where MIX-W, WEAN-W, MXME-W and MXME-C are positioned on the left side of the plot, opposite to CHIC-W, CHIC-E, GRASS-W and GRASS-E lambs, with the first group

having greater FA content (SFA, BCFA, MUFA and PUFA) than the second group. Dim2 separated the animal groups based on the types of fatty acids. MIX-W, WEAN-W, REDC-W and REDC-E showed greater content of PUFA than MXME-W and MXME-C. CHIC-W, CHIC-E, GRASS-W and GRASS-E were intermediate for their PUFA content.

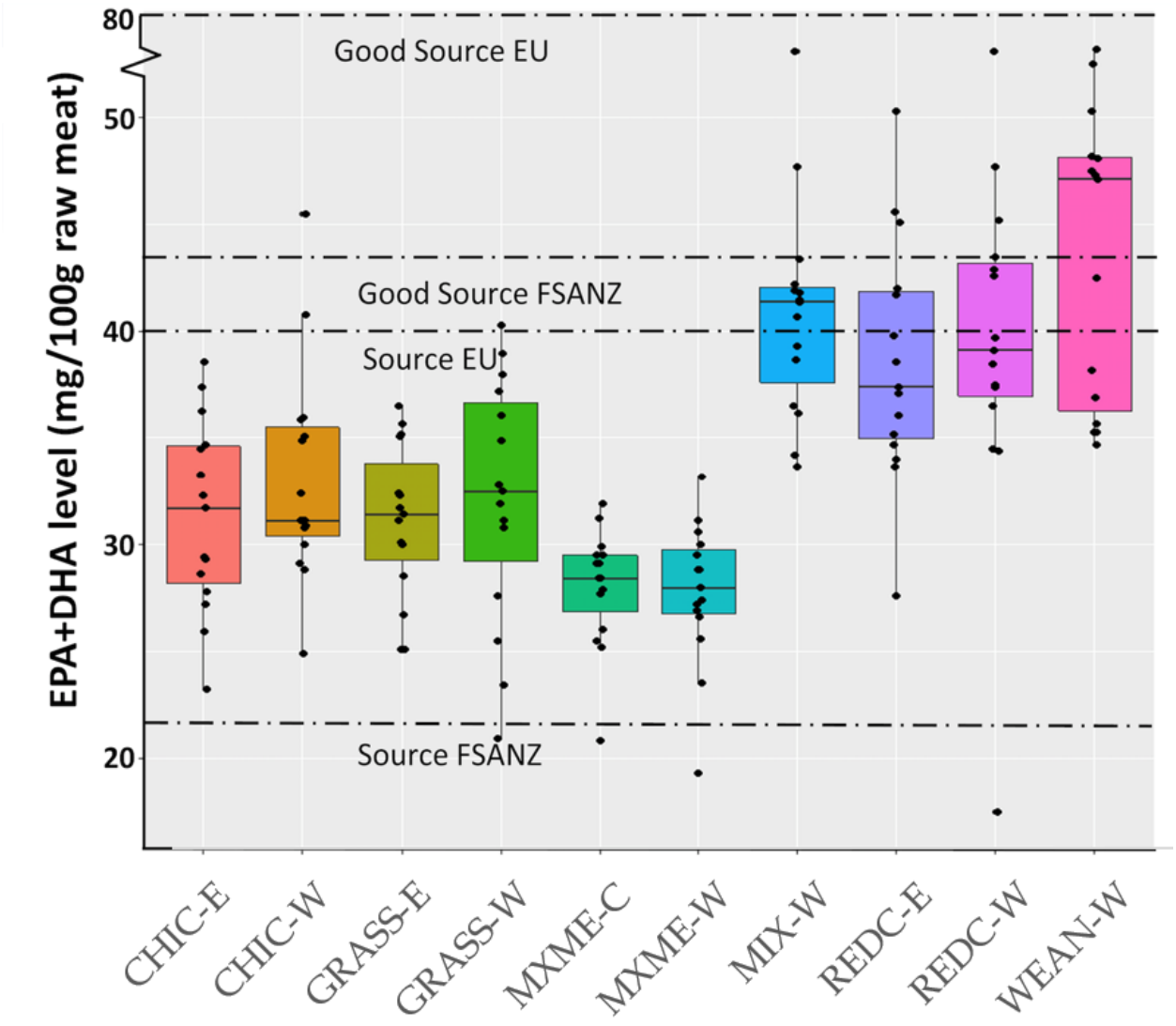

Figure 3.1 Box plot of EPA+DHA content of *m. longissimus thoracis* for each lamb group. Horizontal dash lines indicate minimum values for meat to be classified as a source of omega-3 fatty acids (22 mg/100 g muscle) or a good source (44 mg/100 g muscle) according to food standards of Australia and New Zealand (FSANZ), and minimum values to be classified as a source (40 mg/100 g muscle) and a good source (80 mg/100 g muscle) of omega-3 fatty acids according to the Commission Regulation of European Union. The production systems included: 4-month-old wether lambs of a composite breed at weaning

(WEAN-W), 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing perennial ryegrass-based pasture (GRASS-W and GRASS-E, respectively), 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing chicory (CHIC-W-6-8 and CHIC-E, respectively), 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing red clover (REDC-W and REDC-E, respectively), 6- to 8-month-old wether lambs of composite breed that had been grazing a mixture of perennial ryegrass, red- and white-clover mixed pasture (MIX-W) and 12-month-old wether and cryptorchid Merino lambs that had been grazing a mixed pasture (MXME-W and MXME-C, respectively).

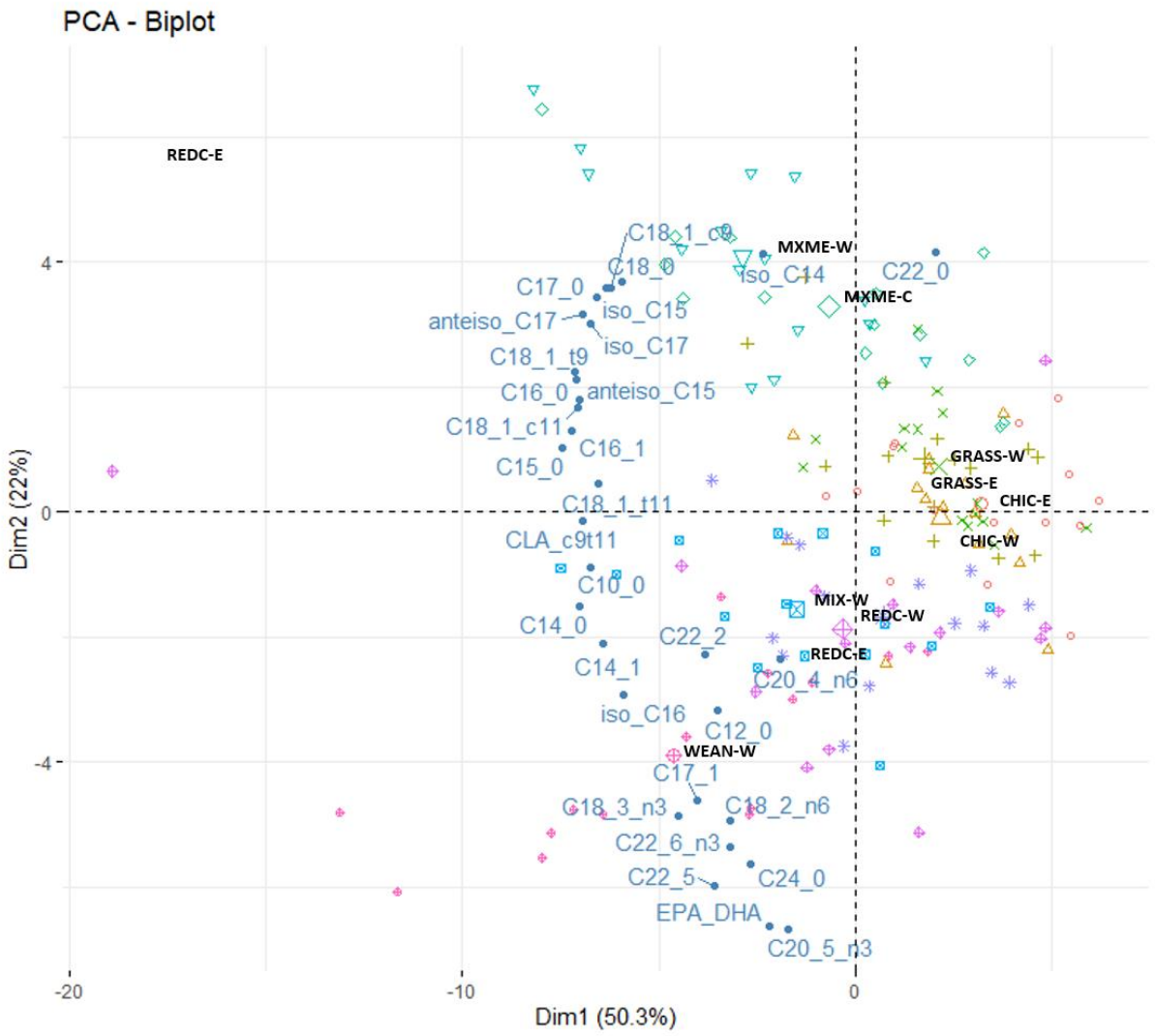

Figure 3.2 Principal component analysis (PCA) biplot of fatty acids content (mg/100g) in raw lean meat as affected by commercial lamb production systems in New Zealand. The production systems included: 4-month-old wether lambs of a composite breed at weaning (WEAN-W), 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing perennial ryegrass-based pasture (GRASS-W and GRASS-E, respectively), 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing chicory (CHIC-W and CHIC-E, respectively), 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing red clover (REDC-W and REDC-E, respectively), 6- to 8-month-old wether lambs of composite breed that had been grazing a mixture of perennial ryegrass, red- and white-clover mixed pasture (MIX-W) and 12-month-old wether and cryptorchid Merino lambs that had been grazing a mixed pasture (MXME-W and MXME-C, respectively).

3.4. Discussion

The main objectives of the present study were to evaluate the fatty acid profile of lamb loins from diverse forage-based production systems in New Zealand and understand the relationships between production systems and the fatty acid profile of lamb meat. Our results indicated that both the content (mg/100g raw meat) fatty acids and proportion of fatty acids in total lipid were affected by the forage production systems evaluated in this study.

3.4.1. Effect of sex, diet, and age at slaughter

Unlike tenderness and juiciness of meat, which can be manipulated via post slaughter process, the fatty acid composition of lamb meat is fixed at the point trucking to the meat processor. Therefore, on farm factors are vital to obtain favourable fatty acid profiles in lamb meat. Lambs slaughtered at weaning clustered with 12:0 and 14:0 in same quadrant of the PCA biplot (Figure 3.2). Which reflects milk intake because studies looking at fatty acids of sucking lambs observed a higher proportion of 12:0 (~3.99%), 14:0 (~10.17%) and 16:0 (~25.10%), and lower proportion of 18:0 (~8.85%, Kęszycka et al., 2013). Bas and Morand-Fehr, 2000 also observed that fatty acid composition of tissues from milk fed lambs was characterised by lower percentages of 18:0 and higher percentages of 14:0 and 16:0. Lambs slaughtered at weaning were associated with higher content of PUFA (Figure 3.2). The 12-month-old Merino lambs had the lowest PUFA and highest SFA, BCFA, and total fatty acid content in the current study (Figure 3.2). Similarly, a negative relationship between PUFA proportion and total lipid content has been reported in a number of bovine and ovine studies (Kazala et al., 1999, Salvatori et al., 2004). In older animals, the PUFA proportion of meat will be lower than younger animals, because the neutral lipids, which has lower proportion of PUFA increased more rapidly during fattening (Warren et al., 2008; Jerónimo et al., 2011). Phospholipid concentrations have been reported to increase slowly as the total fat content

of meat increased (Warren et al., 2008, Jerónimo et al., 2011). The content of linolenic acid (18:3n-3), an important precursor of DHA and EPA (Kitessa et al., 2001) was significantly greater in lambs slaughtered at weaning than 6- to-8-month-old lambs grazed on chicory or perennial ryegrass. Although green pasture or grass can provide higher levels of 18:3n-3 and grain feeds or concentrates provide higher levels of 18:2n−6 (Ponnampalam et al., 2014), subtle differences were observed between pasture diets. It is therefore difficult to explain the greater content and percentage of 18:3n-3 from chicory fed lambs than perennial ryegrass fed lambs because slightly greater herbage 18:3n-3 content (g/100 g) in perennial ryegrass than chicory were reported in both New Zealand (Mangwe et al., 2020) and Australia (Muir et al., 2014). This might be explained by less biohydrogenation took place in rumen when lambs grazed on chicory than perennial ryegrass. Mangwe et al., (2020) reported that milk produced from cows grazing chicory contained greater proportions of omega-3 fatty acids than that from cows grazing on perennial ryegrass, despite a lower omega-3 fatty acid content in the chicory herbage.

It was not surprising that the proportion of c9-18:1 was lowest among lambs slaughtered at weaning and greatest in Merino lambs slaughtered at 12-month-old in the current study. This was likely due to an increase in the activity of stearoyl CoA desaturase on c9-18:1 formation as lambs became older (Wood et al., 2008). This finding was in agreement with Velasco et al. (2001) who reported that the proportion of c9-18:1 and MUFA in the meat from suckling lambs (14-28 kg live weight) was increased along with the state of fatness. In the current study, only BCFA with 14 or greater carbons was identified. Ai-17:0 and i-17:0 were the dominant BCFA found across all groups of meat, with the highest concentrations observed in MXME-W lambs. Volatile fatty acids such 4-methyloctanoic, 4-ethyloctanoic, and 4-methylnonanoic have been reported to be responsible for mutton flavour at low flavour threshold of 0.02–0.6 μg/g, 0.00043–0.0018 μg/g

and 0.65–2.4 μg/ g respectively (Salvatore et al., 2007, Teng et al., 2018). None of these branched chain fatty acids were detected in the current study. Branched-chain fatty acids primarily accumulate in subcutaneous fat (Watkins et al. 2010), which are often below the detection threshold of SPME method in lean meat (Brennand and Lindsay, 1992). The accumulation of BCFA may be related to animal age as Watkins et al. (2014) reported that fat samples from lamb carcasses had lower 4-methyloctanoic and 4-ethyloctanoic concentrations and a higher concentration of 4-methylnonanoic acids in comparison with hogget and mutton.

The diet of the animal, especially the comparison of pasture and concentrate, is generally considered to be the most important environmental factor affecting the proportion of intramuscular fatty acid (Raes et al., 2004). In the current study, however, compared to the differences observed between lambs slaughtered at different ages, where content differences between almost all identified fatty acids were observed, the fatty acid profile difference between pasture diets was small. The limited diet effect was expressed in both dim1 and dim2 in PCA plot, with lambs that grazed chicory and perennial ryegrass in one cluster on the right side, and lambs that grazed on red clover and mixed pasture in the other cluster (Figure 3.2). In general, dim1 indicated that the meat of lambs that grazed red clover or mixed pasture located in the middle of the plot had slightly greater total fatty acids content than perennial ryegrass or chicory grazed lambs located on the right side of plot. Lambs that grazed red clover had higher content of PUFA than lambs that grazed perennial ryegrass despite having similar or slightly greater total fatty acid content in the current study. This was in agreement with Scollan et al. (2006) who reported that the proportions of PUFA in meat were increased when steers were fed silage comprised of red clover than perennial ryegrass due to the inhibition of lipolysis in clover by the plant enzyme polyphenol oxidase (Lee et al., 2004). Lambs that grazed on chicory had lower percentage of BCFA and SFA but greater

percentage of MUFA than lambs grazed on perennial ryegrass. The rumen digesta concentration of PUFA increased when cows grazed on chicory rather than perennial ryegrass, which corresponded with lower rumen pH and lower concentrations of biohydrogenation end-product stearic acid observed in rumen for chicory fed cows (Mangwe et al., 2020). It is therefore reasonable to postulate to chicory diet may affect the rumen fermentation and biohydrogenation of lamb.

No sex or castration effect was found on the fatty acid when comparisons were made between ewe, wether or cryptorchid lambs fed with same diet in the current study. This was not surprising given that the ewe and wether lambs had similar carcass characteristics (Chapter 2) and total intramuscular fat concentration. The influence of sex on fatty acid composition is mainly due to differences in the amount of adipose tissue, with females depositing more fat in the carcass than males (Horcada et al., 1998; Diaz et al., 2003). Some studies have also suggested that the influence of the amount of adipose tissue is small in young lambs (Hopkins and Mortimer, 2014). The PCA plot also indicated that the effect of sex or castration on fatty acid content profiles was not significant. Wether and ewe lambs or wether and cryptorchid lambs that had the same diet tended to cluster together.

3.4.2. Nutritional quality: fatty acid ratios and indices

Excessive intakes of n-6 PUFA together with high n-6:n-3 ratios are commonly found in modern Western diets and have been associated with the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases (Simopoulos, 2008). A dietary n-6:n-3 ratio below 4.0 has been reported to reduce total cardiovascular disease mortalities by up to 70% after two years (De Lorgeril et al., 1994). All lamb production systems

that considered in the current study resulted in lamb meat with a mean n-6:n-3 ratio below 1.5. This is similar to that reported for forage reared lambs in Uruguay (Díaz et al. 2005), but lower than for lambs fed concentrates (Gabriella et al. 2015). The n-6:n-3 ratio is highly influenced by the fatty acid composition of the diet fed to the animal (Raes et al., 2004). Forage reared lambs have a lower n-6:n-3 ratio than those fed concentrates, as 18:3 n-3 is the major fatty acid formed in plant lipids, while 18:2 n-6 predominates in concentrate diets (Díaz et al., 2005).

In the current study, the PUFA:SFA ratio in meat from all production systems was in the range of 0.17-0.35 which is below the recommended value of ≥0.45 for obtaining health benefits (Department of Health United Kingdom, 1994). In human diets, a high SFA intake has been associated with an increase in plasma cholesterol and greater risk of coronary heart disease (World Health Organization, 2008). However, not all saturated fatty acids have the same cholesterol-raising potential (Hunter et al., 2010). Higher amounts of 18:0 in the diet do not elevate plasma low-density lipoprotein-cholesterol because it is poorly digested and can be easily desaturated to c9-18:1 (Bonanome and Grundy 1988). Thus, the atherogenic index and thrombogenic index have been introduced to take into account of the intake of individual fatty acids. Low values of the atherogenic index and thrombogenic index are recommended for a healthy diet (Vacca et al., 2008). In the current study, the greatest atherogenic index of meat from 4-month-old weaning lambs among all production systems was due to the greater proportions of 14:0 and 16:0 fatty acids, whereas the greatest thrombogenic index for 12-month-old Merino lambs was due to their lower proportion of PUFA. The atherogenic index range observed in the current study was similar to lambs reared under extensive conditions and slaughtered at 64 days of age in the study of Salvatori et al. (2004). The thrombogenic index range observed in the current study was similar to previously

reported values for lamb, which was slightly greater than the index reported for pork but lower than milk and cheese (Ulbricht and Southgate, 1991).

The health benefits obtained by human consumption of meat lipids have been associated with LC-n-3-PUFA, mainly EPA and DHA. In the current study, 100 g of fresh lamb muscle could provide 28 to 44 mg EPA plus DHA with the greatest concentration coming from the lambs slaughtered at weaning. Ponnampalam et al. (2014) and Fowler et al. (2019) have reported similar EPA plus DHA content in meat from lambs grazed pasture as seen in the current study. The EPA plus DHA content and proportion observed in the current study, however, were greater than values reported for lambs fed a concentrate diet (Carvalho et al., 2015) or even when concentrate-fed lambs were supplemented with linseed (Realini et al., 2017). However, if lamb meat in the current study is considered the only resource of EPA and DHA, more than 500g of lamb consumption per day is needed in order to reach the nutrition requirement.

Meat from almost all lambs used in the current study could be considered a "source" of n-3 fatty acids by the Australia and New Zealand reference standard (Food Standards Australia New Zealand, 2013), but only the meat from lambs which were slaughtered at weaning could be considered as a "good source" of n-3 fatty acids. However, the potential to aim for nutritional claims on EPA plus DHA is limited by total SFA plus trans-fatty acids in Australia and New Zealand and would depend on the nutritional guidelines and regulations of the export market destinations. When the European standards are considered (Commission Regulation of European Union, 2010), only meat from lambs slaughtered at weaning or finished on mixed pasture could be considered as a "source" of n-3, with none of the production systems providing meat that achieved the reference standard of a "good source" of n-3 fatty acids.

The proportion of CLA isomer (c9t11-18:2) in loin meat samples in the current study were similar to the previous reported values for loin muscle of commercial lambs (Fowler et al., 2019). The CLA isomer c9t11 found in meat has been associated with anticarcinogenic, antiatherogenic and antidiabetic properties (Geay et al., 2001). Current nutrition guidelines do not offer a dietary reference value (Commission Regulation of European Union, 2010) as the potential health benefits in humans associated with CLA intake remain a topic of debate.

3.5. Conclusions

Finishing lambs for a longer period, up to 6- to 8-months after weaning in New Zealand forage systems could result in a lower proportion of SFA in meat as well as a lower atherogenic index compared to younger 4-month-old lambs. Chicory diet increased the proportion of PUFA and decreased the proportion of SFA and BCFA in lean lamb meat compared to perennial ryegrass diet. Further investigation is recommended to ascertain the forage effects on sheep ruminal fermentation characteristics and biohydrogenation. There was generally no sex or castration effects on fatty acid profile of lamb meat. The combined EPA plus DHA content in muscle and the proportion of lambs that met the eligibility criteria claims of EPA plus DHA content were largely determined by production systems. Lambs slaughtered at weaning had the greatest content of EPA plus DHA in the loin, and there is some potential for meat from forage-based lambs to target a premium market by promoting lamb as “healthy” by achieving n-3 fatty acid claims. However, further research is needed to examine if the effect of production systems seen in the current study represents the trends in the wider population of New Zealand lambs and whether these fatty acid composition data are consistent across years.

Chapter 4

Volatile profile of *Longissimus thoracis* from commercial lambs reared in different forage-based production systems

This chapter formed part of the following publication:

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Abstract

Animal production factors can affect the volatile profile of lamb meat. The fatty acid and volatile composition of the *Longissimus thoracis* was evaluated from 150 lambs from 10 groups of commercial lambs differing in age, sex, diet and breed from three farms, which represent typical forage lamb production systems in New Zealand. Volatiles were extracted from the headspace of raw lean meat and 36 volatile compounds were identified. The abundance of 1-pentanol, 1-heptanol, 1-octanol, 2-heptanone, butyrolactone, 2-ethylfuran and Z-2-octene were greater in meat from lambs grazed on mixed pasture than red clover, while the abundance of dimethyl sulfone and acetoin were lower in meat from lambs grazed on mixed pasture than red clover. Chicory diet increased the abundance of hexanoic acid, 1-butanol, 1-penten-3-ol, 1-pentanol, 1-hexanol, 1-octen-3-ol, 1-heptanol, 1-octanol, 2-octen-1-ol, hexanal, octanal, nonanal, 2-pentanone, acetoin and dimethyl sulphide in raw lamb meat compared to perennial ryegrass diet. The only sex effect was observed from lambs grazed on red clover, where the abundance of 1-heptanol, 1-octanol, hexanal, octanal, and nonanal were greater in meat from wethers than ewes. Beta-pinene that accumulate from the forage diets was less abundant in meat from 4-month-old pre-weaning lambs than 6-month-old lambs grazed on chicory or perennial ryegrass.

4.1. Introduction

Flavour is an important aspect for the overall acceptability of meat products, and is considered an important palatability characteristic of cooked sheep meat (Hopkins et al., 2005; Phelps et al., 2018; Thompson et al., 2005). The liking for sheep meat flavour is not universal. Prescott et al., (2001) indicated the presence of volatile branched-chain fatty acids and skatole as one cause of rejection of lamb for Japanese women, while Frank et al., (2016) suggested that up to a certain threshold concentration, the total branched-chain fatty acids play a positive role in defining lamb flavour and acceptance for Chinese and non-Chinese Australian consumers. Pasture-raised lamb dominates New Zealand production systems and has been associated with undesirable barnyard and faecal flavour and odour compared to lamb that is grain fed (Young et al., 2003). The rejection of meat from ruminants finished on pasture is generally a consequence of the consumer being accustomed to the flavour of meat from animals raised on concentrate- or mixed ration-based diets (Young et al., 2003). Although both taste and olfactory are involved in the sensation of flavour, flavour is largely a consequence of volatile chemicals that are perceived in the olfactory epithelium at the back of the nose (Farmer, 1994). Volatile profiles of sheep meat can help identify potential differences in flavour for animals fed different diets and can help reveal the acceptability of meat for to different markets.

Although many volatile compounds responsible for meat flavour arise from heat-induced reactions during cooking, considering volatiles present in raw meat can remain after cooking and affect flavour perception (Gravador et al., 2015). The oxidation derivatives of polyunsaturated fatty acids (PUFA) contribute to the pastoral flavour in meat from ruminant animals (Priolo et al., 2001). Lipid oxidation in meat occurs in phases, where the primary phase involves the removal of hydrogen from a methylene group, and the secondary phase involves the decomposition of

hydroperoxides and leads to the formation of volatile compounds including some aldehydes, alcohols and ketones (Resconi et al., 2013). In raw meat, 2-nonenal was reported as the most potent volatile from oxidation of linoleic acid after 24 h storage at 22-24°C; hexanal was the most potent volatile after 48 h; and hexanal and 2-octenal were the top two potent volatiles after 72 h (Calkins and Hodgen, 2007).

Lamb flavour and volatiles from raw lamb meat have been identified to be influenced by on farm factors including age at slaughter, gender or castration, and diet (Watkins et al., 2013). As a sheep becomes older, the meat becomes more strongly flavoured (Watkins et al., 2014) and the consumer liking scores decline (Hopkins et al., 2006). Lamb was reported to have a lower concentration of 4-ethyloctanoic than mutton, with hogget intermediate and female lambs had a lower 4-ethyloctanoic concentration than male lambs (Watkins et al., 2014). The animal's diet can have direct and indirect effects on meat sensory properties. Directly, plant-derived volatiles such as alpha- and beta-pinene can be transferred into the meat (Chevance and Farmer, 1999; Priolo et al., 2004). Elmore et al., (2005) reported that dietary supplements (linseed oil, fish oil, protected lipid supplement, and marine algae) could alter fatty acid and volatile profile in cooked lamb loin meat.

Commercial production systems for finishing lambs in New Zealand can include different diets, ages at slaughter and sex classifications and suggests that there is potential for variation in volatile profile of the lamb meat. With a better understanding of the volatiles of lamb that has come from different commercial finishing operations it may be possible to use the information to modify forage-based production systems to control sheep meat flavour or designate meat from specific farms to meet the requirements of various consumers. The objective of this study was to evaluate

if there was a difference in the raw meat volatile profiles of lambs from different forage-based production systems in New Zealand.

4.2. Materials and methods

4.2.1 Treatments

To encompass a range of forage-based production systems, 10 groups of lambs (Table 4.1) were sourced from three commercial farms north of Invercargill New Zealand and processed at Alliance Group Ltd. These included 6 to 8-month-old wether and ewe lambs of a composite breed that had been grazing red clover, 6 to 8-month-old ewe lambs of a composite breed that had been grazing perennial ryegrass, red clover and white clover mix pasture (Farm A); 12-month-old, wether and cryptochid Merino lambs that had been grazing a mixed pasture (perennial ryegrass and white clover mix followed by fescue, red and white clover and plantain mix in the 2 weeks prior to slaughter; Farm B); 6 to 8-month-old wether and ewe lambs of a composite breed that had been grazing a predominantly Italian and perennial ryegrass, and red and white clover mix pasture; 6 to 8 month old wether and ewe lambs of a composite breed that had been grazing chicory and; 4-month-old wether lambs of a composite breed at weaning that had grazed a mixed pasture with their dam (Farm C).

4.2.2. Sample acquisition

Lambs were slaughtered within 24 h after leaving the farm following standard practice for commercial processing. The *Longissimus thoracis* was removed with the fat-cap from the left side of each carcass and then vacuum packed and chilled at −1.5°C for 21 days followed by storage at −20°C until further analysis. More details about slaughter and post slaughter processing were described previously in Chapter 2.

Table 4.1 Production systems of animals (n=15) selected according to the pre-slaughter factors (age, sex, diet, breed) included in the study.

