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Effects of dietary supplementation of condensed tannin and calcium nitrate in total mixed rations on long-chain fatty acid metabolism and carcass characteristics of Dohne Merino lambs

By

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DECLARATION

I, Andrea Joyce Hasewinkel, declare that this dissertation, which I hereby submit for the degree of Msc (Agric) Animal Science: Production Physiology and Product Quality at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

PREFACE

This dissertation is based on the work conducted by me. This study was an extension of a nutrition study that evaluated the effects of calcium nitrate (CaN) and Acacia tannin extract on enteric methane emissions, nutrient digestability and the efficiency of animal performance (body weight, average daily gain and feed intake) of growing lambs. For further reading on nutrition and methane emission response data see the PhD thesis conducted by Adejoro (2019): "The use of condensed tannins and nitrate to reduce enteric methane emission and enhance utilization of high-forage diets in sheep". In addition, the research was published in the Animal Feed Science and Technology journal by Adejoro et al. (2020) and titled "Replacing urea with nitrate as a non-protein nitrogen source increases lambs' growth and reduces methane production, whereas acacia tannin has no effect". However, the adaption and sustainability of the methane mitigation strategies are also highly dependent on the minimal effects and/or cobenefits in terms of carcass characteristics and meat quality that will be reported in this research.

This dissertation was prepared using a slight modification of the style and formatting recommended by the South African Journal of Animal Science.

References:

Adejoro, F. A., 2019. The use of condensed tannins and nitrate to reduce enteric methane emission and enhance utilization of high-forage diets in sheep. pHd (Agric) dissertation, University of Pretoria, South Africa.

Adejoro, F. A., Hassen, A., Akanmu, A. M. & Morgavi, D. P., 2020. Replacing urea with nitrate as a non-protein nitrogen source increases lambs' growth and reduces methane production, whereas acacia tannin has no effect. Animal Feed Science and Technology, 259, 114360.

ACKNOWLEDGEMENTS

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Thank you to Proefplaas for supplying the sheep and the facilities, Corlia Swannepoel for any advice or equipment needed, Klipeiland abattoir for giving me the space to remove samples from the carcasses of the sheep brought in over several months, and Nutrilab for providing the equipment and support for the analysis. LECO, Shimadzu, stargate scientific, and too many other generous people and fatty acid experts to mention, thank you for taking time out of your day to answer my many questions in helping me improve the fatty acid method and get the GC up and running.

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"All who have accomplished great things have had a great aim, have fixed their gaze on a goal which was high, one which sometimes seemed impossible."

~ Dr Orison Swett Marden

SUMMARY

For a methane mitigation strategy to be sustainable it must either have no affect on product quality or have an added cobenefit in terms of improving the quality of meat. This study evaluated the effect of *Acacia mearnsii* (as a source of condensed tannin) and calcium nitrate used as anti-methanogenic additives on carcass characteristics and meat quality. This study evaluated the effect of 1% urea or 3.2% calcium nitrate (CaN) as a non-protein-nitrogen (NPN) source in a total mixed ration (TMR) with or without the supplementation of 4.2% *Acacia mearnsii* tannin on carcass characteristics, fatty acid metabolism and hematological parameters of Dhoné Merino lambs. Forty lambs averaging 34.7±4kg were blocked according to weight and sex. Within each block lambs were randomly assigned into one of four treatment groups: Urea-based TMR, Urea-based total mixed ration with tannin, CaN-based TMR and CaN-based total mixed ration with tannin. Two lambs were assigned per pen and each pen was replicated five times. Lambs were adapted to the ration over 21 days. Four ewe lambs from each treatment and four ram lambs from each treatment were randomly assigned to this study and fed beyond 60 days in the trial. On day 61 hematological and serum samples were taken from the jugular vein of those 32 lambs. Lambs were slaughtered between 61 days and 103 days on trial. Four sheep (two ewes and two rams from each treatment group) were slaughtered at a lighter weight (45kg<2n<48kg and 50kg<2n<55kg, respectively) and four sheep (two ewes and two rams from each treatment group) were slaughtered at a heavier weight (48.1kg<2n<51kg and 55.1kg<2n<60kg, respectively).

Acacia Mearnsii, a condended tannin supplement (also referred to throught out the dissertation as 'tannin") increased the days on trial and decreased the cold carcass weight (CCW) and dressing percent (D%; P<0.005) of lambs. The tannin supplement had no effect on body composition, liveweight (LW), subcutaneous fat depth (SCF; over the 8th and 10th ribs), eye muscle area (EMA), carcass pH or temperature (P>0.05). Although tannin treatment had no effect on the percent of moisture and ash in the *longissimus dorsi*, there was an increase in the percent of ether extract (EE%; P<0.01). Calcium nitrate did not affect any of these parameters (P<0.05), except for a decrease in D% (P=0.05). The sex of the lambs affected all parameters except for carcass pH and temperature (P>0.05). Ewes took longer to reach their light and heavy slaughter weights than rams (P<0.05). In terms of body composition, ewes increased fatness and decreased muscle and bone percent (P<0.001). In accordance with this, ewes increased SCF depth over the 8th and 11th ribs (P<0.010 and P=0.001). As a result, moisture was higher in rams (P<0.01) and EE% was higher in ewes (P<0.001). Cold carcass weight (CCW) and EMA were greater in rams and D% was higher in ewes (P<0.005). Slaughter weight had no effect on body composition (P<0.05), D%, carcass pH, temperature or days on trial (P>0.05). However, CCW, EMA and SCF depth were greater in heavier lambs (P<0.001 CCW, P<0.05).

Between day one and six post slaughter, redness (a*) and Chroma (C) increased in the *Longissimus dorsi*, regardless of the treatment and sex (P<0.001). Tannin treatment and CaN had no effect on lightness (L*), redness (a*), yellowness (b*) or hue angle (h) on day one (P>0.05). By day six post slaughter CaN had a lower a* (P<0.05). Tannin had no effect on colour on day six (P>0.05). Lightness (L*), a*, b* and C did not differ in the colour of SCF when tannin was supplemented in the diet. Hue angle (h) increased when CaN was supplemented (P<0.05). Ewes had higher L*, a* and C on day one post slaughter (P<0.05) but by day six there was no difference between the sexes (P>0.05). Heavier lambs had an increase in L* and h on day one (P<0.05 and P<0.01, respectively) but by day six there were no differences between the weights for any of the colour parameters (P>0.05). The colour of SCF was not affected by weight (P>0.05), but a* was higher in ewes (P<0.05) and h was higher in rams (P<0.005).

Calcium nitrate had no effect on albumin and hematocrit, but it increased cholesterol and blood urea nitrogen (BUN) (P<0.05). The adaption of lambs to the diet was sufficient as there were no morbidities or mortalities during the duration of the trial. The cholesterol was within the South African Mutton merino range published by Akanmu et al. (2020) of between 1.42 and 1.53mmol/L.

Tannin did not affect the molar percent (w/w%) or gravimetric concentration (mg/g sample) of total saturated fatty acid (SFA) in subcutaneous fat (SCF), intramuscular fat (IMF), perirenal fat (PRF) and rumen fluid (RF; P>0.05). Monounsaturated fatty acid (MUFA) only decreased gravimetrically in SCF (P<0.05) but not IMF, PRF or rumen fluid (P>0.05). Tannin increased the percent of polyunsaturated fatty acid (PUFA) in SCF and PRF (P<0.05) and increased its gravimetric concentration in IMF and PRF (P<0.05). Tannin increased the PUFA/SFA ratio gravimetrically and as a percent of total fatty acids in IMF, SCF and PRF (P<0.05), apart from the gravimetric concentration in IMF (P>0.05). Fatty acid health indices showed no effect of tannin on the nutritional value of the fatty acid profile of the *longissimus dorsi*. The gravimetric desirable fatty acids were lower (DFA; P<0.05) due to reduced major fatty acid, C18:0 (P<0.05). Both hypercholesterolaemic (h) and hypocholesterolaemic (H) fatty acids were also lower (P<0.05). A lower gravimetric concentration of fat in the SCF could describe these lower indices (P<0.05). Rumen fluid was analysed for fatty acid composition to get an indication of how the treatments were affecting the microbial activity and biohydrogenation patterns. No affect in the biohydrogenation ratios (P>0.05) suggest that rumen microbes were unaffected by the supplementation of *Acacia mearnsii*. No affect of *Acacia Mearnsii* on ezyme indices suggest there was no physiological response to fatty acids within the muscle and fat.

Calcium nitrate did not decrease SFA in SCF, IMF or rumen fluid (P>0.05), but CaN did decrease the SFA percent in IMF (P<0.05). Monounsaturated fatty acids increased as a percent in IMF and

gravimetrically in PRF (P<0.05). Calcium nitrate did not affect PUFA in any of the fatty acid depots (P<0.05). The PUFA/SFA ratio only increased as a molar percent on IMF (P<0.05). There was no affect on any of the health indices in SCF or PRF (P>0.05). Calcium nitrate deceased the atherogenic and thrombogenic fatty acids in IMF resulting in a lower atherogenic index (AI) and thrombogenic index (TI) index (P<0.05 and P<0.001, respectively). Only the molar percent showed a higher hypocholesterolaemic fatty acid index (h) and lower hypercholestrolaemic fatty acid index (H; P<0.05). The h/H index was better in the CaN based diet (P<0.05). An increase in the delta-9 desaturase index suggests the lower percent and concentration of C18:0 in the CaN based diet (P<0.05). A change in the Vaccenic acid /stearic acid ratio in the rumen fluid of CaN supplemented lambs suggests that the microbes in the last step of biohydrogenation were affected by NPN source.

Rams had a lower percent and gravimetric concentration of SFA in IMF and SCF. Monounsaturated fatty acid decreased as a gravimetric concentration in IMF and SCF (P<0.05). Polyunsaturated fatty acid increased as a percent in IMF, SCF and PRF and as a gravimetric concentration in PRF (P<0.05). The PUFA/SFA ratio was higher in all the fatty acid depots (P<0.05). The atherogenic and cholesterolaemic fatty acids decreased AI and H in all fatty acid depot sites (P<0.05). The desirable fatty acid index (DFA) was higher in ewe IMF, lower as a gravimetric concentration in SCF and increased in PRF as a percent (P<0.05). The molar percent h index was higher in IMF and SCF in ewe lambs but lower as a gravimetric concentration in rams (P<0.05). Sex affected the elongase index in IMF and PRF (P<0.05) and delta 9 desaturase activity in IMF, SCF and PRF (P<0.05).

Weight did not affect the nutritional value of the fatty acid profile. There were no changes to the SFA, MUFA or PUFA percent or gravimetric concentrations (P>0.05). Of the individual important fatty acids, C16:0 was consistently lower as a percent and gravimetric concentration in lighter lambs in all the fatty acid depot sites. The elongase activity index suggests that elongase may have reduced C16:0 (P<0.05). The health indecis were not affected by weight (P>0.05).

Rams were superior in both carcass characteristics and fatty acid profile. Weight affected CCW, EMA, SCF depth and individual fatty acids. This indicated the importance of the date and weight at which ewes and rams are slaughtered. Using condensed tannin and CaN (to reduce methane emissions in lamb) influenced some carcass characteristics and the fatty acid metabolism of lamb. Although tannin increased days on feed and decreased CCW and D%, and CaN decreased D%, overall, they can be recommended for their acceptable fatty acid profile.

References: Akanmu, A. M., Hassen, A. & Adejoro, F. A., 2020. Haematology and serum biochemical indices of lambs supplemented with Moringa oleifera, Jatropha curcas and Aloe vera leaf extract as anti-methanogenic additives. Antibiotics, 9(9), 601.

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LIST OF ACRONYMS

a*	Redness
ADG	Average daily gain
AI	Atherogenic index
AOAC	Association of Official Analytical Chemists
b*	Yellowness
BCFA	Branch chain fatty acid/s
BF3	Boron fluoride alcoholate
BMI	Body mass index
BUN	Blood urea nitrogen
C	Chroma
CRBD	Completely randomized block design
CaN	Calcium nitrate
CCW	Cold carcass weight
CH4	Methane
CLA	Conjugated fatty acid
cm	Centimetre
CoA	Coenzyme A
CT	Condensed tannin
Cu	Copper
CVD	Cardiovascular disease
D%	Dressing percent
Da	Dalton/ atomic mass unit
DALRRD	Department of Agriculture, Land Reform and Rural Development
DFA	Desirable fatty acids
DHA	Docosahexanoic acid
DIT	Days in trial
DM	Dry matter
DMI	Dry matter intake
DPA	Docosapentanoic acid
EDA	Eicosadienoic acid
EDTA	Ethaline diamine tetra acetic acid
EE	Ether extract/ lipid

EFSA	The European Food Safety Authority
EMA	Eye muscle area
EPA	Eicosapentanoic acid
EPA	Environmental Protection Agency
ETA	Eicosatrienoic acid
FA	Fatty acid
FAME	Fatty acid methyl esters
FAO	Food and Agriculture Organisation
FCR	Food conversion ratio
FDA	Food and Drug Administration
Fe	Iron
FEC	Fecal egg count
FID	Flame ionisation detector
FRAP	Ferric reducing ability of plasma
g	Gram/s
GHG	Greenhouse gas/es
Gg	Giga gram/s
H	Hypercholesterolaemic fatty acid index
H*	Hue angle
h	Hypocholesterolemic fatty acid index
H^+ or H_2	Hydrogen
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HCl MeOH	Methanolic hydrochloride
HDL	High-density lipoprotein
HSFSA	Heart and Stroke Foundation of South Africa
HT	Hydrolysable tannin
IMD	Intermediate density lipoprotein
IM	Intramuscular
IMF	Intramuscular fat/Intramuscular fatty acid
iTFA	Industrial trans fatty acid
kg	Kilogram
kPA	Kilo pascal
L*	Lightness

L	Litre
LCFA	Long chain fatty acid/s
LD	*Longissimus dorsi*
LDL	Low-density lipoprotein
LNA	Linolenic acid
LW	Live weight
m	Metre
MANOVA	Multivariate Analysis of Variance
mg	Milligram
mL	Millilitre
mm	Millimetre
MMb	Metmyoglobin
mmol	Milli mol
MMT eq	Million metric ton equivalents
mRNA	Messenger RNA
Mt	Megaton
MUFA	Monounsaturated fatty acid
mV	Millivolt
N	No tannin
n-3	Omega 3 fatty acid/s
n-6	Omega 6 fatty acid/s
NA_2SO_4	Sodium sulphate
NAD+	Nicotinamide adenine dinucleotide
NADH	Nictoninamide dinucleotide plus hydrogen
NHANES	The Nutritional Health and Examination Survey
NPN or N	Non protein nitrogen source
NRF	National Research Foundation
OBCFA	Odd branch chained fatty acid/s
OCR	Oxygen consumption rate
OECD	Organisation for the Economic Co-operation and Development
P/S	Polyunsaturated/saturated fatty acid ratio
PCA	Principal component analysis
PKC	Protein kinase C

PR	Perirenal
PRF	Perirenal fat
PUFA	Polyunsaturated fatty acid
RA	Rumenic acid
RA	Rumenic acid
RMRDSA	Red Meat Research and Development South Africa
rpm	Revolutions per minute
RSD	Residual square difference
rTFA	Ruminant trans fatty acid
s	Second/s
SA	South Africa
SA	Stearic acid
SC	Subcutaneous
SCD	Stearoyl coenzyme A desaturase
SCF	Subcutaneous fat
SD	Standard deviation
SFA	Saturated fatty acid
spp.	Species
T	Tannin treatment
TAG	Triacylglycerol
TEAC	Trolex equivalent antioxident capacity
Temp	Temperature
TFA	Trans fatty acid/s
TGF-β	Transforming growth factor beta
TI	Thrombogenic index
TMR	Total mixed ration
TT	Tannin treatment
UFA	Unsaturated fatty acid
µm	Micrometre
µV	Microvolt
VA	Vaccenic acid
VFA	Volatile fatty acid/s
VHDL	Very high-density lipopoprotein
VLDL	Very low-density lipoprotein

w/w%	Molar percent/ percent of detected lipid
WHO	World Health Organisation
Σ	Sum

CHAPTER ONE

1 GENERAL INTRODUCTION

1.1 Introduction and motivation

Consumers worldwide are demanding meat that is both healthy and ecologically friendly, with reduced methane emissions and a lower carbon footprint. However, an increasing global population requires an increase in food production, including food with a good source of protein (Webb & Erasmus, 2013). This puts pressure on the livestock sector to find effective methane mitigation strategies (Black *et al*., 2021). Quality parameters such as colour stability and flavour, both affected by oxidative processes, are also important to consumer purchasing habits (Luciano *et al*., 2009a).

The per capita consumption of mutton in South Africa is 2.6kg, which is lower than beef, pork and poultry (OECD, 2022). Ruminants typically have a greater proportion of saturated fatty acids (SFA) than monogastric species and are a source of trans fatty acids (Webb *et al*., 1994; Enser *et al*., 1996; Jenkins *et al*., 2007; Stender *et al*., 2008). A high amount of SFA and trans fatty acid has historically been associated with a higher rate of coronary heart disease. A study showed that replacing SFA with polyunsaturated fatty acid (PUFA) in the diet reduced the risk of coronary heart disease (Mozaffarian *et al*., 2010; Siri-Tarino *et al*., 2010). However, there is a common misconception that red meat only contains harmful fatty acids, which it does not (Webb, 2021). The type of trans fatty acid and SFA determines its health risk (Stender *et al*., 2008).

Meat from lighter lambs has been reported to have a more acceptable fatty acid profile (Tejeda *et al*., 2008). Sabbioni *et al*. (2019) suggested Cornigliese ewes be slaughtered earlier than rams due to their drop in polyunsaturated fatty acid (PUFA) percent at a heavier weight. The fatty acid profile can also be altered through breed, feeding system and additional dietary supplements like tannin (Diaz *et al*., 2002; Cloete *et al*., 2004; Vasta *et al*., 2009). Therefore, mutton may have the potential to be more competitive in the South African meat market.

Linoleic and linolenic acid, PUFAs commonly found in ruminant diets, are biohydrogenated by rumen microbes to isomerised and reduced fatty acid intermediates until they are fully hydrogenated to stearic acid (Morales & Ungerveld, 2015). These fatty acids are absorbed and undergo further desaturation, elongation and reduction in ruminant tissue (Morales & Ungerveld, 2015). Increasing the absorption of intermediates such as conjugated linoleic acid (CLA) and rumenic acid is advantageous, as it improves the health value of red meat (Morales & Ungerveld, 2015). Therefore, preventing the final step in biohydrogenation to stearic acid is advantageous.