Production systems	Approximate age at slaughter (months)	Sex	Finishing diet[1]	Breed[2]	Farm	Distance to meat plant (km)
REDC-W	6-8	Wethers	Red Clover	Perendale × LambSupreme	A	80
REDC-E	6-8	Ewes	Red Clover	Perendale × LambSupreme	A	80
MIX-W	6-8	Wethers	Pasture	Perendale × Romney	A	80
MXME-W	12	Wethers	Pasture	Merino	B	250
MXME-C	12	Cryptochids	Pasture	Merino	B	250
WEAN-W	4	Wethers	Pre-weaning	Composite	C	100
GRASS-W	6-8	Wethers	Pasture	Composite	C	100
GRASS-E	6-8	Ewes	Pasture	Composite	C	100
CHIC-W	6-8	Wethers	Chicory	Composite	C	100
CHIC-E	6-8	Ewes	Chicory	Composite	C	100

[1] Animal diet: Pre-weaning, suckled and grazing mothers' diet of a chicory (Cichorium intybus) and red clover (Trifolium pratense) mix;
Farm A-Pasture, predominantly Italian and perennial ryegrass (Lolium perenne) and red and white (Trifolium repens) clover mix;
Farm B-Pasture, permanent pasture, perennial ryegrass, red clover and white clover mix;
Farm C-Pasture, ryegrass and white clover mix followed by fescue (Lolium arundinaceum), red and white clover and plantain (Plantago lanceolata) mix during the last 2 weeks.
[2] Composite: Perendale, Texel, Finnish Landrace and Romney genetics; LambSupreme: lean-selected Poll Dorset, Wiltshire, Romney x Dorset, Coopworth, Texel, and high-growth Romney.

4.2.3. Volatile compound analysis

The samples for volatile analysis were analysed in daily batches with 10 samples in each batch. One loin from each production system was randomly chosen for each daily batch. From each loin, duplicate samples of 4 g of frozen lean meat were removed as 5 mm cores and placed in the bottom of a 20-mL solid-phase microextraction (SPME) vial. Samples were kept at 4°C overnight until analysis. Internal standard (50 µL, 1.25 mg/L fenchol in water, 99 % purity, Sigma-Aldrich, Madrid, Spain) was added to a 250 µL insert vial sitting next to the meat within the SPME vials. To minimise the chance of volatile compound changes, samples were held in the auto sampler tray for no longer than 2.5 h. To minimise analysis sequence effects, samples were analysed using a balanced random order, and blanks (empty vials) were also run every day to check background signals.

The vial was equilibrated at 37°C for 5 min in the automated sample preparation unit. Following equilibration, a 50/30 µm Divinylbenzene-Carboxen-Polydimethylsiloxane (DVB/CAR/PDMS) fibre of the auto sampler (PAL RSI 85, Switzerland) was exposed to the headspace of the unstirred sample for 30 min at 37°C and then desorbed directly in the injection port of a 6890N gas chromatograph coupled to a 5975B VL mass spectrometric detection system (Agilent Technologies, Inc, Santa Clara, USA). Desorption occurred for 5 min at 240°C; 2 min in split-less mode followed by 3 min with a purge flow of 60mL/min. Helium was the carrier gas at a flow rate of 1.2 mL/min. A ZB-WAX capillary column (Phenomenex, USA) of 60 m × 0.32 mm I.D. × 0.5 µm film thickness was used for the separation. The oven temperature was initially 50°C for 2 min, then raised by 10°C/min to 240°C and held at this temperature for 10 min. After desorption, the fibre was cleaned for 2 min at 270°C. For mass spectrometry the transfer line temperature was 230°C and the trap temperature was 150°C with an emission current of 34.61 µA.

The global run time was recorded in full scan mode (m/z 29–300 mass range) and the chromatographic data were analysed by MSD Chemstation (version F.01.012317).

Compound identification was carried out by spectra comparison using the Wiley7Nist05 Library (Wiley and Sons Inc., Germany) supported by linear retention indexes (LRI) relative to a series of alkanes (C7-C26) compared to 11th edition NIST Mass Spectral Library. Due to some selected analytes coeluting with other compounds, the selected masses were used for integration. The integrated areas of the selected compound ions were divided by the area of the internal standard ion (m/z 81) to give relative abundances.

4.2.4. Statistical analysis

An analysis of variance (ANOVA) was performed using a general linear model in Genstat 18.1.0 (VSN International Ltd, UK). Sex (wethers vs. ewes) and castration status (wethers vs. cryptorchids) were included in the model as fixed effects for Farm A and B, respectively. Diet was included in the model as fixed effect for wether lambs in farm A (red clover vs pasture). For Farm C, pre-planned non-orthogonal contrasts were used to compare means from 6–8 months old lambs fed pasture vs. chicory (diet) and for wethers vs. ewes (sex) and their interaction. In addition, animal age at slaughter for wether lambs (4 vs. 6–8 months old) was contrasted. Differences among least-square means were assessed according to Tukey posthoc test and were considered significant at a probability level of <0.05.

In order to summarise the relative differences amongst samples in relation to their overall volatile profiles, a Principal Component Analysis (PCA) was performed using the relative abundance of all identified volatiles and SFA, MUFA, PUFA, LC-PUFA, BCFA, and n-3, n-6

fatty acids identified in Chapter 3 using “factoextra” package in RStudio (Version 1.2.5001) for all 10 production systems considered.

4.3. Results

4.3.1. Sex and castration effects

Sex effect on abundance of volatiles was only observed in Farm A (Table 4.2), and no castration (Table 4.3) or sex (Table 4.4) effect on abundance of volatiles were observed in Farm B or C. The top three most abundant volatile acids by peak area were acetic acid, butanoic and hexanoic acid. In Farm A, 1-heptanol, 1-octanol, and hexanal, octanal, nonanal were observed to be more abundant in meat from wether lambs than ewe lambs ($P<0.05$; Table 4.2). While the relative abundance of Z-2-Octene was greater in the meat of ewe lambs than wether lambs that grazed on red clover ($P<0.05$; Table 4.2).

4.3.2. Diet effects (Red clover vs mixed pasture)

In Farm A, 1-pentanol, 1-heptanol, 1-octanol, 2-methylbutanal, 2-heptanone, butyrolactone, Z-2-octene, 2-ethylfuran were more abundant from the meat of 6 to 8-month-old lambs grazed on mixed pasture than lambs that had grazed on red clover ($P<0.05$; Table 4.2). In contrast, raw loin meat of 6 to 8-month-old lambs grazed on red clover had greater abundance of acetoin and dimethyl sulfone than meat from lambs that grazed on mixed pasture ($P<0.05$; Table 4.2).

4.3.3. Diet effects (chicory vs perennial ryegrass)

In Farm C, the relative abundance of hexanoic acid, 1-butanol, 1-penten-3-ol, 1-pentanol, 1-hexanol, 1-octen-3-ol, 1-heptanol, 1-octanol and 2-octen-1-ol, hexanal, nonanal, octanal, 2-pentanone, acetoin, and dimethyl sulphide were greater in the meat of lambs grazed on chicory than perennial ryegrass ($P<0.05$; Table 4.4). While acetone was the only compound that showed greater abundance in meat from lambs grazed on perennial ryegrass than chicory.

Table 4.2 Volatile compounds in the headspace of raw lamb loin meat from animals of different sex (in Farm A) or castration status (in Farm C). The values for different volatile compounds correspond to the peak area (mean ± SEM) of the selected mass ion (ion used) divided by the peak area of the internal standard ion (m/z 81)

Compounds	Calculated RI[1]	Ion used	Wether	Ewe	P-value	Wether	Cryptorchid	P-value
Acids								
Acetic acid	1483	43	6.06±0.40	6.27±0.57	0.773	4.46±0.39	3.60±0.47	0.184
Propanoic acid	1566	74	0.40±0.04	0.35±0.03	0.357	0.23±0.02	0.20±0.03	0.381
Butanoic acid	1654	60	3.32±0.40	3.16±0.33	0.774	1.54±0.23	1.33±0.11	0.462
Pentanoic acid	1765	86	0.12±0.05	0.24±0.14	0.443	0.20±0.07	0.09±0.03	0.195
Hexanoic acid	1872	60	3.35±0.23	3.12±0.29	0.558	2.43±0.23	2.28±0.24	0.669
Heptanoic acid	1979	60	0.38±0.02	0.41±0.05	0.565	0.33±0.03	0.33±0.03	0.986
Octanoic acid	2086	60	0.55±0.06	0.55±0.06	0.992	0.56±0.05	0.58±0.07	0.876
Nonanoic acid	2193	73	0.34±0.06	0.50±0.06	0.086	0.30±0.05	0.33±0.05	0.684
Alcohols								
1-Butanol	1140	56	0.52±0.05	0.42±0.04	0.144	0.85±0.05	0.93±0.05	0.304
1-Penten-3-ol	1155	57	11.56±0.99	8.70±1.04	0.064	4.70±0.92	6.20±1.49	0.417
1-Pentanol	1244	42	5.54±0.39	4.46±0.49	0.107	2.70±0.53	3.38±0.86	0.518
1-Hexanol	1347	56	2.63±0.21	1.97±0.23	0.050	1.54±0.32	2.02±0.72	0.557
2,5-Hexanediol	1418	43	0.14±0.02	0.14±0.02	0.927	0.13±0.02	0.14±0.02	0.882
1-Octen-3-ol	1442	57	6.39±0.61	4.72±0.54	0.058	3.64±0.74	4.50±1.21	0.565
1-Heptanol	1449	70	0.75±0.05	0.59±0.06	**0.049**	0.38±0.07	0.50±0.15	0.492
1-Octanol	1552	56	0.65±0.04	0.52±0.04	**0.044**	0.34±0.06	0.42±0.11	0.542
2-Octen-1-ol	1608	57	0.25±0.02	0.19±0.02	0.092	0.15±0.03	0.18±0.05	0.602
Aldehydes								
2-Methylbutanal	910	43	2.26±0.10	2.04±0.09	0.146	1.95±0.11	1.81±0.08	0.299
Hexanal	1089	43	2.64±0.56	1.12±0.27	**0.025**	0.46±0.13	0.73±0.26	0.375
Heptanal	1193	57	0.11±0.01	0.09±0.01	0.249	0.09±0.01	0.09±0.01	0.998
Octanal	1295	57	0.29±0.04	0.17±0.02	**0.010**	0.08±0.01	0.13±0.04	0.256
Nonanal	1404	57	1.95±0.28	1.25±0.15	**0.040**	0.89±0.11	1.04±0.30	0.653
Ketones								
Acetone	826	70	45.88±4.96	37.68±2.97	0.181	28.31±2.23	32.92±1.96	0.144
2-Butanone	911	72	0.61±0.04	0.55±0.02	0.249	0.54±0.04	0.50±0.04	0.516
2-Pentanone	987	43	2.30±0.22	2.53±0.32	0.581	1.24±0.13	1.56±0.15	0.146
2-Heptanone	1187	43	2.26±0.10	2.04±0.09	0.147	1.95±0.11	1.84±0.08	0.427
3-Octanone	1260	42	0.03±0.004	0.03±0.003	0.448	0.03±0.003	0.03±0.004	0.608
Acetoin	1304	45	6.26±1.72	8.91±3.02	0.468	1.99±1.28	2.46±0.94	0.780
Butyrolactone	1680	42	2.97±0.45	2.82±0.22	0.781	1.16±0.21	1.41±0.20	0.421
Hydrocarbons								
Z-2-Octene	873	43	0.01±0.002	0.02±0.004	**0.032**	0.07±0.01	0.13±0.03	0.053
Isododecane	953	57	1.34±0.23	1.91±0.40	0.243	1.51±0.23	1.28±0.26	0.542
Beta-Pinene	1110	93	0.99±0.09	1.06±0.18	0.741	0.67±0.06	0.60±0.10	0.577
Furans								
2-Ethylfuran	959	81	0.78±0.10	0.53±0.08	0.067	0.14±0.04	0.35±0.13	0.156
Sulphur compounds								
Carbon disulphide	745	76	12.98±4.09	13.10±4.19	0.983	15.33±4.60	20.52±6.44	0.531
Dimethyl sulphide	959	62	1.79±0.16	1.92±0.23	0.658	0.18±0.03	0.14±0.03	0.383
Dimethyl sulfone	1939	79	12.44±0.99	10.11±0.63	0.066	8.00±0.44	8.14±1.35	0.926

1. Calculated RI: calculated linear retention index relative to a series of alkanes C7-C26.

Table 4.3 Volatile compounds in the headspace of raw lamb loin meat from animals of different forage diets in Farm A. The values for different volatile compounds correspond to the peak area (mean ± SEM) of the selected mass ion (ion used) divided by the peak area

Compounds	Red Clover	Mixed pasture	P-value
Acids			
Acetic acid	6.06±0.40	5.48±0.47	0.370
Propanoic acid	0.40±0.04	0.45±0.05	0.465
Butanoic acid	3.32±0.40	2.98±0.28	0.512
Pentanoic acid	0.12±0.05	0.16±0.06	0.613
Hexanoic acid	3.35±0.23	4.13±0.40	0.113
Heptanoic acid	0.38±0.02	0.44±0.04	0.191
Octanoic acid	0.55±0.06	0.61±0.05	0.479
Nonanoic acid	0.34±0.06	0.44±0.06	0.227
Alcohols			
1-Butanol	0.52±0.05	0.97±0.38	0.256
1-Penten-3-ol	11.56±0.99	14.49±1.53	0.132
1-Pentanol	5.54±0.39	7.12±0.61	**0.044**
1-Hexanol	2.63±0.21	3.31±0.29	0.076
2,5-Hexanediol	0.14±0.02	0.14±0.02	0.946
1-Octen-3-ol	6.39±0.61	8.27±0.83	0.087
1-Heptanol	0.75±0.05	1.00±0.07	**0.012**
1-Octanol	0.65±0.04	0.80±0.05	**0.036**
2-Octen-1-ol	0.25±0.02	0.32±0.03	0.081
Aldehydes			
2-Methylbutanal	2.26±0.10	2.70±0.11	**0.010**
Hexanal	2.64±0.56	2.98±0.53	0.680
Heptanal	0.11±0.01	0.11±0.01	0.913
Octanal	0.29±0.04	0.32±0.04	0.616
Nonanal	1.95±0.28	2.41±0.29	0.271
Ketones			
Acetone	45.88±4.96	39.32±2.89	0.279
2-Butanone	0.61±0.04	0.71±0.04	0.124
2-Pentanone	2.30±0.22	1.92±0.10	0.134
2-Heptanone	2.26±0.10	2.70±0.11	**0.010**
3-Octanone	0.03±0.004	0.03±0.003	0.584
Acetoin	6.26±1.72	1.25±0.30	**0.010**
Butyrolactone	2.97±0.45	4.32±0.43	**0.045**
Hydrocarbons			
Z-2-Octene	0.01±0.002	0.02±0.003	**0.017**
Isododecane	1.34±0.23	2.01±0.36	0.137
Beta-Pinene	0.99±0.09	1.01±0.17	0.921
Furans			
2-Ethylfuran	0.78±0.10	1.23±0.18	**0.041**
Sulphur compounds			
Carbon disulphide	12.98±4.09	14.72±4.67	0.789
Dimethyl sulphide	1.79±0.16	2.20±0.47	0.432
Dimethyl sulfone	12.44±0.99	6.11±0.74	**<0.001**

1. Calculated RI: calculated linear retention index relative to a series of alkanes C7-C26.

4.3.4. Age effects

The meat from WEAN-W lambs had greater abundance of octanoic acid, 1-butanol, 2-methylbutanal, 2-butanone, 2-pentanone, 2-heptanone, Z-2-octene and dimethyl sulfone than the meat from GRASS-W and CHIC-W lambs with longer finishing period. Beta-pinene, 1-octen-3-ol, 1-heptanol, 1-octanol, 2-octen-1-ol, and 1-pentanol were present at a greater abundance from the meat of 6- to 8-month-old GRASS-W and CHIC-W lambs compared to 4-month-old WEAN-W lambs ($P<0.05$; Table 4.4).

Table 4.4 Effect of diet, sex, and their interaction, and age at slaughter (weaning at 4 months old vs. 6-8 months old) on peak area of volatiles of meat from lambs from Farm C.

Diet	Pasture		Chicory		Wean	Non-orthogonal contrast[1], P-value			
Sex	Wether	Ewe	Wether	Ewe	Wether	Diet	Sex	Diet x Sex	Age
Acids									
Acetic acid	5.55±0.79	5.93±0.64	6.19±0.55	6.62±.56	6.29±0.73	0.313	0.545	0.965	0.635
Propanoic acid	0.31±0.04	0.35±0.04	0.41±0.04	0.39±0.04	0.41±0.05	0.073	0.880	0.517	0.427
Butanoic acid	3.41±0.84	2.28±0.21	2.82±0.36	3.02±0.31	3.43±0.44	0.881	0.363	0.197	0.675
Pentanoic acid	0.18±0.07	0.22±0.10	0.09±0.04	0.32±0.21	0.91±0.75	0.991	0.298	0.440	0.162
Hexanoic acid	2.72±0.33	2.77±0.26	4.03±0.24	3.59±0.38	3.43±0.24	**0.001**	0.570	0.446	0.895
Heptanoic acid	0.32±0.04	0.40±0.04	0.41±0.03	0.39±0.04	0.43±0.04	0.315	0.591	0.200	0.215
Octanoic acid	0.47±0.06	0.64±0.07	0.64±0.06	0.55±0.06	0.80±0.09	0.542	0.563	**0.037**	**0.010**
Nonanoic acid	0.25±0.04	0.35±0.07	0.37±0.07	0.40±0.06	0.34±0.05	0.194	0.336	0.552	0.678
Alcohols									
1-Butanol	0.36±0.04	0.39±0.02	0.52±0.04	0.47±0.03	0.66±0.04	**<0.001**	0.739	0.278	**<0.001**
1-Penten-3-ol	5.92±0.60	5.37±0.64	11.56±1.54	9.87±1.51	6.47±0.77	**<0.001**	0.413	0.639	0.141
1-Pentanol	4.06±0.40	3.47±0.43	6.61±0.69	5.91±0.70	2.69±0.30	**<0.001**	0.336	0.920	**<0.001**
1-Hexanol	1.41±0.13	1.41±0.19	2.66±0.30	2.36±0.30	1.42±0.17	**<0.001**	0.599	0.552	0.056
2,5-Hexanediol	0.13±0.02	0.14±0.02	0.15±0.02	0.16±0.02	0.13±0.02	0.250	0.604	0.905	0.894
1-Octen-3-ol	4.54±0.58	3.57±0.50	8.01±0.92	6.64±1.04	4.07±0.52	**<0.001**	0.205	0.808	**0.030**
1-Heptanol	0.46±0.04	0.48±0.06	0.68±0.08	0.67±0.08	0.27±0.03	**0.004**	0.959	0.810	**<0.001**
1-Octanol	0.42±0.03	0.46±0.06	0.54±0.06	0.58±0.06	0.26±0.03	**0.036**	0.534	0.942	**<0.001**
2-Octen-1-ol	0.20±0.02	0.15±0.02	0.31±0.03	0.25±0.04	0.17±0.02	**<0.001**	0.147	0.903	**0.024**
Aldehydes									
2-Methylbutanal	1.74±0.14	1.60±0.11	1.43±0.06	1.56±0.08	2.14±0.10	0.101	0.985	0.214	**<0.001**
Hexanal	0.68±0.19	0.54±0.10	4.35±0.96	2.47±0.51	1.47±0.28	**<0.001**	0.143	0.135	0.241
Heptanal	0.07±0.01	0.07±0.01	0.09±0.01	0.09±0.01	0.08±0.01	0.088	0.723	0.780	0.823
Octanal	0.12±0.01	0.11±0.02	0.32±0.05	0.25±0.04	0.12±0.02	**<0.001**	0.426	0.365	0.061
Nonanal	0.82±0.12	0.86±0.15	2.15±0.36	1.76±0.29	0.92±0.14	**<0.001**	0.553	0.422	0.104
Ketones									
Acetone	31.82±4.27	29.65±2.10	21.78±2.66	17.96±1.31	33.93±2.12	**<0.001**	0.352	0.777	0.094
2-Butanone	0.46±0.05	0.39±0.03	0.34±0.03	0.37±0.03	0.56±0.04	0.061	0.648	0.175	**0.003**
2-Pentanone	1.22±0.11	1.18±0.08	2.11±0.22	2.20±0.34	2.31±0.24	**<0.001**	0.913	0.769	**0.025**
2-Heptanone	1.73±0.14	1.61±0.11	1.43±0.06	1.56±0.08	2.10±0.10	0.092	0.972	0.235	**<0.001**

3-Octanone	0.03±0.003	0.03±0.003	0.03±0.003	0.03±0.002	0.03±0.002	0.081	0.358	0.248	0.594
Acetoin	2.29±0.77	2.54±0.65	7.55±1.95	8.15±3.34	2.10±0.95	**0.010**	0.845	0.933	0.126
Butyrolactone	2.99±0.43	2.22±0.22	2.25±0.22	2.49±0.22	2.45±0.25	0.436	0.375	0.097	0.673
Hydrocarbons									
Z-2-Octene	0.02±0.003	0.02±0.003	0.02±0.003	0.02±0.004	0.06±0.01	0.102	0.722	0.698	**<0.001**
Isododecane	1.24±0.30	2.02±0.59	1.04±0.20	1.27±0.26	0.70±0.20	0.218	0.193	0.467	0.145
Beta-Pinene	0.78±0.11	0.94±0.14	0.57±0.10	0.75±0.10	0.30±0.04	0.108	0.154	0.932	**0.002**
Furan									
2-Ethylfuran	0.26±0.04	1.31±0.29	1.06±0.25	0.66±0.14	0.32±0.06	0.743	0.164	**0.001**	0.114
Sulphur compounds									
Carbon disulphide	16.73±5.13	14.54±4.42	15.93±5.43	21.03±7.91	17.04±5.81	0.637	0.809	0.551	0.917
Dimethyl sulphide	0.93±0.10	1.14±0.13	1.44±0.22	2.02±0.25	0.77±0.12	**<0.001**	0.071	0.331	0.051
Dimethyl sulfone	4.48±0.47	6.30±0.98	4.84±0.62	3.68±0.35	7.33±0.87	0.108	0.637	**0.031**	**0.003**

1.Diet: Pasture-Ewe & Pasture-Wethers vs. Chicory-Ewes & Chicory-Wethers; Sex: Pasture-Ewes & Chicory-Ewes vs. Pasture-Wethers & Chicory-Wethers; Diet x Sex: Pasture-Ewes & Chicory-Wethers vs. Pasture-Wethers & Chicory-Ewes); Age: Wean-Wethers vs. Pasture-Wethers & Chicory-Wethers.
2.Calculated RI: calculated linear retention index relative to a series of alkanes C7-C26.

4.3.5. Principle components analysis

To visualise differences in fatty acid content (mg/100g) and volatile profiles among the different lamb groups, PCA biplot is presented in Figure 4.1. The first dimension (Dim1) of PCA explained 27.1% of the total variation in fatty acid and volatile composition, and the second dimension (Dim2) explained 18.2% of the total variation. Dim1 separated the animal groups based on their total fatty acid content, where MIX-W, WEAN-W, MXME-W and MXME-C are positioned on the left side of the plot, opposite to REDC-W, REDC-E, CHIC-W, CHIC-E, GRASS-W and GRASS-E lambs, with the first group having greater FA content (SFA, BCFA, MUFA and PUFA) than the second group. Dim2 separated the animal groups based on the types of fatty acids and the relative abundances of volatile compounds. MIX-W, WEAN-W, REDC-W and REDC-E showed greater content of PUFA and the associated volatiles than MXME-W and MXME-C. CHIC-W, CHIC-E, GRASS-W and GRASS-E were intermediate for their PUFA content and volatiles produced.

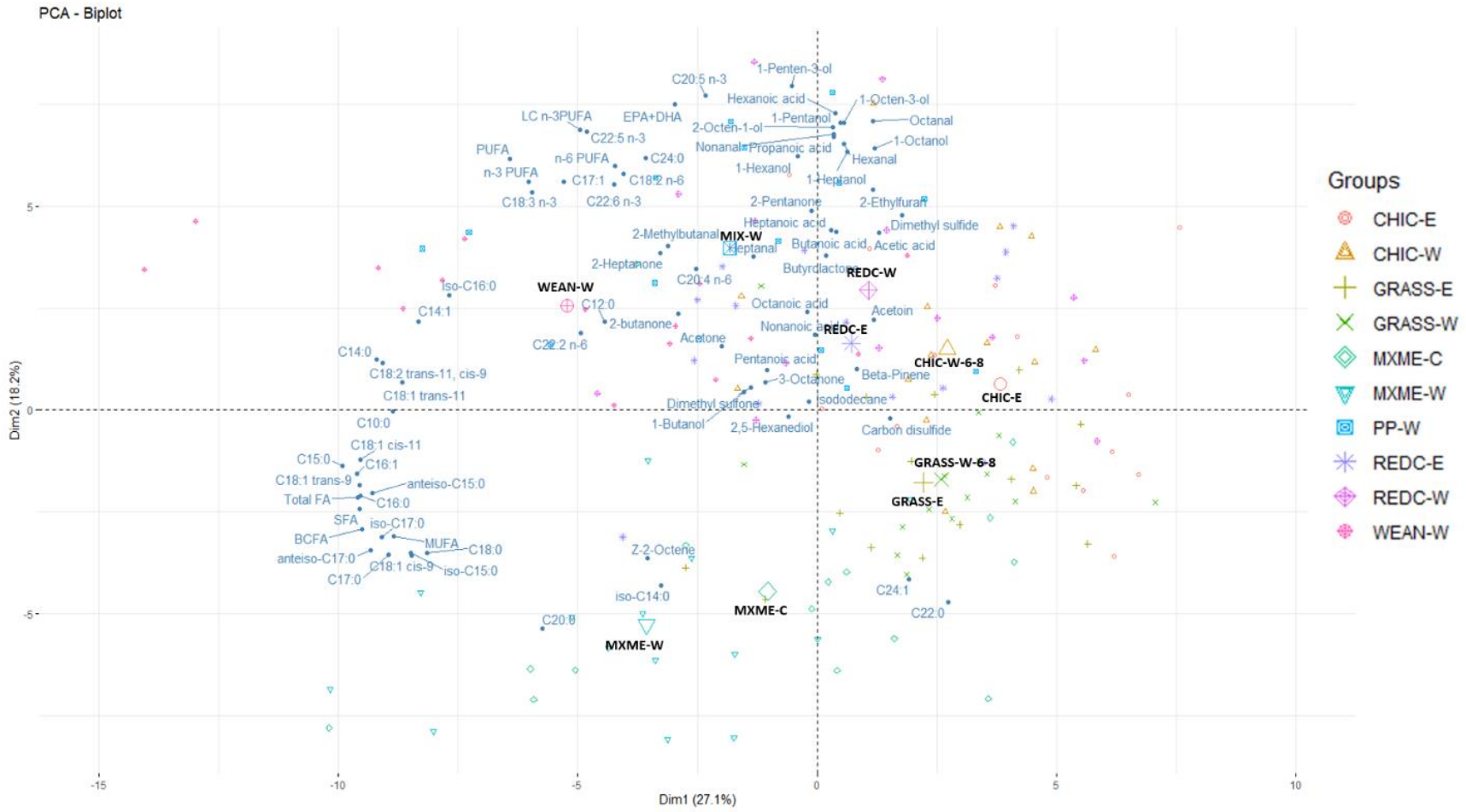


Figure 4.1 Principal component analysis (PCA) biplot of fatty acids (mg/100g raw meat) and volatile compounds identified in raw lean meat as affected by lamb production system. The production systems included: 4-month-old wether lambs of a composite breed at weaning (WEAN-W), 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing

perennial ryegrass-based pasture (GRASS-W and GRASS-E, respectively), 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing chicory (CHIC-W and CHIC-E, respectively), 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing red clover (REDC-W and REDC-E, respectively), 6- to 8-month-old wether lambs of composite breed that had been grazing a mixture of perennial ryegrass, red- and white-clover mixed pasture (MIX-W) and 12-month-old wether and cryptorchid Merino lambs that had been grazing a mixed pasture (MXME-W and MXME-C, respectively).

4.4. Discussion

The main objectives of the present study were to evaluate the volatile profiles of lambs from diverse forage-based production systems in New Zealand, and understand if any relationships between production systems and volatiles of raw lamb meat. Our results indicated that 25 out of 36 identified volatiles in raw lamb meat were affected by forage production systems. However, what it means in terms of flavour was not elucidated in this study because whether these compounds could have an impact on flavour depends on both its concentration and its odour threshold (Farmer, 1994), i.e. how sensitive the human nose is to that compound. Although statistical differences were observed on relative volatile abundance between production systems, this may not associate with a detectible or significant chance in flavour since some volatiles require a large concentration shift to provide a flavour change. In addition, the current study only analysed volatiles from raw meat, which mainly generated from oxidation of lipids during aging and storage (Farmer, 1994). Many volatiles that generated during cooking and play an important part in the overall aroma of cooked meat, such as 1-octen-3-one that derived from the thermal oxidation of polyunsaturated fatty acids; methional that generated from Maillard reaction between amino acids and reducing sugars (Farmer, 1994), were not observed in the current study.