Research has been done on quebracho tannins by a number of authers (Priolo et al., 2005; Luciano *et al*., 2009a; Luciano *et al*., 2009b; López-Andrés *et al*., 2013). Quebracho tannin contains a high proportion of condensed tannin (Luciano *et al*., 2011). Of the 5.91% total phenols extracted, Luciano *et al*. (2011) reported that 4.04% was condensed tannin. Vasta *et al*. (2009) limited the last step of biohydrogenation of vaccenic acid to stearic acid using quebracho tannin to a greater extent than the earlier step in its biohydrogenation to vaccenic acid and CLA (Vasta *et al*., 2009). Condensed tannin may have the ability to limit the last step in biohydrogenation due to the potential to alter the microbial population. Vasta *et al*. (2010) identified a correlation between a reduction in stearic acid and a lower number of *B*. *proteoclasticus* when quebracho tannins were added to the diet (Vasta *et al*., 2010). The fatty acid profile in the intramuscular fat has been shown to reflect the fatty acid profile of rumen fluid (Vasta *et al*., 2009). Thus, the accumulation of vaccenic acid in the rumen is advantageous as it is used as a substrate in muscle to endogenously synthesize rumenic acid, increasing the health value of meat (Sackmann *et al*., 2003; Morales & Ungerveld, 2015). Vasta *et al*. (2009) reported decreased saturated fatty acid (SFA) and increased monounsaturated fatty acids (MUFAs) and PUFAs in both rumen fluid and meat when tannin was supplemented (Vasta *et al*., 2009). Changes in the amount of CLA and long chain n-3 fatty acids have also been documented in *Hedysarum coronarium L.* containing condensed tannin (Priolo *et al*., 2005).

Quebracho tannin is also reported to alter the colour of meat by having more redness (a*) and lower yellowness (b*) and hue (h) upon aging of meat, in comparison to unsupplemented sheep (Luciano *et al*., 2009a). Lower lightness (L) values have also been reported (Priolo *et al*., 2005). It has also been reported to effect metyoglobin, haemoglobin and lipid oxidation in meat (Luciano *et al*., 2009b). Lipid oxidation negatively affects the colour and flavour of meat (Luciano *et al*., 2009a) as the change in colour is correlated to the antioxidant capacity of meat (Luciano *et al*., 2009a; Luciano *et al*., 2011; López-Andrés *et al*.,2013). The higher antioxidant capacity of condensed tannin is advantageous as it increases meat shelf life and stabalises the colour during storage (Luciano *et al*., 2011). The taste of meat is also reported to have altered skatole and indole biosynthesis in the rumen (Tavendal *et al*., 2005).

The major benefit of tannin inclusion is that it reduces methane emissions (Grainger *et al*., 2009; Jayanegara *et al.,* 2009; Tan *et al*., 2011; Malik *et al*., 2017), thus improving energy utilisation where normally 2% to 12% of gross energy (GE) intake is used for methane production (Johnson & Johnson, 1995). It reduces nitrogen loss (Grainger *et al*., 2009) and increases the amount of bypass proteins. This is due to formation of tannin- protein complexes which are most stable at a pH between 4 and 6.5 (Jones & Mangan, 1977). Therefore, good quality proteins will be more available to the ruminant provided the tannin level is not excessively high. However, microbes may require an additional non-

protein nitrogen (NPN) source such as urea or calcium nitrate for growth and production of microbial proteins needed by the ruminant. Calcium nitrate can be used instead of urea as a NPN source with the benefit of lowering methane emissions (Li *et al*., 2012; Adejoro *et al*., 2020). Tannins also have anthelmintic properties and are a natural internal parasite control (Athanasiadou *et al*., 2001: Villalba *et al*., 2010).

Urea improves rumen fermentation, thus increasing microbial nitrogen and rumen volatile fatty acid (VFA) production (Khattab *et al*., 2013), especially propionate, in comparison to other proteins (Belasco, 1954). Nitrate can be used as a NPN source as a replacement for urea (Nolan *et al*., 2010) with the benefit of serving as a free hydrogen sink. Nitrate acts as an electron acceptor in the rumen, thereby restricting the availability of hydrogen for methanogenesis (Guyader *et al*., 2015; Adejoro & Hassen, 2017) and propioanogenesis (Nolan *et al.*, 2010; Van Zijderveld *et al*., 2010). Nitrate alters rumen fermentation patterns by reducing the number of methanogens in the rumen, thereby changing the volatile fatty acid (VFA) ratios (Nolan *et al*., 2010). Propionic acid decreases and acetic and butyric acid increase (Nolan *et al*., 2010; Guyader *et al*., 2015). Acetate is used for the synthesis of lipids (Rittenberg & Bloch, 1945). It has also been reported to alter the bio-hydrogenation of other dietary fatty acids in the rumen (Nolan *et al*., 2010; Guyader *et al*., 2015). Hegarty *et al*. (2016), recently found that calcium nitrate had no significant effect on dressing percent, fat depth or distribution, muscling, eye muscle area and fatness in comparison to urea in feedlot steers. The colour of the meat only differed between calcium nitrate and urea supplemented animals only when both were fed at low concentrations. However, there are few large-scale studies on the effect of calcium nitrate on carcass and meat quality attributes.

There has been a resurgence of interest in the effects of tannin on meat quality. Although the effect of nitrate on rumen volatile fatty acids has been documented, there is little research available on the effect of nitrate with or without tannins on the fatty acid profile of medium and long chain fatty acids, colour and antioxidant status of meat. There has not been extensive research on meat quality and safety when nitrate is supplemented, apart from some interest in the possible danger of methaemaglobinaemia (Van Zijderveld *et al*., 2010).

1.2 Problem statement

Consumers worldwide are demanding red meat that is both healthy and ecologically friendly, with reduced methane emissions, while an ever-increasing global population is demanding an increase in food production and a source of good quality protein (Webb & Erasmus, 2013). Tannin and calcium nitrate supplemented to a total mixed ration (TMR) can be used as part of methane mitigating

strategies to produce mutton at a lower ecological cost. However, the farmer makes less profit when tannin is added to the diet because the length of time the sheep need to reach slaughter weight increases. This may also increase the methane emitted in a sheep's lifetime. Without government subsidies as an incentive to farmers, other benefits need to be considered which may result in an increase in demand or a premium price paid for the carcass. Colour, shelf life, body composition and the nutritional value of the fatty acid profile all affect the desirability of the carcass. The magnitude of change in these factors may have a correlation with the magnitude of change in the microbial population influenced by the effect of urea and calcium nitrate with and without tannin in a TMR. Alternatives to urea need to be considered that can provide a source of NPN while at the same time improving production, reducing methane emissions and improving meat quality. One such possibility to be explored is calcium nitrate.

1.3 Objectives

1. To determine the effect of *Acacia mearnsii* tannin and calcium nitrate (used to reduce the effect of methane emissions) on lamb fatty acid metabolism and carcass characteristics.
2. To compare the differences in fatty acid metabolism and carcass characteristics between treatments: A urea-based TMR, a urea-based TMR with tannin, a calcium nitrate-based TMR and a calcium nitrate-based TMR with tannin.
3. To determine the effect of sex and treatment on fatty acid metabolism and carcass characteristics.
4. To determine the effect of weight and treatment on fatty acid metabolism and carcass characteristics.
5. To determine the health and safety of these dietary tools using blood parameters.

1.4 Hypothesis

H0a: Condensed tannin and calcium nitrate differ significantly in their effect on fatty acid metabolism of adipose tissue, intramuscular fat and biohydrogenation patterns in Dohne Merino lambs.

H1a: Condensed tannin and calcium nitrate do not differ significantly in their effect on fatty acid metabolism of adipose tissue, intramuscular fat and biohydrogenation patterns in Dohne Merino lambs.

H0b: Condensed tannin and calcium nitrate differ significantly in their effect on carcass characteristics in Dohne Merino lambs.

H1b: Condensed tannin and calcium nitrate do not differ significantly in their effect on carcass characteristics in Dohne Merino lambs.

H0c: Condensed tannin and calcium nitrate differ significantly in their effect on haematological parameters in Dohne Merino lambs.

H1c: Condensed tannin and calcium nitrate do not significantly affect haematological parameters in Dohne Merino lambs.

H0d: Dohne Merino lamb sex significantly affects fatty acid metabolism and carcass characteristics.

H1d: Dohne Merino lamb sex did not significantly affect fatty acid metabolism and carcass characteristics.

H0e: Dohne Merino lamb weight significantly affects fatty acid metabolism and carcass characteristics.

He: Dohne Merino lamb weight did not significantly affect fatty acid metabolism and carcass characteristics.

1.5 References

Adejoro, F. A., Hassen, A., Akanmu, A. M. & Morgavi, D. P., 2020. Replacing urea with nitrate as a non-protein nitrogen source increases lambs' growth and reduces methane production, whereas acacia tannin has no effect. Animal Feed Science and Technology, 259, 114360.

Adejoro, F.A. & Hassen, A., 2017. Effect of supplementing or treating *Eragrostis curvula* hay with urea or nitrate on its digestibility and *in vitro* fermentation. South African Journal of Animal Science, 47, 168-177.

Athanasiadou, S., Kyriazakis, I., Jackson, F. & Coop, R.L., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *In vitro* and *in vivo* studies. Veterinary Parasitology, 99, 205-219.

Belasco, I.J., 1954. Comparison of urea and protein meals as nitrogen sources for rumen microorganisms: the production of volatile fatty acids. Journal of Animal Science, 13, 748-757.

Black, J. L., Davison, T. M. & Box, I. 2021. Methane emissions from ruminants in Australia: Mitigation potential and applicability of mitigation strategies. Animals, 11(4), 951.

Cloete, J. J. E., Hoffman, L. C. Cloete, S. W. P. & Fourie, J. E., 2004. A comparison between the body composition, carcass characteristics and retail cuts of South African Mutton Merino and Dormer sheep. South African Journal of Animal Science, 34(1), 44-51.

Dıaz, M. T., Velasco, S., Caneque, V., Lauzurica, S., De Huidobro, F. R., Pérez, C., Gonzalez, J. & Manzanares, C., 2002. Use of concentrate or pasture for fattening lambs and its effect on carcass and meat quality. Small Ruminant Research, 43(3), 257-268.

Enser, M., Hallett, K., Hewitt, B., Fursey, G. A. J. & Wood, J. D., 1996. Fatty acid content and composition of English beef, lamb and pork at retail. Meat Science, 42(4), 443-456.

Grainger, C., Clarke, T., Auldist, M.J., Beauchemin, K.A., McGinn, S.M., Waghorn, G.C. & Eckard, R.J., 2009. Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Canadian Journal of Animal Science, 89, 241-251.

Guyader, J., Eugène, M., Meunier, B., Doreau, M., Morgavi, D.P., Silberberg, M., Rochette, Y., Gerard, C., Loncke, C. & Martin, C., 2015. Additive methane-mitigating effect between linseed oil and nitrate fed to cattle. Journal of Animal Science, 93, 3564-3577.

Hegarty, R.S., Miller, J., Oelbrandt, N., Li, L., Luijben, J.P.M., Robinson, D.L., Nolan, J.V. & Perdok, H.B., 2016. Feed intake, growth, and body and carcass attributes of feedlot steers supplemented with two levels of calcium nitrate or urea. Journal of Animal Science, 94, 5372-5381.

Jayanegara, A., Togtokhbayar, N., Makkar, H.P. & Becker, K., 2009. Tannins determined by various methods as predictors of methane production reduction potential of plants by an *in vitro* rumen fermentation system. Animal Feed Science and Technology, 150, 230-237.

Jenkins, T. C. & Bridges Jr, W. C., 2007. Protection of fatty acids against ruminal biohydrogenation in cattle. European Journal of Lipid Science and Technology, 109(8), 778-789.

Johnson, K.A. & Johnson, D.E., 1995. Methane emissions from cattle. Journal of Animal Science, 73, 2483-2492.

Jones, W.T. & Mangan, J.L., 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia Scop*.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. Journal of Science of Food and Agriculture, 28, 126-136.

Khattab, I.M., Salem, A.Z.M., Abdel-Wahed, A.M. & Kewan, K.Z., 2013. Effects of urea supplementation on nutrient digestibility, nitrogen utilisation and rumen fermentation in sheep fed diets containing dates. Livestock Science, 155, 223-229.

Li, L., Davis, J., Nolan, J. & Hegarty, R., 2012. An initial investigation on rumen fermentation pattern and methane emission of sheep offered diets containing urea or nitrate as the nitrogen source. Animal Production Science, 52, 653-658.

López-Andrés, P., Luciano, G., Vasta, V., Gibson, T.M., Biondi, L., Priolo, A. & Mueller-Harvey, I., 2013. Dietary quebracho tannins are not absorbed, but increase the antioxidant capacity of liver and plasma in sheep. British Journal of Nutrition, 110, 632-639. doi:10.1017/S0007114512005703

Luciano, G., Monahan, F. J., Vasta, V., Biondi, L., Lanza, M. & Priolo, A., 2009a. Dietary tannins improve lamb meat colour stability. Meat Science, 81(1), 120-125.

Luciano, G., Monahan, F., Vasta, V., Biondi, L., Lanza, M., Pennisi, P. & Priolo, A., 2009b. Lamb meat colour stability as affected by dietary tannins. Italian Journal of Animal Science, 8, 507-509.

Luciano, G., Vasta, V., Monahan, F.J., López-Andrés, P., Biondi, L., Lanza, M. & Priolo, A., 2011. Antioxidant status, colour stability and myoglobin resistance to oxidation of *longissimus dorsi* muscle from lambs fed a tannin-containing diet. Food Chemistry, 124, 036-1042.

Malik, P.K., Kolte, A.P., Baruah, L., Saravanan, M., Bakshi, B. & Bhatta, R., 2017. Enteric methane mitigation in sheep through leaves of selected tanniniferous tropical tree species. Livestock Science. 200, 29-34.

Morales, R. & Ungerfeld, E. M., 2015. Use of tannins to improve fatty acids profile of meat and milk quality in ruminants: A review. Chilean Journal of Agricultural Research, 75(2), 239-248.

Mozaffarian, D., Micha, R. & Wallace, S., 2010. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: A systematic review and meta-analysis of randomized controlled trials. PLoS Medicine, 7(3), e1000252.

Nolan, J.V., Hegarty, R.S., Hegarty, J., Godwin, I.R. & Woodgate, R., 2010. Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. Animal Production Science. 50, 801-80.

OECD (2022), Meat consumption (indicator). [Online]: Available from: doi: 10.1787/fa290fd0-en. [Accessed 17.12.2022]

Priolo, A., Bella, M., Lanza, M., Galofaro, V., Biondi, L., Barbagallo, D., Salem, H.B. & Pennisi, P., 2005. Carcass and meat quality of lambs fed fresh sulla (*Hedysarum coronarium L.*) with or without polyethylene glycol or concentrate. Small Ruminant Research, 59, 281-288.

Rittenberg, D. & Bloch, K., 1945. The utilization of acetic acid for the synthesis of fatty acids. Journal of Biological Chemistry, 160, 417-424.

Sabbioni, A., Beretti, V., Zambini, E. M., Superchi, P. & Ablondi, M., 2019. Allometric coefficients for physical-chemical parameters of meat in a local sheep breed. Small Ruminant Research, 174, 141-147.

Sackmann, J. R., Duckett, S. K., Gillis, M. H., Realini, C. E., Parks, A. H. & Eggelston, R. B., 2003. Effects of forage and sunflower oil levels on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. Journal of Animal Science, 81(12), 3174-3181.

Siri-Tarino, P. W., Sun, Q., Hu, F. B. & Krauss, R. M., 2010. Saturated fatty acids and risk of coronary heart disease: Modulation by replacement nutrients. Current Atherosclerosis Report, 12(6), 384-390.

Stender, S., Astrup, A. & Dyerberg, J., 2008. Ruminant and industrially produced trans fatty acids: health aspects. Food & Nutrition Research, 52(1), 1651. DOI: 10.3402/fnr.v52i0.1651

Tan, H.Y., Sieo, C.C., Abdullah, N., Liang, J.B., Huang, X.D. & Ho, Y.W., 2011. Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. Animal Feed Science and Technology, 169, 185-193.

Tavendale, M.H., Lane, G.A., Schreurs, N.M., Fraser, K. & Meagher, L.P., 2005. The effects of condensed tannins from Dorycnium rectum on skatole and indole ruminal biogenesis for grazing sheep. Australian Journal of Agricultural Research, 56, 1331-1337.

Tejeda, J. F., Peña, R. E. & Andrés, A. I., 2008. Effect of live weight and sex on physico-chemical and sensorial characteristics of Merino lamb meat. Meat Science, 80(4), 1061-1067.

Van Zijderveld, S.M., Gerrits, W.J.J., Dijkstra, J., Newbold, J.R., Hulshof, R.B.A. & Perdok, H.B., 2011. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. Journal of Dairy Science, 94, 4028-4038.

Vasta, V., Mele, M., Serra, A., Scerra, M., Luciano, G., Lanza, M. & Priolo, A., 2009. Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. Journal of Animal Science, 87(8), 2674-2684.

Vasta, V., Yáñez-Ruiz, D. R., Mele, M., Serra, A., Luciano, G., Lanza, M., Biondi, L. & Priolo, A., 2010. Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. Applied and Environmental Microbiology, 76(8), 2549-2555

Villalba, J.J., Provenza, F.D., Hall, J.O. & Lisonbee, L.D., 2010. Selections by tannins by sheep in response to gastrointestinal nematode infection. Journal of Animal Science, 88, 2189-2198.

Webb, E. C., 2021. Cis/trans-fatty acid content of red meats and the related effects on meat quality and human health. In: Meat and Nutrition. Ed: Ranabhat, C. L., InechOpen, London, United Kingdom. pp. 121-138.

Webb, E. C., Casey, N. H., & Van Niekerk, W. A., 1994. Fatty acids in the subcutaneous adipose tissue of intensively fed SA Mutton Merino and Dorper wethers. Meat Science, 38(1), 123-131.

Webb, E.C. and Erasmus, L.J., 2013. The effect of production system and management practices on the quality of meat products from ruminant livestock. South African Journal of Animal Science, 43, 413-423.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Introduction

Enteric methane produced by methanogenic bacteria in the rumen account for 30% of methane released into the earth's atmosphere (Black *et al*., 2021). Ruminant derived products are also a significant source of medium chain saturated fatty acid and trans fatty acids due to lipolysis and biohydrogenation of fatty acids by other microbes in the rumen (Lourenço *et al*., 2010; Shingfield *et al*., 2013). Some saturated fatty acids (SFA) and trans fatty acids (TFA) have been associated with cardiovascular disease (Woodside *et al*., 2008).

This literature review looks at meat consumption trends and the impact of ruminant livestock on global warming. It investigates the basics of fat metabolism, fatty acids in lamb meat, and its benefit or detriment of lamb meat fatty acid profile on human health. It compares the fatty acid profile of lamb with other livestock animals and investigates ways in which the fatty acid profile can be improved for human health and acceptability.

Tannin and calcium nitrate are explored as potential ways to reduce methane emissions and improve the fatty acid profile of meat simultaneously. Carcass characteristics (oxidation of meat and colour) and the physiology behind body composition changes are discussed. The weight of sheep and sex, regardless of diet, is discussed as a method to improve the timing of slaughter for the best fatty acid profile and carcass characteristics.

2.2 Meat consumption and trends

The lean part of red meat holds a high nutritional value. It is an excellent source of protein, vitamin B12, niacin, Vitamin B6, iron, zinc and phosphorus. It is relatively low in fat (<7%) and sodium and a source of endogenous antioxidants. It also contains long chain omega 3 fatty acids, riboflavin, pantothenic acid and selenium (Williams, 2007). There is a growing social change amongst people that is setting a trend away from meat culture and tradition because of questions surrounding environmental impact, economic reasons, human health, and ethical obligations that come with the consumption of meat (Leroy & Praet, 2015). There is a metaphorical scale with nutritional value balancing on the one side, and emotional response and health on the other. There has been pressure to lower meat consumption, particularly red and processed meat in higher consuming countries due to its health risks. However, meat in moderation has been proven to have nutritional benefits within a balanced diet (De Smet & Vossen, 2016).

There is an expected 40 megaton (Mt) increase in global meat consumption between 2020 and 2029 largely due to an increase in production as well as economic and population growth in developing countries (OECD/FAO, 2020). However, per capita consumption is leveling off especially in developed countries where consumer's preferences are changing (OECD/FAO, 2020). Developing countries are still increasing consumption whereas developed have reached a saturation point. This "nutritional transition" in developing countries is due to improved income making available a greater amount of money spent on food, followed by changes to the diet (Milford *et al.*, 2019). Several factors are responsible for these nutritional transitions amongst populations such as higher income, urbanization, westernisation of the diet, and most recently environmental awareness, human health and animal welfare (Sans and Combris, 2015).

The major contributors to an increase in meat production lies in the chicken and pig meat sectors (after recovering from the latest African swine flu outbreak) (OECD/FAO, 2020). Chicken has become a better option to many consumers due to its price, health and environmental benefits over red meat (OECD/FAO, 2020). However, sheep production is expected to increase by 2Mt globally with the largest increase coming from China and less developed countries in sub –Saharan Africa (OECD/FAO, 2020). According to the Department of Agriculture Land reform and Rural Development (DALRRD) South Africa had a commercial sheep population of 18651 thousand in 2021 (DALRRD, 2022) and the per capita consumption of sheep, lamb and goats was 2.8kg in the 2020/2021 year (DALRRD, 2022). Sheep meat is characterized as a high fat food high in saturated fatty acid (SFA) and low in polyunsaturated fatty acid (PUFA) with a good n-6/n-3 ratio (Sinclair, 2007). Studies have shown an improvement in the fatty acid profile of sheep fat for human health by feeding fat in the form of protected fat, changing biohydrogenation patterns and increasing the Stearoyl CoA activity (Sinclair, 2007).

2.3 Impact of ruminants on global warming

2.3.1 Global warming

Global warming is caused by greenhouse gases (GHG) trapping heat within the atmosphere (Jeffry *et al*., 2021). It causes an increase in extreme weather patterns such as draughts and heat waves, flooding, freezing conditions and changes in salinity of available water (Zandalinas *et al*., 2021). It is predicted to increase the temperature in summer and winter, increase the length of summer and decrease the length of winter, spring and autumn (Wang *et al*., 2021). Extreme sea levels have also been associated with global warming (Tebaldi *et al*., 2021). These global phenomena could result in changes in the activity and survival of human beings (Jeffry *et al*., 2021) and the extinction of hundreds

of species worldwide (Wiens, 2016). In order to solve the escalating problem, the Paris agreement was established to limit global warming to 1.5°C to 2°C above pre-industrial levels (Tebaldi *et al*., 2021).

2.3.2 *Effect of livestock on global warming*

Livestock production makes up 14.5% of GHG emissions and therefore plays a major role in climate change (Gerber *et al*., 2013). Small ruminants make up 6.5% of the livestock sectors GHG emissions (Gerber *et al*., 2013). Methane is the most important GHG in small ruminants as it makes up 57% emissions for milk production and 55% in meat production (Opio *et al*., 2013). Methane has a 23% greater heat trapping potential than CO_2 (Tan *et al*., 2011). Therefore, it is the responsibility of the livestock sector to find strategies and ways to reduce it. Not only is methane a GHG but it takes away 2 to 12% of energy from feed (Johnson & Johnson, 1995) that could otherwise be used for physiological functions such as animal growth, milk production, maintenance, and pregnancy (Piñeiro-Vázquez *et al*., 2015; Matthews *et al*., 2019).

The FAO (2017) reported a global aggregate methane output of 177206801015 kg CO_2 eq. The total livestock methane emissions in the United States for 2022 was 175.2 million metric tons of carbon dioxide equivalents (MMT CO_2 eq.), of which sheep produced 1.2 MMT $C0_2$ equivalents (EPA, 2022). Livestock production is the only option on about 70% of agricultural land in South Africa (Scholtz *et al.,* 2013). Therefore, methane is one of the major anthropogenic greenhouse gas producers in South Africa with an annual average between 1990 and 2014 of 1227.96 Giga grams (Gg), of which sheep produced 18.54% of the methane (Moeletsi et al., 2017). In 2010 sheep produced 167 Gg of methane of which commercial sheep produced 8.5kg/head/year and communal sheep produced 6.1kg/head/year (Du Toit *et al.,* 2013). This is well above the 5kg/head/year calculated for other developing contries (Du Toit *et al.,* 2013). However, a more recent study shows that GHG emissions in Southern Africa have not increased in the last 20 years as the result of a 40% reduction per kg animal product (Scholtz et al., 2023). This would suggest tangible improvements are already being made in livestock efficiency and breeding practices within Southern Africa (Scholtz et al., 2023).

2.3.3 *Methanogens*

Over 90% of methane emissions from livestock come from rumen fermentation (McAllister *et al*., 2015). Monomers from the degradation of carbohydrates, proteins and organic polymers are further converted to volatile fatty acids (VFA), carbon dioxide ($C0_2$) and hydrogen (H^+; Morgavi *et al*., 2010). If H^+ is not controlled, the pH of the rumen would drop and affect fermentation, fibre digestion and microbial growth (Callaghan *et al*., 2014; Matthews *et al*., 2019). To overcome this problem, a group of archaea called methanogens scavenge H^+ as an energy source and CO_2 as an electron acceptor in

the synthesis of methane (Janssen, 2008; Hill *et al*., 2016) to prevent the detrimental effect of H^+ build up in the rumen (Morgavi *et al*., 2010). Therefore, H^+ is eructated in the form of methane when four moles of H^+ are used to reduce one mole of CO_2 (Callaghan *et al*., 2014). Some major methanogenic pathways and their microbes are as follows (Hill *et al*., 2016):

Hydrogenotrophic: $\mathbf{CO_2} + \mathbf{4H_2} \rightarrow \mathbf{CH_4} + 2H_2O$

Methylotrophic: $CH_3OH + \mathbf{H_2} \rightarrow \mathbf{CH_4} + H_2O$

$4CH_3OH \rightarrow \mathbf{3CH_4} + CO_2 + 2H_2O$

$CH_3NH_2 + \mathbf{H_2} \rightarrow \mathbf{CH_4} + NH_3$

Aceticlastic: $CH_3COOH \rightarrow CO_2 + \mathbf{CH_4}$ (minor in the rumen)

Although this microbial evolution efficiently solves the problem of H^+ build up in the rumen, methane is a significant source of GHG emissions (Min *et al*., 2020). Studies have shown that methane can be reduced safely without a negative effect on fibre digestion or lactation (Brask *et al*., 2013). Vaccines, microbial enzyme inhibitors, breeding for smaller rumen size and additives have been studied (Matthews *et al*., 2019). Other methods include chemical compounds targeting methanogens; nitrate and nitro-compounds reducing methanogens and competing for H^+ used in the synthesis of methane; ionophores reducing H^+ producing bacteria; oils inhibiting methanogens and protozoa, anti-protozoal agents; and plant extracts (Ungerfeld, 2015). A strategy to reduce methane emissions while simultaneously having other advantages such as improved meat quality, product shelf life, improved animal health and performance would be a gold standard for both farmers and ecologists.

2.4 Acceptability of lamb in terms of oxidative stability and colour

2.4.1 *General mechanism*

Oxidation is typically associated with fats and oils (Gray & Monahan, 1992) but also affects meat colour, protein and vitamins (Dominguez *et al*., 2019). Colour, texture and appearance are the first to change but oxidation can also cause disease (Dominguez *et al*., 2019). There are three main reactions; auto-oxidation, enzymatic oxidation and photo-oxidation (Amaral *et al*., 2018). When oxygen reacts with unsaturated fatty acids (UFA) via a free radical, hydroperoxides are formed. It is very unstable and gives rise to hydrocarbons, ketones, alcohols, esters, acids and most importantly aldehydes (also react with proteins), which reduce the sensory quality of meat through a characteristic off odour and flavour (Dominguez *et al*., 2019).

The colour of fresh meat is an important characteristic influencing consumer purchasing habits (Faustman & Cassens, 1990). Colour stability of meat is affected by the oxygen consumption rate (OCR)

and metmyoglobin reductase activity (Jacob, 2020). A study conducted by Ledward *et al*. (1992) suggested that a high OCR in steaks up until 96 hours post-mortem affected meat redness by preventing it from fully oxygenating. The red colour during blooming is maximised if mitochondrial oxidation is moderate; too high and it doesn't fully bloom or too low and metmyoglobin (and yellowness) increases (Ramanathan *et al*., 2019). When meat begins to age the OCR drops, resulting in a change in colour (Hood, 1980). The colour of fresh meat changes from purple to bright red when myoglobin is oxygenated to oxymyoglobin on exposure to air (MacDougall, 1981). The colour is determined by the depth of oxygen diffusion and the extent of myoglobin oxidation (Ramanathan *et al*., 2019). Oxymyoglobin formation is altered by temperature, oxygen partial pressure and pH of meat (Mancini & Hunt, 2005). The redness is reduced, and browning commences when the iron atom in the myoglobin (in the ferrous state) is oxidised to metmyoglobin (in the ferric state) (MacDougall, 1982; Bekhit & Faustman, 2005). The reduction of metmyoglobin occurs until it cannot keep up anymore due to the depletion of the nicotineamide dinucleotide plus hydrogen (NADH) pool and muscle's oxygen scavenging and reducing enzyme activity (MacDougall, 1982; Bekhit & Faustman, 2005). Variations in colour stability are possible due to differences in extrinsic factors like post-mortem age, ultimate carcass pH, rigour temperature, genotype, antioxidants (Jacob, 2020) and muscle group (Mckenna *et al*., 2005).

Colour has been stabilised using different feeding strategies such as feeding tannin (Luciano *et al*., 2009b; Luciano *et al*., 2011), vitamin E (Calnan *et al*., 2019: Leal *et al*., 2020) and grazing sheep on pasture (Gao *et al*., 2014).

2.4.2 *Effect of diet and dietary supplements*

Feeding soybean, typically high in polyunsaturated fatty acids (PUFA), will increase the PUFA profile of meat. Unsurprisingly, it will also increase the susceptibility of muscle and subcutaneous tissue to lipid oxidation (Ladeira *et al*., 2014). This is because phospholipids carry the majority of PUFAs (37 to 47% in comparison to only 4.5 to 14% in triglycerides) and their oxidative catalytic sites are closer together, making them very reactive to oxygen molecules (Ladeira *et al*., 2014). They also have a 90% contribution to malonaldehyde, a lipid oxidation product (Dominguez *et al*., 2019).

The incorporation of natural supplements to the diet can better protect the fat from lipid oxidation after slaughter. Jeronimo *et al*. (2020) successfully supplemented *Cistus Ladanifer* (a plant particularly high in phenolic compounds such as condensed tannins and α- tocopherol (Vitamin E)) to increasing levels of PUFA rich vegetable oils in a total mixed ration (TMR) ration. It was suggested that increased levels of α-tocopherol improved fatty acid protection in muscle. This is in accordance with Kasapidou

et al. (2012) who found a minimum of 1.9ug/ml α- tocopherol supplementation to be sufficient in significantly preventing oxidation of lipids in sheep meat. However, Luciano *et al*. (2011) suggested that a quebracho tannin-containing di*et al*so has the potential to successfully increase phenol in muscle and improve antioxidant capacity. This also resulted in a more stable colour profile with a low increase in hue angle and metmyoglobin percent (Luciano *et al*., 2011).

2.4.3 *Preservatives*

Another method of reducing oxidation is by preserving meat. This is traditionally done with nitrate and nitrite (Flores & Toldra, 2021). These methods bring with them health concerns, although the risk of inappropriate levels remain low if used correctly (The European food safety authority EFSA, 2017; Flores & Toldra, 2021). For this reason, other more natural products are gaining traction, for example plant extracts rich in polyphenols (Efenberger-Szmechtyk *et al*., 2021).

2.5 **Body composition, lipid deposition and storage**

2.5.1 Body composition

The health-conscious consumer is reluctant to eat very fatty meat. However, a small amount of fat is advantageous for a moist and tasty product (Wood, 1984). Body composition changes as an animal grows and reaches maturity. Brand *et al*. (2018) fed SA Mutton Merino sheep in a feedlot for 0 to 105 days and reported a decrease in lean muscle percent, a decrease in bone percent and an increase in fat percent. On day 0 the muscle: bone: fat percent were 49.9±1.01, 22.9±0.75 and 26.2±1.39 respectively and by day 105 they were 31.3± 1.05, 15.1± 0.76 and 53.4±1.41, respectively.

Growth starts in neural tissue and then advances to bone and muscle (Owens, 1993). Adipose tissue is the last to develop (Owens, 1993). By the finishing phase of the lamb's growth in a feedlot, the percent of bone and muscle is decreasing, and adipose tissue is increasing (Haugebak *et al*., 1974). Skeletal muscle is made up of myofibres, connective tissue, adipocytes, vascular tissue and nervous tissue (Lefaucheur, 2010). On average, it contains about 75% water, 19% protein, 0.5 to 8% lipid and 1% glycogen (Lefaucheur, 2010). A young animal's meat is wetter, less firm and the fat is pinker due to more water and connective tissue and a lower percent of fat (Wood, 1984). Muscle mass grows at a consistent rate after birth until maturity (Prache *et al*., 2021). However, as body weight increases and the lamb begins to mature, fat deposition occurs. This leads to a decreasing percent of muscle relative to an increasing percent of fat (Prache *et al*., 2021). The meat becomes firmer when lipid replaces water in muscle and there is less space between fat cells (Wood, 1984; Prache *et al*., 2021).

Lipid accumulation appears early, medium or late depending on where it is deposited (Owens, 1993). Wood (1984) suggested that differences in the rate of accumulation of fat between depots is more specifically as a result of differences in relative growth rate at the different sites (omental fat> subcutaneous> intermuscular> peri-renal> mesenteric> intramuscular). Thus, the speed of accumulation of fat differs between depots. For example, subcutaneous fat has a higher relative allometric growth rate than intermuscular fat (Wood, 1984). According to later studies, in the early days postpartum intramuscular fat does not increase immediately, but as the animal begins to grow a period of linear deposition occurs, followed by a plateau when the animal reaches its mature size and fat deposition reaches capacity (Pethick *et al*., 2004: Hocquette *et al*., 2010). Within the depot site, there may also be differences in allometric growth depending on its anatomical position on the carcass (Kempster *et al*., 1981).

Breed, sex and birth weight will affect the body composition ratio at a given body weight (Prache *et al*., 2021). Therefore, slaughter weight will vary depending on its sex and whether it is an early or late maturing breed (Brand *et al*., 2018). Composition is also affected by correlations between weight and sex or diet (Wood, 1984). In South Africa, it is important to know the fat deposition trends based on the above to determine the slaughter weight needed to reach an A2 carcass (Brand *et al*., 2018), after which the animal will become over fat and the value of the carcass will diminish (Prache *et al*., 2021). The way fat is partitioned will also affect its classification and quality (Brand *et al*., 2018).

2.5.2 Lipid deposition during growth

Two mechanisms are important for tissue growth; hyperplasia (increase in cell number) early in development and hypertrophy (increase in cell size) later in life (Owens *et al*., 1993). Hypertrophy occurs before hypoplasia to accommodate an increase in fat storage (Jo *et al*., 2009) but hyperplasia can continue throughout life in adipose tissue (Owens *et al*., 1993). In addition, hypertrophy is strongly correlated to diet whereas hyperplasia is affected by the interaction between diet and genetics (Jo *et al*., 2009). Differences in cell size between different depots have also been recorded in 17-month-old cattle by Cianzio *et al*. (1985), where kidney fat had the greatest cell size followed by mesenteric, subcutaneous, intermuscular, intramuscular and brisket, respectively.

2.5.3 Fatty acid storage

Fat can be stored within (intramuscular) or around (intermuscular) muscle. However, intermuscular fat (for example subcutaneous fat) can easily be trimmed. Intramuscular fat, in other words marbling, is the fat between muscle bundles in close proximity to blood capillaries in the connective tissue (Hocquette *et al*., 2010). Intramuscular fat is predominantly structural lipids, phospholipids and

triglycerides (storage lipids as energy reserves) (Hocquette *et al*., 2010; Listrat *et al*., 2016). Triglyceride, the largest concentration of the three, is predominantly stored in adipocytes between muscle fibres or perimysium surrounding muscle bundles (Lefaucheur, 2010). A much smaller amount is stored in the lipid droplets of predominantly oxidative type 1 myofibres (Lefaucheur, 2010). Phospholipid, the main constituent of cell membranes, is 30% higher in oxidative muscle fibres (red) than glycolytic fibres (white) due to its proximity to mitochondria. There is much debate surrounding this topic as not all researchers have found an increase in intramuscular fat in oxidative as opposed to glycolytic muscle fibres, particularly in pigs (Lefaucheur, 2010). However, other researchers have found a significant correlation between oxidative fibres and increased marbling (Calkins *et al*., 1981).

2.6 **Functional role of fatty acids in the human diet**

Fatty acids can either be saturated (single bond between carbon atoms), monounsaturated (one double bond between carbon atoms) or polyunsaturated (more than one double bond between carbon atoms; Makumbo & Muchenje, 2016). The double bonds can be in a cis or trans configuration depending on if they are pointing in the same or opposite directions (Wood *et al*., 2008). Fatty acids are part of phospholipids, sphingolipids (the building blocks of cell membranes) (Beitz, 2004) triacylglycerol (TAG), and fatty acid derivatives able to regulate cell signaling (De Carvalho & Caramujo, 2018).