4.4.1 Sex and castration effects

In Farm C, no volatiles abundance difference between meat from wether and ewe lambs grazed on perennial ryegrass or chicory diet was observed in the current study. This is not surprising because the ewe and wether lambs had similar carcass characteristics (Chapter 2) and fatty acid profiles (Chapter 3) in the current study. This was in agreement with some studies that suggested the influence of sex on the amount of adipose tissue is small in young lambs (Hopkins and Mortimer, 2014). Tejeda et al., (2008) reported that the sex of Merino lambs did not affect the

fatty aicd composition and sensory quality of meat when slaughtered at young age (live weight of 24 or 29 kg). When older (22-month-old) lambs or entire male lambs were considered, the effect of sex seems to be more obvious (Salvatore et al., 2007). Meat of ram lambs score higher for undesirable aroma (Gkarane et al., 2017) and lipid oxidation products (Gkarane et al., 2018b) than meat of wether lambs according to a trained panel and SPME-GC-MS. The PCA biplot also indicated that the sex or castration status of the lamb is unlikely to be associated with the volatile profile of the raw meat as wether and ewe lambs or wether and cryptorchid lambs with the same diet tended to cluster together.

In Farm A, the greater relative abundance of 1-octanol, 1-heptanol and hexanal, octanal, and nonanal observed in meat from wether lambs than ewe lambs is hard to explain because their content of PUFA was similar in loins (Chapter 3). These alcohols are mainly formed when amino acids and ribose interact in the lipid autoxidation pathway (Resconi et al. 2018). 1-Hexanol could degrade from homologous aldehydes such as hexanal during lipid and amino acid oxidation (Garcia et al. 1991), which has a herbal and fatty odour that is correlated to rancid odour (Resconi et al. 2018). While 1-octanol has a fatty, waxy and citrus odour (Calkins and Hodgen, 2007). Similarly, straight chain aldehydes including hexanal, octanal, and nonanal are also lipid oxidation products, which are characterized to have green-grass and soapy odours (Calkins and Hodgen, 2007).

4.4.2. Diet effects

The diet of the animal, is generally considered to be the most important environmental factor affecting volatiles (Raes et al., 2004). The diet effects on most of volatile compounds identified in the current study were mainly expressed via differences in fatty acid profiles except some

hydrocarbons that can accumulate in lamb tissue directly (Elmore et al., 2000). The greater abundance of 1-pentanol, 1-heptanol, 1-octanol, 2-methlybutanal, 2-heptaone, butyrolactone, 2-ethylfuran in the meat of lambs grazed on mixed pasture than lambs grazed on red clover can be a potential consequence of their greater fatty acid content (Chapter 3). Similarly, the greater abundance of alcohols, aldehydes and ketones in the meat of lambs grazed on chicory than lambs grazed on perennial ryegrass can be a potential consequence of their greater PUFA content (Chapter 3) because these volatiles are commonly reported as lipid oxidation products (Elmore et al., 2005). The alcohols (1-butanol, 1-penten-3-ol, 1-pentanol, 1-hexanol, 1-octen-3-ol, 1-heptanol, 1-octanol, 2-octen-1-ol) mainly formed when amino acids and ribose interact in the lipid autoxidation pathway (Resconi et al. 2018). These alcohols were known to contributed to different flavours in cooked meat. 1-Hexanol has a herbal and fatty odour that is correlated to rancid odour (Resconi et al. 2018), whereas 1-pentanol has a pleasant, sweet or fruity odour (Calkins and Hodgen, 2007). 1-Octen-3-ol is characterized by a mushroom-like, grassy odour (Yvon and Rijnen, 2001) and commonly reported as an oxidation product from several precursors including linoleic and arachidonic acids.

Chicory diet increased the abundance of acetoin in raw lamb loins in comparison with perennial ryegrass diet. Acetoin has been reported to associated with fat oxidation and animals with fat-enriched diets (Elmore et al., 2005). In addition, bacteria (e.g., Serratia, Erwinia, Enterobacter) could also produce acetoin or its oxidation (diacetyl) or reduction (2,3-butanediol) products though different pathways (Moat et al., 2003). Many linear acids such as hexanoic acid observed in meat are likely to be a result of bacterial activities (Resconi et al., 2018). It is inevitable that meat will carry some level of microbial contamination during production, and storage (Biss

and Hathaway, 1998). However, since all samples were handled under same process, it is not surprising that very little differences in abundance of linear acids was found in the current study.

Chicory diet also increased the abundance of linear aldehydes in raw lamb meat in comparison with perennial regress diet. Among them, octanal that imparts lemon and floral flavour is vital to the flavour because it is one of top 15 chemicals that contributed to cooked lamb baseline aroma according to gas-chromatography-olfactometry studies (Watkins et al., 2013). Hexanal is one of the major secondary products from the oxidation of linoleic acid, it has an odour detection threshold of 5.87 mg /L in lean ground beef and imparts a fresh, green, grass flavour (Casaburi et al., 2015). The concentration of hexanal is frequently used as a predictor of meat lipid oxidation, and has been reported to negatively correlated with meat acceptability in pork (Karabagias, 2018). Elmore et al., (2005) reported that the lambs fed protected lipid supplement high levels of hexanal and scored highest for grassy flavour, which was probably related to its high content of C18:2 n-6 in meat. In addition, a hexanal to nonanal ratio equal or lower than 2.45 has been proposed as an indicator of lamb meat freshness and overall quality during storage (Karabagias, 2018). Based on this criterion, meat from lambs grazed on all types of forages in the current study can be considered as fresh. Whether increasement of PUFA content and lipid oxidation compounds by forage diets would associate with greater off flavour requires further research. 2-Methylbutanal, which was described as "mushroom," "malty" odour with a low odour threshold (3 μg/L), was known to contributed to grilled lamb baseline aroma (Gravador et al., 2015). Unlike straight chain aldehydes which originate from fatty acids oxidation, 2-methylbutanal is a Strecker reaction product from amino acids (Resconi et al. 2010), which had the greater abundance in the meat from lambs grazed on a mixed pasture diet than red clover. A hypothesis is that the increment of pH in meat increased the production of the Strecker aldehydes (Gravador et al., 2015), however, this does not explain

the observation in the current study since the pH was similar between lambs grazed on mixed pasture and red clover (Chapter 2).

Lower abundance of 2-heptanone, which has a rancid and slightly fruity aroma (Resconi et al. 2012), was observed for the meat from lambs that grazed on mixed pasture compared to meat from lambs that grazed on red clover. This is difficult to explain because 2-heptanone is mainly derived from the decomposition of 18:2 n-6 fatty acid (Elmore et al., 2005), which was of similar content between chicory and red clover fed lambs (Chapter 3).

Sulphur compounds originate from the degradation of cysteine and methionine, two sulphur-containing amino acids (Mottram, 1998). Dimethyl sulfone, which has an unfavourable "milky" flavour in meat of pasture-fed sheep (Young et al., 2003), was more abundant in lambs that grazed on red clover compared to lambs grazed mixed pasture in Farm A. Stødkilde et al., (2020) reported that the crude protein extracted from grass-clover had a higher methionine content, but lower lysine and total sulphur-containing amino acids than that in soybean. Thus, in the current study the sulphur-containing amino acids may also differ between different types of pastures and potentially affect the level of sulphur compounds in lamb meat. In Farm C, dimethyl sulphide, which has a sulphurous, onion, and green odour in raw meat (Casaburi et al. 2014) was more abundant in lambs grazed on chicory than perennial ryegrass. Dimethyl sulphide was also commonly observed in grilled lamb meat (Gkarane et al., 2019; Pavan et al., 2021). This diet effect on abundance of dimethyl sulphide was likely to retain in cooked lamb meat (Elmore et al., 2000), although how the abundance of sulphur compounds changes from raw to cooked lamb meat, and how they effect the meat flavour requires further research.

Terpenes such as alfa pinene and the p-cymene are synthesized exclusively in the vegetal regnum, and they have been shown to be transferred from green herbage to animal tissue (Vasta

et al., 2012; Vasta and Priolo, 2006). Cornu et al. (2001) suggested that Beta-pinene in beef could be used to determine the region that an animal came from. No difference in abundance of beta-pinene and isododecane, however, was observed from the meat of lambs grazed on different diets. Our results suggest that hydrocarbons from different production system treatments did not differ in New Zealand from the farms investigated. This might because Farm A, B and C were not sufficiently geographically distanced or that the diets grazed on the farms were not diversely different to create differences in terpenes.

Lastly, thermal treatments cannot be neglected when considering the effect of lamb grazing diets on the flavour of meat ready to be served. Domínguez et al., (2014) reported that the abundance of aldehydes, especially hexanal increased significantly in foal meat during cooking (approximately 55 times in grilled meat and 76 times in roasted meat). Therefore, although forage diets had some influence on the abundance of aldehydes in raw lamb meat, types of thermal treatments may play the dominant role in abundance of aldehydes in cooked meat.

4.4.3. Age effects

The meat of 4-month-old pre-weaning lambs had a lower abundance of Beta-pinene than meat from 6-month-old lambs that grazed perennial ryegrass or chicory, which probably due to its shorter finishing period. This is not surprising as terpenes were considered as green forage indicators and can transfer from grass to animal tissue directly (Chevance and Farmer, 1999; Priolo et al., 2004). The abundance of Z-2-octene, on the contrary, decreased in lamb loins when finishing time increased from 4-month to 6- to 8-month. The abundance of Z-2-octene in lamb perirenal fat was reported to increase in lamb subcutaneous fat with pasture grazing time and reached the peak

at 51 days of pasture grazing (Sivadier et al., 2009), and then started decreasing, although the mechanism behind is not clear.

The meat from lambs slaughtered at weaning that had greater content of PUFA (Chapter 3) as well as greater abundance of octanoic acid, 1-octen-3-ol, 1-butanol, 1-heptanol, 1-octanol, 2-octen-1-ol and 1-octen-3-ol, 2-methylbutanal, 2-butanone, 2-pentanone, 2-heptanone and dimethyl sulfone than older lambs that grazed on perennial ryegrass or chicory. Many previous studies have suggested different results of age effect on fatty acids and volatile content because various lamb production systems and volatiles analysis techniques has been applied. Some studies reported that the content of intramuscular fat as well as lipid oxidation compounds increased with the finishing period (Díaz et al., 2005; Hopkins and Mortimer, 2014). Other studies indicated there was no consistent age effect on the relative abundance of volatiles (Gkarane et al., 2018b), or age of the animals (9-month vs 12-month) had no statistical significance in the intramuscular fatty acids. Under ideal management conditions in the current, loins of lambs slaughtered at 4-month-old could have greater abundance of PUFA and lipids oxidation volatiles than 6- to 8-month-old lambs.

4.4.4. Overall production system effects on volatile compounds and flavour

The abundance of many volatiles differed between raw lamb meat from ten investigated New Zealand commercial forage production systems, which implicated that the flavour of cooked meat from these lambs may differ. However, explain how these differences in volatiles abundance could influence the flavour of cooked meat is probably far more complex. Firstly, integrated flavour perception is brought about by the interaction of non-volatile (i.e glycogen, lactic acid, amino acids, fatty acids) and volatile components with human chemosensory receptors, including taste and olfactory receptor cells as well as other sensory networks flavour in a food product is a combination

of both aroma and taste (Watkins et al., 2013). There is an inherent limitation that non-volatiles in lamb meat were not considered in the current study however, the literature does not indicate the extent to which these non-volatiles influence flavour, given they are non-volatile it is likely there is minimal impact. Secondly, within the top 15 impact compounds in cooked lamb baseline aroma reported by Watkins et al., (2013) via a meta-analysis, only octanal was identified from raw meat in the current study. These 15 compounds listed in decreasing rank as: 4-ethyloctanoic acid, 1-octen-3-one, (E,E)-2,4-decadienal, (Z)-2-nonenal, 2-acetyl-1-pyrroline, furaneol, (E)-2-heptenal, methional, 2,3-diethyl-5-methylpyr-azine, dimethyl trisulfide, (E)-2-nonenal, decanal/2,4-(E,E)-heptadienal, 4-methylphenol, octanal, and (E)-2-octenal. Nevertheless, some these volatiles from raw meat may be important precursors involved in chemical changes during cooking or remained in cooked meat as "warmed-over flavour" (Farmer, 1994). Lastly, different thermal treatments have significant influence on formation of volatile compounds and aroma. Domínguez et al. (2014) reported that thermal treatments (microwaved, fried, grilled, roasted) significantly increased the level of aldehydes in raw foal meat steak and decreased the level of esters. The formation of volatile compounds also related to the temperature reached by the samples during cooking, pyrazines for example, were only observed in meat when fired in high temperature oil (Domínguez et al., 2014). The changes in the relative ratio and type of lamb aroma volatile compounds may result in noticeable sensory differences in the final cooked meat (Watkins et al., 2013).

On farm production systems had little effect on the abundance of carboxylic acids in raw meat in general. This is because carboxylic acids in raw meat can be derived from several pathways, including enzymatic and chemical reactions and from action of spoilage bacteria on lipids, amino acids or carbohydrate (Resconi et al., 2013). Butanoic acid can be formed from fermentative metabolism during aging and frozen storage in vacuum packed meat (Broda et al., 1996) and has

lower odour detection threshold (240 μg/l in water) compared to acetic acid and is associated with rancid, sharp and cheesy odours (Casaburi et al., 2015). Pentanoic and heptanoic acids are also associated with imparting fatty, gamey, cheesy and dairy odours and are highly correlated with rancid odour scores according to a trained sensory panel (Resconi et al., 2018; Casaburi et al., 2015). However, these volatiles were present at similar abundance for the lambs across the production systems in this study and therefore, were unlikely to differentiate lamb meat across the different systems used in this study. No branched chain volatile fatty acids such as 2- and 3-methyl butanoic acids were found in the current study as expected, since they were specifically associated with the presence of *Br.thermosphacta.* and only reported in meat stored aerobically (Casaburi et al., 2015).

Different volatile profiles observed between studies could also due to the different methodologies applied (Resconi et al. 2018). For example, static extraction methods such as the SPME were reported to have lower recovery yields compared to dynamic headspace extraction in general (Rivas-Cañedo et al., 2011). The CAR-PDMS-DVB fibre that used in current study has been reported to have better performance for long chain fatty acids and some alcohols than CAR-PDMS or PDMS-DVB fibre (Reis et al., 2016), while CAR-PDMS fibre was known to have better performance on aldehydes and ketones. When similar head space GC-MS analysis was applied for grilled lamb loin steaks (cooked in clamshell with internal temperature of 70°C), nonanoic acid was the only identified volatile acids (Gkarane et al., 2019). This implied that the abundance of the volatile acids observed from raw lambs in the current study may decrease during cooking.

4.4.5. Overview by PCA

The PUFA were clustered in the same quadrant as 2-heptanone, acetone, 2-methylbutanal, butyrolactone, and 2-butanone (Figure 4.1) which implicated that PUFA are more susceptible to oxidation (Howes et al., 2015) and contributed to the generation of these aldehydes, ketones and alcohols volatiles associated with lipid oxidation volatiles (Calkins and Hodgen, 2007). In general, lambs that grazed on red clover pastures and mixed pasture have higher content of PUFA and overall volatiles than lambs grazed on perennial ryegrass (Figure 4.1), despite having similar or slightly greater total fatty acid content (Chapter 3) in the current study. Similar results were reported by Vipond et al., (1993) that increased content of total PUFA was observed in lean meat of lambs finished on swards containing white clover compared to lambs finished on grass. In Farm C, the greater abundance of lipid oxidation compounds in meat from lambs grazed on chicory than perennial ryegrass may also be a result of greater level of PUFA in meat from lambs grazed on chicory (Chapter 3).

4.5. Conclusions

Most of these alcohols, ketones and aldehydes identified in the current study were known as the results of lipid oxidation during storage. Meat from lambs that grazed on chicory had greater abundance of lipid oxidation compounds including hexanal, octanal, nonanal, 2-pentanone and 1-penten-3-ol and 1-octen-3-ol than meat from lambs grazed on perennial ryegrass, which is likely due to its greater content of PUFA (Chapter 3). It is not possible to draw a conclusion whether chicory is better than pasture on flavour because we don't have quantitative data. i.e., some of the volatiles reported may be below detection threshold of consumers, and do not affect the flavour at all.

There was no consistent sex or castration effect on the relative abundance of volatiles. Increase the age of slaughter from 4-month-old to 6- to 8-month-old decreased the content of PUFA (Chapter 3) and relative lipid oxidation compounds. There was little evidence that different pastures diets investigated in the current study had significant effect on accumulated hydrocarbons. Whether the effect of production systems on volatile compounds of raw meat would influence the flavour scores of cooked lambs will be discussed in Chapter 6

Chapter 5

Proteomic profile of *Longissimus thoracis* from commercial lambs reared in different forage production systems

This chapter is going to be submitted to Food Research International

Abstract

New Zealand climate favours pasture growth throughout the year, and a variety of lamb production systems that differ in animal age, sex, diet and breed are used. Manipulation of production system factors has been shown to influence protein profile of meat that may have an impact on meat quality. This study compared the protein composition of *M. longissimus thoracis* of lambs from 6 commercial forage production systems in New Zealand. In total, 286 proteins were identified with at least two unique peptides per protein in all the six production systems based on liquid chromatography-tandem mass spectrometry. Two approaches were taken: first, a binomial model was used to see if protein differences in meat could be detected between the six production systems. Results showed that most proteins were similar, but different lamb production groups could be distinguished based on different ($P<0.05$) abundances of 16 proteins. Second, pair-wise comparisons were performed to search for protein abundance differences in meat due to animal gender (ewe vs. wether), diet (perennial ryegrass vs. chicory) and age (4 vs. 6-8 months old) within a single farm. Several insights were obtained, such as greater ($P<0.05$) abundance of some myofibrillar (actin, troponin C, myosin-2, myosin regulatory light chain 2, myosin light chain 1) and sarcoplasmic (immunoglobulin lambda-1 light chain-like, glutathione S-transferase P) proteins and lower ($P<0.05$) abundance of stromal proteins (collagen alpha-3(VI) chain) in loins from ewe compared to wether lambs grazed on chicory, that can be associated with differences in muscle fibre type and collagen levels. Chicory diet showed lower ($P<0.05$) abundance of some myofibrillar proteins (myosin-2, myosin regulatory light chain 2 and actin) in lamb loins compared to perennial ryegrass, possibly due to greater proportion of muscle glycolytic fibres from faster growing lambs when grazed on chicory and heavier carcasses at slaughter. Similarly, the abundance of myofibrillar proteins in loins was lower ($P<0.05$) in lambs slaughtered at 6- to 8-

month-old than 4-month-old, which was probably due to faster growth rate of glycolytic muscle fibres than oxidative fibres during finishing. Results showed that most muscle proteins did not differ for meat from animals reared under 6 different forage production systems. However, significant differences were obtained in the abundances of some proteins due to animal sex, diet and age at slaughter, that could be further investigated to understand their potential for influencing meat quality.

5.1. Introduction

Red meat exports from New Zealand generated $8.39 billion revenue in total for the 2019-20 season (Beef and Lamb New Zealand, 2020). Improvement in meat quality to satisfy consumers and entice repurchase is considered key for maintaining markets (Jiang et al., 2015). The meat quality attributes of lamb are influenced by several production system factors including animal age at slaughter, forage type and breed (Hopkins and Mortimer, 2014; Ye at al, 2020). Increasing animal age at slaughter was reported to be favourable for meat juiciness and flavour due to greater intramuscular fat concentration, but unfavourable for tenderness (Gagaoua et al., 2018b). Young animals produce meat that is lighter and less red in colour compared to older animals (Mashele et al., 2017). Furthermore, at the same age of slaughter, females could provide a more flavourful, tender, intense coloured meat than males (Picard et al., 2019).

Being able to modify meat quality via production systems is dependent on understanding the intrinsic components of meat that can influence key quality attributes such as tenderness, flavour, colour, and juiciness (Clerens et al., 2016). Previous studies have shown links between protein profiles and meat quality characteristics (Picard et al., 2019). The texture and tenderness of meat is directly related to its protein matrix, and is affected by post-translational changes such as glycosylation, protein backbone cleavage, aggregation, oxidation and crosslinking as well as the crosslinking of collagen (Koohmaraie and Geesink, 2006; Nishimura, 2010). Several myofibrillar proteins, such as actin and myosin regulatory light chain 2 have been reported as biomarkers for tenderness (Picard and Gagaoua, 2020). An important contributor towards meat colour is the abundance of myoglobin (Mancini and Hunt, 2005). Myosin regulatory light chain 2, aldose reductase, and β-enolase are also positively associate with redness of meat colour (Canto et al., 2015; Paredi et al., 2012). In addition, troponin and myosin light chain with a dense hydrophobic

region are negatively associated with water holding ability of meat (Wei et al., 2019). Creatine kinase has a greater expression in the muscle of animals that have experienced stress, and has a role in maintaining the water holding capacity of meat (Daroit and Brandelli, 2008). Merinos have a propensity to produce meat with a higher pH because they lose greater amounts of muscle glycogen than other breeds under pre-slaughter stress (Hopkins and Mortimer 2014). Therefore, the abundance of creatine kinase may also differ between different lamb breeds. Currently, however, there is still a knowledge gap on how production systems influence these traits. The broad range of forage-based commercial production systems utilized in New Zealand suggests that there is potential for variation in protein profiles of lamb meat. Additionally, a deeper understanding of these variations in protein profiles offers potential to improve meat quality. To gain further insights, the objective of this study was to compare protein profiles of meat from six types of typical commercial New Zealand forage lamb production systems.

5.2. Materials and methods

5.2.1. Animals and management

To encompass a range of forage-based production systems, lambs were sourced from three commercial farms north of Invercargill, New Zealand. The six production systems (Table 5.1) included: 4-month-old wether lambs of a composite breed at weaning (WEAN-W), 6 to 8-month-old wether lambs of a composite breed that had been grazing perennial ryegrass based pasture (GRASS-W); 6 to 8-month-old wether and ewe lambs of a composite breed that had been grazing chicory (CHIC-W and CHIC-E, respectively); 6- to 8-month-old wether lambs of a composite breed that had been grazing red clover (REDC-W); and 12-month-old wether Merino lambs that had been grazing a mixed pasture (MXME-W). The groups of lambs were reduced due to the limits

of proteomics analysis capability. Eight lambs from each production system were randomly selected and identified at slaughter from a larger group of lambs.

Table 5.1 Production systems of animals (n=8) selected according to the pre-slaughter factors (age, sex, diet, breed) included in the study.

Production systems	Approximate age at slaughter (months)	Sex	Finishing diet[1]	Breed[2]	Farm
REDC-W	6-8	Wethers	Red Clover	Perendale × LambSupreme	A
MXME-W	12	Wethers	Pasture	Merino	B
WEAN-W	4	Wethers	Pre-weaning	Composite	C
GRASS-W	6-8	Wethers	Pasture	Composite	C
CHIC-W	6-8	Wethers	Chicory	Composite	C
CHIC-E	6-8	Ewes	Chicory	Composite	C

[1] Animal diet: Pre-weaning, suckled and grazing mothers' diet of a chicory (Cichorium intybus) and red clover (Trifolium pratense) mix;
Farm A-Pasture, predominantly Italian and perennial ryegrass (Lolium perenne) and red and white (Trifolium repens) clover mix;
Farm B-Pasture, permanent pasture, perennial ryegrass, red clover and white clover mix;
Farm C-Pasture, ryegrass and white clover mix followed by fescue (Lolium arundinaceum), red and white clover and plantain (Plantago lanceolata) mix during the last 2 weeks.
[2] Composite: Perendale, Texel, Finnish Landrace and Romney genetics; LambSupreme: lean-selected Poll Dorset, Wiltshire, Romney x Dorset, Coopworth, Texel, and high-growth Romney.

5.2.2. Meat sampling and storage

The *Longissimus thoracis* (loin) muscle was removed from the carcass, vacuum packed and chilled at -1.5°C for 21 days then frozen at -20°C until further analysis. A detailed description of animal groups, sample collection and carcass and meat quality characteristics corresponding to this study were previously reported in Chapter 2. Prior to proteomics analysis all samples were freeze-dried (Cuddon Freeze Drier, Blenheim, NZ) and ground into a fine (<1mm) powder.

5.2.3. Protein extraction

200 mg frozen grounded lamb tissue were soaked in 2 ml lysis buffer (7 M Urea, 2M Thiourea,1% freshly-added dithiothreitol and 1 protease inhibitor tablet in 50 mL buffer) and homogenised in a hand-held, serrated pestle homogeniser over ice for four cycles of 1 min grinding and 10 s pause as previously described by Yu et al. (2016). After vortexing the homogenate for 30 min at 4°C, the insoluble material was pelleted by centrifugation of the homogenate at 15000g for

30 min at 4°C. The pellet was discarded, and the supernatant was collected and stored at −80 °C. The protein concentrations were determined using the 2D-Quant kit according to the manufacturer's instructions (GE Healthcare, USA). 150 µg protein in each samples were extracted from supernatant using chloroform-methanol precipitation method based on Wessel and Flügge (1984).

5.2.4. Protein digestion

Protein pellets generated from chloroform-methanol precipitation were resuspended in 100 µl 0.1M ammonium bicarbonate containing 1% sodium deoxycholate (SDC), then reduced with 20 µl of 50 mM dithiothreitol in 0.1 M ammonium bicarbonate at 56°C for 45 min. Alkylation was performed by adding 20 µl of 150 mM iodoacetamide in 0.1M ammonium bicarbonate solution and vortexed for 30 min in the dark at room temperature. Protein digestion was performed by adding 2 µg MS-grade trypsin (Promega, Madison, WI, USA) and 10% acetonitrile, vortexing briefly and incubating at 37 °C for 18 h. The SDC was precipitated and removed by adding 5% formic acid and centrifuged. The resulting peptides were desalted with C18 spin columns according to the manufacturer's instructions (Thermo Scientific, USA), dried by a centrifuge concentrator and resuspended in 0.1% formic acid prior to LC-MS/MS analysis.

5.2.5. Mass spectrometric analysis

Liquid chromatography-mass spectrometry (LC-MS) was performed on a nanoflow Ultimate 3000 Dionex UPLC (Thermo Scientific) coupled to an Impact HD mass spectrometer equipped with a CaptiveSpray source (Bruker Daltonik, Bremen, Germany). For each sample, 1 µL of the sample was loaded on a C18 PepMap100 nano-Trap column (300 µm ID x 5 mm, 5 micron 100Å) at a flow rate of 3000 nl/min. The trap column was then switched in line with the analytical column ProntoSIL C18AQ (100 µm ID x 150 mm 3 micron 200Å). The reverse phase elution gradient was

from 2% to 20% to 45%B over 60 min, total 85 min at a flow rate of 1000 nL/min. Solvent A was LCMS-grade water with 0.1% Formic acid; solvent B was LCMS-grade acetonitrile with 0.1% FA.

The LC was directly interfaced with a captive spray ion source (3.0 L/min dry gas, operated at 1500 V) to a high-resolution Impact HD quadrupole-time-of-flight (Q-TOF) (Bruker Daltonik) mass spectrometer. To profile protein expression patterns, the analytes were detected via MS-only mode in positive ion mode, with a mass range between 130 – 2200 m/z and a sampling rate of 2 Hz. To link the expression levels with identifications, a pool of per treatment was created, and these pooled samples were run via LC-MS/MS with data-dependent auto-MS/MS mode with the following settings: the same LC parameters as described before, a full scan spectrum, with a mass range of 350-2200 m/z, was followed by a maximum of ten collision-induced dissociation (CID) tandem mass spectra at a sampling rate of 2 Hz for MS scans and 1 to 20 Hz for MS/MS. Precursors with charges 2+ to 3+ were preferred for further fragmentation and a dynamic exclusion of 60 sec was set. Following the LC-MS run, the Q-TOF data were further analysed with Compass DataAnalysis 4.4 software (Bruker Daltonik) to evaluate the LC chromatogram and the overall quality of both MS1 and MS2 spectra.