Lipids are important for the oxidation of fatty acids to $C0_2$ for the production of metabolizable energy (Beitz, 2004). Therefore, they serve as an alternative energy source in aerobic tissues and have important biological functions (De Carvalho & Caramujo, 2018). Triglycerides are neutral fats consisting of three long chain fatty acids (LCFA) attached to a glycerol backbone and are most commonly stored for energy in adipose tissue (Dzoyem *et al*., 2014; Kaçar *et al*., 2019). Phospholipids make up the structural matrix of cell membranes (Lodish & Rothman, 1978). They are amphiphilic molecules where the glycerol backbone is attached to a hydrophilic head and two hydrophobic fatty acyl tails (Sebaaly *et al*., 2019). The glycerophospholipid is the main type of phospholipid in eukaryotes and is esterified to a choline, ethanolamine, serine or inositol molecule (Van Meer, 2008).

Other functions of fatty acids include alveolar integrity, solubilisation of non-polar compounds, cell and tissue metabolism, function, and response (Beitz, 2004; Calder, 2015). They have been reported to regulate transcription factors, gene expression and protein production (Calder, 2015).

In a healthy person, the majority of fatty acids consumed in the diet are made available through digestion and absorption into the blood stream (Calder, 2015). The total amount of fat consumed by

the South African population is within range (20% to 30%) but the quality of fats still needs to be improved (Vorster & Badham, 2013). According to the South African Nutritional Guidelines, foods with the right types of fats and oils should be selected and consumed in moderation. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and trans fatty acids should make up 7% to 10% (depending on cardiovascular health), 10% to 12%, 6% to no more than 10%, and 1% of the total energy intake per day, respectively (Vorster & Badham, 2013). Lean red meat (with trimmed fat and skin) can be consumed safely at 90g per day (Vorster & Badham, 2013).

Not all fatty acids can be synthesized *de novo*, but are required in the diet for metabolic functioning, somatic growth and reproduction (De Carvalho & Caramujo, 2018). These essential fatty acids, such as Omega-3 and Omega-6 fatty acids, are a functional component of the diet due to their health benefits and body's inability to synthesize them (Kaur *et al*, 2014). α-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are such fatty acids (Kaur *et al*, 2014). Ruminant meat is a source of essential fatty acids as they provide linoleic acid, α-linolenic acid, C20 and C22 PUFAs to the diet (Vorster & Badham, 2013). In the Australian diet omega 3 fatty acids makes up the second greatest contribution of n-3 fatty acids after fish (Williams, 2007). Some other fatty acids with health promoting benefits found in ruminant meat are branch chain fatty acids, vaccenic acid and rumenic acid (Vahmani *et al*., 2020).

2.7 **Fatty acids linked to disease**

In 2016 cardiovascular disease (CVD) accounted for 17.9 million global deaths (World health statistics, 2021). According to the Heart and Stroke Foundation of South Africa (HSFSA, 2016), one in six deaths are caused by CVD in South Africa. Hypertension, overweightness and obesity are also a problem amongst the South African population. One in three South Africans 15 years and older suffer from hypertension, which increases the risk of CVD, heart attacks and strokes (HSFSA, 2016).

2.7.1 *Saturated fatty acids*

There is a correlation between populations with a lower intake of saturated fatty acids, but higher intake of polyunsaturated and MUFAs with lower incidence of CVD (Sacks *et al*., 2017). According to the South African National Health and Nutrition examination survey, one out of four people have an abnormally high total serum and low-density lipoprotein (LDL) cholesterol level (Shisana *et al*., 2014). A lowering of blood glycerol levels is associated with reduced LDL cholesterol and has been proven to prevent and regress atherosclerosis in nonhuman primates (Sacks *et al*., 2017).

2.7.2 *Trans fatty acids*

Trans fatty acids have at least one double bond in a trans configuration (FDA, 2013). The FDA do not include conjugated fatty acids as part of this definition (FDA, 2013). According to the World Health Organistion (WH0), the Codex commission defines them as "all the geometrical isomers of monounsaturated and polyunsaturated fatty acids having non-conjugated, interrupted by at least one methyl group, carbon-carbon double bonds in the trans configuration", and does not include conjugated trans fatty acids, like conjugated linoleic acid (CLA), commonly found in ruminant fats (WHO, 2004). They are either sourced commercially by partial hydrogenation of vegetable or marine oils, or naturally in smaller quantities in ruminant meat and milk (Parodi, 2004).

According to a European study spanning 14 countries published in 1998 called the TRANFAIR study, margarines and low-fat spreads typically had between 0.1 and 17% trans fatty acids, and vegetable oils typically had less than 1% (Aro *et al*., 1998a). The major trans-fat found in dietary fats and oils was C18:1; comprising 94% in hardened vegetable oils, 52 to 68% in butter, and 28 to 42% in fish oil. Fish oil had the greatest distribution of monoenoic fatty acids (C16:1, C22:1 and C18:1; Aro *et al*., 1998a).

Replacing butter and lard, typically high in saturated fatty acids, with alternatives high in trans fatty acids proved to have serious health implications (Mozaffarian & Stampfer, 2010) which led to a global drive to reduce trans fatty acids in margarines, shortenings and cooking fats (Presch & Molkentin, 2000). In 2004, following health warnings, Denmark was the first to implement a law to restrict industrially produced trans fats to a maximum of 2% of oils and fats to be used for human consumption (Leth *et al*., 2006; Stender *et al*., 2006). This legislation was implemented for fat in any food products (Leth *et al*., 2006), except for oils and fats from animal origin (Stender *et al*., 2006). Three years after implementation, CVD deaths were reduced by as much as 14.2 people per 100,000 per year from its original annual death rate of 441.5 per 100,000 people when compared to a synthetic OECD group (Restrepo & Rieger, 2016). Research by Spruk & Kovac (2020) also found substantial health benefits from the legislation. Following these positive results 46 other counties, including South Africa, have according to the Global database on the Implementation of Nutritional Action (GINA) implemented the "Best-practice TFA policy" which limits industrially produced TFAs in foods, fats and oils (GINA, 2022). At present, 42.98% of the world's population is protected by mandatory TFA limits (GINA, 2022). The World Health Organisation (WHO) recommend that TFAs should be limited to less than 1% total energy intake, which equates to less than 2.2g per day when consuming a 2000 calorie diet (WHO, 2018). Other countries are now also starting to see a reduction in trans fatty acid intake and reduced CVD related deaths following its implementation (Restrepo, 2016).

Brouwer *et al.* (2010) studied the effect of trans fatty acids on the ratio of low-density-lipoprotein (LDL) and high-density-lipoprotein (HDL) plasma cholesterol levels from 23 industrial trans fatty acid (iTFA) studies and 5 ruminant (milk) trans fatty acid studies. The studies showed that fatty acids with at least one double bond in the trans configuration increased the ratio, thereby increasing the risk of coronary heart disease, regardless of whether they come from industrial or ruminant origin. However, there may be a threshold limit to which changes in the ratio occur. Lacroix *et al*. (2012) found that rumen TFAs (rTFAs) from butter, equivalent to 1% daily energy intake in woman, had no significant effect on the ratio. However, they found that predisposing factors such as a high BMI, in conjunction with consuming rTFA at 1% daily energy, resulted in a reduced plasma high density lipoprotein (HDL) cholesterol concentration. The Transfair study also concluded that European intake levels were low enough not to affect the serum lipid profile low density lipoprotein (LDL), high density lipoprotein (HDL) and LDL/HDL ratio (Van de Vijver *et al*., 2000). Where high TFA diets are consumed, there has been evidence of increased cholesterol in plasma membranes due to suppression of the transforming growth factor beta (TGF-β), thereby increasing the risk of atherosclerosis (Chen *et al*., 2011).

The association between trans fatty acids with stroke and myocardial infarcation have also been documented. Kiage *et al*. (2014) found that in a fully adjusted model (adjusted for covariates) for every 2g/day increase in TFA intake, there was a 14% risk in males but not females having a stroke. However, other research has shown both males and females responding three or more years after governmental restrictions with significantly reduced combined myocardial infarcation and stroke hospital admissions. This resulted in a 6.2% additional significant decline beyond temporal trends in populations coming from countries with restrictions (Brandt *et al*., 2017).

Trans fatty acids can also cause serious metabolic syndromes such as systemic inflammation, endothelial dysfunction, visceral adiposity, weight gain, insulin resistance and diabetes mellitus (Micha & Mozaffarian, 2009). The functioning of hepatocytes, adipocytes, macrophages and endothelial cells can also be affected (Micha & Mozaffarian, 2009). In a survey carried out by the National Health and Nutrition Examination Survey (NHANES) in the years 1999/2000 and 2009/2010 plasma TFAs were successfully reduced by 54%. Although this was a significant reduction, there was no threshold (apart from blood pressure) to which TFAs did not cause metabolic risks (Zhang *et al*., 2017). Some studies have shown that TFA effect blood pressure (Wang *et al*., 2010), whereas an earlier study found no association (Mensink *et al*.,1991). Therefore, other factors may also be involved.

Trans fatty acids have also been shown to affect fertility and fetal development. When men have an intake of greater than 1% energy, they risk infertility due to a marked decrease in sperm concentration

and semen quality. Infertility in woman is also prevalent when consuming concentration of more than 2% of total energy (Çekici & Akdevelioğlu, 2018).

2.8 **Fatty acid composition of farm animals**

Fatty acids in meat are predominantly medium and long chain fatty acids between 12 and 22 carbon atoms in length, with sheep meat having a small amount of short chain fatty acids between 8 and 10 carbons in length (Wood *et al*., 2008). Approximately 40% of meat is SFAs, 40% MUFAs and 2 to 25% PUFAs (Wood *et al*., 2008).

The transfer of fats from the diet to the animal product is indicative of the digestive system, and thus differs between ruminant and monogastric animals (Woods & Fearon, 2009). Ruminant fats are higher in saturated fatty acids and lower in polyunsaturated fatty acids in comparison to the leaner meat of monogastric animals (Table 2.8.1). Oleic acid (C18:1n9c) and palmitic acid (C16:0) make up the largest portion of fat, followed by stearic acid (C18:0; Table 2.8.1). Table 2.8.1 also shows that myristic (C14:0) and elaidic acid (C18:1n9t) are found in higher concentrations in 100g of South African lamb than beef, chicken and pork, and stearic (C18:0) acid is higher in ruminants than monogastric animals. Chicken and pork are higher in C16:1, C18:2, Omega 3 (n-3) and Omega 6 (n-6) fatty acids. Pork has about 250 times more conjugated linoleic acid (CLA) than ruminants. The fatty acid profile is also more complex in ruminants with higher proportions of trans, odd, conjugated and branch chain fatty acids (BCFAs; Wood *et al*., 2008; Lourenço *et al*, 2010; Buccioni *et al*, 2012). Therefore, because of their symbiotic relationship with the microbial population in the rumen, the fatty acid profile of ruminant adipose tissue and intramuscular fat is more of a challenge to change through diet than monogastric animals (Wood *et al*, 2003). There are also differences within ruminants; the rate of passage through the rumen is faster in sheep than cattle due to the size of the rumen, resulting in differences in the extent of biohydrogenation (Nudda *et al*., 2014; Chikwanah *et al*., 2018). This in turn, favours higher quantities of n-3 PUFAs, n-6 PUFAs, conjugated linoleic acid (CLA), rumenic acid (cis-9, trans-11 CLA), vaccenic acid (C18:1n9t) and branch chain fatty acids (BCFA) in sheep meat (Chikwanah *et al*., 2018).

Table 2.8.1: Fatty acid profile of IM fat in comparison to other animals

g/100g meat	Lamb1[1]	Mutton1[2]	Beef A2[3]	Chicken (white) V.H[4]	Chicken (dark) V.H[4]	Pork4 (av shoulder, loin, leg)[5]
FAT	**9.01**	**8.98**	**14.5**	**2.91**	**8.91**	**5.20**
SFA	**4.27**	**4.50**	**4.39**	**3.024**	**2.85**	**2.08**
C14:0	0.57	0.25	0.28	0.08	0.08	0.08
C16:0	2.22	2.29	2.15	2.16	2.06	1.24
C18:0	1.46	1.94	1.76	0.72	0.64	0.68
C20:0	0.02	0.02	0.05	0	0	0
MUFA	**3.62**	**3.77**	**3.02**	**4.163**	**4.28**	**2.15**
C16:1	0.19	0.15	0.17	0.452	0.49	0.12
C18:1n9t	0.31	0.2	0.03			0.06
C18:1n9c	3.06	3.42	2.58	3.695	3.76	1.90
PUFA	**0.27**	**0.22**	**0.19**	**2.188**	**2.470**	**1.00**
C18:2	0.27	0.22	0.17	2.058	2.31	0.88
CLA	5.6[6]	NA	0.02	NA	NA	5.23
N-9	3.43	3.62	2.81	NA	NA	1.96
N-3	NA	NA	0.01	0.108	0.138	0.05
N-6	0.27	0.22	0.19	0.001	0.001	0.91

Source: [1]Van Heerden *et al*., 2007; [2]Schönfeldt *et al*., 2011; [3] Schönfeldt & Hall., 2015; [4]Van Heerden *et al*., 2002; [5]Van Heerden & Smith., 2013; Chin et el., 1992 (mg/g fat).

2.9 **Fatty acid composition of ruminants**

Ruminant fatty acids not only originate from the diet, but also from fatty acid products derived from rumen microbial biohydrogenation of dietary lipids (Bucciani *et al*., 2012). They can also be synthesized *de novo* from acetate, glucose, lactate and propionate (Garton *et al*., 1972; Smith & Cruise, 1984).

2.9.1 *Saturated fatty acids*

Saturated fatty acids are fats with single carbon-to-carbon bonds. In nature they are usually unbranched with an even number of carbon atoms. They are divided into short (C8:0 to C13:0), medium (C8:0 to C16:0), long (C14:0 to C20:0) and very long chain fatty acids (Ratnayake & Galli, 2009). They can be even chained, odd chained, branched or unbranched.

a) Branch chain fatty acids

Dairy products and ruminant meat are good sources of branch chain fatty acids (BCFAs) (Ran-Ressler *et al*., 2014). Branch chain fatty acids are predominantly saturated with one or more methyl groups attached to the carbon chain. The number of methyl groups determines whether they are mono-, di-

, or multi methyl. If the terminal end is an isopropyl (methyl group one from the end) it is referred to as 'iso', and if it terminates with an isobutyl (methyl two from the end) it is referred to as 'anti-iso' (Ran-Ressler *et al*., 2014; Taormina *et al*., 2020).

There has not been substantial research on the risk and health benefits of BCFAs, but *in vitro* research has shown them to have potential benefits in protecting against inflammation, cancer and metabolic disorders due to their structural properties (Taormina *et al*., 2020). BCFAs are particularly important for sheep meat consumer satisfaction. Three BCFAs have been isolated as major contributors towards the odour of cooked sheep meat; 4- methyloctanoic acid (MOA), 4- elthyloctanoic acid (EOA) and 4-methylnonanoic acid (MNA) (Watkins *et al*., 2014). A younger animal is favoured because of its lower MOA and EOA and higher MNA concentrations (Watkins *et al*., 2010). The ruminanant's diet prior to slaughter also impacts BCFAs found in meat (Watkin's *et al*., 2010), and pasture-based diets have higher MOA and lower MNA than maize and lucerne based diets (Young *et al*., 2003).

The availability of propionate is correlated with the amount of odd and BCFAs found in adipose tissue (Garton *et al.*, 1972). Therefore, grain diets typically have a prominent lamb "flavour" because of the high concentration of propionate available from the fermentation of carbohydrates (Priolo *et al*., 2002). Typically, propionate is converted to succinyl CoA via methylmalonyl CoA in the liver, and used for gluconeogenesis (Scaife *et al*., 1978). When propionate is produced faster than the liver can metabolise, there is a surplus of propionyl CoA and methylmalonyl CoA (Garton *et al*., 1972; Scaife *et al*., 1978). The additional propionyl CoA is used for odd and straight chain fatty acid synthesis in the liver, and the methylmalonyl CoA (some of which is converted from propionyl CoA) is used to synthesise BCFAs (Scaife *et al*., 1978). The methylmalonyl CoA competes with malonyl CoA for fatty acid synthesis, resulting in an increase in unusual branch chained fatty acids (Scaife *et al*., 1978). Small amounts of iso and ante-iso BCFAs from the rumen are also transferred to meat. These fatty acids are as a result of oxidative deamination of branch chain amino acids (valine, leucine and isoleucine) by rumen bacteria and protazoa (Vasta & Priolo, 2006) to form iso-14:0 and iso-16:0 fatty acids from valine, iso-15:0 and iso-17:0 from leucine, and anteiso-15:0 and anteiso 17:0 from isoleucine (Taormina *et al*., 2020). Branch chain amino transferase removes the amine group, resulting in a branch chain α-ketoacid, which is decarboxylated, and then elongated with malonyl CoA (Taormina *et al*., 2020). Phytanic and pristanic acid are polymethyl BCFAs synthesized in the rumen (Taormina *et al*., 2020).

b) Even chain fatty acids and odd chain fatty acids

Even chained fatty acids are the most common saturated fatty acid (SFA) in sheep meat. An indigenous South African sheep breed such as the dorper is reported to be 52.8% saturated, of which 46.0% of

that is palmitic acid (C16:0) followed by 27.3% stearic acid (C18:0) (Tshabalala *et al*., 2003). SFAs are important for the provision of energy, and the structural component of cell membranes (Hunter *et al*., 2010). They also improve the consumer's eating experience by improving the texture and palatability of meat (Hunter *et al*., 2010).

Palmitic acid (C16:0) is an important fatty acid supplied in the diet or synthesised *de novo*, provided it is not consumed in excess (Agostoni *et al*., 2016) or exacerbated by other factors such as a diet too high in energy or with a poor palmitic acid/ PUFA ratio (Carta *et al*., 2017). It is an essential component of cell membranes, secretory and transport lipids, protein palmitoylation and it's signaling molecules (Agostoni *et al*., 2016). In a diet too high in carbohydrate, the energy balance will shift, and the palmitic acid can become detrimental. It can cause diseases such as dyslipidemia, hypoglycemia, inflammation and weight gain (Carta *et al*., 2017). A poor homeostatic balance in conjunction with high palmitic acid can increase the risk of atherosclerosis, neurodegenerative diseases and cancer (Carta *et al*., 2017).

Stearic acid (C18:0) has a lower CVD risk because of its lower LDL cholesterol levels in comparison to other saturated and trans fatty acids (Hunter *et al*., 2010). Animal products (meat, poultry, fish, eggs and dairy products) make up 60% of dietary stearic acid (Hunter *et al*., 2010). Increased visceral adipose tissue is associated with cancer survival and risk. Dietary stearic acid is able to reduce visceral adipose tissue by apoptosis of adipocytes in nude mice (Shen *et al*., 2014). *In vitro* studies have also found stearic and oleic acid to have cytotoxic effects on cancer colonies (Fermor *et al*., 1992). It protects against breast cancer by inhibiting the proliferation and invasion of cancer cells and increasing diacylglycerol (DAG), which activates protein kinase C (PKC) and induces apoptosis (Evans *et al*., 2009). Significantly higher prostatic tissue stearic acid levels in hyperplasia patients but not cancer patients support the theory that it plays a role in mitogenesis and malignancy (Mamalakis *et al*., 2002). The lower stearic acid to oleic acid ratio observed in cancer patients may be due to an increased delta 9 desaturase activity (Mamalakis *et al*., 2002). The palmitoleic acid in cancer patients was also three times higher (Mamalakis *et al*., 2002). Stearic acid at an amount of 19g/day (6.6% of energy) reduces thrombogenic and atherogenic risk in comparison to palmitic acid and trans fatty acids (Kelly *et al*., 2001). However, in a randomized control study undertaken by Baer *et al*. (2004), where the diet contained 8% of energy from stearic acid, they identified higher fibrinogen levels and increased inflammatory response, perhaps due to higher intake levels amongst other dietary factors. Dietary stearic acid's involvement with mitochondrial regulation through mitofusion activity might also explain why it reduces the risk of CVD in comparison to palmitic acid (Senyilmaz-Tiebe *et al*., 2018).

2.9.2 *Monounsaturated fatty acids*

The major MUFA in sheep meat is oleic acid (Table 2.8.1). Webb *et al*. (2022) found oleic acid to make up between 40% and 44% of the subcutaneous fatty acid profile of SA Mutton Merino sheep, and Cloete *et al*. (2004) reported oleic acid to make up 36.5% of total fatty acids in the *longissimus thoracis* muscle of SA Mutton Merino sheep. Oleic acid in ruminants is hydrogenated by microbes to stearic acid in the rumen (Smith *et al*., 2006). Therefore, its concentration is dictated by delta 9 desaturase activity in adipose tissue (Smith *et al*., 2006). Smith *et al*. (2020) reported no increased risk of cardiovascular disease in beef containing high oleic acid concentrations as a result of a corn-based finishing diet. It has also been associated with reduced blood lipids, cholesterol, LDL cholesterol and TAG (Lopez-Huertas, 2010). 3.9% of MUFA in the *longissimus dorsi* of lambs is made up of vaccenic acid (C18:1n11t) and 1.0% is made up of C16:1 (Webb, 2021). Although vaccenic acid is a trans fatty acid it is a precursor of rumenic acid and has health promoting properties (Stender *et al*., 2008; Field *et al*., 2009).

2.9.3 *Polyunsaturated fatty acids*

The PUFAs contain two or more double bonds along a hydrocarbon chain (Minihane & lovegrove, 2006). They are defined as either n-3 or n-6 fatty acids depending on the position of the double bond from the methyl end (Minihane & lovegrove, 2006). The fatty acid structure is predominantly in *cis* configuration with meat and milk containing only 2 to 5% in *trans* configuration (Minihane & lovegrove, 2006). For optimum health the n-3 PUFA concentration should be between 1.8 and 1.9g/day and the n-6/n-3 ratio should be below 4:1 (Ma *et al*., 2016). This ratio is usually good in ruminants (Wood *et al*., 1999). It is particularly good in lamb when on a grass as opposed to concentrate feeding system due to the high concentration of C18:3n3 in grass (Wood *et al*., 1999; Boughalmi & Araba, 2016). Common PUFAs include, linoleic acid, α-linolenic acid, β- linolenic acid, CLA, arachidonic acid (AA), eicosapentanoic acid (EPA), docosapentanoic acid (DPA) and docosahexanoic acid (DHA) (Minihane & lovegrove, 2006). Humans have a limited capacity to synthesize longer chain PUFAs, such as EPA and DHA, and thus require it in their diet (Williams & Burdge, 2006). Lean red meat, particularly lamb, is a good source of DPA. Although fish is a lot higher, it is still a better source of EPA and DHA than pork and poultry (Howe *et al*., 2007).

Linoleic acid and α-linolenic acid are essential fatty acids required in a mammal's diet for the synthesis of longer chain n-3 and n-6 fatty acids, as they do not contain the desaturase enzymes necessary to insert double bonds beyond carbon 9 (Minihane & lovegrove, 2006). Concentrate based diets are high in linoleic acid and grass-based diets are higher in linolenic acid (Aldai *et al*., 2011). This in turn is

replicated in the fatty acid profile of meat (Aldai *et al*., 2011). Due to grass, plants and green leafy vegetables being higher in n-3 fatty acids (Williams and Burdge, 2006), cattle and sheep grazed on grass have a higher concentration of C18:3n3 (linolenic acid) and C20:5n3 (EPA) fatty acids than those fed concentrates (Wood *et al*., 2003). The best source of α-linolenic acid is oily fish, but it is also found in common oils such as soybean oil, rapeseed oil, linseed and linseed oil (Wood *et al*., 2003; Williams & Burdge, 2006). A higher n-6/n-3 ratio typical of Western diets can lead to cardiovascular disease, cancer and autoimmune diseases (Simopoulas, 2002). Therefore, supplementing a grain-based diet with linseed or linseed oil (better still if it is rumen protected) will increase the amount of n-3 fatty acids deposited in ruminant fat (Wood *et al*., 2003) and improve the healthfulness to the consumer.

There is competition between the synthesis of fatty acids from these precursor fatty acids as they share the same elongation and desaturation enzymes (Williams & Burdge, 2006). Although α-linolenic acid is the preferred substrate for the synthesis of longer chain fatty acids like EPA and DHA, a higher concentration of linoleic acid in the diet leads to the synthesis of more n-6 PUFAs like arachidonic acid (Williams & Burdge, 2006).

Australia and New Zealand are the only countries that provide recommendations for DPA (Byelashov *et al*., 2015). Other countries, and organisations like the FDA, do not acknowledge it in their dietary guidelines (Howe *et al*., 2007). The "Food standards code" qualifies a good source of omega-3 to have ≥ 60mg EPA + DHA/ serving (Howe *et al*., 2007). Therefore, red meat omega-3 fatty acids are not acknowledged as a substantial source. Although EPA and DHA have been well documented as biologically important fatty acids, DPA is only starting to gain recognition as a beneficial omega-3 fatty acid with important health benefits. Researchers are finding an association of DPA with reduced platelet aggregation, improved lipid metabolism, endothelial cell migration, and anti-inflammatory responses, thereby lowering the risk of atherosclerosis and improving cardiovascular health (Byelashov *et al*., 2015). It also improves neural health, and mental and cognitive function (Byelashov *et al*., 2015). It can be used for the synthesis of EPA and DHA, as it can elongate to DHA and convert back to EPA (Byelashov *et al*., 2015). Presence in human milk has biological importance for early infant development (Liu *et al*., 2016).

However, the PUFA/ SFA (P/S) ratio in ruminant meat is typically low in comparison to monogastric animals due to biohydrogenation (Enser *et al*., 1998). Where pork could have a ratio of 0.58, sheep have been reported to have a ratio of 0.15 (Enser *et al*., 1998). This is below the recommended dietary ratio of above 0.45 (Enser *et al*., 1998). However, unsaturated fatty acid also increases the risk of off odour and flavour in meat and causes colour deteriation (Wood *et al*., 2004).

2.9.4 *Trans fatty acids*

Following restrictions, the Transfair study found that ruminant derived fats (milk and meat) contributed between 28 and 79% total TFAs in the diet (Hulshof *et al*., 1999). Since then, more countries have successfully reduced industrial trans fatty acids (iTFAs; Downs *et al*., 2013). Therefore, a larger percent is coming from natural sources in these countries. The majority of natural TFAs come from ruminant meat and milk (Sommerfeld, 1983). Generally, 1-5% (<0.5% total energy intake) of total fatty acids in ruminant fat is TFA (Micha & Mozaffarian, 2009), and lamb and mutton have higher levels than both beef and veal (Williams, 2007). Industrial trans fatty acids are much more varied and can contribute anything between 2 and 10% energy intake (Micha & Mozaffarian, 2009).

Ruminants have higher quantities of trans fatty acids than monogastric animals due to partial hydrogenation of linoleic and linolenic acid by microbes in the rumen (Sommerfeld, 1983). In comparison, small amounts of trans fatty acids deposited in non-ruminant fat is more typical of the diet (Sommerfeld, 1983). The major trans fatty acid contributor in commercial and ruminant products is C18:1, and its isomers differ according to the source. Although there are several isomers ranging from trans-6 to trans-16, the most prominent in ruminant meat and milk products are trans-11 vaccenic acid (C18:1n11t) followed by elaidic acid (C18:1n9t) and C18:1n10t (Parodi, 2004; Sommerfeld, 1983 & Aro *et al*., 1998b). Vaccenic acid is also used for the synthesis of rumenic acid in tissue (Stender *et al*., 2008).

There is some controversy surrounding the effect of rTFAs and heart disease. Some authors go as far as to say there is no substantial evidence to substantiate the claim that there is an association between ruminant trans fatty acids (rTFA) and CVD (Jakobsen *et al*., 2008; Brendsen *et al*., 2011). Ruminant fat contains approximately 8% TFA in comparison to 50% in partially hydrogenated vegetable oils (Kuhnt *et al*., 2016). This would indicate that a lot more would need to be consumed in order to reach an inacceptable level (Brendson *et al*., 2011; Kuhnt *et al*., 2016). Motard- Bélanger *et al*. (2008) studied 38 men in Quebec City between the ages of 18 and 65 years old. Where men consumed rTFA or industrial trans fatty acids (iTFA) at 10.2g/2500 kcal diet (3.6% energy intake), they significantly increased their cardiovascular risk. However, rTFAs consumed at 4.2g/2500 kcal (1.5% energy intake), well above the normal upper limit, had neutral effects on plasma lipids and other risk factors. A meta-analysis performed on 13 trials by Gayet-Boyer *et al.* (2014) on healthy people, found no adverse effects with levels as high as 4.19% energy intake. In most European countries, intake levels are low to moderate, and thus no threat to human health (Kuhnt *et al*., 2016). Some studies have even shown benefits at low intake levels. One author found an inverse relationship between rTFA intake and change in weight with a levelling off at 0.4% energy intake (Hansen *et al*., 2012).

It is well understood that iTFAs and rTFAs not only differ in concentration but isomer distribution (Kuhnt *et al*., 2016). Different isomers of C18:1 may have varying effects on human health, some even positive. Vaccenic acid is one such fatty acid that is converted to rumenic acid, an important conjugated linoleic acid (CLA), by Δ^9-desaturase activity in tissue (Mosley *et al*., 2006; Turpeinen *et al*., 2002). There are 28 geometric and positional isomers of CLA, of which cis-9 trans-11, trans-10 cis 12, and trans-9 trans-11 have known biological activities (Collomb *et al*., 2004).

Ruminant derived products are a rich source of CLA in comparison to chicken, pork and fish (Chin *et al*., 1992). Lamb is an excellent source of rumenic acid and other CLAs (Chin *et al*., 1992). Vaccenic acid, the major trans fatty acid in ruminant fat, accumulates in the rumen as a biohydrogenation intermediate (Griinari *et al*., 2000; Field *et al*., 2009). Studies suggest it to have important health benefits (Field *et al*., 2009). It is also converted by Δ^9-desaturase in adipose tissue and mammary glands to CLA (Griinari *et al*., 2000). Important health benefits of CLA at low concentrations include weight loss, insulin sensitivity, reduced diabetes, atherosclerosis and inflammation, improved immunity and blood lipid profile, is anti-carcinogenic and is cardioprotective (Noone *et al*., 2002; Song *et al*., 2005; Toomey *et al*., 2006; Lee *et al*., 2007; Zhou *et al*., 2008; Dilzer & Park, 2012; Den Hartigh *et al*., 2017; Mohammadi *et al*., 2020). However, epidemiological studies have been inconsistent, possibly because of isomer variations (Tricon & Yaqoob., 2006). The fatty acid profile, including CLAs of meat and milk, can be altered through feeding practices. Ruminants grazed on green forages, grasses or herbage will seasonally have a typically higher CLA content than animals fed concentrate (Alfaia *et al*., 2009) due to its higher *α*-linoleic acid content (Antongiovanni *et al*., 2003). Milk and meat are also typically lower in C12, C14 and C16 saturated fatty acids and higher short chain fatty acids, unsaturated fatty acids, CLA and n-3 fatty acids (Antongiovanni *et al*., 2003).

2.10 **Fatty acid metabolism in ruminants**

2.10.1 *Lipolysis*

Fatty acids absorbed by ruminants come from lipolysis and biohydrogenation of fatty acid esters by *de novo* synthesis of fatty acids by microorganisms in the rumen (Bauchart *et al*., 1993). Lipolysis is the first step by which fatty acid ester linkages of triacylglycerol (TAG), phospholipids and glycolipids are hydrolysed by microbial lipase into free fatty acids (FFA) and glycerol, thereby making the carboxyl end available for subsequent biohydrogenation (Shingfield *et al*., 2010; Buccioni *et al*., 2012). Microorganisms and their lipases are specific to the fatty acid. Therefore, *Anaerobic lipolytica* hydrolysis diacylgycerol and triacylglycerol bonds typical of concentrate feeds, whereas *Butyrivibrio spp*. hydrolyse the ester bonds on galacto- and phospholipids commonly found in the grazing

ruminant's diet (Lourenc¸o *et al*., 2010). Protozoa are also responsible for lipolysis but to a much smaller extent (Buccioni *et al*., 2012). Non-esterified fatty acids (NEFAs) are adsorbed onto feed particles and biohydrogenated or become part of bacterial lipids (Shingfield *et al*., 2010).

2.10.2 *Biohydrogenation*

Changes in the fatty acid composition of the diet has been a topic of interest for many years. Wu *et al*. (1991) found that some fatty acids increased between the rumen and the duodenum, whereas others did not. This was indicated by a reduction in C16:0, C14:0, C16:1, C18:2 and C18:3 fatty acids. Other fatty acids like C18:0 (stearic acid) increased significantly. Although to a lesser extent, C18:1, branched and odd chain fatty acids (C15, C16 and C17) also increased. This was hypothesised to be as a result of biohydrogenation and selective synthesis of fatty acids by microorganisms in the rumen (Wu *et al*., 1991).

Ruminant meat and milk have higher amounts of saturated fatty acids than monogastric species (Shingfield *et al*., 2010). Although the n-3/n-6 ratio differs significantly in ruminant meat between the different feeding systems (Nuernberg *et al*., 2005), most linoleic acid and α-linolenic acid is not directly transferred to ruminant tissue due to biohydrogenation by microbes in the rumen (Maia *et al*., 2007). Biohydrogenation, the mechanism by which microorganisms convert unsaturated fatty acids to saturated fatty acids, is a microbial protective mechanism to reduce the toxicity of polyunsaturated fatty acids in the rumen (Maia *et al*., 2007).

The sensitivity of microorganisms to the toxicity of linoleic acid and PUFAs are similar, but α-linolenic acid appears to be more toxic (Maia *et al*., 2007). Maia *et al*. (2007) found that cellulolytic microflora such as *Ruminococcus* and *F. succinogenes spp*. are extremely vulnerable. Other microorganisms such as *Butyrivibrio spp.* (especially *B. Hungatei*), *C. Proteclasticum* and *E. Ruminantium* are also sensitive to PUFAs. These microorganisms desaturate these fats to more saturated, less toxic forms. Linoleic acid is isomerised and biohydrogenated to less saturated CLA by *Proteoclasticum* P-18. *Proteoclasticum* P-18 and B316 produce the saturated fatty acid, stearate (Maia *et al*., 2007).

The first step in the biohydrogenation of linoleic acid and α-linolenic acid is isomerisation (Shingfield *et al*., 2010). Under normal conditions biohydrogenation is predominantly generated by two microbial populations that are partial to a specific portion of the biohydrogenation pathway. This pathway isomerises the linoleic acid and α-linolenic acid cis-12 double bond to C18:2 and C18:3 conjugated fatty acid isomers, respectively (Shingfield *et al*., 2010). The most common isomer for linoleic acid is cis-9, trans-11 CLA and that of α-linolenic acid is cis-9, trans-11, cis-15 which is then reduced in the next step to trans-11, cis-15. These isomers are reduced by microbial reductase enzymes to more

saturated C18:1 fatty acids, the primary pathway producing vaccenic acid (C18:1n11t) (Shingfield *et al*., 2010). The final product of complete biohydrogenation is C18:0 (Shingfield *et al*., 2010). The last step is rate limiting, explaining accumulation of C18:1 isomers in the rumen. However, the metabolic pathway can vary, resulting in the accumulation of different trans C18:1, C18:2 and C18:3 isomers (Shingfield *et al*., 2010).

2.10.3 *Digestion*

The omasum removes water and absorbs volatile fatty acids (VFAs). The abomasum's epithelial cells secrete HCl, pepsin and mucus that decreases the pH resulting in the death of microbes and protozoa in the digesta. The small intestine secretes bile and pancreatic secretions which also digest protozoa and microbes. This digestion of the microbial population increases odd chain fatty acids and double bond positional isomers in ruminant tissue (Harfoot, 1981).

The phospholipid fatty acid profile of the microbial membranes come from *de novo* synthesis of C16:0 and C18:0 fatty acids as well as the uptake of PUFAs. Thus, the composition of the diet can influence the composition of the microorganisms (Bauman *et al*., 2003).

2.10.4 *Absorption*

Volatile fatty acids (VFAs) are transported across the caecal wall and into the blood stream by simple diffusion (Harfoot, 1981). Once the medium and long chain fatty acids flow into the omasum and abomasum there is little change in composition and absorption (Bauman *et al*., 2003) as only a negligible amount is absorbed before the small intestine (Noble., 1981). The amount of C16:0 and C18:0 fatty acids entering the small intestines is greater than that in the diet due to microbial activity and biohydrogenation in the rumen (Bauman *et al*., 2003). Approximately 80% to 90% of fatty acids are in the form of free fatty acids (FFA)/ None esterified fatty acids (NEFA) attached to feed particles and the rest are present in microbial phospholipids, triglycerides and glycolipids (Bauman *et al*., 2003). None esterified fatty acids are absorbed onto the surface of feed particles when the acidic conditions of the abomasum and the duodenum (pH 2 to 2.5) protonate them (Bauchart, 1993). Esterified fatty acids must first be hydrolysed by intestinal and pancreatic lipases to NEFAs (Bauman *et al*., 2003). Triglycerides from protected fats can also attach to solid particles (Bauchart, 1993).