5.2.6. Protein identification

The PEAKS X+ Studio data analysis software package (Bio informatic Solutions Inc, Waterloo, Canada) was used to analyse the LC-MS/MS data. The raw data were refined by a built-in algorithm which allows association of chimeric spectra. The proteins/peptides were identified with the following parameters: a precursor mass error tolerance of 10 ppm and fragment mass error tolerance of 0.05 Da were allowed, the UNC01_Ovis aries database (v2020.06, 53326 sequences) was used, the cRAP database was used as contaminant database, semi-trypsin was specified as digestive enzyme and up to 2 missed cleavages were allowed. Carbamidomethylation of cysteine was set as fixed modification. Both oxidation and deamidation are chosen as variable

modifications in Peaks DB, and in the optimized Peaks PTM search Pyro-Glu from Q, amidation, and carbamidomethylation were added to the variable modification list. A maximum of 3 post-translational modifications (PTMs) per peptide was permitted. False discovery rate (FDR) estimation was made based on decoy-fusion. An FDR of < 1% with a peptide hit threshold of -10 log p> 19.3 and a PTM A-score of 100 was considered adequate for confident peptide identification. To allow for confident protein identification and relative quantification, at least two unique peptides per protein were required.

5.2.7. Label free quantification

To quantify the protein expression levels, label-free quantification (LFQ) was performed using the quantitation node of Peaks Studio X+ software. Here, expression levels between all samples were compared. The following parameters were included: a mass tolerance error of 15 ppm and a retention time shift tolerance of 2 min was allowed. To determine the relative protein and peptide abundance in the retention time aligned samples, peptide feature based quantification was performed. Relative comparison between samples is based on the area under the curve and to get this cumulative area for each protein, only unique peptides that are assigned to a particular protein were selected.

5.2.8. Statistical analysis

The relative abundance estimates resulting from the LFQ analyses were used to select proteins with a measured abundance in at least 75% of the samples, prior to scaling to zero means and unit variances. Supervised Partial Least Square Discriminant Analysis (PLS-DA) were performed on the scaled LFQ results to identify clusters of samples using mixOmics software (version 6.12.2, Rohart et al., 2017) and R (version 4.0.2).

Eight samples from each of the six treatment groups (REDC-W, MXME-W, GRASS-W, CHIC-W, CHIC-E, WEAN-W) were sent for mass spectrometry analysis. The samples were

randomly assigned to six batches for analysis, with duplicate samples present in each batch, along with standards and quality control samples. During quality control of the samples, a distinct batch effect was detected for the samples assigned to the first batch (group B1, supplementary Figure S1). The batch effect was detected by performing an analysis of variance on the first three components of the PCA. Significant differences between the batches were detected in all three components ($P < 0.05$). From the 11 samples present in the first batch of analysis, three samples were duplicated in different batches. By removing the samples from batch 1 from the analysis, no significant batch effect was then detectable between the batches (supplementary Figure S2). After quality control, 7 samples processed in the first batch of the analysis were removed from the dataset, reducing the number of samples to 41 across the six production groups (n=8 for REDC-W, n=7 for MXME-W, n=6 for GRASS-W, n=6 for CHIC-W, n=7 for CHIC-E, n=7 for WEAN-W).

A negative binomial model, with the abundance level as the response level and the interaction between the protein and each production system, was applied to identify proteins of lamb loin muscle that were significantly different between commercial forage production systems. Using the binomial model, additional pairwise comparisons were made between lamb groups differing in sex (CHIC-E vs CHIC-W), diet (GRASS-W vs CHIC-W), and age (CHIC-W vs WEAN-W) for each of the proteins. For all models, p-values were adjusted for multiple testing using the Benjamini-Hochberg correction and p-values smaller than 0.05 were deemed significant.

5.3. Results

5.3.1. Differences in protein profiles in meat from lambs reared in six forage production systems

In total, 286 proteins were identified across all samples with at least two unique peptides per protein. Of these, 281 proteins were detected and quantified in at least 75% of all samples. The

PLS-DA plot indicated that there are some differences and also similarities between the overall protein profiles in meat from six groups of lambs (Figure 5.1). The first component (x-variate) and second component (y-variate) of the PLS-DA explained 26% and 13% of the data variation, respectively. The CHIC-W lambs formed a cluster that diverged from CHIC-E lambs in both x- and y-variate although some overlap was observed. Among meat from all 6 production systems, REDC-W lambs showed the lowest variation in protein profiles measured.

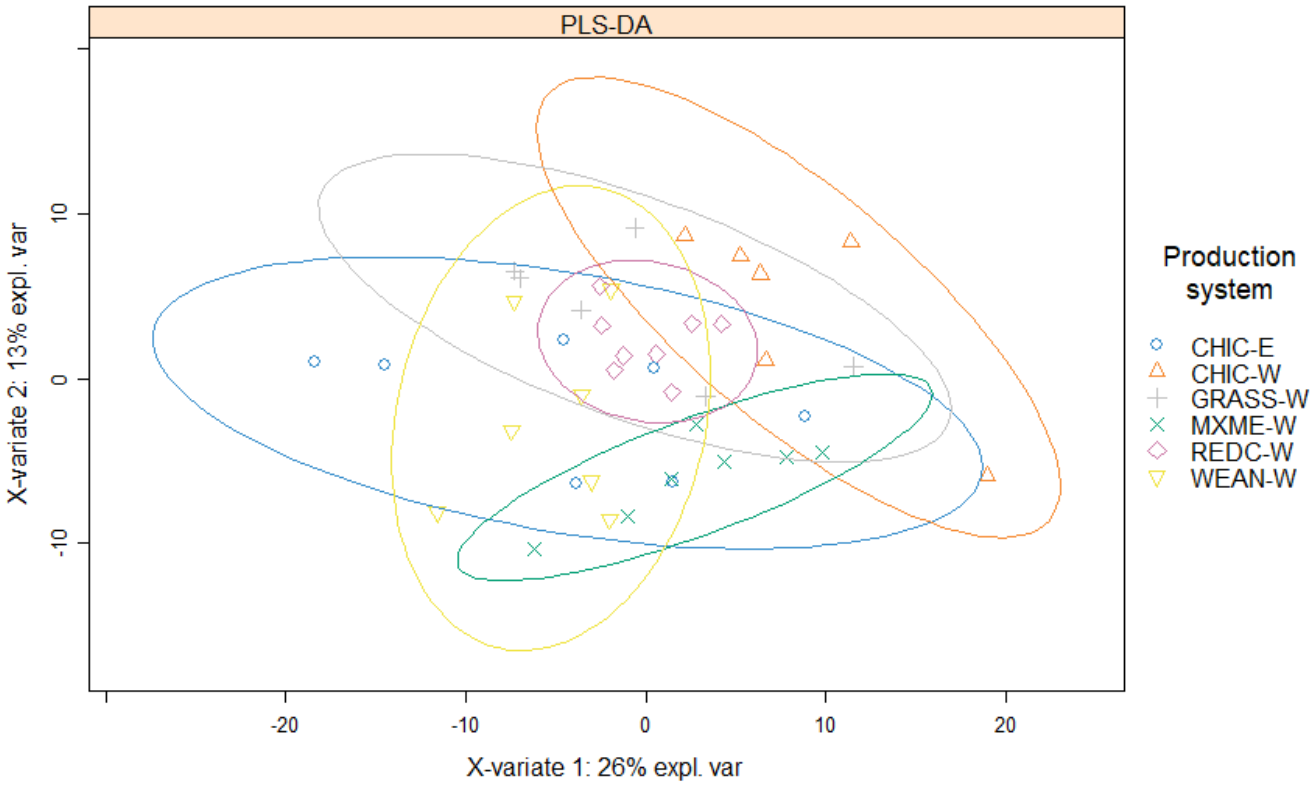


Figure 5.1 Partial least squares discriminant analysis of all identified proteins in raw lamb loins from six New Zealand commercial production systems (4-month-old wether lambs of a composite breed at weaning, WEAN-W; 6- to 8-month-old wether lambs of a composite breed that had been grazing red clover, REDC-W; 6- to 8-month-old wether lambs of a composite breed that had been grazing perennial ryegrass-based pasture, GRASS-W; 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing chicory, CHIC-W and CHIC-E; 12-month-old wether Merino lambs that had been grazing a mixed pasture, MXME-W). n=8 for REDC-W, n=7 for MXME-W, n=6 for GRASS-W, n=6 for CHIC-W, n=7 for CHIC-E, n=7 for WEAN-W.

A further look to see what drives these subtle differences between the protein profiles in the different groups is performed by fitting a negative binomial model to the interaction between the production systems and each of the proteins, predicting the abundance levels. The mean abundance level for each protein was then predicted using the generated model, followed by a comparison between the group means of the production system.

Overall, 16 proteins were identified by the negative binomial model as being significantly different between the production systems (Table 5.2). Within the seven myofibrillar proteins, the CHIC-W lambs exhibited the most unique protein profile, with 4 proteins differing from the other five production systems. Meat from CHIC-W lambs differed from CHIC-E and WEAN-W in Troponin C abundance, from REDC-W in myosin 8 abundance, and from CHIC-E in Myosin light chain 1 abundance.

Within the six identified sarcoplasmic proteins, the most unique protein profile was observed in loins from REDC-W lambs, which differed from all other animal groups in Heat shock proteins family A (Hsp70) and cognate 71 kDa abundances. The highest abundance variation among all production systems was observed for the immunoglobulin lambda-1 light chain-like protein. Creatine kinase U-type mitochondrial protein in meat was significantly different between MXME-W lambs and all other animal groups.

No unique protein profile was observed for stromal proteins, but there was a difference in the collagen proteins between lamb loins from CHIC-W and CHIC-E production systems. A large variation was observed between lamb loins from different production systems for heparan sulphate proteoglycan 2 protein.

Table 5.2 Identified myofibrillar, sarcoplasmic, stromal and proteoglycan proteins from raw lamb *longissimus lumborum* muscle that significantly differed between commercial forage production systems using a negative binomial model. A complete list of all identified proteins is presented as supplementary material (Table S1). n=8 for REDC-W, n=7 for MXME-W, n=6 for GRASS-W, n=6 for CHIC-W, n=7 for CHIC-E and n=7 for WEAN-W.

Protein	REDC-W	MXME-W	GRASS-W	CHIC-W	CHIC-E	WEAN-W	Accession number
Myofibrillar proteins[1]							
Troponin C, skeletal muscle isoform X1	AB	AB	AB	B	A	A	XP_027832152.1
Actin, alpha skeletal muscle isoform X4	A	A	A	B	A	A	XP_004021390.1
Myosin 2	A	A	A	B	A	A	XP_027830685.1
Myosin 8	A	AB	AB	B	AB	AB	XP_027830687.1
Myosin light chain 1	AB	A	AB	A	B	AB	A0A0H3V384
Myosin regulatory light chain 2, skeletal muscle isoform	A	A	A	B	A	A	NP_001138655.1
Myosin regulatory light chain 2, skeletal muscle isoform isoform X1	A	A	A	B	A	A	XP_011959304.1
Sarcoplasmic proteins[1]							
Hemoglobin subunit beta	AB	AB	A	AB	B	A	NP_001091117.1
Heat shock protein family A (Hsp70)	A	B	B	B	B	B	W5NPN4
Heat shock cognate 71 kDa protein	A	B	B	B	B	B	XP_011951023.2
Creatine kinase U-type, mitochondrial	B	A	B	B	B	B	XP_011954300.1
Creatine kinase B-type	A	A	AB	B	AB	AB	XP_027813246.1
Immunoglobulin lambda-1 light chain-like	BCD	A	ABC	CD	AB	D	XP_027812629.1
Stromal proteins[1]							
Collagen alpha-3(VI) chain	AB	AB	AB	A	B	AB	XP_027823071.1
Collagen type VI alpha 3 chain	AB	AB	AB	A	B	AB	W5QCP9
Others[1]							
Heparan sulfate proteoglycan 2	AB	A	ABC	C	BC	AB	W5PEL7

[1] A, B, C, D In the same row, letters represent the difference in predicted means of the protein between groups. The same letter indicates that there is no significant difference between the groups, while a different letter indicates a significant difference with a p-value < 0.05 (Benjamini-Hochberg adjusted).

5.3.2. Pairwise comparison between the meat proteome from ewe and wether lambs grazed on chicory (CHIC-E vs. CHIC-W)

The abundances of all myofibrillar, sarcoplasmic and stromal proteins that were significantly different between ewes and wethers that grazed on chicory are presented in Table 5.3. The abundances of all myofibrillar and sarcoplasmic proteins from this table were higher in meat from CHIC-E than CHIC-W lambs. Collagen alpha-3 chain was the only protein that was more abundant in meat from wether than ewe lambs that grazed on chicory.

Table 5.3 Identified myofibrillar, sarcoplasmic and stromal proteins that significantly differed between raw lamb *longissimus lumborum* muscle from 6- to 8-month-old ewe and wether lambs grazed on chicory. n=6 for CHIC-W and n=7 for CHIC-E.

Protein	Fold change (ewe/wether)	Adjusted *P* value	Coverage (%)	Unique Peptides	Accession
Myofibrillar proteins[1]					
Actin, alpha skeletal muscle isoform X4	12.88	<0.001	76	3	XP_004021390.1
Myosin-2	5.48	<0.001	61	3	XP_027830685.1
Myosin regulatory light chain 2, skeletal muscle isoform	5.12	<0.001	85	6	NP_001138655.1
Myosin regulatory light chain 2, skeletal muscle isoform isoform X1	9.27	<0.001	99	3	XP_011959304.1
Troponin C, skeletal muscle isoform X1	2.58	0.038	80	9	XP_027832152.1
Myosin light chain 1	2.61	0.005	78	2	A0A0H3V384
Sarcoplasmic proteins[1]					
Immunoglobulin lambda-1 light chain-like	2.51	0.007	35	2	XP_027812629.1
Glutathione S-transferase P	3.07	0.003	1	2	W5QCP9
Stromal proteins[1]					
Collagen alpha-3(VI) chain	0.33	<0.001	1	2	XP_027823071.1

[1] P-value cut-off of 0.05 with Benjamini-Hochberg correction.

5.3.3. Protein comparison between meat from wether lambs grazed on perineal ryegrass and chicory (GRASS-W vs. CHIC-W)

The abundances of only 4 myofibrillar proteins differed (Table 5.4) between the loins of 6-8-month-old wether lambs that grazed on perennial ryegrass (*Lolium perenne*) compared to chicory (*Cichorium intybus*). The abundance of alpha skeletal muscle isoform X4 of actin was 17.22-fold greater in loins from lambs grazed on perennial ryegrass than chicory. The abundance of skeletal muscle isoform of myosin regulatory light chain 2, skeletal muscle isoform X1 of myosin regulatory light chain 2, and myosin-2 were 5.22, 6.21 and 3.70-fold greater in loins from lambs grazed on perennial ryegrass than chicory, respectively.

Table 5.4 Identified myofibrillar proteins that significantly differed between raw lamb *longissimus lumborum* muscle from 6- to 8-month-old ewe lambs grazed on perennial ryegrass and chicory. n=6 for GRASS-W and n=6 for CHIC-W.

Protein	Folder change (grass/chicory)	Adjusted *P* value	Coverage (%)	Unique Peptides	Accession
Myofibrillar proteins[1]					
Actin, alpha skeletal muscle isoform X4	17.22	<0.001	76	3	XP_004021390.1
Myosin regulatory light chain 2, skeletal muscle isoform	5.22	<0.001	85	6	NP_001138655.1
Myosin regulatory light chain 2, skeletal muscle isoform isoform X1	6.21	<0.001	99	3	XP_011959304.1
Myosin-2	3.70	<0.001	61	3	XP_027830685.1

[1] P-value cut-off of 0.05 with Benjamini-Hochberg correction.

5.3.4. Protein comparison between meat from wether lambs slaughtered at 4-months of age and 6- to 8-months of age (WEAN-W vs. CHIC-W)

Alpha skeletal muscle isoform X4 of actin was 16.66-fold more abundant in the loin meat from 4-month-old pre-weaning lambs than 6- to 8-month-old lambs grazed on chicory (Table 5.5). The abundances of the other myofibrillar proteins in meat, Myosin regulatory light chain 2, Myosin-2, Myosin-8, and Troponin C, were also greater in loins form 4-month-old than 6- to 8-month-old lambs. Other proteins such as Heparan sulfate proteoglycan 2 was more abundant in loins from 4-month-old than 6- to 8-month-old lambs.

Table 5.5 Identified myofibrillar and proteoglycan proteins that significantly differed (P< 0.05) between raw lamb *longissimus lumborum* muscle from 4-month-old pre-weaning lambs and 6-8-month-old wether lambs grazed on chicory. n=7 for WEAN-W and n=6 for CHIC-W.

Protein	Folder change (wean/chicory)	Adjusted *P* value	Coverage (%)	Unique Peptides	Accession
Myofibrillar proteins[1]					
Actin alpha skeletal muscle isoform X4	16.66	<0.001	76	3	XP_004021390.1
Myosin regulatory light chain 2 skeletal muscle isoform	6.25	<0.001	85	6	NP_001138655.1
Myosin regulatory light chain 2 skeletal muscle isoform X1	5.26	<0.001	99	3	XP_011959304.1
Myosin-2	5.26	<0.001	61	3	XP_027830685.1
Myosin-8	2.33	0.032	42	7	XP_027830687.1
Troponin C skeletal muscle isoform X1	2.86	0.002	80	9	XP_027832152.1
Others[1]					
Heparan sulfate proteoglycan 2	2.77	0.003	1	2	W5PEL7

[1] P-value cut-off of 0.05 with Benjamini-Hochberg correction.

5.4. Discussion

The objective of the present study was to explore the protein profiles of meat from six types of typical commercial New Zealand lamb production systems that included animals of different sex, genetics, age at slaughter and grazed on different forage diets. Based on the 286 proteins that were identified across all samples, 270 proteins (supplementary Table S1) that contributed to various functions in lamb loin muscle were similar between all 6 production systems. This indicated that the expressions of more than 90% of proteins identified in lamb loins in the current study are relatively stable under different forage production systems. This is in agreement with Picard et al. (2019) who indicated that diet composition, gender and rearing practices had few effects on proteins abundances of cattle muscles. However, 16 proteins were found in this study to enable different groupings of loins from lambs reared in the different production systems. These 16 proteins included structural myofibrillar proteins, sarcoplasmic proteins involved in biological pathways in the muscle related to energy metabolism and cell turnover, and stromal proteins associated with muscle cell structure.

Myofibrillar proteins are the most abundant proteins in lamb muscle, constituting 65–75% of the total muscle proteins. Myosin and actin are the major myofibrillar proteins accounting for approximate 45% and 25% of the total myofibrillar proteins in muscle (Lametsch et al., 2003). Seven out of 16 of the discriminating proteins from the six production system groups belong to this class. Interestingly, based on these proteins except myosin light chain 1, CHIC-W lambs could be clearly distinguished from the other five production systems. A similar clear difference between groups is seen when two sarcoplasmic proteins are considered. Heat shock protein family A (Hsp70) and heat shock cognate 71 kDa protein, showed a greater expression in wether lambs grazed on red clover (REDC-W) than lambs from other production systems. Heat shock proteins are associated with impeding apoptosis including the activity of proteolytic enzymes associated

with post-mortem aging of meat (Lomiwes et al., 2014; Luca et al., 2013; Zhang et al., 2019). As such, the expression of heat shock proteins has been correlated to a reduction in myofibrillar protein degradation and leading to meat toughness (Lomiwes et al., 2014; Picard and Gagaoua, 2020). However, meat from REDC-W lambs showed similar ($P>0.05$) shear force values to meat from the other types of lamb except for MXME-W, which showed a higher shear force value (Chapter 2). The differences in the abundances of these proteins may not be large enough to have a significant impact on meat quality such as tenderness for lambs slaughtered at 6- to 8-month-old. Mitochondrial creatine kinase U-type is another sarcoplasmic protein that enables grouping of the production systems. The main function of creatine kinase is to catalyse the reversible phosphotransferase reaction between creatine and ATP, which is required when high ATP regeneration is demanded. In the post-mortem muscle, creatine kinase is used to maintain ATP concentration when it cannot be regenerated through oxidative metabolism (Beldarrain et al., 2018). Due to its ability to slow the decline in post-mortem muscle pH, creatine kinase has a role in maintaining the water holding capacity of meat (Daroit and Brandelli, 2008). The relative abundance of creatine kinase in meat from MXME-W was significantly different from the other animal groups. Meat from MXME-W lambs showed significantly higher muscle pH than meat from the other animal groups in this study (5.66-5.78 vs.5.39-5.47, respectively; Chapter 2). All other proteins couldn't specifically differentiate just one production system.

To gain further insights, pairwise comparisons were performed to evaluate the effect of gender (ewe vs. wether), diet (perennial ryegrass vs. chicory) and age at slaughter (4 vs. 6- to 8-months old) on the relative abundance of proteins in meat from animals within a single farm.

5.4.1. Proteome abundance differences due to gender (CHIC-E vs. CHIC-W)

Among the 4 myosin types (myosin-2, -6, -7, -8) observed in the current study, only myosin-2 showed greater abundance in meat from ewe lambs (CHIC-E) than wether lambs (CHIC-W). One molecule of myosin (223kDa) consists of two myosin heavy chains (56kDa), two myosin regulatory light chain 2 (18.8KDa), two essential light chains and other fragments (Hopkins and Geesink, 2009). Likewise, myosin regulatory light chain 2 was also more abundant in the loin of ewe compared to wether lambs. Both myosin-2 and myosin regulatory light chain 2 are more abundant in slow-twitch oxidative type muscles than the fast-twitch glycolytic type (Clerens et al., 2016; Kim et al., 2018). For most mammals, females have more slow oxidative fibres in muscles than males (Schiaffino and Reggiani, 2011). For cattle a greater abundance of myosin-2 was associated with tender beef (mean shear force 27.9N) rather than tough beef (mean shear force 69.6N, Beldarrain et al., 2018). Another myofibrillar protein, the alpha skeletal muscle isoform X4 of actin, was 12.88-fold more abundant in loins from ewe than wether lambs. The abundance of alpha actin was reported to be 5.5 fold higher in the high-quality beef than low-quality beef, and positively correlated to meat tenderness and redness (Gagaoua et al., 2021b; Kim et al., 2008). However, tenderness and colour did not differ ($P>0.05$) for meat from ewe (mean shear force 26.4 N) lambs compared to wether lambs in the current study (mean shear force 26.4 N, Chapter 2). The differences in the abundance of these myofibrillar proteins may not be large enough to be reflected in differences in meat shear force or colour between ewe and wether lambs slaughtered at 6-8-month-old.

Glutathione S-transferase P is the only enzyme in our study that differed between ewe and wether lambs. This enzyme plays a role in mechanisms of cellular detoxification and cellular resistance to oxidative damage by catalysing the conjugation of glutathione to potentially toxic compounds such as reactive oxygen species (Hamelin et al., 2006). Greater abundance of

glutathione S-transferase P was found in double-muscled Belgian Texel lambs than Romanov lambs but was not correlated to any meat quality characteristics (Hamelin et al., 2006). This enzyme could have an impact on colour and lipid stability and therefore meat shelf-life. However, these parameters were not measured in the samples from the current study. Immunoglobulin lambda-1 light chain was more abundant in loins from ewe lambs than wether lambs and is a small polypeptide subunit of an antibody (immunoglobulin). Likewise, immunoglobulin lambda-1 light chain has not been associated with any meat quality traits and any differences in abundance are likely to reflect individual lamb immunological responses (Ferreira et al., 2017).

Collagen alpha-3 (VI) chain is a stromal protein in connective tissue, which is the only protein that had greater abundance in loins from wether lambs than ewe lambs in the current study. Similar results were reported by Monteschio et al. (2018) where both soluble collagen and total collagen content were greater in loins from wether lambs than ewe lambs, likely because of the increased collagen synthesis in males at puberty (Young et al., 1993a).

5.4.2 Proteome abundance differences due to diet (GRASS-W vs. CHIC-W)

Lamb production systems in New Zealand have traditionally relied on perennial ryegrasses. There has, however, been an increase in the use of alternative forage types such as chicory in order to offer a diet with a higher nutritive value than traditional pastures for faster lamb liveweight gains (Somasiri et al., 2015). The chicory diet decreased the abundance of actin, myosin-2 and myosin regulatory light chain 2 but did not affect the abundance of sarcoplasmic or other proteins. Chicory diets are associated with faster growth rates and lambs that are heavier at slaughter (Somasiri et al., 2015). This means that lambs grazed on chicory, tend to be more physiologically mature at slaughter. As the degree of maturity increases in production animals, there is an increased proportion of fast glycolytic relative to slow oxidative fibres in the muscle (Schreurs et al., 2008).

The decreased abundance of actin, myosin-2 and myosin regulatory light chain 2 in chicory fed lambs is likely a consequence of a shift towards more fast glycolytic fibres in the loin muscle, which is associated with less amount of these myofibrillar proteins (Clerens et al., 2016; Kim et al., 2018). Interestingly, the chicory diet did not affect the abundance of sarcoplasmic or stromal proteins.

5.4.3. Proteome abundance differences due to age (WEAN-W vs. CHIC-W)

Loins from lambs slaughtered at a younger age (4-month-old) had a greater abundance of myosin-2, myosin-8, myosin regulatory light chain 2, actin and troponin C compared to lambs slaughtered at 6- to 8-month-old, which might also be due to the differences in maturity and muscle fibre types. For sheep aged from 4- to 22-month-old, the proportion of oxidative fibres in the loin decreased with animal age due to the relatively faster development of glycolytic fibres (Greenwood et al., 2007). Therefore, the proportion of slow oxidative fibres is likely to be lower in the muscle of the older 6 to 8-month-old lambs, which resulted in lower abundance of myofibrillar proteins, compared with lambs slaughtered at 4-month-old in the current study. Heparan sulfate proteoglycan is a linear polysaccharide that binds to a variety of protein ligands and regulates a wide range of biological activities, including developmental processes, angiogenesis, blood coagulation, and tumour metastasis (Velleman et al., 1996). The heparan sulfate proteoglycan 2 gene in chromosomes SSC6 that encodes a large proteoglycan was positively associated with marbling score in pork (Choi et al., 2012). Similarly, meat from WEAN-W lambs showed a greater abundance of heparan sulfate proteoglucan 2 protein and a higher intramuscular fat percentage than meat from CHIC-W in the current study (3.2% vs. 2.6%, respectively; Chapter 2). Further investigation is needed to evaluate whether heparan sulfate proteoglycan could be considered as a potential indicator of fat deposition in meat producing animals.

5.5. Conclusions

286 proteins were identified across lamb loin samples from 6 New Zealand forage production systems using liquid chromatography-tandem mass spectrometry. Based on a negative binomial model, most proteins expressed similar abundance, while 16proteins showed different abundance in meat from the different animal groups. These 16 proteins included structural myofibrillar proteins, sarcoplasmic proteins, and stromal proteins involved in biological pathways in the muscle related to energy metabolism, cell turnover and muscle cell structure. The abundance of some of those proteins such as creatine kinase, was significantly different in meat from MXME-W lambs which tend to have higher ultimate pH than meat from other breeds.

Greater abundance of myofibrillar proteins and lower abundance of stromal proteins were observed in ewes than wethers, which was likely due to differences in the proportions of muscle fibre types and collagen. Chicory diet decreased the abundance of myosin-2, myosin regulatory light chain 2 and actin in lamb loins compared to traditional perennial ryegrass, possibly due to greater proportion of muscle glycolytic fibres from faster growing lambs and heavier carcasses at slaughter. Similarly, the abundance of myofibrillar proteins in loins decreased when lambs were slaughtered at 6- to 8-month-old compared to 4-month-old, which was probably due to faster growth rate of glycolytic fibres than oxidative fibres during the finishing period. Overall, these results provide a deeper understanding of the effect of production factors on the relative abundance of meat proteins. Quantification of the contribution of individual proteins in the current study to meat quality characteristics requires further investigation. However, the sensory impact which results from both chemical and physical characteristics of the meat was investigated in Chapter 6

Chapter 6

Sensory properties of *Longissimus thoracis* from commercial lambs reared in different forage systems evaluated by New Zealand and Chinese Consumers

This chapter formed part of the following publication:

Pavan, E., Ye, Y., Eyres, G.T., Guerrero, L., Reis, M.G., Silcock, P., Johnson, P.L., Realini, C.E., 2021. Relationships among consumer liking, lipid and volatile compounds from New Zealand commercial lamb loins. Foods 1143.