Long chain fatty acids are non-polar, and for them to become soluble for absorption, they need to attach to micelles (Bachart, 1992). Ruminant micelles are very small molecules consisting of LCFAs, bile salts, phospholipids and water (Bauchart, 1993). The micelle is round, polar on the outside and non-polar with cholesterol and fat-soluble vitamins on the inside (Ratnayake *et al*., 2009).

In a review by Bauman *et al*. (2003) it was well established that micelle formation is not possible without bile salts and lecithin released from bile, and pancreatic enzymes and bicarbonate from the pancreas. Bicarbonate raises the pH (Bauman *et al*, 1992) increasing ionisation and thus solubility of fatty acids in micelles (Leat & Harrison, 1969). Pancreatic enzymes, namely phospholipase A2, reacts with lecithin to form lysolecithin (Bauchart, 1993). Leat & Harrison (1969) emphasised the importance of pancreatic juice for its interaction with lecithin to form lysolecithin needed for optimal micelle formation and absorption of fatty acids. Lysolecithin and bile salts are important for the removal of fatty acids from feed particles and bacteria for the synthesis of micelles (Bauman *et al*., 2003).

Bile salts, lysolecithin and the acidic conditions of the duodenum and jejunum make the ruminant system ideal for the absorption of saturated fatty acids (Bouchart, 1993). The high efficiency of SFA absorption is amplified by the continual flow of small amounts of fatty acids into the duodenum, most of which are in a FFA form (Lock *et al*., 2006). Micelle formation starts in the duodenum (only 5%), increasing as it moves through the jejunum (20 and 25%), with the reminder forming in the ilium (Bauchart, 1993). The soluble fats in micelles move through the water layer between microvilli and are absorbed by the intestinal mucosal cells (Bauchart, 1993).

2.10.5 *Transport*

After diffusing into the epithelial cells (Ratnayake *et al*., 2009) lipid is transported in lymph from the mucosal intestinal cells, through the thoracic duct and into the blood plasma (Noble, 1981). Under normal conditions in sheep, lipids are (mostly) absorbed as FFAs (NEFAs) in the duodenum and upper jejunum, enter the mucosal cells, and are re-esterified to triacylglycerol (TAG) through the α-glycerophosphate pathway in the rough endoplasmic reticulum (RER) and Golgi (Noble, 1981). Phospholipids are also synthesised, with the majority most likely coming from bile lecithin (Harrison & Leat, 1975). Leat & Harrison (1973) reported that thoracic duct lymph in sheep consisted of 77% TAG and 18% Phospholipid (PL), both of which have an affinity for carrying different types of fatty acids.

In sheep, lipids of the thoracic lymph are predominantly part of very low density lipoproteins (VLDL) (73%) and less so in chylomicrons (27%) (Noble, 1981), evident by the high content of phospholipid in the thoracic duct lymph (Leat *et al*., 1974). Unsaturated LCFAs and saturated LCFAs stimulate the synthesis of Chylomicron and VLDL synthesis from the intestine respectively (Hocquette & Bauchart, 1999). Chylomicrons are secreted from the small intestine and transport TAG, whereas VLDLs also transport phospholipids (Bauchart 1993). Bauchart (1983) separated lipoproteins into 5 classes, namely Chylomicron, VLDL, intermediate density lipoproteins (IMD), LDL, HDL and very high density

lipoprotein (VHDL). The major lipid component of lipoproteins are cholesterol esters and phospholipids with smaller amounts of cholesterol, TAG and free fatty acids (NEFAs). However, TAG is high in VLDLs and NEFA are high in VHDLs.

Medium chain fatty acids (<12 carbons in length) are absorbed and secreted into the blood as NEFAs, whereas the longer chain fatty acids are absorbed by the epithelial cells and re-esterified and transported by chylomicrons and VLDLs or travel through the portal vein in the event of high lipid absorption (Hocquette & Bauchart, 1999). Very low density lipoproteins, synthesised in the liver and to a lesser extent in the small intestine, transport fat to extrahepatic tissue where it is hydrolysed from TAG by the enzyme lipoprotein lipase (Beitz, 2004). The NEFA is rapidly absorbed by the tissue and either esterified or oxidised (Beit, 2004). Once the hydrolysis is complete, the remainder of the VLDL becomes an IDL and is converted to LDL in blood plasma which is directed back to the liver (Beitz, 2004).

High density lipoprotein (HDL), synthesised by the liver and intestines, carries cholesterol from the extrahepatic tissue to the liver (Beitz, 2004), where it is used for bile excretion and resynthesis of VLDLs (Bauchart, 1993). It is also important for the delivery of cholesterol for steroidogenesis and membrane synthesis (Bauchart, 1993).

The NEFAs have three fates, they are either stored as TAG in the liver or adipose tissue, oxidised in tissues such as the liver, heart and muscles, or recycled back to the liver (Hocquette & Bauchart, 1999). Therefore, the LCFAs are either esterified to TAG and stored in the cytosol or they enter the microsomes where they become part of VLDLs which enters the circulation. However, fats can also undergo oxidation as a source of energy in peroxisomes and mitochondria (Hocquette & Bauchart, 1999).

The major lipid in adipose tissue is TAG, and in muscle a significant portion is in phospholipid. PUFAs are predominantly in the PL fraction. The phospholipid fraction decreases as a proportion of the total lipid fraction when the animal gets fatter, with an increased concentration of MUFA and SFA. Triacylglycerol predominates in the fatter carcass due to an increase in oleic acid and a decrease in the proportion of linoleic acid (Wood *et al*., 2008). The type of muscle fibre also determines the amount of PUFA present. Fast twitch muscle fibres are higher in phospholipids and therefore higher in PUFA concentration (Wood *et al*., 2003).

2.10.6 *Oxidation of fatty acids*

Long chain fatty acids depend on the carnitine transport system, whereas SCFAs and MCFAs are carnitine independent (Schönfeld & Wojtczak, 2016). The latter can permeate through the inner

mitochondrial membrane and are activated to their coenzyme A (CoA) derivative in the mitochondrial matrix, to be used as substrates in mitochondrial β-oxidation and citric acid cycle (Schönfeld & Wojtczak, 2016).

2.10.7 *De novo* fatty acid synthesis

De novo lipogenesis is the endogenous synthesis of fatty acids (Steenson *et al*., 2017) predominantly in the adipose tissue of ruminants but also in the liver and mammary gland (Laliotis *et al*., 2010). These products are stored as triacylglycerols in lipid droplets and oxidised for energy at a later stage, incorporated into structural lipids or undergo further modifications (Wallace & Metallo, 2020). Biosynthesis of long chain fatty acids is under nutritional, hormonal and metabolic control (Beitz, 2004).

Unlike monogastrics, due to low ruminant activity of ATP citrate lyase and malate dehydrogenase, they can't use glucose as a major source of carbon in *de novo* fatty acid synthesis (Laliotis *et al*., 2010). Therefore, ruminants have adapted by using acetate and to a lesser extent buterate and propionate made widely available by microbes (Beitz, 2004; Laliotis *et al*., 2010) from their breakdown of carbohydrate in the rumen (McDonald, 2011).

Palmitate, synthesised from Acetyl-CoA and malonyl-CoA, is catalysed by fatty acid synthase in the cytosol (Laliotis *et al*., 2010). The overall reaction is as follows: Acetyl-CoA + 7 malonyl-CoA +14 NADPH + 14 H^+ -> Palmitate + 14 $NADP^+$ + 8 CoA + 7 $CO_2{}^+$ + 6H_2O (Laliotis *et al*., 2010). In ruminants, the major source of acetyl CoA comes from acetate absorbed through the gut wall and activated by acetyl CoA synthetase (McDonald, 2011). Acetyl CoA initiates a new acyl chain and is elongated by Malonyl CoA (Wallace & Metallo, 2020). It is also used by the rate limiting enzyme, acetyl CoA carboxylase, for carboxylation of acetyl CoA to malonyl CoA (Cambell & Farrell, 2010; Laliotis *et al*., 2010). In sheep, most of the NADPH comes from the pentose phosphate pathway and the remainder from cytosolic NADP isocitrate dehydrogenase (Laliotis *et al*., 2010). The C15:0 and C17:0 odd chain fatty acids are synthesised when acetyl CoA is replaced by propionyl CoA, which is then elongated as before using malonyl CoA (Wallace & Metallo, 2020).

Palmitate can undergo further elongation and / or desaturation in the endoplasmic reticulum (Laliotis *et al*., 2010). Stearoyl- CoA desaturase (delta 9 desaturase) is a rate limiting enzyme that inserts a double bond at the 9^{th} carbon in C16 and C18 saturated fatty acids to form palmitoleic (C16:1n7) and oleic (C18:1n9) MUFAs respectively (Park, 2018). Fatty acid elongase can also produce eicosenoic (C20:1n9), erucic (C22:1n9) and nervonic acid (C22:1n9) from oleic acid (Ratnayake & Galli, 2009), and

vaccenic acid from palmitoleic acid in beef (Burns *et al*., 2012). Stearic acid can also be synthesised from palmitic acid by fatty acid elongase (Steenson, 2017).

Stearoyl CoA desaturase is also used in adipose tissue for the synthesis of cis-9 trans -11 CLA from vaccenic acid, a major trans fatty acid biohydrogenation intermediate (Wood *et al*., 2008). Oleic acid (C18:1n-9), vaccenic acid and CLA are stored in greater amounts in the neutral lipid than phospholipid fraction (Wood *et al*., 2008). They are therefore more prevalent in adipose tissue than muscle (Wood *et al*., 2008).

Palmitoleic acid (C16:1) and oleic acid (C18:1) MUFAs and polyunsaturated fatty acids (linoleic and linolenic) undergo further desaturation by delta 5 and delta 6 desaturase enzymes and elongation by elongase enzymes. Oleic acid initiates the n-9 series, palmitoleic acid the n-7 series, linoleic acid the n-6 series and α linolenic acid the n-3 series (Ratnayake & Galli, 2009). Muscles contain a significant amount of C20 to C22 PUFAs, like arachidonic acid in the n-6 series and eicosapentanoic acid in the n-3 series (Wood *et al*., 2008). It is important for mammals to obtain linoleic and linolenic acid from the diet for the synthesis of metabolically important fatty acids in the n-6 and n-3 pathway, as they lack the delta 12 and delta 15 enzymes to synthesise them (Park, 2018). There is competition between the n-6 and the n-3 pathways because the enzymes are shared. However, C18:3n-3 appears to be the preferred substrate, although due to higher amounts of C18:2n6 there are more n-6 series products (Wood *et al*., 2007).

2.11 **Manipulating fatty acid composition in meat**

2.11.1 *Sex*

A trend in research indicates that the sex of a sheep has an influence on its fatty acid profile, and therefore its nutritional value. In various studies the intact and vasectomised ram were shown to be the nutritionally superior sex because of its significantly higher PUFA profile in comparison to the castrate or the ewe (Okeudo & Moss, 2007; Villalobos-Villalobos *et al*., 2014).

c) Intramuscular fat

Okeudo *et al*. (2007) studied sex affects between castrates, entire rams, vasectomised rams and ewes between 32 and 52kg. The major saturated fatty acids found in intramuscular fat were palmitic acid (21 to 24%) and stearic acid (16 to 19%) (Okeudo *et al*., 2007). Monounsaturated fatty acids made up the largest percent of fat, with oleic acid (C18:1) contributing between 46 and 48%. Polyunsaturated fatty acids are present in much smaller amounts but are mostly made up of n3 and n6 fatty acids (C18:2 and C18:3). They only contribute 0.7 to just over 1% respectively (Okeudo *et al*., 2007). The

vasectomised and entire rams had a similar fatty acid profile besides total PUFA. Ewes contained significantly more C14:0 (on an equal age basis) and C16:0 fatty acids but less C18:0 and C18:3 than entire and vasectomised rams, whereas castrates were somewhere in between. Kemp *et al*. (1981) observed differences in C16:1, C18:2 and C18:3 fatty acids (P<0.01) where the wether had more of the monounsaturated fatty acid, but the ram had more of the polyunsaturated fatty acids. The ewes and the wethers were very similar except for more C18:0 than the former (P<0.05). Gravador *et al*. (2018) also found significant differences in total PUFA, C16:1 and C18:2 but not C18:3 in rams and castrates. However, MUFAs were also significant (P<0.01) in the castrate due to higher t10 and c9-C18:1 (P<0.001). Rams also had more C15:0 (P<0.05), P/S ratio (P<0.01) and n-6 PUFAs (P<0.05). In contrast, Cloete *et al*. (2004) investigated breed and sex effects of SA Mutton Merino and Dorper sheep and concluded that sex had very little effect on intramuscular fatty acid composition at 18 months of age when the average weight of the ewe was 44.6kg and that of the ram was 64.9kg. The only exception was higher C18:3 and P/S ratio in the ram.

d) Subcutaneous fat

Saturated fatty acids make up 53% of subcutaneous fat with over whelming majority coming from C16:0 and C18:0 fatty acids, which account for 22 and 23% respectively. Monounsaturated fatty acids constitute 40% of the fatty acid profile with the main contribution coming from C18:1 (37%). Polyunsaturated fatty acids make up a small portion (4.62%) and predominantly come from C18:2 (3.97%) and C18:3 (0.74%) fatty acids (Webb & Casey, 1995). Stearic acid is lower in rams in comparison to wethers (Crouse *et al*., 1972; Busboom *et al*., 1981; Kemp *et al*., 1981; Solomon *et al*., 1990; Sañudo *et al*., 1998; Villalobos *et al*., 2014), but not significantly lower in Kryptorchids (Solomon *et al*., 1990) or ewes (Sañudo *et al*., 1998; Diaz *et al*., 2003). Some studies have experienced lower palmitic acid in rams than wethers (Busboom *et al*., 1981; Safari *et al*., 1988) but higher quantities in rams than ewes (Diaz *et al*., 2003 & Sañudo *et al*., 1998). On the other hand, PUFAs are significantly higher in rams than wethers (Solomon *et al*.,1990; Villalobos., 2014). Although authers such as Busboom *et al*. (1981) and Crouse *et al*. (1972) found significant differences in individual unsaturated fatty acids like C18:1 (Busboom *et al*. (1981) and Crouse *et al*. (1972) as well as C18:2 and C18:3 fatty acids (Cruise *et al*., 1972), it was not enough to show a significant difference between the total PUFA between rams and wethers. Ewes have also indicated a tendency to have higher total unsaturated fatty acids (Kemp *et al*., 1981; Sañudo *et al*., 1998,) or higher total PUFAs (Diaz *et al*., 2003) than rams. As unsaturation increases, melting point declines affecting the firmness/ hardness of fat (Wood *et al*., 2003) resulting in a softer ram carcass (Kemp *et al*.,1981). Busboom *et al*. (1981) also found increasing correlations between C10:0, C14:0, C16:0 and C18:0 SFAs with fat firmness. A decrease in C18:0 and increase in odd and branch chain fatty acids (C10 to C17) resulted in softer oilier carcasses. Therefore,

heavy ram lambs had softer carcasses than heavy wether lambs with diet and sex having an additive effect.

e) Perirenal fat

Crouse *et al*. (1972) determined similar fatty acid trends to subcutaneous fat in perirenal fat with significantly higher UFA in rams due to higher quantities of C18:2 and C18:3 ($P<0.01$) fatty acids and lower quantities of C16:0. They also determined that smaller quantities of C18:2 were present in fatter carcasses. In contrast, Kemp *et al*. (1981) experienced no significant differences between ewes and wethers, and rams and wethers. However, there was a trend $P<0.06$) indicating higher UFAs in rams than wethers due to insignificantly higher C18:1 and C18:2 fatty acids.

2.11.2 *Age and weight*

The form and composition of an animal's body changes at it grows; and different parts of the body and their tissues grow at different rates. The maximum growth rate of bone is attained earlier than muscle, while fat is the latest to develop (Hammond, 1950). The rate in change of the growth curve to reach its mature weight and therefore the age at which fattening occurs, is affected by plane of nutrition during the different phases of growth (Hammond, 1950), breed (early vs late maturing) and sex (Lopez *et al*., 2018; Van der Merwe *et al*., 2019). A ewe is smaller than a ram, and therefore when comparing them at the same weight the ewe is expected to be fatter; reaching her mature state earlier (Pannier *et al*., 2014). Thus, ewes tend to accumulate fat at an earlier age, but due to their slower growth rate they tend to be slaughtered later than rams (Diaz *et al*., 2003). Allometric coefficients from Sabbioni *et al*. (2019) indicated that a change in body weight from 60 and 80kg reduced PUFAs in ewes from 5.01 to 4.87% in comparison to males that increased from 5.54 to 5.83%. Therefore, Sabbioni *et al*. (2019) suggested slaughtering females earlier than males to better their PUFA deposition relative to body weight.

An increase in carcass weight is associated with a higher percent of SFA and MUFA, but lower amount of PUFA (della Malva *et al*., 2016). As the animal becomes fatter, heavier or older the proportion of fatty acids change, but not necessarily in a linier or identical fashion (Okeudo & Moss, 2007). Okeudo and Moss (2007) noted a quadratic decrease in intramuscular C16:0, C14:0 and C18:3 fatty acids with increasing slaughter weight. However, C18:0 had a negative linear correlation with slaughter weight whereas C18:1 was positively correlated (Okeudo & Moss, 2007). Equations used to predict the change in IMF between 35 and 55kg animals indicated an increase in oleic acid by 10%, but a decrease in C18:3 (by 41%), followed by C14:0 (39%), C18:0 (17%) and C16:0 (5%) fatty acids while total IMF increased by as much as 61%. SA Mutton Merino subcutaneous fat of wethers slaughtered at 37 and 43kg did

not show significant molar differences in these fatty acids. However, the gravimetric fatty acid content (mg/g) of these fats (as well as C16:1, MUFA and UFA) increased significantly with increased fatness (Webb & Casey, 1995). If carcass fatness were included as a covariate, only C16:0, C18:1 and MUFA would remain significant.

Gravador *et al*. (2018) suggested the accumulation of C18:1 and MUFAs in IMF of animals of increasing maturity, is due to the increase in activity and accumulation of enzymes responsible for fatty acid biosynthesis. Stearoyl coenzyme A desaturase 1 (SCD1) mRNA levels are one of the lipogenic enzymes and/or regulators contributing toward *de novo* fatty acid biosynthesis (Bartoň *et al*., 2011). It is proved to differ between age, sex and fat depot of the animal (Bartoň *et al*., 2011). The delta 9 desaturase enzyme, encoded by the SCD gene, synthesises MUFA from SFA and converts trans-vaccenic acid (C18:1 trans-11) to CLA cis-9, trans-11 C18:2 (Smith *et al*., 2006; Gravador *et al*., 2018).