Abstract

One hundred and sixty New Zealand consumers were recruited to assess the flavour, tenderness and juiciness and overall liking of lamb loins from lambs from six production systems. In addition, Chinese consumers (n=159) were recruited to evaluate the eating quality of loins from lambs reared in three of the six production systems. Tenderness, flavour, and overall-liking scores of New Zealand consumers (n=160) differed ($p<0.05$) between meat samples from the six groups evaluated, but juiciness scores did not differ ($p>0.05$). The highest overall-liking scores were observed for meat from wether lambs grazing chicory, while meat of Merino lambs was least favoured. Among Chinese consumers (n=159), tenderness, flavour and overall scores did not differ between the three evaluated production systems. One cluster of Chinese consumers (n = 51) showed a linear decrease in overall-liking with increasing intramuscular fat. Another cluster of Chinese consumers (n=54) showed a decrease in overall liking with increasing content of polyunsaturated fatty acids and abundance of lipid oxidation compounds. A cluster of New Zealand consumers (n = 75) had greater overall liking scores for loin meat with increasing content of polyunsaturated fatty acids and abundance of lipid oxidation compounds. The fatty acid profile and the volatile compounds associated with the oxidation of the meat before cooking had an influence on the liking of lamb for some, but not all consumers Chinese and New Zealand consumers.

6.1. Introduction

Consumers are the final step in the meat production chain, and meeting their expectations is an important part of their satisfaction and shopping behaviour. The New Zealand red meat industry is export focused and built primarily on grazing systems (Loughlin and Ritchie, 2018). In 2019/20 season, 209,386 tonnes of sheep meat was exported to China, which constituted approximately 54% of New Zealand's total sheep meat exports during the year (Meat Industry Association, 2020). Thus, a key goal of the New Zealand lamb industry is to consistently produce meat with highly desirable eating quality characteristics that satisfy both New Zealand and overseas consumers (Craigie et al., 2017). Acceptability of lamb meat also depends on regional and cultural factors which differ between countries (Oliver et al., 2006; Sanudo et al., 1998). Japanese consumers have been reported to be sensitive to "mutton" flavours generated by branched-chain fatty acids which may be a barrier to their acceptance of sheep meat (Prescott et al., 2001; Watkins et al., 2010). According to Font-i-Furnols and Guerrero (2014), the factors affecting consumer purchasing behaviour for meat products can be divided into three types: psychological (lifestyle and values, socio-cultural effect), sensory (in-mouth texture, flavour, visual appearance) and marketing (price, brand, availability). More recently, a range of psychological attributes such as animal welfare, sustainability and health concerns have become increasingly important in meat purchasing decisions although consumers do not appear to be willing to compromise on eating quality (Realini et al., 2021; Verbeke, 2006).

The eating quality of cooked meat is determined by a combination of flavour, tenderness and juiciness (Miller, 2020). Tenderness is considered the most important descriptive attribute driving consumer liking for beef, while flavour was considered as the most important attribute for lamb, particularly when acceptable tenderness (shear force <40N) was achieved (Hopkins et al., 2006;

Miller, 2020). Intramuscular fat content has been reported to have a positive effect on eating quality as increasing levels in meat are associated with increasing tenderness, flavour, and juiciness (Lambe et al., 2017; Realini et al., 2021). Fat also has the capacity to act as a solvent for volatile compounds, and its role in the release of thermal oxidative compounds in meat products during cooking has been investigated in various studies (Farmer, 1994). In addition, the level of volatile compounds in cooked meat also depends on cooking method and the internal temperature of cooked meat. For example, pyrazines were only observed when meat was fried in oil (Domínguez et al., 2014). Nevertheless, many volatiles have been observed in both raw and cooked lamb meat for example dimethyl sulfone which contributes to an unfavourable "milky" flavour in meat from pasture-fed sheep (Young et al., 2003).

To achieve high quality meat products, knowledge of animal production system factors on eating quality attributes, especially the flavour of lamb meat is essential. The objective of this chapter was to investigate the eating quality, as assessed by both New Zealand and Chinese consumers, of meat from New Zealand lambs finished in commercial production systems and evaluate the role of fatty acid and volatile composition on the sensory scores.

6.2. Material and methods

6.2.1. Treatments

In Chapters 2, 3, and 4, the differences between meat quality and the fatty acid and volatile profiles of raw meat from 10 forage production systems were investigated. Due to the limits of analysis capability, proteomic analysis in Chapter 5 was reduced to 6 groups. These same 6 groups were assessed by New Zealand consumers for sensory evaluation in this chapter. Three production systems, GRASS-W, CHIC-W and MXME-W were assessed during Chinese consumer

evaluations. Further reductions in the number of production system groups assessed were made for the Chinese consumer sensory testing due to a limited quantity of meat sample that remained. Samples were prioritised to be allocated to the New Zealand consumer study and also to the objective meat quality, fatty acid, volatile and proteomics testing (Chapters 3-5). Details of lamb production system groups and the slaughter and post slaughter processing were described previously in Chapter 2.

6.2.2. New Zealand consumer sensory evaluation

One hundred and sixty New Zealand consumers were recruited in Dunedin (New Zealand) in June 2018 by the Product Development Research Centre at the University of Otago to assess and score consumers liking of flavour, degree of tenderness, degree of juiciness and overall liking of lamb samples from six production systems. The production systems included: 4-month-old wether lambs of a composite breed at weaning (WEAN-W-4); 6- to 8-month-old wether lambs of a composite breed that had been grazing red clover (REDC-W); 6- to 8-month-old wether lambs of a composite breed that had been grazing perennial ryegrass-based pasture (GRASS-W); 6- to 8-month-old wether and ewe lambs of a composite breed that had been grazing chicory (CHIC-E and CHIC-W); 12-month-old wether Merino lambs that had been grazing a mixed pasture (MXME-W). Consumers were selected from a database following a stratified random sampling procedure to balance by gender and age to match the distribution of the New Zealand national population (Stats NZ Tatauranga Aotearoa, 2020). A total of 8 sensory sessions were carried out over a period of 3 days. Each session included 20 consumers randomly chosen from the initial group of participants, with each consumer participated in only one session. A signed consent form was obtained from each participant before each session (see consent form for New Zealand

consumers in Appendix). For each of selected production systems, 8 vacuum-packaged loin samples were randomly chosen from the total 15 samples, and were randomly allocated to one of 8 sensory study sessions.

Meat samples (15 cm sections of loin) were thawed at 4°C for 24 h and then repacked in food degree sous vide vacuum bags (Sunbeam FoodSaver®) and cooked at 57°C for 1 h. After cooking, the loins were removed from the sous vide bag, dried using paper towels and rested for 3 min. Loins were then grilled (Blue Seal Hot Plate) on a 170°C hot plate for 5 min with the fat side down followed by 3 min on the other side. The average recorded core temperature was 60°C (equivalent to a medium degree of doneness). Each loin was cut into 1.5 cm slices using a cutting guide, and then were cut in half again in order to obtain 20 portions per loin. Portions were kept warm at 40°C in a Bain Marie prior to serving on plates labelled with a random 4-digit code.

The evaluation of the samples was performed in individual sensory booths that had controlled environmental conditions. To avoid first order and carryover effects, samples were presented to consumers in different orders according to a Williams square design (Macfie et al., 1989). Consumers were given a cup of tap water, diluted juice and crackers to cleanse their palate between each sample. Each consumer rated their overall liking of each sample, their liking of the flavour, the degree of juiciness and the degree of tenderness of the six lamb samples (WEAN-W, REDC-W, GRASS-W, CHIC-W, CHIC-E, MXME-W) using a 100 mm non-structured line scale anchored at each end (0: “dislike extremely”, “not juicy”, “not tender” to 100: “like extremely”, “very juicy”, “very tender”).

6.2.3. Chinese consumer sensory evaluation

One hundred and sixty Chinese consumers were recruited by Plant & Food Research in Auckland (New Zealand) in January 2019 to evaluate the eating quality of meat samples from three production systems (GRASS-W, CHIC-W, MXME-W). Consumers were asked to score their liking of flavour, degree of tenderness degree of juiciness and overall liking. Consumers were selected from a database using a stratified random sampling procedure to balance for gender and age to represent the distribution of the national population in China (Ritchie, 2019). All consumers spoke Mandarin as their first language and had lived in New Zealand for no more than 5 years. A total of eight tasting sessions were carried out over 3 days. Each session included 20 consumers randomly chosen from the initial group of participants, with each consumer only participant in one session. A signed consent form was obtained from participants before each session (See consent form for Chinese consumers in Appendix).

Loins samples for each production system were taken from the same 8 lambs as selected for New Zealand consumer trials. The samples were prepared in the same manner as the samples for New Zealand consumers, however, a higher degree of doneness was used for Chinese consumers to avoid potential rejection caused by appearance (i.e pink colour and appearance of blood), as Chinese consumers have been reported to be more familiar with a high degree of doneness for sheep meat (Mao et al., 2016). The loins were grilled in the same way as for the New Zealand consumers, on a 170°C plate for 5 min with the fat side down and 3 min on the other side. An average core temperature recorded of 71°C was achieved. Samples were served on plates with a random 3-digit code, and each consumer was asked to score their overall liking, liking of flavour, the degree of juiciness and the degree of tenderness using the same unstructured scale as New Zealand consumers.

6.2.4. Volatile and fatty acid analysis

The analysis of fatty acids (Chapter 3) and volatiles in the raw meat (Chapter 4) have been decribed in previously.

6.2.5. Statistical analysis

To identify clusters of consumers among the New Zealand and Chinese consumer studies an agglomerative hierarchical cluster analysis was performed by applying the Ward aggregation method on the squared Euclidean distance. Two clusters of New Zealand consumers (Cluster-1 and Cluster-2) and three clusters of Chinese consumers were identified based on their overall liking scores using XLSTAT (2017). Individual animal means were then generated for each sensory trait evaluated either by all consumers or for only the consumers in each cluster, Cluster-1 or Cluster-2. The effects of the commercial lamb production system on tenderness, juiciness, flavour liking, and overall liking scores were evaluated using an ANOVA with a completely randomized design in GENSTAT (18th edition, VSN International Ltd, Hemel Hempstead, UK). The model included production system as a fixed effect and consumer as a random effect; meat samples from individual lambs were considered as the experimental units.

Relationships between volatile compounds from raw meat, and total fatty acids content (based on the 8 individual lambs which were considered as the experimental units), sensory traits scores from all consumers, consumers in clusters were assessed using GENSTAT (18th edition, VSN International Ltd, Hemel Hempstead, UK) to generate Pearson correlation coefficients.

6.3. Results

6.3.1. New Zealand consumers

6.3.1.1. NEW ZEALAND CONSUMER SENSORY SCORES

When considering all the New Zealand consumers (n=160), tenderness, flavour, and overall-liking scores differed between meat samples from the 6 groups of commercial lambs, but juiciness scores did not differ (P=0.551; Table 6.1). Tenderness scores were higher (P<0.05) for meat from REDC-W, CHIC-E and CHIC-W lambs than MXME-W lambs. Flavour-liking scores were higher (P<0.05) for meat from REDC-W and CHIC-W lambs than WEAN-W and MXME-W lambs. Overall-liking scores were higher (P<0.05) for meat from CHIC-W lambs than MXME-W and WEAN-W lambs. Overall-liking scores of GRASS-W and CHIC-E lambs did not differ (P>0.05) from any other group.

Cluster-1 included about half of the evaluated New Zealand consumers (n=85) who preferred meat from GRASS-W lambs compared to WEAN-W, REDC-W and CHIC-E lambs as determined from their overall liking scores (Table 6.1). In cluster-1 consumer tenderness scores did not differ between the different types of lamb (P=0.14). The highest juiciness, flavour liking, and overall liking scores were observed for meat from GRASS-W lambs. The lowest juiciness and flavour liking scores were observed for meat from REDC-W and CHIC-E lambs, respectively (Table 6.1).

For the remaining consumers (cluster-2, n=75), meat from REDC-W lambs was the most preferred and meat from MXME-W lambs was the least preferred in overall liking. In cluster-2, consumer scores for tenderness and juiciness liking were highest for CHIC-E and lowest for MXME-W lambs. Consumer scores for flavour liking were highest for meat from REDC-W lambs and lowest for meat from MXME-W lambs.

Table 6.1 Consumer scores (0-100, mean and SEM) from sensory panel evaluation of grilled lamb loins (*Longissimus thoracis*) from 6 typical New Zealand commercial animal groups.

Descriptor	WEAN-W	REDC-W	GRASS-W	CHIC-E	CHIC-W	MXME-W	SEM	P-value
All Consumers (n = 160)								
Tenderness	70.0^{ab}	75.6^{a}	71.4^{ab}	75.1^{a}	74.9^{a}	67.4^{b}	1.5	<0.001
Juiciness	62.3	64.1	65.8	65.3	64.9	63.1	1.5	0.551
Flavour Liking	65.0^{b}	71.1^{a}	68.2^{ab}	68.3^{ab}	72.2^{a}	64.3^{b}	1.4	<0.001
Overall Liking	66.9^{bc}	72.0^{ab}	69.1^{abc}	69.3^{abc}	73.1^{a}	65.6^{c}	1.4	0.001
Cluster 1 (n = 85)								
Tenderness	70.8	71.3	76.2	69.8	74.2	71.7	1.9	0.141
Juiciness	63.8^{ab}	61.1^{b}	70.6^{a}	61.8^{b}	65.5^{ab}	68.6^{ab}	1.9	0.002
Flavour Liking	64.1^{c}	66.3^{bc}	75.6^{a}	62.9^{c}	71.9^{ab}	70.2^{abc}	1.9	<0.001
Overall Liking	67.4^{bc}	66.8^{bc}	76.6^{a}	63.2^{c}	73.2^{ab}	72.2^{ab}	1.7	<0.001
Cluster 2 (n = 75)								
Tenderness	69.2^{bc}	80.4^{a}	65.9^{c}	81.0^{a}	75.7^{ab}	62.6^{c}	2.2	<0.001
Juiciness	60.5^{ab}	67.5^{a}	60.3^{ab}	69.3^{a}	64.2^{ab}	57.0^{b}	2.2	<0.001
Flavour Liking	65.9^{bc}	76.6^{a}	59.8^{cd}	74.2^{a}	72.6^{ab}	57.7^{d}	2.0	<0.001
Overall Liking	66.3^{bc}	77.7^{a}	60.5^{c}	76.1^{a}	72.9^{ab}	58.3^{c}	2.0	<0.001

Different letters within the same row denote significant difference between means ($P < 0.05$).
SEM: standard error of the means.

6.3.1.2 CORRELATION BETWEEN SENSORY SCORES AND FATTY ACID PROFILES

Fatty acid composition of the raw meat and total fatty acid content (mg/g raw meat) were not correlated with overall liking or flavour liking when considering the scores from all the New Zealand consumers (n=160) or the consumers segregated into cluster-1 (n=85) ($P>0.05$). For consumers in cluster-2, negative correlations were observed between i-15:0 ($P<0.05$, Table 6.2), ai-17:0 ($P<0.01$), c9-18:1 ($P<0.05$), branched chain fatty acids (BCFA, $P<0.05$), monounsaturated fatty acids (MUFA, $P<0.01$) and overall liking and flavour liking scores. In addition, positive correlations were observed between 17:1 ($P<0.01$), 18:3 n-3 ($P<0.05$), 20:5 n-3 ($P<0.01$), 22:5 n-3 ($P<0.01$), 22:6 n-3 ($P<0.05$), polyunsaturated fatty acids (PUFA, $P<0.001$) and overall liking scores and flavour-liking scores.

Table 6.2 Pearson correlation coefficients of overall liking scores from all New Zealand consumers (n=160), cluster-1 (n=85), cluster-2 (n=75) and fatty acids composition of lamb loins (*Longissimus thoracis*) from the six typical New Zealand commercial animal groups assessed.

Fatty acids, % of total FA	Pearson correlation coefficients, N=48 (6 groups * 8 loins per group)					
	All 160 consumers		Cluster-1		Cluster-2	
	Overall liking	Flavour liking	Overall liking	Flavour liking	Overall	Flavour liking
10:0	0.02	-0.01	-0.01	-0.07	0.07	0.08
12:0	0.04	0.05	-0.13	-0.11	0.18	0.19
14:0	-0.02	-0.04	-0.06	-0.10	0.03	0.06
15:0	-0.05	-0.10	0.09	0.03	-0.07	-0.05
16:0	-0.03	0.01	0.01	0.01	-0.01	-0.01
17:0	-0.13	-0.19	0.14	0.12	-0.28	-0.30
18:0	0.07	0.07	0.21	0.25	-0.08	-0.11
i-15:0	-0.09	-0.11	0.26	0.25	-0.32*	-0.33*
ai-15:0	-0.04	-0.07	0.21	0.20	-0.20	-0.20
i-16:0	-0.01	0.01	-0.10	-0.08	0.25	0.28
i-17:0	-0.11	-0.11	0.27	0.29	-0.19	-0.20
ai-17:0	-0.20	-0.24	0.21	0.19	-0.43**	-0.45**
16:1	-0.14	-0.17	-0.04	-0.11	0.011	0.02
17:1	0.08	0.08	-0.25	-0.25	0.39**	0.44**
t9-18:1	0.01	-0.05	0.05	0.00	-0.12	-0.12
t11-18:1	0.16	0.09	-0.05	-0.11	0.05	0.07
c9-18:1	-0.26	-0.26	0.01	0.00	-0.29*	-0.32*
c11-18:1	0.09	0.09	-0.07	-0.05	0.23	0.24
18:2 n-6	0.23	0.23	-0.07	-0.06	0.20	0.23
18:3 n-3	0.23	0.24	-0.16	-0.14	0.30*	0.34*
c9t11-18:2	0.08	0.03	-0.09	-0.14	0.25	0.26
20:4 n-6	0.12	0.13	0.08	0.10	0.26	0.25
20:5 n-3	0.17	0.20	-0.13	-0.09	0.45**	0.47***
22:5 n-3	0.18	0.19	-0.07	-0.04	0.44**	0.45**
22:6 n-3	0.23	0.25	0.00	0.02	0.39*	0.40*
BCFA	-0.14	-0.16	0.24	0.23	-0.36*	-0.39**
SFA	0.00	0.01	0.20	0.22	-0.23	-0.22
MUFA	-0.26	-0.27	-0.02	-0.04	-0.45**	-0.46***
PUFA	0.23	0.24	-0.08	-0.07	0.52***	0.52***
Total FA	-0.21	-0.22	-0.08	-0.10	-0.34*	-0.34*

P < 0.05; **, P < 0.01, ***, P < 0.001

6.3.1.3 CORRELATION BETWEEN SENSORY SCORES AND VOLATILES

Pearson correlation coefficients of the mean overall liking scores from all (n=160) New Zealand consumers and from cluster-1 (n=85) and cluster-2 (n=75) identified the volatile compounds in raw lamb loin that appear to be associated with sensory characteristics (Table 6.3). When considering the sensory responses from all the consumers in the trial, three volatile compounds, 1-butanol, 2-butanone, and carbon disulphide were negatively correlated ($P<0.05$,) with overall liking and flavour liking scores (Table 6.3). Consumers in cluster-1 appear to have a general dislike of the volatiles from lamb meat as negative correlations were observed for most of volatiles, however, 2,5-hexanediol was the only compound that was negatively correlated ($P<0.05$) with overall liking scores. In contrast, for the consumers in cluster-2, the alcohols: 2,5-hexanediol, 1-penten-3-ol, 1-hexanol, 1-octen-3-ol, 1-heptanol were all positively correlated ($P<0.05$) with the overall and flavour liking scores. In addition, the aldehydes: octanal, nonanal, 2-pentanone, acetoin, 2-ethylfuran and dimethyl sulphide were also positively correlated ($P<0.05$) with overall and flavour liking scores for consumers. In general, the correlations seen for cluster-1 consumers were the converse of cluster-2 in terms of overall and flavour liking scores with the abundance of acids, alcohols, and aldehydes, although not all correlations were statistically significant.

Table 6.3 Pearson correlation coefficients (n=48, 6 groups * 8 loins per group) of overall liking scores from all New Zealand consumers (n=160), cluster-1 of New Zealand consumers (n=85) and cluster-2 of New Zealand consumers (n=75) and volatile compounds detected in the headspace of raw lamb loins (*Longissimus thoracis*) from the six typical New Zealand commercial production groups assessed.

Pearson correlation coefficients, n=48 (6 groups * 8 loins per group)						
Compounds	All 160 consumers		Cluster 1		Cluster 2	
	Overall	Flavour liking	Overall liking	Flavour liking	Overall liking	Flavour liking
Acids						
Acetic acid	-0.09	-0.11	-0.18	-0.19	0.11	0.10
Propanoic acid	0.08	0.08	-0.12	-0.11	0.29*	0.31*
Butanoic acid	-0.01	-0.05	-0.05	-0.07	0.14	0.10
Pentanoic acid	-0.25	-0.29	-0.16	-0.19	-0.28	-0.26
Hexanoic acid	-0.02	-0.01	-0.15	-0.15	0.25	0.29*
Heptanoic acid	-0.16	-0.17	-0.17	-0.23	0.04	0.07
Octanoic acid	-0.08	-0.09	-0.03	-0.05	0.02	0.01
Nonanoic acid	0.12	0.12	-0.02	0.05	0.16	0.12
Alcohols						
1-Butanol	-0.31*	-0.36*	-0.16	-0.22	-0.34*	-0.30*
1-Penten-3-ol	0.17	0.17	-0.09	-0.10	0.34*	0.41**
1-Pentanol	0.24	0.24	0.06	0.04	0.34*	0.39**
1-Hexanol	0.15	0.15	-0.02	-0.05	0.30*	0.38**
2,5-Hexanediol	-0.12	-0.05	-0.08	0.01	-0.09	-0.07
1-Octen-3-ol	0.17	0.17	-0.02	-0.03	0.28	0.34*
1-Heptanol	0.26	0.26	0.05	0.05	0.37*	0.42**
1-Octanol	0.20	0.21	-0.01	0.01	0.36*	0.40**
2-Octen-1-ol	0.14	0.14	0.01	0.01	0.26	0.32*
Aldehydes						
2-Methylbutanal	-0.28	-0.32*	-0.33*	-0.36*	-0.07	-0.05
Hexanal	0.28	0.28	0.04	0.03	0.46***	0.46***
Heptanal	0.09	0.03	-0.03	0.03	0.21	0.10
Octanal	0.24	0.24	-0.02	-0.01	0.48***	0.47***
Nonanal	0.20	0.18	-0.09	-0.07	0.48***	0.45**
Ketones						
Acetone	-0.16	-0.14	-0.16	-0.11	-0.06	-0.10
2-Butanone	-0.35*	-0.38*	-0.28	-0.33*	-0.20	-0.18
2-Pentanone	0.14	0.11	-0.17	-0.15	0.48***	0.43**
2-Heptanone	-0.28	-0.31*	-0.33*	-0.35*	-0.07	-0.05
3-Octanone	-0.13	-0.12	0.13	0.16	-0.21	-0.26
Acetoin	0.09	0.06	-0.13	-0.09	0.42**	0.33*
Butyrolactone	0.14	0.16	0.10	0.13	0.13	0.13
Hydrocarbons						
Z-2-Octene	0.06	-0.03	0.12	0.05	-0.11	-0.16
Isododecane	0.18	0.17	0.20	0.19	0.07	0.04
Beta-Pinene	0.35*	0.36*	0.29*	0.26	0.18	0.25
Furan						
2-Ethylfuran	0.20	0.24	-0.03	-0.05	0.40**	0.47***
Sulphur compounds						
Carbon disulphide	-0.29	-0.31	-0.22	-0.20	-0.19	-0.24
Dimethyl sulphide	0.27	0.29*	-0.18	-0.13	0.52***	0.51***
Dimethyl sulfone	-0.06	-0.02	-0.18	-0.15	0.01	0.07

*, $P < 0.05$

6.3.2. Chinese consumers

6.3.2.1. CHINESE CONSUMER SENSORY SCORES

Among the Chinese consumers (n=159), tenderness, flavour and overall scores did not differ between the 3 types of commercial lamb included in the assessment (P>0.05; Table 6.4). Greater juiciness scores were observed for meat from GRASS-W lambs than CHIC-W and MXME-W lambs.

Overall liking scores showed that there were three clusters of consumers using the agglomerative hierarchical cluster analysis. Cluster-1 (n=51) favoured meat from GRASS-W compared to MXME-W lambs while CHIC-W was intermediate (P<0.05) for all sensory traits that were evaluated. Cluster-2 (n=54) preferred meat from CHIC-W lambs when compared to MXME-W lambs and GRASS-W lambs. The meat from MXME-W lambs was also more preferred than GRASS-W lambs for flavour and overall liking for cluster 2 consumers. Cluster-3 (n=54) gave similar sensory scores for meat from GRASS-W and MXME-W lambs (P>0.05), which were higher than meat from CHIC-W lambs (P<0.05, Table 6.4).

Table 6.4 Consumer scores (0-100) from sensory panel evaluation of grilled lamb loins (*Longissimus thoracis*) from 3 typical New Zealand commercial production groups evaluated by Chinese consumers in Auckland, New Zealand.

Descriptor	GRASS-W	CHIC-W	MXME-W	SEM	P-value
All Consumers (n = 159)					
Tenderness	66.5	63.4	61.4	1.1	0.142
Juiciness	49.1[b]	43.0[a]	42.8[a]	1.1	<0.05
Flavour Liking	62.0	59.6	57.0	1.0	0.108
Overall Liking	63.1	61.5	59.1	1.0	0.232
Cluster 1 (n =51)					
Tenderness	73.2[b]	63.1[b]	52.3[a]	1.9	<0.001
Juiciness	56.6[c]	41.0[b]	34.7[a]	1.7	<0.001
Flavour Liking	72.1[c]	58.8[b]	42.1[a]	1.8	<0.001
Overall Liking	73.4[c]	61.4[b]	43.6[a]	1.7	<0.001
Cluster 2 (n = 54)					
Tenderness	57.1[a]	72.4[b]	62.9[ab]	1.7	<0.001
Juiciness	35.7[a]	52.2[b]	41.0[a]	1.8	<0.001
Flavour Liking	48.7[a]	71.9[c]	59.3[b]	1.6	<0.001
Overall Liking	48.5[a]	75.8[c]	61.9[b]	1.5	<0.001
Cluster 3 (n =54)					
Tenderness	69.6[b]	54.5[a]	69.3[b]	1.9	<0.001
Juiciness	55.5[b]	35.3[a]	52.6[b]	1.9	<0.001
Flavour Liking	65.7[b]	48.5[a]	68.4[b]	1.7	<0.001
Overall Liking	67.9[b]	47.7[a]	70.8[b]	1.6	<0.001

1. Different letters within the same row denote significant difference between means ($P < 0.05$).
2. SEM: standard error of the means.

6.3.2.2. CORRELATION BETWEEN SENSORY SCORES AND FATTY ACIDS

Overall liking scores of all Chinese consumers (n=159) were negatively correlated with c11-18:1 ($P < 0.05$, Table 6.5). Cluster-1 consumers, who preferred meat from GRASS-W lambs showed a negative correlation of total intramuscular fat ($P<0.05$, Table 6.5), monounsaturated fatty acids ($P<0.05$), c9-18:1 ($P<0.05$), 17:0 ($P<0.05$), ai-17:0 ($P<0.05$) with overall liking and flavour liking scores. Positive correlations were found between polyunsaturated fatty acids ($P<0.05$), 18:2 n-6 ($P<0.05$), 18:3 n-3 ($P<0.05$), 20:5 n-3 ($P<0.01$), 22:5 n-3 ($P<0.01$), 22:6 n-3 ($P<0.01$), 12:0 ($P<0.05$) and overall liking and flavour liking scores.

Cluster-2 consumers, who preferred meat from CHIC-W lambs, showed negative correlations between 15:0 ($P<0.05$, Table 6.5), 17:0 ($P<0.05$), i-15:0 ($P<0.05$), i-17:0 ($P<0.05$), branched chain fatty acids ($P<0.05$) and overall liking and flavour liking scores.

Cluster-3 consumers, who preferred meat from MXME-W lambs, showed negative correlations between 17:1 ($P<0.05$), t11-18:1 ($P<0.05$), polyunsaturated fatty acids ($P<0.01$), 18:2 n-6 ($P<0.001$), 18:3 n-3 ($P<0.001$), 20:5 n-3 ($P<0.05$) with overall liking and flavour liking scores. Positive correlations were observed between 17:0 ($P<0.001$), i-17:0 ($P<0.01$), ai-17:0 ($P<0.01$), c9-18:1 ($P<0.01$), branched fatty acids ($P<0.05$), monounsaturated fatty acids ($P<0.01$) and overall liking and flavour liking scores.

Table 6.5 Pearson correlation coefficients of overall liking scores from all Chinese consumers (n=159), cluster-1 (n=51), cluster-2 (n=54), cluster-3 (n=54) with fatty acids content of lamb loins (*Longissimus thoracis*) from the three typical New Zealand commercial animal groups assessed.