2.11.3 *Diet*

a) Roughage and pasture-based diets

Diaz *et al*. (2002) reported that not only was it more cost effective to have lambs on pasture, but when they were slaughtered at a lower 24kg weight, they also had an acceptable n-6/n-3 ratio. Linolenic acid (LNA) and linoleic acid (LA) are the predominant unsaturated fatty acids in forages, and palmitic acid the highest saturated fatty acid. In grasses 16% of the fatty acid profile is made up of C18:3, 13% C18:2 and 14% C16:0. However, C18:3 varies more in other forages. As plants age there is a decline of fatty acids over time, although the proportional contribution of each fatty acid remains relatively unchanged (Clapham *et al*., 2005). Grazing on pasture tends to increase SFA ($P<0.05$), decrease MUFAs ($P<0.05$) and has no effect on total PUFA ($P>0.05$) contents of IMF. However, it still has the potential to improve the dietetic quality of meat by reducing the total content of lipid, as well as increase healthy n-3 PUFAs ($P<0.01$), while simultaneously lowering the n-6/n-3 PUFA ratio (Popova *et al*., 2015). Forages alter the microbial population in the rumen which is why there is an increase vaccenic (t11-C18:1) acid outflow, and a higher content of CLA c9t11 in meat and milk (Lourenço *et al*., 2008).

The type of pasture can affect the fatty acid composition of meat (Casey *et al*., 1988), brain, heart and lung (Leiber *et al*., 2019). Lambs grazing leguminous rich pastures, for example lucerne or red clover, have higher total PUFA (due to more linoleic and linolenic acid) in their IMF and SCF than animals on pastures like ryegrass (Fraser *et al*., 2004; Lourenço *et al*., 2007). It has been widely observed that subcutaneous fat has greater sensitivity to dietary fatty acid changes than IMF (Lourenço *et al*., 2007). Casey *et al*. (1988) identified differences in the fatty acid composition of SA Mutton Merinos grazed on five different winter (maize stubble, triticale, midmar rye grass, nui rye grass and cocksfoot) and

three different summer pastures (smuts finger, couch and lucerne) in South Africa. The major fatty acids in subcutaneous fat were the same; C18:1, C18:0 and C16:0 in decreasing amounts respectively. C18:1 was higher due to the biohydrogenation of C18 PUFAs. As ether extract (EE) in pastures increased, so did the palmitoleic acid (C16:1) content in subcutaneous fat. It also reduced stearic acid (C18:0), Oleic acid (C18:1) and Linoleic acid (C18:2), although not significantly. The type of pasture affected C14:0, C17:1, C18:2 and stearic acid (C18:0). There was variability between pastures in the amount of C18:2 that was able to bypass the rumen microbes for absorption further down the digestive tract (Casey *et al*., 1988). Rumen biohydrogenation intermediates and odd branch chain fatty acids (OBCFA) are often used as indicators of change in the rumen microbial population brought about by dietary change (Lourenço *et al*., 2008). Evidence suggests that reduced lipolysis is possible as a result of plant factors. For example, polyphenoloxidase is an enzyme present in red clover that increases the outflow of C18:3 from the forestomach of ruminants by inhibiting lipolysis (Lourenço *et al*., 2008) through the formation of protein-bound phenols and encapsulation in protein phenol complexes (Van Ranst *et al*., 2011).

The way the forage is presented also impacts the fatty acid profile. Santos-Silva *et al*. (2004) found significant differences in the fatty acid profile when feeding hay, ground or pelleted lucerne. Green and dry forages also differ in their fatty acid profile.

b) Concentrate based diets

Lambs fed concentrate diets generally have higher average daily gains, heavier carcass weights and reach their target weight sooner than pasture fed lambs (De Brito *et al*., 2016). Degree of finishing, fat depth and rib eye area are other carcass characteristics increased through concentrate-based diets (Realini *et al*., 2004). South Africa is unique in that grain-fed red meat is lower in saturated fatty acids in comparison to grass-fed, whereas internationally it is often higher (Schönfeldt & Hall, 2015). Concentrate has the benefit of reducing SFAs, increasing the PUFA:SFA ratio and increasing MUFAs in lamb. However, this needs to be weighed against the lower C18:3 fatty acids and higher C18:2, n-6/n-3 ratio and TFAs in concentrate fed lambs (Diaz *et al*., 2002). Realini *et al*. (2004) research resulted in less C20:4, C20:5, C22:5, CLA, CLA cis9 trans 11, LNA, LA, total PUFAs, UFAs and n-3 fatty acids in concentrate fed Uruguayan beef in comparison to pasture. Cereal rich concentrate diets increase the outflow of t10-C18:1 at the expense of t11-C18:1 (Bessa *et al*., 2015). When the rumen outflow of vaccenic acid is replaced by t10-C18:1 a trans- 10 shift occurs in the rumen biohydrogenation pathway. Trans vaccenic acid (t11-C18:1) makes up 55 to 60% trans monoenes in roughage and pasture-based diets whereas the opposite is true for high starch low roughage diets (Bessa *et al*., 2015). Therefore, the trans-10 shift inadvertently reduces c9, t11 CLA in meat due to its lacking vaccenic acid precursor. This is amplified by supplementing concentrate with PUFAs (Bessa *et al*., 2015).

c) *Protected fats*

Altering ruminant meat and milk fatty acid profile through the supplementation of protected lipid has potential (Shingfield *et al*., 2013). Bhatt *et al*. (2020) successfully used calcium salts to improve the fatty acid profile of meat by decreasing SFAs, increasing MUFAs, increasing cis-9, trans-11 C18:2, decreasing the n-6:n-3 ratio and decreasing the atherogenic index (AI) in intramuscular fat; and increasing PUFA, cis-9, trans-11 C18:2, and thrombogenic index (TI) in adipose tissue (Bhatt *et al*., 2020). Calcium salts associate with free carboxyl groups required by microbial isomerases for biohydrogenation (Jenkins, 1993) and are later released for absorption by acidic dissociation in the abomasum (Jenkins & Bridges Jr, 2007). Goodridge *et al*. (2001) identified an 8-fold increase in LNA in milk when flaxeed protected by formaldehyde was supplemented to a dairy ration, and when Linola (also protected by formaldehyde) was supplemented it improved LA from 4.8% to 10.3% in milk. Formaldehyde also has the potential to provide CLA with 70% protection, thereby increasing the amount available for absorption in the intestine by 3.5 to 4%, ultimately resulting in a 10-fold increase in CLA concentration in goat milk (Gulati *et al*., 2000).

Rumen protected fats bypass the rumen by resisting microbial biohydrogenation, thereby increasing the post-ruminal flow of one or more UFAs (Jenkins & Bridges Jr, 2007) with the intention of improving production and quality of meat and milk, as well as reproductive performance (Gulati *et al*., 2005) without the danger of UFA toxicity to microbes resulting in unfavourable effects on fermentation and production (Behan *et al*., 2019). Therefore, rumen undegradable fats not only increase the energy density of the diet, but also the direct absorption of long chain PUFAs and important bioactive fatty acids such as LA, LNA, CLA, n-3/n-6 FAs and n-9 FAs (Gulati *et al*., 2005).

Depending on its intention, a good bypass fat should be over 75% protected (Gulati *et al*., 2005) for improved, consistent and predictable flow into the duodenum, efficiently release UFAs in the intestines for absorption, and have minimal effect on fermentation (Jenkins & Bridges Jr, 2007). There are two major types of lipid protection. Polyunsaturated fatty acids can become encapsulated inside a microbial resistant shell using formaldehyde, lipid encapsulation with high melting point SFA, non-enzyme browning, using tyrosine cross-linkage, or lipid composite gel to name a few (Jenkins & Bridges Jr, 2007; Gadeyne *et al*., 2017). Alternatively, the structure of fatty acids can be altered to block the free carboxyl end, thereby resisting microbial enzyme attack, for example by associating with calcium salts or fatty amides (Jenkins & Bridges Jr, 2007; Gadeyne *et al*., 2017). Prilled fat and extrusion of vegetable oilseed are also used to protect fats (Lee *et al*., 2007). Although whole seeds provide some protection against biohydrogenation, raw seeds provide the most protection followed by processed oils and vegetable oils respectively (Chikwanha *et al*., 2018). However, there is a large variation

between and within techniques; although formaldehyde and lipid composit gels seemed most effective in transferring fatty acids to milk, formaldehyde can be toxic. Calcium salts, fatty acyls and lipid encapsulation are more practical, but they can be pricy or have lower transfer rates (Gadeyne *et al*., 2017).

d) Essential oils

Essential oils (EO) from plants are a diverse group of secondary metabolites classified as terpenoids (mono- and sesquiterpenoids) and phenylpropanoids (Calsamiglia *et al*., 2007). Essential oils are known for their strong antiseptic and antimicrobial properties but also have a variety of health benefits including antioxidant and free radical scavenging activities (Calsamiglia *et al*., 2007). Essential oils have the potential as an alternative to antibiotics for growth promotion due to their improved average daily gain (ADG) without affecting dry matter intake (DMI) and food conversion ratio (FCR), although more research needs to be done (Andri *et al*., 2020).

Essential oils interact with the microbial cell membrane, proteins or enzymes causing conformational changes which result in fluidification of the cell membrane, loss of membrane stability and ultimately leakage of ions across the cell membrane (Calsamiglia *et al*., 2007). Bacterial growth rate is slowed, changing fermentation patterns (Calsamiglia *et al*., 2007). This gives them the potential to reduce methane emissions *in vitro* in a dose dependant manner, although *in vivo* results are more variable possibly due to microbial adaption and smaller practical dose (Benchaar & Greathead, 2011). Due to its antimicrobial properties, Parver *et al*. (2018) suggested that *Ferulago angulate* essential oil improves meat quality by inhibiting biohydrogenation, thereby increasing PUFAs and n-3 fatty acids, and decreasing the n-6:n-3 ratio and SFAs. Other researchers have also found positive effects on fatty acid quality (Smeti *et al*., 2018).

e) Saponins

Saponins are a secondary metabolite common in plants and who's name comes from the stable soap-like foam that it forms in aqueous solution (Francis *et al*, 2002). They are steroids or triterpenoids that consist of a sugar moiety linked to a hydrophobic aglycone (which can vary in structure) with one or more unsaturated bonds (Francis *et al.*, 2002). Oligosaccharides may arise at position C3, and in some cases C26 or C28. There has been great interest in them because of cases of increased membrane permeability, immunostimulatory and anticarcinogenic properties (Francis *et al.*, 2002). Although they also reduce protazoal numbers, protein digestion and vitamin and mineral absorption as well as increase hypoglycaemia (Francis *et al.*, 2002). In a study conducted by Brogna *et al*. (2011) the overall fatty acid profile and cholesterol in lamb meat was unaffected by saponin supplementation to the diet

at 30, 60 and 90ppm of *Quillaja*. However, although Brogna *et al*. (2011) found no differences in biohydrogenation intermediates and BCFAs, they did record less cis 9 C14:1 concentration in saponin supplemented lamb meat. As this is formed in muscle by delta 9 desaturation of C14:0 via stearoyl CoA desaturase enzyme (Palmquest *et al*., 2004) suggested it may affect the enzyme's activity. In addition, an increase in C20:4n6c, mostly synthesized by elongation and desaturation of LA, suggested an influence on LA metabolism (Brogna *et al*., 2011). Similarly, saponins did not modify the fermentation pattern, biohydrogenation pathway or extent of biohydrogenation in a study done by Lourenço *et al*. (2008). In comparison, Wang *et al.* (2017) found possibilities of microbial adaption of dairy cow's rumen microbes to tea saponins after four weeks of supplementation at their highest dosage of 40g/day. Prior to this C16:1 cis 9, C18:1 cis9 and UFAs were higher in milk. This might be explained by Makker & Becker (1997) who suggested that after initial adaption, microbes may produce enzymes capable of saponin degradation, after they experienced increasing reduction of Quillaja saponin between 9 and 24h incubation. In a review by Patra & Saxena (2009) inconsistencies were found in rumen fermentation due to chemical structure of saponins, dosage, diet, and microbial community and how they adapt. Patra & Saxena (2009) also found that some individuals in the microbial community may be more resistant to saponins than others.

2.12 **Advantages in using tannin and calcium nitrate as methane mitigation strategies**

2.12.1 *Tannin*

a) Overview

Tannins are a group of plant secondary metabolites originally used for the tanning of leather that are now being studied for their potential to reduce methane emissions (Frutos *et al*., 2004; Min *et al*., 2020). They have also shown potential to improve the fatty acid profile of meat and milk, improve the oxidative stability of meat on top of being a natural anthelmintic (Addis *et al*., 2005; Alonso-Díaz *et al*., 2010; Valenti *et al*., 2019; Futos *et al*., 2020). They are synthesised during the normal development of plants and increase in response to stress (Teixeira *et al*., 2016). They are water soluble polyphenolic compounds of differing molecular weights and complexites (Makkar, 2003; Goel *et al*., 2005). When consumed by ruminants they form reversable and irreversible bonds with proteins in aqueous solution, and to a lesser extent bind to polysaccharide, alkaloids, nucleic acids, minerals (Frutos *et al*., 2004), amino acids and metal ions (Makkar, 2003). The strength of binding is dependent on molecular weight (condensed tannin or hydrolysable tannin), isoelectric point and binding sites (Śliwiński *et al*., 2002). Condensed tannin binds strongly to protein when pH is between 4.0 and 7.0, which makes the rumen ideal for the formation of tannin-protein complexes (pH 5.6 to 6.8 on pasture). When the pH

drops below 4.0 in the abomasum (pH 2.5) the complex dissociates and protein is made available for degradation by acidic proteases in the duodenum (Jones & Mangan, 1977). Therefore, what is absorbed is a closer resemblance to the feed supplied by bypassing the rumen.

b) Classification of tannins

Tannins are classified into two groups: hydrolysable (HT) and condensed tannin (CT)s. Hydrolysable tannins are potentially toxic to microbes but are degradable in the rumen (Waghorn, 2008). They have a molecular weight of 500 to 3000 Da and consist of a carbohydrate core with hydroxyl groups that esterify with phenolic acids, predominantly gallic and hexahydroxydiphenic acid (Frutos *et al*., 2004).

Unlike hydrolysable tannins, condensed tannins are not susceptible to anaerobic enzyme degradation (Waghorn, 2008). They have a molecular weight of 1000 to 20000 Da, are also called proanthocyanidins, and consist of non-branched oligomers (2 to 10 monomers) or polymers (>10 monomers) of flavanol units, predominantly flavan-3-ol (Frutos *et al*., 2004; Girard & Bee, 2020) linked by carbon-carbon bonds (Waghorn, 2008). The number of flavanol units as well as the position and linkage type between them differ (Waghorn, 2008). Oxidative coupling or phenyl ether bonds link flavanol units (Duvel & Averous, 2016). A-type linkages consist of C4-C8 bonds and C2-O-C7 or C2-O-C5 ether bonds, whereas B-type linkages consist of C4-C8 or C4-C6 bonds (Girard & Bee, 2020). The monomer consists of two phenyl rings (A and B) and one hetrocyclic ring (C) (Teixeira *et al*., 2016). Differences in structure can also occur in the number and position of the hydroxyl group at the substitution site of the A and B rings (Waghorn, 2008; Duvel & Averous, 2016; Girard & Bee, 2020) as well as the stereochemistry at position 2,3 and 4 on the C ring (Waghorn, 2008). Common flavan-3-ol units include (epi) chatechin, (epi) afzelechin, (epi) gallocatechin, (epi) fisitinidol and (epi) robinetinidol which link up to form different polymers such as procyanidin, prodelphinidin, profusetinidin, prorobinetinidin and propelargonidin. Therefore, procyanidin for example refers to polymers of catechin and epicatechin stereoisomers (Waghorn, 2008; Girard & Bee, 2020). These structural differences are important because it affects the reactivity of the tannin (Waghorn, 2008). Therefore, tannins can have adverse or advantageous effects on animals depending on the animal species, physiological state, diet, as well as tannin dose and nature (Makker, 2003).

c) *Use of tannin in the mitigation of methane*

Researchers suggests the reduction of methane by tannin is dependent on the type of tannin, concentration, ruminant's diet and the type of ruminant it is offered to (Aboagye & Beauchemin, 2019). Some hypothesised mechanisms of action are 1) directly targeting methanogens and protozoa associated with methanogenesis, 2) act on fibrolytic bacteria, and/ or 3) function as a hydrogen sink (Aboagye & Beauchemin, 2019). Cieslak *et al*. (2012) demonstrated that the propionate synthesis

pathway competes with that of methane synthesis. Tannin supplementation has been associated with an increase in propionate production and a decrease in acetate production in the rumen (Jayanegara *et al*., 2015). This is advantageous as the synthesis of acetate in the rumen produces hydrogen, whereas propionate utilises hydrogen (Jayanegara *et al*., 2015) that would otherwise be available to methanogenic microbes in the rumen. Cieslak *et al*. (2012) suggested the increase in propionate production was because of the substrates available for microbial digestion and the effect of tannin on the microbial population in the rumen.

In many cases CT indirectly affect methane production by reducing fibre digestion, whereas HT directly inhibit growth and activity of methanogens and hydrogen producing bacteria (Goel & Makkar., 2012). Hydrolysable tannins are more consistent in its ability to reduce methane emissions due to its gallic acid subunit and less variations in structural diversity in comparison to the less toxic CT (Aboagye & Beauchemin, 2019). Denninger *et al*. (2020) experienced an immediate decrease in methane emissions of dairy cows fed *Acacia mearnsii* (CT) by potentially affecting methanogens, protozoa and fibre degrading bacteria. However, some researchers have been less successful (Beauchemin *et al*., 2007). In this case, Quebrecho tannin supplemented at 1.8% DM on a forage-based diet was not enough, although some protein binding effect was identified. When it was fed above 60g/kg DM it affected feed intake (Lima *et al*., 2019). At impractically high concentrations for ruminants, it has been reported to be toxic (Hervás *et al*., 2003), reduce palatability, reduce fibre digestibility by inhibiting enzyme degradation, bind to carbohydrates increasing rumen fill, decrease intake and reduce production (Aboagye & Beauchemin, 2019).

d) *Effect of tannin on fatty acid composition*

I. Overview

In a series of studies by Vasta and colleagues (Vasta *et al*., 2007; Vasta *et al*., 2010), CT was identified as having the ability to alter microbial biohydrogenation and thus fatty acid profile of meat. Vasta *et al*. (2007) compared a commercial maize-based concentrate diet with carob pulp (high in CT), and carob pulp with polyethyline glycol (PEG) (PEG binds to the CT and deactivates it). It was reported that CT successfully reduced biohydrogenation. C18:0 (a major SFAs in meat and the final product of biohydrogenation) was higher in concentrate fed lambs in comparison to carob pulp and PEG supplemented lambs. Another indicator of microbial response to CT was a 77% increase in the biohydrogenation intermediate C18:1n9t (vaccenic acid) and a 31% increase in conjugated linoleic acid (CLA) in the IMF of lambs supplemented with PEG in comparison to carob pulp. The effect of tannin on microbial biohydrogenation was confirmed in a follow up study by Vasta *et al*. (2010), who identified changes in the ruminal microbial populations and ruminal fatty acid profile in lambs fed a

barley-based concentrate when supplemented with quebracho tannins (9.57% DM). The saturated fatty acid (SFA)/vaccenic acid ratio in rumen fluid was lower (P<0.005), and vaccenic acid accumulated possibly as a result of the lower conversion of vaccenic acid to stearic acid, indicating inhibition of the last step of biohydrogenation. Rumenic acid was only detected in the rumen of lambs fed tannin, possibly as a result of a lower conversion of rumenic acid to vaccenic acid. Mirroring these findings was a significant (P<0.05) increase in *B. fibrisolvens* (converts linoleic acid to rumenic acid) in the first steps of biohydrogenation and a trend towards a decrease of *B. proteoclasticus* (converts vaccenic acid to stearic acid) responsible for the last step of biohydrogenation. There was also an unexpected increase in total protozoal numbers. The lower iso C15:0/odd and BCFA ratio also supports the hypothesis that cellulolytic bacteria enriched with iso C15:0 was depressed.