Fatty acids, % of total FA	Pearson correlation coefficients, N=24 (3 groups * 8 loins per group)							
	All 159 consumers		Cluster-1		Cluster-2		Cluster-3	
	Overall	Flavour	Overall	Flavour	Overall	Flavour	Overall	Flavour
10:0	-0.17	-0.14	0.34	0.31	-0.07	-0.05	-0.23	-0.28
12:0	-0.03	0.06	0.42*	0.44*	-0.26	-0.15	-0.10	-0.17
14:0	-0.11	0.00	0.39	0.40	-0.23	-0.15	-0.08	-0.17
15:0	-0.37	-0.28	-0.07	-0.06	-0.43*	-0.36	0.34	0.24
16:0	0.33	0.29	0.02	-0.02	0.34	0.39	-0.08	-0.05
17:0	-0.24	-0.23	-0.52*	-0.47*	-0.44*	-0.46*	0.66***	0.63**
18:0	-0.10	-0.20	-0.27	-0.24	-0.20	-0.36	0.35	0.33
i-15:0	-0.29	-0.23	-0.04	-0.05	-0.48*	-0.42*	0.39	0.28
ai-15:0	-0.35	-0.25	0.00	-0.02	-0.35	-0.28	0.24	0.20
i-16:0	-0.36	-0.31	0.27	0.26	-0.28	-0.27	-0.06	-0.11
i-17:0	-0.35	-0.30	-0.17	-0.13	-0.53*	-0.52*	0.53**	0.40
ai-17:0	-0.30	-0.28	-0.54*	-0.53*	-0.29	-0.29	0.60**	0.55**
16:1	-0.02	0.02	-0.11	-0.13	-0.10	-0.03	0.25	0.20
17:1	-0.09	0.00	0.35	0.34	0.10	0.14	-0.41*	-0.38
t9-18:1	-0.11	-0.07	-0.07	-0.11	-0.36	-0.24	0.31	0.20
t11-18:1	0.01	0.04	0.05	-0.03	0.34	0.41*	-0.41*	-0.33
c9-18:1	0.16	0.11	-0.46*	-0.43*	-0.15	-0.17	0.55**	0.56**
c11-18:1	-0.45*	-0.39	0.10	0.11	-0.11	-0.10	-0.12	-0.20
18:2 n-6	-0.13	-0.07	0.43*	0.41*	0.26	0.30	-0.69***	-0.65***
18:3 n-3	0.00	0.03	0.46*	0.46*	0.37	0.36	-0.73***	-0.69***
c9t11-18:2	0.00	0.06	0.23	0.20	0.14	0.21	-0.33	-0.37
20:4 n-6	-0.37	-0.28	0.34	0.33	-0.26	-0.20	-0.19	-0.24
20:5 n-3	-0.09	-0.06	0.54**	0.54**	0.06	0.05	-0.47*	-0.49*
22:5 n-3	-0.13	-0.06	0.57**	0.57**	-0.11	-0.09	-0.37	-0.37
22:6 n-3	-0.06	-0.03	0.53**	0.55**	-0.02	-0.03	-0.32	-0.34
BCFA	-0.36	-0.31	-0.21	-0.21	-0.47*	-0.43*	0.51*	0.42*
SFA	0.04	-0.03	-0.14	-0.14	-0.14	-0.20	0.36	0.31
MUFA	0.16	0.11	-0.49*	-0.48*	-0.11	-0.12	0.53**	0.55**
PUFA	-0.15	-0.08	0.49*	0.47*	0.16	0.19	-0.61**	-0.60**
Total FA	0.23	0.18	-0.43*	-0.41*	0.07	0.10	0.36	0.35

P < 0.05; **, P < 0.01, ***, P < 0.001

6.3.2.3. CORRELATION BETWEEN SENSORY SCORES AND VOLATILES

When the responses from all Chinese consumers were analysed, none of the volatile compounds were correlated with overall liking or flavour liking scores ($P>0.05$, Table 6.6). The overall liking and flavour liking scores of cluster-1 consumers (n=51, Table 6.6) were negatively correlated with 1-butanol ($P<0.01$), Z-2-octene ($P<0.001$) and dimethyl sulfone ($P<0.05$) and positively correlated with butyrolactone ($P<0.05$). The overall liking scores of cluster-2 consumers (n=54) were negatively correlated to pentanoic acid ($P<0.05$) and acetone ($P<0.05$) and positively correlated to nonanal ($P<0.05$). In addition, 2-butanone was negatively correlated with flavour-liking scores.

For cluster-3 consumers (n=54), negative correlations were identified between overall liking and flavour-liking scores with most of the alcohol and aldehyde compounds including 1-penten-3-ol ($P<0.01$), 1-pentanol ($P<0.001$), 1-hexanol ($P<0.01$), 1-octen-3-ol ($P<0.01$), 1-heptanol ($P<0.01$), 1-octanol ($P<0.05$), 2-octen-1-ol ($P<0.05$), hexanal ($P<0.05$), octanal ($P<0.01$), nonanal ($P<0.01$) as well as 2-pentanone ($P<0.05$), dimethyl sulphide ($P<0.01$) and 2-ethylfuran (0.01). Positive correlations were found between overall liking scores and pentanoic acid ($P<0.05$), Z-2-octene ($P<0.05$), 2-butanone ($P<0.05$) and dimethyl sulfone ($P<0.01$). In general, cluster-1 consumers were the opposite of cluster-3 consumers for their correlation of overall and flavour liking scores with dimethyl sulphide, 2-ethylfuran, Z-2-octene and dimethyl sulfone volatiles identified from the raw lamb.

Table 6.6 Pearson correlation coefficients of overall liking scores from all Chinese consumers (n=159), cluster-1 (n=51), cluster-2 (n=54) and cluster-3 (n=54) and volatile compounds detected in the headspace of raw lamb loins (*Longissimus thoracis*) from the three typical New Zealand commercial animal groups assessed.

Pearson correlation coefficients, N=24 (3 groups * 8 loins per group)								
Compounds	All 159 consumers		Cluster-1		Cluster-2		Cluster-3	
	Overall liking	Flavour liking	Overall liking	Flavour liking	Overall liking	Flavour liking	Overall liking	Flavour
Acids								
Acetic acid	-0.07	-0.03	0.32	0.35	-0.24	-0.20	-0.05	-0.19
Propanoic acid	-0.06	-0.01	0.21	0.25	0.04	0.08	-0.32	-0.41
Butanoic acid	-0.07	-0.05	0.35	0.37	-0.29	-0.30	-0.01	-0.16
Pentanoic acid	-0.04	-0.07	-0.19	-0.18	-0.45*	-0.49*	0.46*	0.45*
Hexanoic acid	-0.24	-0.27	0.22	0.21	0.10	0.09	-0.39	-0.51*
Heptanoic acid	-0.17	-0.22	0.16	0.20	0.05	-0.02	-0.21	-0.37
Octanoic acid	-0.31	-0.38	-0.02	0.03	-0.01	-0.13	-0.06	-0.23
Nonanoic acid	-0.24	-0.30	-0.29	-0.33	0.30	0.24	-0.22	-0.21
Alcohols								
1-Butanol	-0.14	-0.20	-0.57**	-0.54*	0.07	0.02	0.31	0.25
1-Penten-3-ol	-0.14	-0.14	0.27	0.28	0.21	0.22	-0.56**	-0.60**
1-Pentanol	-0.12	-0.06	0.35	0.33	0.21	0.25	-0.64***	-0.62***
1-Hexanol	-0.23	-0.23	0.09	0.06	0.26	0.27	-0.53**	-0.52*
2,5-Hexanediol	0.00	-0.02	-0.23	-0.26	-0.08	0.02	0.33	0.27
1-Octen-3-ol	-0.30	-0.29	0.17	0.15	0.19	0.20	-0.53**	-0.56**
1-Heptanol	-0.08	-0.08	0.27	0.24	0.19	0.21	-0.54**	-0.51*
1-Octanol	-0.15	-0.16	0.27	0.26	0.06	0.05	-0.42*	-0.45*
2-Octen-1-ol	-0.29	-0.27	0.23	0.21	0.11	0.13	-0.49*	-0.53**
Aldehydes								
2-Methylbutanal	-0.05	-0.13	0.07	0.10	-0.36	-0.40	0.37	0.15
Hexanal	-0.13	-0.06	0.16	0.15	0.36	0.37	-0.56**	-0.46*
Heptanal	-0.01	0.08	-0.07	-0.05	0.03	0.14	0.10	0.17
Octanal	-0.14	-0.08	0.21	0.21	0.35	0.37	-0.60**	-0.53**
Nonanal	-0.08	0.03	0.18	0.18	0.35	0.42*	-0.53**	-0.44*
Ketones								
Acetone	-0.24	-0.26	0.10	0.14	-0.41*	-0.44*	0.24	0.01
2-Butanone	-0.09	-0.15	0.04	0.08	-0.39	-0.47*	0.44*	0.26
2-Pentanone	-0.27	-0.26	0.15	0.16	0.34	0.29	-0.45*	-0.45*
2-Heptanone	-0.05	-0.13	0.07	0.10	-0.36	-0.40	0.37	0.15
3-Octanone	0.17	0.20	0.25	0.22	-0.08	0.01	0.09	0.10
Acetoin	-0.11	-0.07	0.16	0.15	0.25	0.25	-0.37	-0.28

Butyrolactone	0.12	0.15	0.45*	0.46*	-0.33	-0.34	-0.05	-0.14
Hydrocarbons								
Z-2-Octene	-0.28	-0.31	-0.66***	-0.64***	0.04	0.03	0.49*	0.47*
Isododecane	-0.22	-0.31	-0.26	-0.28	0.18	0.14	0.02	-0.04
Beta-Pinene	-0.01	-0.01	0.01	-0.05	-0.15	-0.12	0.17	0.24
Furan								
2-Ethylfuran	-0.20	-0.13	0.21	0.20	0.30	0.35	-0.60**	-0.58**
Sulphur compounds								
Carbon disulphide	-0.24	-0.31	-0.11	-0.13	-0.10	-0.15	0.03*	0.01*
Dimethyl sulphide	0.26	0.28	0.39	0.40	0.37	0.33	-0.64***	-0.60**
Dimethyl sulfone	-0.23	-0.21	-0.48*	-0.42*	-0.19	-0.21	0.44*	0.39

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

6.4. Discussion

There were two main objectives of the present study: firstly, to evaluate New Zealand and Chinese consumer preferences for the meat of lambs reared in New Zealand commercial forage production systems that varied by diet, gender and age at slaughter; and secondly, examine the association between consumer liking scores and other characteristics such as fatty acid and volatile profiles.

6.4.1. New Zealand consumer preferences

The results of the current study indicated that New Zealand consumers could differentiate the meat from lambs from different types of commercial production systems in terms of overall liking, flavour liking and tenderness. This suggests that in the future there may be potential to create a premium lamb market or produce lines of lambs to meet consumer preferences. In the current study, loins from wether lambs that grazed chicory were given the highest overall and flavour liking scores by all New Zealand consumers. It was not, however, rated the most preferred product by any of the two clusters. Meat from wether lambs that grazed perennial ryegrass received the highest overall liking scores and flavour liking scores from Cluster-1 while for Cluster-2 the highest scores were for meat from ewe lambs that grazed chicory. This indicated that when the tenderness was acceptable (shear force values <40N, Hopkins et al., 2006), New Zealand consumers are not homogeneous in their preference for lamb meat flavour and liking.

Although not statistically significant, New Zealand consumers from both Cluster-1 and Cluster-2 showed negative correlations between IMF content and overall liking scores. This finding was somewhat surprising as a positive relationship has been reported in a number of studies (Hopkins et al., 2006; Pannier et al., 2014; Phelps et al., 2018; Realini et al., 2021). The IMF percentage of the loins assessed in the current study ranged from 1.6% to 4.4% which was

comparable with the IMF percentages previously reported for commercial lambs grown in New Zealand forage-based systems (Craigie et al., 2017). The negative association between IMF content and consumer liking scores seen in the current study may be partially explained by the preference for moderate amounts (2.5%-3.5%) of IMF for some consumers in the panel. Realini et al., (2021) suggested that the IMF preference in pasture fed lamb of New Zealand consumers could be divided into two clusters: two thirds were 'IMF lovers: the more the better', who showed a linear increase in overall liking scores with increasing IMF%; and one third were 'IMF optimizers' who preferred 2.5–3.5% IMF.

Significant correlations between individual fatty acids and overall liking or flavour liking scores were only observed in Cluster-2. New Zealand consumers in Cluster-2 showed a negative correlation between overall and flavour liking scores and content of MUFA and BCFA; and a positive correlation with the content of PUFA. This matches the observation that meat from MXME-W lambs, which had the greatest content of BCFA and lowest content of PUFA, was the least preferred product by consumers in Cluster-2. BCFA generates undesirable 'waxy' and 'fatty' flavours for some consumers (Chambers IV and Koppel, 2013), which consumers in Cluster-2 maybe more sensitive to compared to consumers in Cluster-1. More comprehensive fatty acid analysis is needed to further quantify the content of volatile BCFA in meat and investigate their influence on the acceptability of lamb meat for New Zealand consumers.

Negative correlations were observed between flavour liking scores and pentatonic acid, 1-butanol, 2-butanone, carbon disulphide and dimethyl sulphide for all New Zealand consumers. These volatile compounds are primarily generated by lipid degradation and result in rancidity in raw meat or 'warmed-over flavour' in cooked meat (Farmer, 1994) so, the negative association with flavour liking was anticipated. During cooking the abundance of aldehydes and alcohols,

especially hexanal increase significantly in meat (Domínguez et al., 2014). Among the 36 volatile compounds identified in head space of raw lamb meat in Chapter 4, 24 were also identified in cooked lamb meat (Pavan et al., 2021). Therefore, although correlations of consumer scores and volatiles from raw meat were assessed, it is likely that the volatiles were relevant to those released with cooking. It is also unknown which volatile compounds reached sensory thresholds as semi quantitative volatile analysis was utilised for the analysis in the current study. Further quantitative research or GC-O would be needed to confirm the percentage of the volatiles identified in raw lamb meat that remained in cooked meat after different types of thermal treatments and to identify which volatiles reach sensory thresholds and drive preferences for lamb flavour.

When a cluster analysis was applied, the flavour liking scores of New Zealand consumers in Cluster-2 were positively correlated with the abundance of 1-pentanol and 1-pentanol-3-ol which have been noted to have 'green' and 'fruity' odour descriptors (Sutherland and Ames, 1995). Prescott, Young, and O'Neill (2001) observed that flavour descriptors such as 'green', 'fruity', and 'sweet' had positive impacts on meat liking scores for both New Zealand and Japanese consumers. The positive correlation between hexanal and flavour liking scores of New Zealand consumers from Cluster-2 was unexpected as hexanal has been reported to be negatively correlated with meat acceptability in pork (Nissen et al., 2004). The consumer detection threshold of hexanal in lamb, however, is not known and it is possible that concentrations were not at a level that would create flavour issues. Hexanoic acid has been associated with goat/mutton-like odour (Brennand et al., 1989; Bueno et al., 2014), and showed a positive corelation with flavour liking scores of New Zealand consumers in Cluster-2. Dimethyl sulphide which was the least abundant in meat from Merino lambs (Chapter 3) has a 'sulphurous', 'onion', 'green' odour in raw meat was positively correlated to flavour liking scores of consumers in Cluster-2. Overall, PUFA and lipid

oxidation volatiles had a positive role in defining lamb flavour and acceptance for New Zealand consumers in Cluster-2. In contrast to Cluster-2, neither the overall nor flavour liking scores from consumers in Cluster-1 were significantly correlated with aldehydes, ketones or sulphur compounds, which implicates that those consumers in Cluster-2 may prefer a fuller flavoured lamb meat product. Thus, the fatty acid profile and the volatile compounds derived from their oxidation during cooking appear to be a stronger driver of overall liking of lamb for some consumers than others. A more dedicated sensory study is required to compare the concentration of volatiles in lamb and different consumer preferences to fully elucidate the desirable volatiles for improving the eating experience of lamb for New Zealand consumers.

6.4.2. Chinese consumer preferences

When responses from all Chinese consumers were considered, no differences were observed in flavour, tenderness, and overall liking scores, however, a cluster analysis identified three distinct clusters. Consumers in Cluster-1 scored all sensory attributes (overall liking, flavour liking, tenderness, and juiciness scores) the highest for meat from GRASS-W lambs. Cluster-2 scored meat from CHIC-W lambs had the highest for all sensory scores, and Cluster-3 the highest for meat from MXME-W lambs.

When the responses from all Chinese consumers were considered, IMF showed a positive relationship with overall liking scores. This was in agreement with a number of previous studies that reported that IMF contributed to meat eating quality (Hopkins et al., 2006; Pannier et al., 2014; Phelps et al., 2018; Realini et al., 2021). Chinese consumers in Cluster-1, however, showed a negative correlation of IMF with overall liking scores. Therefore, it was not surprising that the meat from the relatively lean GRASS-W lambs was most preferred by consumers in this cluster.

Positive correlations between overall and flavour liking scores with PUFA were observed for Chinese consumers in Cluster-1.

Consumers in Cluster-2 showed a significant negative correlation between overall and flavour liking scores and BCFA. This cluster of consumers showed no preference for IMF, MUFA and PUFA, but appeared to be more sensitive towards BCFA as the meat from the CHIC-W lambs had the lowest content of BCFA and had the highest preference score from consumers in Cluster-2. Chinese consumers in Cluster-2 reflected the response found in a study with Japanese consumers, where a low BCFA concentration in sheep meat was associated with higher (more favourable) sensory scores (Prescott et al., 2001). For Asian consumers sensitive to the sensory impact of BCFA that are present in the meat of forage-fed lamb, a lamb finishing diet of chicory may provide a solution. This concept would be interesting to validate in further research.

Positive correlations of IMF with flavour and overall liking scores for Chinese consumers in Cluster-3 indicated that these consumers may be "IMF lovers". These consumers also showed a positive correlation between flavour liking scores and MUFA; and negative correlation between flavour liking scores and PUFA. This is likely to be partly driven by the fact that PUFA concentration in meat decreases as the IMF content increases (Realini et al., 2021). The positive and negative correlations between PUFA and consumer liking scores in Cluster-1 and -3 in the current study suggest that fatty acid profile affects flavour liking, although the effect varied between consumer clusters. Moderate (Karamichou et al., 2007; Realini et al., 2021), weak (Gravador et al., 2020) or no associations (Naja et al., 2012; Ponnampalam et al., 2014) between fatty acid profiles and sensory properties of meat have all been previously reported but, the current research highlights that subgroups of consumers maybe more discerning of fatty acids on eating quality of lamb.

6.4.3. Comparison of New Zealand and Chinese consumers

In the current study, when New Zealand consumers were considered as a single group, they appeared to be more sensitive to differences between lambs reared in different forage production systems compared to Chinese consumers. Once the cluster analysis had been applied it became evident that Chinese consumers contained subgroups of consumers that diverged in their sensory perception of lamb from different forage-based production subsystems. This divergence maybe a consequence of Chinese consumers being less familiar with the flavour of pasture reared lamb than New Zealand consumers due to the per capita sheep meat consumption per year in China in 2017 being 3.25 kg compared to 19.00 kg in New Zealand (Ritchie, 2017).

Meat from wether Merino lambs received lowest tenderness and overall liking scores by both New Zealand and Chinese consumers, although the mean shear force value of merino lamb meat was lower than for pre-weaned lambs (Chapter 2). These lambs were older at slaughter than the other lamb treatments, indicating an importance of age at slaughter on lamb eating quality.

IMF content of the lamb meat samples was negatively correlated with overall liking scores among New Zealand consumers but positively correlated to overall liking scores for Chinese consumers. This suggests that New Zealand and Chinese consumers respond differently to increased IMF in lamb and this may be partly attributed with differences in fatty acid profiles at different IMF concentrations that are altering flavour profile (Realini et al 2021). This is likely as consumer response to IMF is known to vary due to multiple factors including the influence on mouthfeel, perception of succulence and ability to provide a sensation of juiciness (Shahrai et al., 2021).

For both New Zealand and Chinese consumers, the fatty acid profile and the volatile compounds derived from their oxidation upon cooking seem to be a stronger driver of consumer

liking of lamb for some consumers than others. Cluster- 2 of New Zealand consumers and Cluster-3 of Chinese consumers may have greater sensitiveness as they are more influenced by fatty acid and volatile composition of lamb meat. Overall, the loins from 6-8-month old lambs had greater eating quality compared to loins from 4-month-old lambs or 12-month-old Merino lambs and should be the target slaughter age for both NZ and Chinese consumers.

Chapter 7

General discussion

7.1. Introduction to General Discussion

A plethora of research has shown that different pasture production systems result in variation in lamb carcass and meat quality (Chapter 1; Table 1.1). However, production factors such as the breed of the sheep, its diet, the target slaughter weight, and age at slaughter differ globally. In Mediterranean countries, for example, suckling and light lamb (carcass weight <7 kg and 5–13 kg, respectively) production have been prioritized for many years (Bravo-Lamas et al., 2018). While British lambs were typically reared on a grass-based system using strategic concentrate supplementation and slaughtered at heavier carcass weight (Díaz et al., 2005). Female lambs deposit greater fat in the carcass than males, but some studies also suggested that this influence is small in young lambs (Hopkins and Mortimer, 2014). Therefore, there is no universal answer to how sex, age, diet, and breed influence lamb carcass and meat quality characteristics as each case can be different. The collection of data and information on the meat quality, composition and sensory characteristics of meat obtained from lambs from a range of commercial production systems utilized in New Zealand may help farmers and processors make better decisions on what production system aspects are useful to achieve specific characteristics for discerning markets or alternatively identify lambs which can have their meat directed to meet the requirements of specific markets.

The physical and chemical properties of lamb meat can be affected by production factors. Lambs grazing herbs and alternative forages (plantain and chicory) have been reported to have a greater ability to deposit fat and achieved heavier carcass weights than lambs grazing ryegrass pastures (Somasiri et al., 2015). Meanwhile, a greater fat deposition can result in greater tenderness of meat as well as a more saturated fatty acid profile of meat (Wood et al., 2008). A greater level

of fat deposition in lamb especially the polyunsaturated fatty acids can contribute to stronger pastoral flavour of meat due to greater abundance of lipid oxidation volatiles (Priolo et al., 2001).

In contrast with the lipid profile of meat which has had substantial investigation, very few studies have investigated and correlated meat quality attributes with the proteomic profiles of lamb meat. Alpha-actin (structural protein), glyceraldehyde-3-phosphate dehydrogenase (metabolomic enzyme), and μ-calpain (proteolysis) have been reported to effect the tenderness of meat (Huang et al., 2020). However, some contradictory results have been reported, for instance, heat shock protein 27 is negatively correlated with the shear force in Chianina beef (D'Alessandro et al., 2012), but was positively correlated in Korean beef (Kim et al., 2008). The abundance of these protein biomarkers is affected by breed, age, sex, feeding patterns, muscle type, which often vary greatly between studies, and therefore, cannot yet be used to predict meat quality in industry accurately and consistently.

This general discussion outlines the major findings of each experimental chapter. The overall aim of the work was to characterize lamb meat, produced from different forage-based production systems, in terms of its meat quality, fatty acid profile, volatile profile, proteomic profile and its sensory impact for New Zealand and Chinese consumers. Methodological considerations and limitations of the experimental designs reported will be summarised. Finally, the implications of this study will be contemplated and considerations for further research suggested.

7.2. Role of lamb production systems for meat quality

The results of chapter 2 indicated that sending lambs with suitable live weights for processing at weaning (4-month-old) will result in a heavier carcass and meat that has a greater intramuscular fat content than finishing lambs for an additional period post-weaning (6- to 8-month-old). For lambs slaughtered at 6- to 8-month-old, lambs finished on chicory had a greater carcass weight (18.1±0.1kg vs. 16.9±0.1kg) and greater intramuscular fat in the loin (2.0-2.6% vs. 1.3-1.6%), compared to the perennial ryegrass diet. Instrumental measurement of meat quality (shear force, pH, colour, and water holding capacity) with lamb loins showed that animal age at slaughter and diet had a greater effect on meat quality than sex. Some differences in meat colour could be attributed to the effect of age at slaughter or diet. Castrated Merino lambs (wether) had greater IMF in the loin compared to cryptorchid lambs (4.4 ± 0.2% vs 3.2 ± 0.2%, respectively).

The chapter 3 showed that finishing lambs in New Zealand forage systems at 12 months-of-age compared to 4- to 8-months-of-age resulted in lower proportions of n-3 fatty acids in meat (2.99-3.41% vs 4.79-5.86%) as well as a lower PUFA:SFA ratio (0.17-0.19 vs 0.27-0.35) however, this result was also confounded by breed differences which cannot be ruled out as having an effect. A chicory diet increased the proportion of PUFA and decreased the proportion of SFA and BCFA in lean lamb meat compared to perennial ryegrass diet. The combined EPA plus DHA content in the muscle of the lambs fed chicory could be considered a ‘source’ or ‘good source’. The potential to for nutritional claims on EPA plus DHA, is limited by the total SFA plus trans fatty acids and would depend on the nutritional guidelines and regulations of the export markets.

Many of the volatiles identified in the current study (chapter 4) were lipid oxidation compounds. The differences in the volatiles identified production systems were due primarily to changes in fatty acid composition as discussed in Chapter 3 (Figure 7.1 Graphic review). The 12-

month-old Merino lambs had the lowest abundance of the straight chain volatile acids and aldehydes, including acetic acid, butanoic acid, hexanoic acid, hexanal, and octanal as a result of its lower PUFA content. No consistent sex or castration effects on the relative abundance of volatiles was identified. Meat from lambs that grazed chicory had greater abundance of lipid oxidation compounds including hexanal, octanal, nonanal, 2-pentanone and 1-penten-3-ol and 1-octen-3-ol than meat from lambs grazed on perennial ryegrass, which is likely due to greater content of PUFA in meat from lambs that grazed chicory. There was, however, little evidence that the pasture-based diets investigated in the current study could affect the abundance of volatiles that accumulate directly from the diet.

Of the 286 proteins identified across raw lamb loins from the six New Zealand forage production systems investigated, only 16 proteins showed significant differences in abundance between production systems. Greater abundance of myofibrillar proteins (actin, troponin C, myosin-2, myosin regulatory light chain 2, myosin light chain 1) was observed in the meat from ewe lambs than wether lambs that grazed chicory. The chicory diet appeared to decrease the abundance of myosin-2, myosin regulatory light chain 2 and actin in lamb loins compared to perennial ryegrass diet but did not affect sarcoplasmic proteins. The abundance of myofibrillar proteins in loins was lower when lambs were slaughtered at 6- to 8-month-old compared to 4-month-old, which was speculated to be due to more glycolytic fibres than oxidative fibres. Overall, our results indicated that the abundance of myofibrillar proteins in lamb loins was affected by animal age, diet and age at slaughter, but the expression of most proteins (>90%) was relatively stable and rarely effected by the production system.

Among the New Zealand consumers (n=160), the highest overall liking scores were observed for meat from wether lambs grazing chicory, while meat of 12-month-old Merino lambs was the

least favoured. Among the Chinese consumers (n=159), scores for tenderness, flavour and overall did not differ between the three production systems that were evaluated. This may have been due to the Chinese consumers being less familiar or sensitive to the flavour of pasture reared lamb than New Zealand consumers. In one cluster of Chinese consumers (n = 51) there was a linear decrease in the overall liking scores with increasing amounts of intramuscular fat. The other cluster of Chinese consumers (n=54) showed a decrease in overall liking scores with increasing amounts of polyunsaturated fatty acids and abundance of lipid oxidation compounds. In contrast, one New Zealand consumer cluster (n = 75) showed an increase in overall liking with increasing content of polyunsaturated fatty acids and abundance of lipid oxidation compounds.

7.2.1. Do ewe or wether lambs have any benefits for meat quality?

In this thesis, wether and ewe lambs slaughtered at less than 12-month-old did not differ in carcass characteristics, meat quality, fatty acid and volatile profile and sensory properties. Previous studies have suggested that male lambs can have greater carcass weight than females but that females have meat that is more flavourful, tender, intense in colour than males at the same age (Picard et al., 2019). The published research, however, often analysed older lambs (2-year-old) or entire male animals rather than wethers. The sex effects observed were minor for New Zealand commercial lambs which had a target slaughter weight of 32-38kg. Ewe lamb loins had a higher abundance of myofibrillar proteins than males which might be a consequence of a greater proportion of slow oxidative fibre in the loin muscle. This result requires further research but is unlikely to contribute to any meat quality differences.

7.2.2. Does a chicory diet have any benefits in meat quality than perennial ryegrass diet for lambs?

There were some production efficiency and meat quality benefits to providing lambs a chicory diet. The chicory diet increased the carcass weight, loin yield, and loin intramuscular fat content compared to a perennial ryegrass diet. While meat colour, shear force, water holding capacity remained similar between loins from lambs grazed on different diets. lambs grazed on chicory had greater content of SFA, MUFA, and PUFA as a result of a greater total fat content. Likewise, the volatile compounds that are mainly generated from the oxidation of PUFA were more abundant in meat from lambs grazed on chicory than perennial ryegrass. The CHIC-W lambs received the greatest overall liking scores when evaluated by New Zealand consumers, and the greater abundance of lipid oxidation volatiles were linked to lamb that was considered more favourably by one cluster of New Zealand consumers (Figure 7.1 Graphic review). Overall, chicory diet did not affect the tenderness, juiciness or abundance of volatiles that accumulate directly from the diet but elevated the intramuscular fat content and overall eating quality.

7.2.3. Do 4-month-old lambs slaughtered directly from their dams at weaning have meat that is different compared to 6- to 12-month-old lambs?

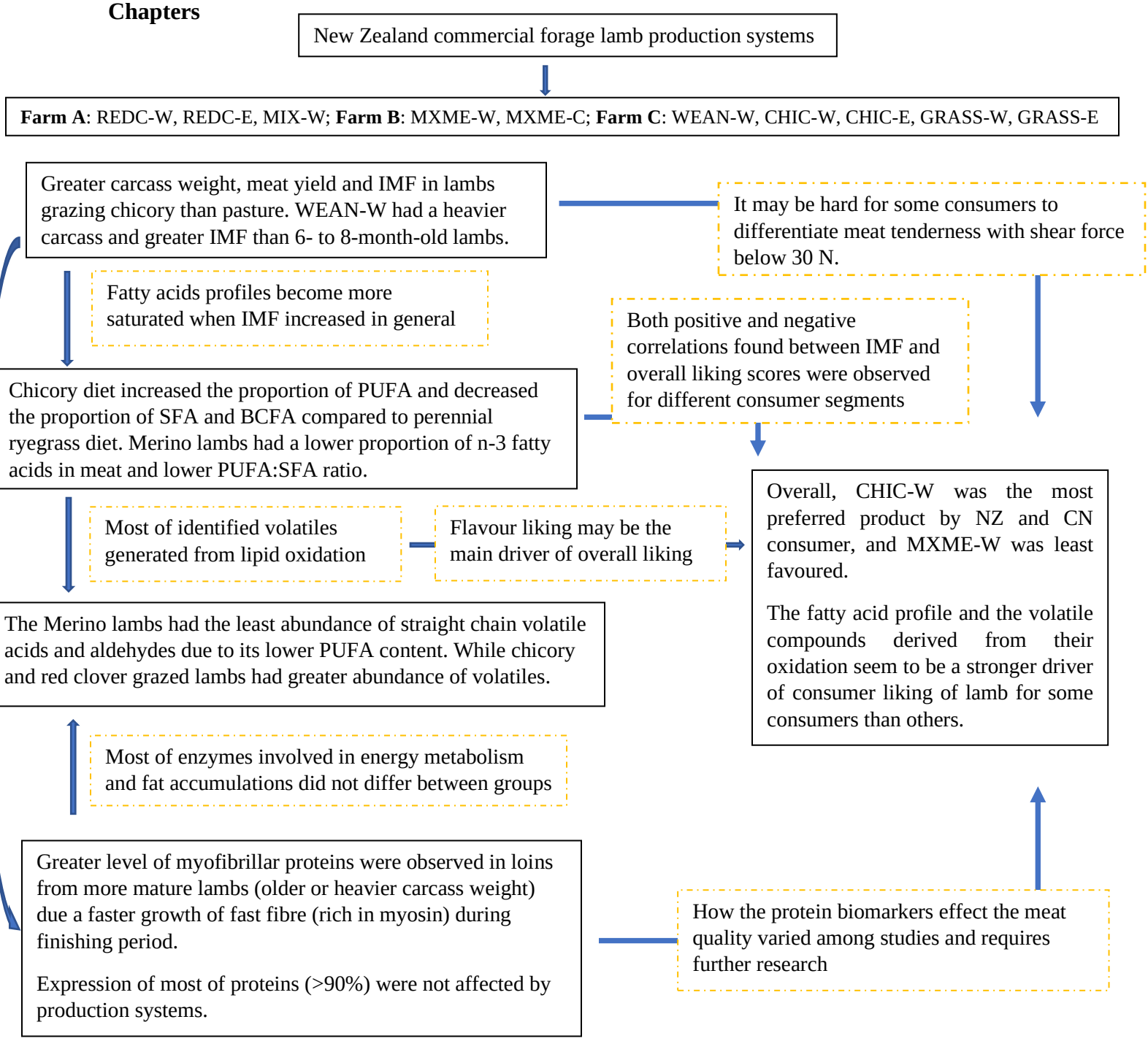
Sending lambs with suitable live weights for processing at weaning resulted in a heavier carcass and meat that had a greater intramuscular fat content than finishing lambs for an addition period post-weaning. Greatest EPA plus DHA content (43.52mg/100g raw meat) was observed in the loins of lambs slaughtered at 4-month-old resulting in this being the only group of lambs that were considered a ‘good source’ of n-3 fatty acids according to the Food Standards Australia New Zealand. The shear force of the loins of lambs slaughtered at weaning (4-months-of-age) was

greater than lambs slaughtered at 6- to 8-month-old, although, and correspondingly the lambs slaughtered at weaning had a numerically lower tenderness and overall liking scores than 6- to 8-month-old lambs when evaluated by New Zealand consumers. This suggests that there are some disadvantages in meat eating quality for early season lambs compared to lambs finished for longer period, although the absolute differences were small and suggesting eating quality difference are unlikely to be a substantial issue.

7.2.4. Do late season Merino lambs differ in meat quality compared to early and mid-season lambs?

The average shear force value of loins from 12-month-old Merino lambs was slightly higher than lambs slaughtered between 6- to 8-month-old but, remained below 30N which is considered the threshold for meat to be considered as tender. Finishing lambs for a longer period of up to 12 months in New Zealand forage systems was found to result in a lower proportion of n-3 fatty acids in loin meat, as well as a lower PUFA:SFA ratio, compared to 4- to 8-month-old lambs. Compared to lambs from other production system groups, the 12-month-old Merino lambs also had the lowest content and proportion of EPA plus DHA and a low abundance of straight-chain volatile acids and aldehydes, including acetic acid, butanoic acid, hexanoic acid, hexanal and octanal, as a result of its lower PUFA content. The Merino lambs in this thesis had the lowest tenderness, flavour and overall liking scores evaluated by both New Zealand and Chinese consumers. Overall, our results indicated that the overall meat quality and nutritional values of late season Merino lambs was lower than younger lambs.

Figure 7.1 Graphic review: overall findings of the current thesis and linking between each Chapters



7.3. Methodological considerations and future research

This section covers some methodological considerations of the experiments and the limitation of current study and alludes to future work.

7.3.1. Experimental design

The lambs that formed the basis of this series of studies were collected from three commercial farms; therefore, direct meat quality comparisons could only be made within each farm although the farm managements differences might be small. To some extent some of the treatment groups were confounded by different farms being used, each with their unique breeds and forage diets. However, an important aspect of this work was to use commercial lambs and ascertain the extent to which meat quality, volatiles, proteins and consumer sensory scores could be altered by farm systems used in New Zealand to produce lamb. To allow for a comparison without confounding effects, future studies would need to consider important factors to focus on and lambs on lambs from controlled treatments from the same farm.

Further research using a farmlet type design with a greater number of animals per treatment and having replicated treatments in neighbouring paddocks is required to substantiate the effect of diet and understand the variation. For factors such as sex, breed and age at slaughter studies with lambs of similar background replicated across farms or repeated across years and seasons on the same farm would help to elucidate the true variation. Given that in the current study the sex of commercial lambs less than 12-months of age had little effect on meat quality, comparing diet, age or breed treatments could use lambs of the same sex.

The cooking method should be consistent in future research in order to better associate the subjective shear force values with objective tenderness scores. In the current study, shear force values were measured on meat that cooked in a water bath at 70°C for 90 min. While the meat

evaluated by New Zealand and Chinese consumers were cooked in a water bath at 57°C and 68°C for 60 min respectively, and finished on a hot plate. In addition, the top three consumption pattens of sheep meat in China are stew, hot pot and roasting (Mao et al., 2016). These cooking methods can have significant effects on volatile and protein profiles, and this requires a new set of experiments to investigate.

7.3.2. Accuracy and precision of analyses

The fatty acid extraction method used in the current study was based on sulphuric acid and methanol solution (American Oil Chemists' Society 6th edition, Ce 1f-96), which could not identify 4-methyloctanoic acid, 4-ethyloctanoic acid and 4-methylnonanoic acid. The recovery rates of these branched chain fatty acids are low because the branched chain fatty acid concentration is low in the lean meat of young lambs however, even at a low concentration, 4-methyloctanoic acid, 4-ethyloctanoic acid and 4-methylnonanoic acid can have a significant impact on flavour (Watkins et al., 2014). A two-step extraction with ethanol, diethyl ether, and petroleum ether mixer has been recommended by Teng et al., (2018) to extract 4-alkyl-branched chain fatty acids in goat milk powders that are present at a low concentration. This maybe a feasible method to recover 4-alkyl-branched chain fatty acids from meat of young lambs in further research.

In general, the methods used to analyse volatiles in the head space of raw meat had a suitable level of accuracy and precision. The ZB-WAX capillary column (Phenomenex, USA) used in the current study had consistent and high recovery rate of internal standard throughout the test. Analytical variation was smaller than that of within-treatment biological variation, making it possible to detect genuine treatment differences. The use of different types of gas chromatography

columns such as CAR-PDMS may allow the detection of some volatiles that were missed or detected in low mass spectrum quality in the current study.

Of the volatiles identified in the current study, a full quantitative analysis could have been conducted which, has some advantages. Firstly, quantitative analysis would require the use of volatile standards which can give more confidence in identification of compounds since the semi-quantitative identification in this study was based on retention index and mass spectrum matching. Secondly, quantitative data makes it easier to connect odour perception with chemical analysis because the odour thresholds can vary significantly among volatiles. For focus in studies of volatiles in lamb, volatile abundances in cooked lamb meat that are below their odour thresholds could be removed from consideration be neglected in future research. Gas-chromatography-olfactometry is another valuable method that could be used to relate the information supplied by chemical characterization with odour perception.

For further studies of volatiles and proteomics in lamb meat it would be imperative to consider of cooked meat samples. During cooking, new volatiles can be formed from compounds and molecules present within the raw meat. Also, because volatiles are released to a greater extent with cooking and consumer will be perceiving volatile from cooked meat it is logical that volatiles from cooked meat are explored and related to sensory attributes. The increased temperature associated with cooking results in the loss of tertiary and secondary structures of meat proteins. The unfolded proteins may aggregate, and can cross-link with other polypeptides, which can change the texture of the meat. In addition, a meta-analysis using data from the current and previous studies could better associate the proteomic profile with various meat quality traits. Future investigations could be conducted including correlation heatmaps, protein-protein interaction network maps, and forest

plots indicating the sample size of previous studies, confidence interval and heterogeneity of the data.

7.3.3 Sensory study

In order to avoid Chinese consumers rejecting loin samples caused by a pink appearance, the degree of doneness of lamb loins was increased. Similarly, the medium degree of doneness was applied for New Zealand consumers to avoid rejection caused by overcooking. This difference in cooking, however, prevented a direct preference comparison between the preferences of New Zealand and Chinese consumers. Future research should be conducted to examine the effect of cooking temperature on the preferences of both of these consumer groups. A rate-all-that-apply questionnaire, with questions such as 'what is the level of importance of meat marbling on a 0-10 scale' would help researchers and the New Zealand meat industry to better understand consumer demands. These types of questionnaires can be conducted online with large numbers of consumers from different regions. This knowledge will help the industry to implement the best set of management policies to gain additional value from the lamb meat that is produced.

7.3.4. Future research for meat quality from lamb production systems

The research in this thesis identified that there were some subtle differences in meat quality, profiles of fatty acids, volatile and proteins for lamb produced from the different commercial production systems but that all overall, all the lamb meat considered could be classed as being of premium quality. Sensory differences in the meat were perceived but, the difference was dependent on the consumer cluster and hence the sensory response to lamb meat from different production systems is individualised. The results in this thesis highlight that in order to meet the requirements of different consumers, production systems need further consideration to ensure that a premium

product with no negative attributes is being produced. Regenerative agriculture techniques are being considered for New Zealand forage-based animal production (Grelet and Lang, 2021). Regenerative agriculture is considered to meet consumer preferences for food products that are safer, healthier, and produced in a system that is environmentally friendly, and it will be important to consider the effects that regenerative agriculture has on product quality utilising similar testing procedures as used in this thesis.

In this thesis, chicory was associated with the highest intramuscular fat content in lamb meat and produced lamb meat that had the most desirable fatty acid profile for nutritive value. These effects of chicory will be important to validate under different farming conditions (climate, topography, grazing intensities) but it will also be useful to undertake a controlled study to fully quantify the extent to which chicory can be used to modify intramuscular fat content and composition compared to perennial ryegrass. There are a range of forages used in New Zealand for finishing lambs. It maybe that other forages can influence fat and fatty acid deposition in lamb meat. Controlled farmlet studies to consider the effect that different alternative forages have on meat quality and modifying intramuscular fat deposition and fatty acid composition will identify further options for production systems to achieve the desirable characteristics that are required for discerning markets.

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Appendix

Supplementary Table S1 List of total 286 identified proteins in raw lamb loins from six (WEAN-W, GRASS-W, REDC-W, CHIC-W, CHIC-E, MXME-W) New Zealand productions systems

Accession Number	Significance	Coverage (%)	Total Peptides	Unique Peptides	Post-translational modification	Description
XP_011992886.2	1.63	12	78	77	Carbamidomethylation; Deamidation (NQ); Pyro-glu from Q	LOW QUALITY PROTEIN: nebulin [Ovis aries]
XP_027824589.1	6.88	41	76	69	Carbamidomethylation; Deamidation (NQ); Phosphorylation (STY); Dehydration; Dethiomethyl	LOW QUALITY PROTEIN: filamin-C [Ovis aries]
W5PX04|W5PX04_SHEEP	2.24	49	50	49	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; Pyro-glu from Q	Myosin binding protein C fast type OS=Ovis aries OX=9940 GN=MYBPC2 PE=4 SV=1
XP_027815274.1	1.36	72	63	47	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Dehydration; Pyro-glu from Q	alpha-actinin-3 isoform X2 [Ovis aries]
XP_011957551.3	1.36	69	63	47	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Dehydration; Pyro-glu from Q	alpha-actinin-3 isoform X1 [Ovis aries]
sp|O18751|PYGM_SHEEP	1.4	48	45	36	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Pyro-glu from Q; Formylation	Glycogen phosphorylase muscle form OS=Ovis aries OX=9940 GN=PYGM PE=2 SV=3
XP_012036281.1	4.43	60	34	33	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Dehydration; Pyro-glu from Q; Formylation	pyruvate kinase PKM isoform X1 [Ovis aries]
XP_004021419.1	1.76	57	53	33	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Pyro-glu from Q	alpha-actinin-2 [Ovis aries]
XP_012045938.1	4.26	80	59	31	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 2 more	LOW QUALITY PROTEIN: creatine kinase M-type [Ovis aries]
XP_014959134.2	1.82	22	28	28	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Pyro-glu from Q	myomesin-1 isoform X1 [Ovis aries]
W5QDF3|W5QDF3_SHEEP	2.87	49	107	26	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 3 more	Myosin heavy chain 7 OS=Ovis aries OX=9940 GN=MYH7 PE=3 SV=1
XP_004010374.1	2.87	49	107	26	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 3 more	myosin-7 [Ovis aries]
XP_012040978.1	0.33	73	40	26	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration	beta-enolase isoform X1 [Ovis aries]
XP_012032382.1	4.02	26	25	25	Carbamidomethylation; Deamidation (NQ)	glycogen debranching enzyme [Ovis aries]
NP_001177319.1	3.31	74	30	24	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Dehydration	glyceraldehyde-3-phosphate dehydrogenase [Ovis aries]
W5PDG3|W5PDG3_SHEEP	3.31	73	30	24	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Dehydration	Glyceraldehyde-3-phosphate dehydrogenase OS=Ovis aries OX=9940 GN=GAPDH PE=3 SV=1
NP_001135988.1	1.05	60	23	23	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Pyro-glu from Q	phosphoglycerate kinase 1 [Ovis aries]
XP_004006621.1	0.88	62	22	22	Deamidation (NQ); Oxidation (M)	ATP synthase subunit beta mitochondrial [Ovis aries]
XP_004005001.1	1.95	51	24	21	Carbamidomethylation; Deamidation (NQ); Pyro-glu from Q	desmin [Ovis aries]
XP_004006413.2	2.79	45	19	19	Carbamidomethylation; Deamidation (NQ); Oxidation (M)	glycerol-3-phosphate dehydrogenase [NAD(+)] cytoplasmic isoform X1 [Ovis aries]
W5PW05|W5PW05_SHEEP	2.36	45	19	19	Carbamidomethylation; Deamidation (NQ); Dehydration; Formylation	Malate dehydrogenase 2 OS=Ovis aries OX=9940 GN=MDH2 PE=4 SV=1
XP_004021309.2	2.36	54	19	19	Carbamidomethylation; Deamidation (NQ); Dehydration; Formylation	malate dehydrogenase mitochondrial [Ovis aries]
XP_004020913.1	1.01	31	28	19	Carbamidomethylation; Deamidation (NQ); Oxidation (M)	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1 [Ovis aries]
W5NVR1|W5NVR1_SHEEP	1.01	32	28	19	Carbamidomethylation; Deamidation (NQ); Oxidation (M)	Calcium-transporting ATPase OS=Ovis aries OX=9940 GN=ATP2A1 PE=3 SV=1
XP_004020912.1	1.01	31	28	19	Carbamidomethylation; Deamidation (NQ); Oxidation (M)	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X2 [Ovis aries]
XP_027823736.1	0.89	76	19	19	Carbamidomethylation; Deamidation (NQ)	triosephosphate isomerase [Ovis aries]
XP_012030289.1	1.38	15	19	18	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Dehydration; Formylation	myosin-binding protein C slow-type isoform X2 [Ovis aries]
XP_012030288.1	1.38	15	19	18	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Dehydration; Formylation	myosin-binding protein C slow-type isoform X1 [Ovis aries]
XP_004009106.3	2.67	50	22	17	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; Formylation	creatine kinase S-type mitochondrial [Ovis aries]
W5P323|W5P323_SHEEP	3.18	45	16	16	Deamidation (NQ); Oxidation (M)	Glucose-6-phosphate isomerase OS=Ovis aries OX=9940 GN=GPI PE=3 SV=1
XP_004015200.2	3.18	45	16	16	Deamidation (NQ); Oxidation (M)	glucose-6-phosphate isomerase [Ovis aries]
XP_004020569.1	0.59	37	16	16	Deamidation (NQ)	ATP synthase subunit alpha mitochondrial [Ovis aries]
A0A3Q9U3M0|A0A3Q9U3M0_SHEEP	4.03	25	16	14	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Pyro-glu from Q	ATP-dependent 6-phosphofructokinase OS=Ovis aries OX=9940 GN=PFKM PE=2 SV=1

XP_004006455.2	4.03	25	16	14	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Pyro-glu from Q	LOW QUALITY PROTEIN: ATP-dependent 6-phosphofructokinase muscle type [Ovis aries]
W5QDD4|W5QDD4_SHEEP	4.03	25	16	14	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Pyro-glu from Q	ATP-dependent 6-phosphofructokinase OS=Ovis aries OX=9940 GN=PFKM PE=3 SV=1
XP_004002712.1	1.99	48	14	14	Deamidation (NQ); Dehydration	calsequestrin-1 [Ovis aries]
XP_014953428.1	1.04	66	14	14	Carbamidomethylation; Deamidation (NQ)	carbonic anhydrase 3 [Ovis aries]
XP_027823951.1	2.52	23	13	13	Deamidation (NQ); Pyro-glu from Q	aconitate hydratase mitochondrial [Ovis aries]
NP_001159670.1	1.53	46	13	13	Carbamidomethylation; Deamidation (NQ)	four and a half LIM domains protein 1 [Ovis aries]
XP_027818556.1	1.53	48	13	13	Carbamidomethylation; Deamidation (NQ)	four and a half LIM domains protein 1 isoform X3 [Ovis aries]
XP_027815619.1	0.57	61	17	13	Carbamidomethylation; Deamidation (NQ); Pyro-glu from Q	L-lactate dehydrogenase A chain isoform X1 [Ovis aries]
W5PDD8|W5PDD8_SHEEP	4.13	55	12	12	Deamidation (NQ)	Myozenin 1 OS=Ovis aries OX=9940 GN=MYOZ1 PE=4 SV=1
XP_027817273.1	3.8	83	12	12	Pyro-glu from Q	heat shock protein beta-1 [Ovis aries]
XP_004015091.1	3.94	29	10	10	Carbamidomethylation; Deamidation (NQ)	aspartate aminotransferase mitochondrial [Ovis aries]
XP_004020149.1	1.21	31	10	10	Carbamidomethylation; Deamidation (NQ)	aspartate aminotransferase cytoplasmic [Ovis aries]
XP_004004324.1	0.97	58	27	10	Carbamidomethylation; Dehydration; Pyro-glu from Q	tropomyosin beta chain isoform X1 [Ovis aries]
W5PVY5|W5PVY5_SHEEP	0.94	68	15	10	Carbamidomethylation; Deamidation (NQ); Oxidation (M)	Phosphoglycerate mutase OS=Ovis aries OX=9940 GN=PGAM2 PE=3 SV=1
NP_001155354.1	0.29	63	11	10	Carbamidomethylation; Deamidation (NQ); Oxidation (M)	adenylate kinase isoenzyme 1 [Ovis aries]
XP_011955483.2	2.01	27	9	9	Carbamidomethylation; Deamidation (NQ)	cytochrome b-c1 complex subunit 1 mitochondrial [Ovis aries]
XP_004004940.1	1.21	85	34	9	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 2 more	myosin light chain 1/3 skeletal muscle isoform isoform X1 [Ovis aries]
XP_004018103.4	0.86	25	9	9	Carbamidomethylation; Deamidation (NQ)	isocitrate dehydrogenase [NADP] mitochondrial [Ovis aries]
W5PR04|W5PR04_SHEEP	0.86	26	9	9	Carbamidomethylation; Deamidation (NQ)	Isocitrate dehydrogenase [NADP] OS=Ovis aries OX=9940 GN=IDH2 PE=3 SV=1
XP_027832152.1	0.73	80	10	9	Carbamidomethylation; Deamidation (NQ); Oxidation (M)	troponin C skeletal muscle isoform X1 [Ovis aries]
XP_004004668.2	0.6	12	9	9	Carbamidomethylation; Deamidation (NQ)	kelch-like protein 41 [Ovis aries]
W5NZL8|W5NZL8_SHEEP	0.6	12	9	9	Carbamidomethylation; Deamidation (NQ)	Kelch like family member 41 OS=Ovis aries OX=9940 GN=KLHL41 PE=4 SV=1
W5PI38|W5PI38_SHEEP	0.11	20	9	9	Carbamidomethylation	Citrate synthase OS=Ovis aries OX=9940 GN=CS PE=3 SV=1
XP_004006633.1	0.11	20	9	9	Carbamidomethylation	citrate synthase mitochondrial [Ovis aries]
XP_004017458.1	2.63	20	8	8	Carbamidomethylation	aldehyde dehydrogenase mitochondrial [Ovis aries]
XP_027815150.1	2.38	50	8	8	Carbamidomethylation; Deamidation (NQ)	phosphatidylethanolamine-binding protein 1 [Ovis aries]
XP_027817851.1	5.03	77	30	7	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; Pyro-glu from Q	fructose-bisphosphate aldolase A [Ovis aries]
XP_027815667.1	3.62	44	10	7	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Pyro-glu from Q; 2 more	troponin I fast skeletal muscle [Ovis aries]
XP_027824959.1	1.43	30	7	7	Carbamidomethylation; Deamidation (NQ)	voltage-dependent anion-selective channel protein 1 isoform X1 [Ovis aries]
XP_004015238.1	1.05	67	7	7	Carbamidomethylation	cytochrome c oxidase subunit 6B1 [Ovis aries]
XP_027830687.1	0.96	42	135	7	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 2 more	myosin-8 [Ovis aries]
XP_027831980.1	0.47	20	7	7	Carbamidomethylation; Deamidation (NQ)	myosin-binding protein H [Ovis aries]
XP_004020879.1	0.23	23	7	7	Carbamidomethylation; Deamidation (NQ); Pyro-glu from Q	cytochrome b-c1 complex subunit 2 mitochondrial [Ovis aries]
XP_027817094.1	0.14	20	7	7	Carbamidomethylation	tripartite motif-containing protein 72 [Ovis aries]
W5Q0R4|W5Q0R4_SHEEP	3.54	37	6	6	Deamidation (NQ); Pyro-glu from Q	Alpha-crystallin B chain OS=Ovis aries OX=9940 GN=CRYAB PE=3 SV=1
W5PF65|W5PF65_SHEEP	3.17	9	6	6	Carbamidomethylation	Transferrin OS=Ovis aries OX=9940 GN=TF PE=3 SV=1
XP_027816111.1	3.17	9	6	6	Carbamidomethylation	serotransferrin [Ovis aries]
XP_027821559.1	3.13	48	6	6	Carbamidomethylation; Deamidation (NQ)	galectin-1 isoform X1 [Ovis aries]
W5PWZ2|W5PWZ2_SHEEP	3.13	60	6	6	Carbamidomethylation; Deamidation (NQ)	Galectin OS=Ovis aries OX=9940 GN=LGALS1 PE=4 SV=1
W5PFT7|W5PFT7_SHEEP	3.01	35	6	6	Carbamidomethylation; Deamidation (NQ)	Fructose-bisphosphatase 2 OS=Ovis aries OX=9940 GN=FBP2 PE=3 SV=1
NP_001119823.1	2.79	71	32	6	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 2 more	tropomyosin alpha-1 chain [Ovis aries]
W5Q8N4|W5Q8N4_SHEEP	2.19	51	6	6	Deamidation (NQ)	Myosin light chain 2 OS=Ovis aries OX=9940 GN=MYL2 PE=2 SV=1

W5PP37|W5PP37_SHEEP	1.55	45	6	6	Carbamidomethylation	ATP synthase subunit d mitochondrial OS=Ovis aries OX=9940 GN=ATP5PD PE=3 SV=1
NP_001136363.1	1.55	45	6	6	Carbamidomethylation	ATP synthase subunit d mitochondrial [Ovis aries]
XP_027820491.1	1.54	17	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X10 [Ovis aries]
XP_027820483.1	1.54	15	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X2 [Ovis aries]
XP_027820501.1	1.54	20	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X20 [Ovis aries]
XP_027820492.1	1.54	18	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X11 [Ovis aries]
XP_027820498.1	1.54	19	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X17 [Ovis aries]
XP_027820488.1	1.54	17	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X7 [Ovis aries]
XP_027820484.1	1.54	16	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X3 [Ovis aries]
XP_027820482.1	1.54	15	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X1 [Ovis aries]
XP_027820499.1	1.54	19	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X18 [Ovis aries]
XP_027820489.1	1.54	17	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X8 [Ovis aries]
XP_027820495.1	1.54	18	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X14 [Ovis aries]
XP_027820496.1	1.54	18	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X15 [Ovis aries]
XP_027820490.1	1.54	17	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X9 [Ovis aries]
XP_027820485.1	1.54	16	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X4 [Ovis aries]
XP_027820504.1	1.54	21	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X23 [Ovis aries]
XP_027820497.1	1.54	19	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X16 [Ovis aries]
XP_027820502.1	1.54	20	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X21 [Ovis aries]
XP_027820493.1	1.54	18	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X12 [Ovis aries]
XP_027820487.1	1.54	17	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X6 [Ovis aries]
XP_027820503.1	1.54	21	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X22 [Ovis aries]
XP_027820494.1	1.54	18	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X13 [Ovis aries]
XP_027820500.1	1.54	20	6	6	Deamidation (NQ)	myc box-dependent-interacting protein 1 isoform X19 [Ovis aries]
XP_027820594.1	1.11	28	754	6	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 3 more	LOW QUALITY PROTEIN: titin [Ovis aries]
XP_004010842.2	0.93	14	6	6		dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex mitochondrial [Ovis aries]
W5NTS6|W5NTS6_SHEEP	0.93	14	6	6		Dihydrolipoamide S-succinyltransferase OS=Ovis aries OX=9940 GN=DLST PE=4 SV=1
XP_027829100.1	0.9	1	6	6		plectin isoform X2 [Ovis aries]
XP_027829099.1	0.9	1	6	6		plectin isoform X1 [Ovis aries]
XP_027829103.1	0.9	1	6	6		plectin isoform X4 [Ovis aries]
XP_027829102.1	0.9	1	6	6		plectin isoform X3 [Ovis aries]
W5QFQ1|W5QFQ1_SHEEP	0.71	22	6	6	Carbamidomethylation; Deamidation (NQ)	Malate dehydrogenase OS=Ovis aries OX=9940 GN=MDH1 PE=3 SV=1
W5QFQ0|W5QFQ0_SHEEP	0.71	23	6	6	Carbamidomethylation; Deamidation (NQ)	Malate dehydrogenase OS=Ovis aries OX=9940 GN=MDH1 PE=3 SV=1
XP_004005894.2	0.71	23	6	6	Carbamidomethylation; Deamidation (NQ)	malate dehydrogenase cytoplasmic [Ovis aries]
XP_027830504.1	0.66	24	6	6	Deamidation (NQ)	tropomodulin-4 [Ovis aries]
NP_001138655.1	0.64	85	36	6	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 3 more	myosin regulatory light chain 2 skeletal muscle isoform [Ovis aries]
XP_004022002.1	4.45	24	5	5	Carbamidomethylation; Deamidation (NQ)	pyruvate dehydrogenase E1 component subunit alpha somatic form mitochondrial [Ovis aries]
W5PIG6|W5PIG6_SHEEP	4.31	40	15	5	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration	Enolase 1 OS=Ovis aries OX=9940 GN=ENO1 PE=3 SV=1
XP_027831475.1	4.31	42	15	5	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration	alpha-enolase isoform X2 [Ovis aries]
XP_004002406.2	3.43	7	5	5		AMP deaminase 1 isoform X1 [Ovis aries]
XP_004002407.1	3.43	7	5	5		AMP deaminase 1 isoform X2 [Ovis aries]
W5QFJ2|W5QFJ2_SHEEP	3.43	7	5	5		AMP deaminase OS=Ovis aries OX=9940 GN=AMPD1 PE=3 SV=1

W5PNI5|W5PNI5_SHEEP	3.28	8	16	5	Carbamidomethylation; Deamidation (NQ); Formylation	Myosin heavy chain 7B OS=Ovis aries OX=9940 GN=MYH7B PE=3 SV=1
sp|P62896|CYC_SHEEP	2.02	50	6	5		Cytochrome c OS=Ovis aries OX=9940 GN=CYCS PE=1 SV=2
XP_011982042.2	1.54	22	5	5	Pyro-glu from Q	peroxiredoxin-1 [Ovis aries]
XP_004002090.1	1.06	66	31	5	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Pyro-glu from Q	phosphoglucomutase-1 isoform X2 [Ovis aries]
XP_004007775.1	1.04	4	5	5		collagen alpha-2(I) chain [Ovis aries]
XP_027812976.1	0.91	54	5	5	Deamidation (NQ)	cytochrome c oxidase subunit 5A mitochondrial [Ovis aries]
W5NXT8|W5NXT8_SHEEP	0.91	68	5	5	Deamidation (NQ)	Cytochrome c oxidase subunit 5A OS=Ovis aries OX=9940 GN=COX5A PE=1 SV=1
XP_004013798.1	0.57	38	5	5	Carbamidomethylation	protein/nucleic acid deglycase DJ-1 [Ovis aries]
sp|P02190|MYG_SHEEP	5.91	62	16	4	Deamidation (NQ); Oxidation (M); Dehydration; Formylation	Myoglobin OS=Ovis aries OX=9940 GN=MB PE=1 SV=2
XP_027818348.1	4.14	18	4	4	Carbamidomethylation	PDZ and LIM domain protein 3 isoform X2 [Ovis aries]
XP_027827422.1	2.47	37	89	4	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 2 more	myosin-6 isoform X2 [Ovis aries]
A0A1X9H6E5|A0A1X9H6E5_SHEEP	2.32	21	4	4	Carbamidomethylation	Troponin I type 1 variant X2 OS=Ovis aries OX=9940 GN=TNNI1 PE=2 SV=1
XP_027831854.1	2.32	22	4	4	Carbamidomethylation	troponin I slow skeletal muscle [Ovis aries]
W5PEA4|W5PEA4_SHEEP	1.89	8	4	4	Carbamidomethylation	Succinate--CoA ligase [ADP-forming] subunit beta mitochondrial OS=Ovis aries OX=9940 GN=SUCLA2 PE=3 SV=1
W5PEA2|W5PEA2_SHEEP	1.89	7	4	4	Carbamidomethylation	Succinate--CoA ligase [ADP-forming] subunit beta mitochondrial OS=Ovis aries OX=9940 GN=SUCLA2 PE=3 SV=1
XP_027829360.1	1.89	8	4	4	Carbamidomethylation	succinate--CoA ligase [ADP-forming] subunit beta mitochondrial [Ovis aries]
W5PH95|W5PH95_SHEEP	1.18	21	4	4	Carbamidomethylation; Deamidation (NQ)	Uncharacterized protein OS=Ovis aries OX=9940 PE=4 SV=1
XP_027817699.1	0.98	6	4	4		sarcalumenin isoform X1 [Ovis aries]
W5NYM5|W5NYM5_SHEEP	0.98	6	4	4		Sarcalumenin OS=Ovis aries OX=9940 GN=SRL PE=3 SV=1
XP_004021201.1	0.98	11	4	4		sarcalumenin isoform X2 [Ovis aries]
XP_004007802.1	0.82	46	4	4		cytochrome c oxidase subunit NDUFA4 [Ovis aries]
NP_001091117.1	0.79	86	12	4	Carbamidomethylation; Deamidation (NQ)	hemoglobin subunit beta [Ovis aries]
XP_012038833.2	0.52	18	4	4	Carbamidomethylation; Deamidation (NQ)	cytochrome c1 heme protein mitochondrial [Ovis aries]
NP_001138659.1	0.45	59	4	4	Carbamidomethylation	cytochrome b-c1 complex subunit 6 mitochondrial [Ovis aries]
W5NPN4|W5NPN4_SHEEP	0.26	18	9	4	Carbamidomethylation; Deamidation (NQ)	Heat shock protein family A (Hsp70) member 8 OS=Ovis aries OX=9940 GN=HSPA8 PE=3 SV=1
XP_011951023.2	0.26	18	9	4	Carbamidomethylation; Deamidation (NQ)	heat shock cognate 71 kDa protein [Ovis aries]
NP_001155363.1	0.26	15	4	4	Deamidation (NQ)	fumarate hydratase mitochondrial [Ovis aries]
XP_027833508.1	0.01	20	4	4		cytochrome c oxidase subunit 4 isoform 1 mitochondrial [Ovis aries]
W5PPE8|W5PPE8_SHEEP	0.01	20	4	4		Cytochrome c oxidase subunit 4I1 OS=Ovis aries OX=9940 GN=COX4I1 PE=1 SV=1
W5PAN7|W5PAN7_SHEEP	0	33	6	4	Carbamidomethylation; Deamidation (NQ); Dehydration	Myosin light chain 3 OS=Ovis aries OX=9940 GN=MYL3 PE=4 SV=1
XP_027813530.1	0	33	6	4	Carbamidomethylation; Deamidation (NQ); Dehydration	myosin light chain 3 [Ovis aries]
XP_027836013.1	14.12	9	3	3	Carbamidomethylation; Deamidation (NQ)	succinate dehydrogenase [ubiquinone] flavoprotein subunit mitochondrial [Ovis aries]
W5Q216|W5Q216_SHEEP	14.12	9	3	3	Carbamidomethylation; Deamidation (NQ)	Succinate dehydrogenase [ubiquinone] flavoprotein subunit mitochondrial OS=Ovis aries OX=9940 PE=3 SV=1
XP_011950844.2	4.38	10	3	3	Carbamidomethylation; Deamidation (NQ)	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex mitochondrial [Ovis aries]
W5Q2C5|W5Q2C5_SHEEP	4.38	10	3	3	Carbamidomethylation; Deamidation (NQ)	Acetyltransferase component of pyruvate dehydrogenase complex OS=Ovis aries OX=9940 PE=3 SV=1
XP_004008028.1	4.37	3	3	3		2-oxoglutarate dehydrogenase mitochondrial isoform X4 [Ovis aries]
XP_004008027.1	4.37	3	3	3		2-oxoglutarate dehydrogenase mitochondrial isoform X3 [Ovis aries]
XP_012032355.1	4.37	3	3	3		2-oxoglutarate dehydrogenase mitochondrial isoform X2 [Ovis aries]
XP_012032354.1	4.37	3	3	3		2-oxoglutarate dehydrogenase mitochondrial isoform X1 [Ovis aries]
XP_027832746.1	3.91	15	5	3	Deamidation (NQ)	elongation factor 1-alpha 2 [Ovis aries]

W5PN24|W5PN24_SHEEP	3.91	14	5	3	Deamidation (NQ)	Eukaryotic translation elongation factor 1 alpha 2 OS=Ovis aries OX=9940 GN=EEF1A2 PE=3 SV=1
XP_004008889.1	3.68	6	3	3		stress-70 protein mitochondrial [Ovis aries]
XP_027813217.1	3.3	4	3	3		heat shock protein HSP 90-alpha [Ovis aries]
XP_004009741.1	2.15	14	3	3		PDZ and LIM domain protein 5 isoform X7 [Ovis aries]
XP_027826671.1	2.15	7	3	3		PDZ and LIM domain protein 5 isoform X4 [Ovis aries]
XP_004009740.1	2.15	13	3	3		PDZ and LIM domain protein 5 isoform X6 [Ovis aries]
XP_004009744.1	2.15	6	3	3		PDZ and LIM domain protein 5 isoform X3 [Ovis aries]
XP_004009748.1	2.15	6	3	3		PDZ and LIM domain protein 5 isoform X2 [Ovis aries]
XP_004009739.1	2.15	5	3	3		PDZ and LIM domain protein 5 isoform X1 [Ovis aries]
XP_004021390.1	1.87	76	54	3	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 3 more	actin alpha skeletal muscle isoform X4 [Ovis aries]
XP_027813246.1	1.61	22	7	3	Carbamidomethylation; Deamidation (NQ); Phosphorylation (STY); Formylation	creatine kinase B-type [Ovis aries]
XP_011959304.1	1.47	99	33	3	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Dehydration; Pyro-glu from Q; 2 more	myosin regulatory light chain 2 skeletal muscle isoform isoform X1 [Ovis aries]
XP_027830685.1	1.02	61	199	3	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 3 more	LOW QUALITY PROTEIN: myosin-2 [Ovis aries]
NP_001120750.1	0.92	29	10	3	Deamidation (NQ)	ADP/ATP translocase 1 [Ovis aries]
XP_014958051.2	0.76	9	3	3		uncharacterized protein LOC101108868 [Ovis aries]
XP_027820543.1	0.76	14	3	3		uncharacterized protein LOC114113063 [Ovis aries]
XP_027814900.1	0.76	9	3	3		uncharacterized protein LOC101120961 [Ovis aries]
W5QFN7|W5QFN7_SHEEP	0.76	27	3	3		Histone H4 OS=Ovis aries OX=9940 PE=3 SV=1
W5QFN8|W5QFN8_SHEEP	0.76	27	3	3		Histone H4 OS=Ovis aries OX=9940 PE=3 SV=1
W5QFP4|W5QFP4_SHEEP	0.76	28	3	3		Histone H4 OS=Ovis aries OX=9940 PE=3 SV=1
W5PHH3|W5PHH3_SHEEP	0.76	28	3	3		Histone H4 OS=Ovis aries OX=9940 PE=3 SV=1
W5PHL0|W5PHL0_SHEEP	0.76	28	3	3		Histone H4 OS=Ovis aries OX=9940 PE=3 SV=1
W5QFR6|W5QFR6_SHEEP	0.76	28	3	3		Histone H4 OS=Ovis aries OX=9940 PE=3 SV=1
W5PHM4|W5PHM4_SHEEP	0.76	27	3	3		Histone H4 OS=Ovis aries OX=9940 GN=LOC101123670 PE=3 SV=1
W5QFN3|W5QFN3_SHEEP	0.76	27	3	3		Histone H4 OS=Ovis aries OX=9940 PE=3 SV=1
XP_004008824.1	0.6	38	3	3		ATP synthase subunit delta mitochondrial [Ovis aries]
W5P642|W5P642_SHEEP	0.36	26	3	3		Cytochrome b-c1 complex subunit 7 OS=Ovis aries OX=9940 GN=UQCRB PE=1 SV=1
XP_027829005.1	0.36	25	3	3		cytochrome b-c1 complex subunit 7 isoform X1 [Ovis aries]
XP_004002472.1	0.35	20	3	3		histone H2B type 2-F [Ovis aries]
XP_004019125.1	0.35	20	3	3		histone H2B type 1-M [Ovis aries]
XP_004019076.1	0.35	20	3	3		histone H2B type 1-N [Ovis aries]
XP_027814886.1	0.35	20	3	3		histone H2B type 1-K [Ovis aries]
W5NRX3|W5NRX3_SHEEP	0.35	19	3	3		Histone H2B OS=Ovis aries OX=9940 PE=3 SV=1
W5NS71|W5NS71_SHEEP	0.35	18	3	3		Histone H2B OS=Ovis aries OX=9940 PE=3 SV=1
W5Q9Y2|W5Q9Y2_SHEEP	0.35	18	3	3		Histone H2B OS=Ovis aries OX=9940 PE=3 SV=1
W5NR98|W5NR98_SHEEP	0.35	18	3	3		Histone H2B OS=Ovis aries OX=9940 PE=3 SV=1
W5QAH2|W5QAH2_SHEEP	0.35	18	3	3		Histone H2B OS=Ovis aries OX=9940 GN=LOC101119426 PE=3 SV=1
W5NR06|W5NR06_SHEEP	0.35	18	3	3		Histone H2B OS=Ovis aries OX=9940 PE=3 SV=1
W5QA24|W5QA24_SHEEP	0.35	18	3	3		Histone H2B OS=Ovis aries OX=9940 PE=3 SV=1
W5QAW7|W5QAW7_SHEEP	0.35	18	3	3		Histone H2B OS=Ovis aries OX=9940 PE=3 SV=1
XP_004019124.2	0.35	17	3	3		histone H2B type 1 [Ovis aries]
W5QC76|W5QC76_SHEEP	0.33	2	6	3	Carbamidomethylation	Myosin heavy chain 15 OS=Ovis aries OX=9940 GN=MYH15 PE=3 SV=1

XP_027817063.1	0.29	4	3	3		collagen alpha-2(VI) chain isoform X1 [Ovis aries]
XP_004005716.1	0.22	20	3	3	Carbamidomethylation; Deamidation (NQ)	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 [Ovis aries]
XP_027820863.1	0.15	32	3	3		10 kDa heat shock protein mitochondrial isoform X2 [Ovis aries]
W5Q1T4|W5Q1T4_SHEEP	0.15	32	3	3		Uncharacterized protein OS=Ovis aries OX=9940 PE=3 SV=1
A0A0H3V384|A0A0H3V384_SHEEP	5.64	78	20	2	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; Formylation	Myosin light chain 1 OS=Ovis aries OX=9940 GN=MYL1 PE=2 SV=1
XP_004007919.2	5.04	13	2	2		F-actin-capping protein subunit alpha-2 [Ovis aries]
W5PCG7|W5PCG7_SHEEP	4.19	14	2	2		Uncharacterized protein OS=Ovis aries OX=9940 GN=MYOZ3 PE=4 SV=1
XP_027823071.1	3.03	1	2	2		LOW QUALITY PROTEIN: collagen alpha-3(VI) chain [Ovis aries]
W5QCP9|W5QCP9_SHEEP	3.03	1	2	2		Collagen type VI alpha 3 chain OS=Ovis aries OX=9940 GN=COL6A3 PE=4 SV=1
XP_027814043.1	2.9	8	2	2	Carbamidomethylation; Deamidation (NQ)	pyruvate dehydrogenase E1 component subunit beta mitochondrial [Ovis aries]
W5PRM8|W5PRM8_SHEEP	2.9	8	2	2	Carbamidomethylation; Deamidation (NQ)	Pyruvate dehydrogenase E1 component subunit beta OS=Ovis aries OX=9940 PE=4 SV=1
W5PTW1|W5PTW1_SHEEP	2.51	12	2	2	Carbamidomethylation	Uncharacterized protein OS=Ovis aries OX=9940 PE=3 SV=1
XP_027815272.1	2.51	12	2	2	Carbamidomethylation	glutathione S-transferase P [Ovis aries]
W5NQP9|W5NQP9_SHEEP	2.38	15	6	2	Carbamidomethylation; Deamidation (NQ)	Fructose-bisphosphate aldolase OS=Ovis aries OX=9940 GN=ALDOC PE=3 SV=1
XP_014954022.2	2.38	19	6	2	Carbamidomethylation; Deamidation (NQ)	fructose-bisphosphate aldolase C [Ovis aries]
XP_011954300.1	2.16	11	7	2	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Formylation	creatine kinase U-type mitochondrial [Ovis aries]
XP_027829423.1	1.93	12	2	2	Deamidation (NQ)	ubiquitin-40S ribosomal protein S27a [Ovis aries]
sp|P0C276|RL40_SHEEP	1.93	20	2	2	Deamidation (NQ)	Ubiquitin-60S ribosomal protein L40 OS=Ovis aries OX=9940 GN=UBA52 PE=2 SV=2
W5Q9W5|W5Q9W5_SHEEP	1.93	5	2	2	Deamidation (NQ)	Ubiquitin C OS=Ovis aries OX=9940 GN=UBC PE=4 SV=1
XP_027812205.1	1.93	3	2	2	Deamidation (NQ)	LOW QUALITY PROTEIN: polyubiquitin-C [Ovis aries]
XP_004005993.3	1.93	16	2	2	Deamidation (NQ)	ubiquitin-40S ribosomal protein S27a [Ovis aries]
sp|P0CG55|UBB_SHEEP	1.93	8	2	2	Deamidation (NQ)	Polyubiquitin-B OS=Ovis aries OX=9940 GN=UBB PE=2 SV=1
sp|P09670|SODC_SHEEP	1.64	24	2	2	Deamidation (NQ)	Superoxide dismutase [Cu-Zn] OS=Ovis aries OX=9940 GN=SOD1 PE=1 SV=2
NP_001138657.1	1.64	24	2	2	Deamidation (NQ)	superoxide dismutase [Cu-Zn] [Ovis aries]
W5QBF5|W5QBF5_SHEEP	1.56	10	2	2		NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10 mitochondrial OS=Ovis aries OX=9940 GN=NDUFA10 PE=1 SV=1
XP_004001808.2	1.56	10	2	2		NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10 mitochondrial isoform X1 [Ovis aries]
XP_027822837.1	1.56	11	2	2		NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10 mitochondrial isoform X2 [Ovis aries]
W5PEL7|W5PEL7_SHEEP	1.4	1	2	2		Heparan sulfate proteoglycan 2 OS=Ovis aries OX=9940 GN=HSPG2 PE=4 SV=1
XP_027821348.1	1.4	1	2	2		LOW QUALITY PROTEIN: basement membrane-specific heparan sulfate proteoglycan core protein [Ovis aries]
XP_014950281.1	1.19	17	3	2	Carbamidomethylation	myosin light chain 6B [Ovis aries]
XP_027812629.1	1.06	35	5	2	Carbamidomethylation; Deamidation (NQ)	immunoglobulin lambda-1 light chain-like [Ovis aries]
XP_027835867.1	0.98	31	17	2	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 2 more	beta-actin-like protein 2 isoform X2 [Ovis aries]
XP_012033947.2	0.96	5	2	2	Carbamidomethylation	myotilin isoform X2 [Ovis aries]
W5Q1Q5|W5Q1Q5_SHEEP	0.96	5	2	2	Carbamidomethylation	Myotilin OS=Ovis aries OX=9940 GN=MYOT PE=4 SV=1
XP_004008869.2	0.96	5	2	2	Carbamidomethylation	myotilin isoform X1 [Ovis aries]
XP_027834603.1	0.91	14	3	2		troponin T slow skeletal muscle isoform X5 [Ovis aries]
XP_014955600.2	0.91	14	3	2		troponin T slow skeletal muscle isoform X4 [Ovis aries]
XP_011950163.2	0.91	14	3	2		troponin T slow skeletal muscle isoform X3 [Ovis aries]
XP_027834602.1	0.91	14	3	2		troponin T slow skeletal muscle isoform X2 [Ovis aries]
W5NUR7|W5NUR7_SHEEP	0.91	13	3	2		Troponin T1 slow skeletal type OS=Ovis aries OX=9940 GN=TNNT1 PE=4 SV=1

XP_027834601.1	0.91	13	3	2		troponin T slow skeletal muscle isoform X1 [Ovis aries]
A0A1X9H6F1|A0A1X9H6F1_SHEEP	0.91	13	3	2		Troponin T type 1 variant X1 OS=Ovis aries OX=9940 GN=TNNT1 PE=2 SV=1
XP_004014229.1	0.79	8	2	2		ATP synthase subunit gamma mitochondrial isoform X1 [Ovis aries]
W5P340|W5P340_SHEEP	0.74	13	2	2		Superoxide dismutase OS=Ovis aries OX=9940 PE=3 SV=1
NP_001267632.1	0.74	13	2	2		superoxide dismutase [Mn] mitochondrial [Ovis aries]
W5PUX0|W5PUX0_SHEEP	0.71	8	2	2	Deamidation (NQ)	NADH dehydrogenase [ubiquinone] flavoprotein 1 mitochondrial OS=Ovis aries OX=9940 GN=NDUFV1 PE=1 SV=1
XP_027815074.1	0.71	8	2	2	Deamidation (NQ)	NADH dehydrogenase [ubiquinone] flavoprotein 1 mitochondrial [Ovis aries]
XP_027816001.1	0.66	23	2	2		cytochrome c oxidase subunit 5B mitochondrial [Ovis aries]
W5PSQ7|W5PSQ7_SHEEP	0.52	30	5	2	Carbamidomethylation; Deamidation (NQ)	Uncharacterized protein OS=Ovis aries OX=9940 PE=4 SV=1
XP_004021550.1	0.37	7	2	2		voltage-dependent anion-selective channel protein 2 [Ovis aries]
W5QGQ8|W5QGQ8_SHEEP	0.35	7	2	2	Carbamidomethylation	Succinate--CoA ligase [ADP/GDP-forming] subunit alpha mitochondrial OS=Ovis aries OX=9940 GN=SUCLG1 PE=3 SV=1
XP_004007304.4	0.35	8	2	2	Carbamidomethylation	succinate--CoA ligase [ADP/GDP-forming] subunit alpha mitochondrial [Ovis aries]
XP_014957448.2	0.31	5	2	2		isocitrate dehydrogenase [NAD] subunit alpha mitochondrial [Ovis aries]
W5NR14|W5NR14_SHEEP	0.31	5	2	2		Isocitrate dehydrogenase [NAD] subunit mitochondrial OS=Ovis aries OX=9940 GN=IDH3A PE=3 SV=1
XP_004014945.1	0.25	8	2	2		cytochrome b-c1 complex subunit Rieske mitochondrial [Ovis aries]
XP_004013127.3	0.24	47	33	2	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 2 more	actin cytoplasmic 2 [Ovis aries]
sp|P60713|ACTB_SHEEP	0.24	47	33	2	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 2 more	Actin cytoplasmic 1 OS=Ovis aries OX=9940 GN=ACTB PE=2 SV=1
XP_027834968.1	0.23	7	2	2		acetyl-CoA acetyltransferase mitochondrial [Ovis aries]
W5PN85|W5PN85_SHEEP	0.23	7	2	2		Acetyl-CoA acetyltransferase 1 OS=Ovis aries OX=9940 GN=ACAT1 PE=3 SV=1
XP_027830506.1	0.21	5	7	2		collagen alpha-1(I) chain isoform X1 [Ovis aries]
W5P481|W5P481_SHEEP	0.21	5	7	2		Collagen type I alpha 1 chain OS=Ovis aries OX=9940 GN=COL1A1 PE=4 SV=1
XP_004012685.1	0.18	4	2	2	Deamidation (NQ)	very long-chain specific acyl-CoA dehydrogenase mitochondrial [Ovis aries]
XP_004007902.1	0.16	5	2	2		dihydrolipoyl dehydrogenase mitochondrial [Ovis aries]
sp|P00922|CAH2_SHEEP	0.13	7	2	2		Carbonic anhydrase 2 OS=Ovis aries OX=9940 GN=CA2 PE=1 SV=2
XP_027829052.1	0.13	7	2	2		carbonic anhydrase 2 [Ovis aries]
W5PTU7|W5PTU7_SHEEP	0.13	6	2	2		Carbonic anhydrase 2 OS=Ovis aries OX=9940 GN=CA2 PE=3 SV=1
W5PXG3|W5PXG3_SHEEP	0.11	20	2	2		COX6C domain-containing protein OS=Ovis aries OX=9940 PE=1 SV=1
W5Q754|W5Q754_SHEEP	0.09	29	750	2	Carbamidomethylation; Deamidation (NQ); Oxidation (M); Phosphorylation (STY); Dehydration; 3 more	Titin OS=Ovis aries OX=9940 GN=TTN PE=4 SV=1
W5PTA0|W5PTA0_SHEEP	0.07	12	2	2		Uncharacterized protein OS=Ovis aries OX=9940 GN=ATP5PO PE=3 SV=1
W5PT98|W5PT98_SHEEP	0.07	11	2	2		Uncharacterized protein OS=Ovis aries OX=9940 GN=ATP5PO PE=3 SV=1
XP_027815230.1	0.06	34	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X25 [Ovis aries]
XP_027815256.1	0.06	43	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X50 [Ovis aries]
XP_027815254.1	0.06	43	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X48 [Ovis aries]
XP_027815252.1	0.06	43	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X46 [Ovis aries]
XP_027815248.1	0.06	42	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X43 [Ovis aries]
XP_027815247.1	0.06	41	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X42 [Ovis aries]
XP_027815245.1	0.06	41	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X40 [Ovis aries]
XP_027815241.1	0.06	40	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X36 [Ovis aries]
XP_027815240.1	0.06	40	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X35 [Ovis aries]
XP_027815233.1	0.06	38	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X28 [Ovis aries]
XP_027815232.1	0.06	37	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X27 [Ovis aries]

XP_027815250.1	0.06	36	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X45 [Ovis aries]
XP_027815243.1	0.06	34	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X38 [Ovis aries]
XP_027815239.1	0.06	34	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X34 [Ovis aries]
XP_027815237.1	0.06	34	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X32 [Ovis aries]
XP_027815235.1	0.06	33	14	2	Deamidation (NQ); Oxidation (M); Pyro-glu from Q	troponin T fast skeletal muscle isoform X30 [Ovis aries]

Consent Form for New Zealand Consumers

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

1. My participation in the project is entirely voluntary;

2. I am free to withdraw from the project at any time without any disadvantage;

3. Personal identifying information will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for five years, after which they will be destroyed;

4. I understand there no health risks are anticipated as a result of this research.

5. I understand that my participation in this survey will enable the fundraising group to which I belong will be paid $20 per participant for the particular organisation (e.g. Parent Teachers Association), and that this payment will be made to the organisation after all the survey forms have been completed.

6. The results of the project may be published but every attempt will be made to preserve my anonymity.

I agree to take part in this project.

…..... ……

(Signature of participant) (Date)

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

[Reference Number 15/092]

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Consent Form for Chinese Consumers

同意书

食品及饮料的消费者研究

（请圈出每项相应的答复）

我已阅读并了解本研究院发放的参与者资讯（21/01/2019）。 是 / 否

我了解我将需要品尝烹饪好的羊肉和不同风味的饮料，并且我确认我对这些食品没 是 / 否
有任 何已知的过敏或不耐受。

我明白参与本研究全属自愿（个人意愿），并且我可以在任何时间选择退出本研 是 / 否
究，并 不需说明理由。

我明白我的隐私将受到保护，在本研究中任何能确认我身份的资料将不会在任何交 是 / 否
流中 使用。

我明白新西兰皇家植物与食品研究院正在进行施工，相应的防范措施已实施以确保 是 / 否
在场 人员的风险降至最低。

我已获得机会讨论本研究，并且我很满意我所得到的答案。 是 / 否

本人 _____ （中文全名） _____ （拼音）在此确认我已阅读本同意书，同意其内容，
并同意参与本次研究。

签名: _____ 日期: _____