Condensed tannin and HT change the fatty acid profile in different ways. A case study by Costa *et al*. (2018) compared *Acacia mearnsie* (CT) with chestnut (a typical HT) on rumen biohydrogenation patterns, and found a greater reduction in rumen fermentation and a lower abundance of fribrolytic bacteria and biohydrogenation in the former.

II. Effect of tannin when added to roughage or concentrate based diets

The feed tannin is added to affects the improvement in the fatty acid profile. Vasta *et al*. (2009) discovered differences in the effect of quebracho tannins (at 4% DM) added to a concentrate or fresh herbage diet. Like the previous studies the last step of biohydrogenation was affected, indicated by the stearic acid/vaccenic acid ratio. Vaccenic acid accumulated, and there was an increase in PUFA and a decrease in SFA in IMF. However, the effect of tannin was greatest when added to the concentrate-based diet. In the concentrate diet, tannin decreased the rumen fluid concentration of stearic acid by 49% and increased vaccenic acid by 97%. Vaccenic acid is a precursor for endogenous synthesis of rumenic acid, thus IMF accumulated two times more rumenic acid than the concentrate-free diet.

III. *Dosage and source of tannin*

The ability of tannin to modulate ruminal biohydrogenation and fatty acid profile is controversial, possibly due to the dosage and source of tannin (Carreño *et al*., 2015). The dose of tannin supplied in the diet effects rumen biohydrogenation and fatty acid profile of meat (Min *et al.*, 2015; Kamel *et al.*, 2018; Alves *et al*., 2017). Priolo *et al.* (2005) reported that Sulla, a legume grass containing a low percent of CT, did not affect meat quality and fatty acid profile. Min *et al*. (2015) experienced an increase in MUFA and PUFA with increasing levels of pine bark (1.63%DM vs 3.2% DM), and Kamel *et al*. (2018) found improved CLA content when Quebracho tannin was increased from 20 to 40g/kg in a diet containing vegetable oil. Delta 9 desaturase, a regulatory enzyme involved in the biosynthesis of

MUFAs and conversion of vaccenic acid to rumenic acid in muscle, is also reported to increase with increasing supplantation of tannin; influenced by lower SFA, and higher MUFA and PUFAs (Rana *et al*, 2012). Therefore, improving CLA content in meat (Rana *et al*, 2012). Differences in *in vitro* biohydrogenation of UFAs from a selection of tannin sources (Quebracho, Grape, Chestnut and Oak) at increasing doses (20, 40, 60 and 80g/kg diet DM) showed an interaction between tannin, dose and biohydrogenation pathway in a study by Carreño *et al*. (2015). Although there were some similarities in response to incremental levels of different tannin extracts (C17:0 and C18:0 decreased and C18:2n6 and C18:3n3 increased) other extracts differed in response (t-10 and t-11 C18:1 or cis-9, trans-11 C18:2). The study suggested these responses were a consequence of chemical and structural differences (procyanidin/prodelphinidin ratios, degree of galloylation and/or molecular weight) altering its binding capacity or effect on microbes in the rumen. All extracts elicited a response at impractically high quantities for *in vivo* studies, except for oak (20g/kg diet DM) which increased PUFA, C18:3n3, C18:2n6 and t11 C18:1 while decreasing t10 C18:1 and C18:0 *in vitro* without negatively affecting rumen fermentation.

e) *The effect of tannin on oxidative stability of meat*

Some researchers have reported that tannin has the potential to improve the oxidative stability of meat. Tannin from different plant species generally has a similar effect on lamb meat colour (Priolo *et al*., 2007). It affects colour stability, shelf life and consumer satisfaction during storage (Luciano *et al*., 2009b). Storage time increases yellowness (b^*) and hue (H^*) at the expense of redness (a^*) (Luciano *et al*., 2009a). However, when tannin is added to the diet, a^* decreases at a slower rate, b^* increases more slowly and H^* delays the extent of colour deterioration and meat browning (Luciano *et al*., 2009a; Luciano *et al*., 2009b). The rate at which myoglobin is oxygenated to metmyoglobin is also slower in tannin supplemented diets (Luciano *et al*., 2011). There is a strong positive correlation between metmyoglobin percent (MMb%), which is also affected by tannin supplementation, and change in H^* (Luciano *et al*., 2011). Haem pigment also decreases with increasing storage time but is higher in supplemented animals (Luciano *et al*., 2009a). Highlighting its antioxidant ability, Luciano *et al*. (2011) obtained a 16.81% improvement in reducing power (ferric reducing ability of plasma; FRAP) and a 24.81% improvement in its radical scavenging ability (trolex equivalent antioxidant capacity; TEAC) when supplementing quebracho tannin (8.96% DM) to a barley-based concentrate diet.

There are direct and indirect hypothesis on the mechanism of action of tannins (López-Andrés *et al*., 2013). The mechanism of action used by tannins to reduce oxidation include antioxidant free radical activity, chelation of transition metals (Fe and Cu), inhibition of prooxidative enzymes and lipid peroxidation (Koleckar *et al*., 2008). The absorption of tannin to carry out its anti-oxidative properties

is controversial. Some researchers believe that the absorption of tannin is dependent on its degree of polymerisation and molecular weight. Deprez *et al*. (2001) research suggested that proanthocyanidin dimers and trimers are absorbed through intestinal epithelium. However, the absorption decreases with the higher level of polymerisation, unless they are degraded to monomers or oligomers in the stomach or aromatic compounds in the colon (Deprez *et al*., 2001). Although López-Andrés *et al*. (2013) quebracho tannin supplementation did improve the antioxidant capacity in lamb liver and plasma, tannin and phenol were not detected. As a result, it probably indirectly affected the tissue's endogenous antioxidant system or increased the regeneration of antioxidants. Priolo & Vasta (2007) suggested that haem pigment was lower in tannin supplemented lambs because of reduced microbial biosynthesis of vitamin B12 needed for its synthesis. In addition, tannins may have a protective effect against myoglobin damage (Luciano *et al*., 2009a). Priolo & Vasta (2007) also suggested that tannin likely prevented the utilisation of Fe for haemoglobin synthesis resulting in changes in haemoglobin but no change in blood Fe concentrations.

f) *Other uses of tannin*

Tannins have the potential to have anti-biotic, anti-parasitic, anti-viral, antioxidant, anti-inflammatory and immunomodulatory properties (Huang *et al*., 2018). For this reason, it also has the potential to be used as a preservation method for food (Wonghirundecha & Sumpavapol, 2012). Fraquelli *et al*. (2015) successfully used it as a sustainable management strategy for coccidiosis, a common disease in sheep. The source of tannin and chemical structure has an influence in its antimicrobial activity as evidence by Min *et al*. (2008) who found oak tannin to exhibit the best antimicrobial resistance to mastitis pathogens, in comparison to other tannin sources. However, these large variations in molecular weight, chemical compositions, chemical structures and the effect of different growing conditions can all be used as an advantage against microbial resistance (Huang *et al*., 2018)

Tannin can act directly on parasitic nematodes or indirectly by improving the host's resistance (Hoste *et al*., 2006). Lima *et al*. (2019) infected Santa Ines sheep with *T. colubriformis* (inhabits the small intestine but exsheaths in the forestomach) and *H. contortus* (resides in the abamasum). Acacia mearnsii bark (CT) highlighted its anthelmintic potential by improving blood parameters and reducing faecal egg count (FEC) and fertility, suggesting either a direct effect on the gastrointenstinal nematodes (GIN) or improved immune response due to important proteins reaching the small intestines. It was particularly effective in reducing *T. colubriformis* worm burden by 38% (by inhibiting larval exsheathment in the abomasum), and affecting *H. contortus* by reducing the fertility, FEC and eggs per female worm. Further research is needed for tannin to be used as a practical application in infected ruminants (Hoste *et al*., 2006; Saric *et al*., 2015).

Studies have shown potential in tannins to improve wool production, ovulation, lambing percent and reduce bloat risk (Min *et al*., 2003). Min *et al*. (1999; 2001) suggested that improved utilisation of ingested nutrients increased efficiency in reproduction and wool production due to an increase in plasma sulfur amino acids and essential amino acid available for wool growth and an increase in branch chain amino acids important for reproduction. As little as 1.0mg CT/g DM is sufficient to form complexes with soluble proteins responsible for the formation of frothy bloat in the rumen.

2.12.2 *Non-protein nitrogen (NPN) supplementation*

There are two types of proteins supplied in the diet; rumen degradable and rumen undegradable proteins (Goff, 2015). Rumen undegradable proteins are identical to that found in the diet and are digested in the small intestines. Rumen degradable proteins are used by microbes (in conjunction with carbon chains from the digestion of carbohydrates) for the synthesis of amino acids that are a very similar to those in muscle and milk (Goff, 2015).

Microbial degradation of dietary protein and NPN also produces amino acids, oligopeptides and ammonia important for microbial protein synthesis (Janssen, 2010). Urea is the most available form of NPN. It is broken down to ammonia by microbial urease and is used by microbes for microbial protein synthesis, amino acid synthesis and growth (Callaghan *et al*., 2014; Matthews *et al*., 2019). Non-protein nitrogen is particularly important in low protein diets and poor-quality roughages to increase intake, thereby improving liveweight gain and reduce mortality particularly in the dry season (Callaghan *et al*., 2014).

Calcium nitrate, an alternative NPN source, can reduce nitrate to nitrite to ammonia in the rumen (Matthews *et al*., 2019). Li *et al*. (2012) demonstrated that 3% calcium nitrate can replace 1.5% urea to meet nitrogen requirements of lambs without effecting production, and with the added benefit of reducing methane emission. When nitrate and nitrite are reduced to ammonia the electrons (hydrogen) are used at the expense of methanogenesis, hence the reducing methane production (Matthews *et al*., 2019). The reduction of nitrate to ammonia consumes 8 electrons, so theoretically for every 1 mole of nitrate, 1 mole of methane is reduced (Van Zijderveld *et al*., 2010). This agrees with Adejoro *et al*. (2020) who reported a 20% reduction in methane (g/day) emissions as well as a 20% improved ADG when replacing urea with calcium nitrate in lambs receiving a TMR ration. This would suggest a change in the microbial activity in the rumen.

There is limited research available on the effect of calcium nitrate on fatty acid metabolism. Studies have indicated an increase in acetate production at the expense of propionate when nitrate is used instead of urea as an NPN source (Nolan *et al*., 2010; Davis *et al*., 2012). Efficient removal of hydrogen

by nitrate supplementation decreases NADH from cells and increases nicotinamide adenine dinucleotide (NAD+), which is more favorable for the synthesis of acetate (Ungerfeld & Kohn, 2006). An increase in acetate could result in an increase in *de novo* fatty acid synthesis, of which C16:0 is the end product (Shingfield *et al*., 2013). Palmitic acid (C16:0) is not advantageous as it is linked to increased risk of cardiovascular disease (Shramko *et al*., 2020). Almeida *et al*. (2022) reported that when calcium ammonium nitrogen was used as a NPN source in dairy feed, the milk fatty acid profile improved with a decrease in SFA, increase in MUFA and increase in omega 3 fatty acids. Research is lacking in the identification of any improvement in the fatty acid profile of meat when calcium nitrogen is used as a NPN source.

2.13 Conclusion from literature review

Literature indicates a growing pressure on the livestock industry to reduce enteric methane emissions from ruminants as a means to slow down global warming. Methane is produced by microbes as a mechanism to remove H^+ in the rumen. Consumers also perceive ruminant meat as less healthy due to its higher concentration of saturated and trans fatty acids due to the reduction of PUFA by microbes in the rumen. The sex of sheep and managing the weight at which they are slaughtered can be used as a method to improve the fatty acid profile. Feeding roughage, protected fats, essential oils and saponins have also shown promise. However, literature shows the potential of tannin to affect both methanogens and biohydrogenation bacteria in a positive way, by reducing methane emissions and improving the fatty acid profile in lamb. The effect on the fatty acid profile can also has also shown positive implications on the oxidative stability of meat. Calcium nitrate has been used as a method for reducing methane emissions with success. This may increase acetate production, which could increase palmitic acid, a major fatty acid in ruminant meat associated with CVD risk. In contrast, another researcher found positive effects in milk. Therefore, more research is required to determine its effect on meat quality. This study explores South African Mutton Merino ewe and ram lambs slaughtered at two different weights fed tannin and calcium nitrate based total mixed rations, and its effect on carcass characteristics, colour, blood parameters and fatty acid metabolism.

2.14 References

Aboagye, I. A. & Beauchemin, K. A., 2019. Potential of molecular weight and structure of tannins to reduce methane emissions from ruminants: A review. Animals, 9(11), 856.

Addis, M., Cabiddu, A., Pinna, G., Decandia, M., Piredda, G., Pirisi, A. & Molle, G., 2005. Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid cis-9, trans-11. Journal of Dairy Science, 88(10), 3443-3454.

Adejoro, F. A., Hassen, A., Akanmu, A. M. & Morgavi, D. P., 2020. Replacing urea with nitrate as a non-protein nitrogen source increases lambs' growth and reduces methane production, whereas acacia tannin has no effect. Animal Feed Science and Technology, 259, 114360.

Agostoni, C., Moreno, L. & Shamir, R., 2016. Palmitic acid and health: Introduction. Critical Reviews in Food Science and Nutrition, 56(12), 1941-1942.

Aldai, N., Dugan, M. E. R., Kramer, J. K. G., Martínez, A., López-Campos, O., Mantecón, A. R. & Osoro, K., 2011. Length of concentrate finishing affects the fatty acid composition of grass-fed and genetically lean beef: An emphasis on trans-18: 1 and conjugated linoleic acid profiles. Animal, 5(10), 1643-1652.

Alfaia, C. P., Alves, S. P., Martins, S. I., Costa, A. S., Fontes, C. M., Lemos, J. P., Bessa, R. J. B. & Prates, J. A., 2009. Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. Food Chemistry, 114(3), 939-946.

Almeida, K. V., Santos, G. T., Daniel, J. L. P., Osorio, J. A. C., Yamada, K. L. G., Sippert, M. R., Cabral, J. F., Marchi, F. E., Araujo, R. C. & Vyas, D., 2022. Effects of calcium ammonium nitrate fed to dairy cows on nutrient intake and digestibility, milk quality, microbial protein synthesis, and ruminal fermentation parameters. Journal of Dairy Science, 105(3), 2228-2241.

Alonso-Díaz, M. A., Torres-Acosta, J. F. J., Sandoval-Castro, C. A. & Hoste, H., 2010. Tannins in tropical tree fodders fed to small ruminants: A friendly foe? Small Ruminant Research, 89(2-3), 164-173.

Alves, S. P., Francisco, A., Costa, M., Santos-Silva, J. & Bessa, R. J., 2017. Biohydrogenation patterns in digestive contents and plasma of lambs fed increasing levels of a tanniferous bush (*Cistus ladanifer L.*) and vegetable oils. Animal Feed Science and Technology, 225, 157-172.

Amaral, A. B., Silva, M. V. D. & Lannes, S. C. D. S., 2018. Lipid oxidation in meat: Mechanisms and protective factors–a review. Food Science and Technology, 38, 1-15.

Andri, F., Huda, A. N. & Marjuki, M., 2020. The use of essential oils as a growth promoter for small ruminants: A systematic review and meta-analysis. F1000Research, 9, 486.

Antongiovanni, M., Buccioni, A., Petacchi, F., Secchiari, P., Mele, M. & Serra, A., 2003. Upgrading the lipid fraction of foods of animal origin by dietary means: rumen activity and presence of trans fatty acids and CLA in milk and meat. Italian Journal of Animal Science, *2*(1), 3-28.

Aro, A., van Amelsvoort, J., Becker, W., van Erp-Baart, M. A., Kafatos, A., Leth, T. & van Poppel, G., 1998a. Trans fatty acids in dietary fats and oils from 14 European countries: The TRANSFAIR study. Journal of Food Composition and Analysis, 11(2), 137-149.

Aro, A., Kosmeijer-Schuil, T., van de Bovenkamp, P., Hulshof, P., Zock, P. & Katan, M. B., 1998b. Analysis of C18: 1cis and trans fatty acid isomers by the combination of gas-liquid chromatography of 4, 4-dimethyloxazoline derivatives and methyl esters. Journal of the American Oil Chemists' Society, 75(8), 977-985.

Baer, D. J., Judd, J. T., Clevidence, B. A. & Tracy, R. P., 2004. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: A randomized crossover study. The American Journal of Clinical Nutrition, 79(6), 969-973.

Bartoň, L., Bureš, D., Kott, T. & Řehák, D., 2011. Effect of sex and age on bovine muscle and adipose fatty acid composition and stearoyl-CoA desaturase mRNA expression. Meat Science, 89(4), 444-450.

Bauchart, D., 1993. Lipid absorption and transport in ruminants. Journal of Dairy Science, 76(12), 3864-3881.

Bauman, D. E., Perfield, J. W., De Veth, M. J. & Lock, A. L., 2003. New perspectives on lipid digestion and metabolism in ruminants. In: Proceedings of the Cornell Nutritional Conference, Cornell University. New York, USA, 65. 175-189.

Beauchemin, K. A., McGinn, S. M., Martinez, T. F. & McAllister, T. A., 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. Journal of Animal Science, 85(8), 1990-1996.

Behan, A. A., Loh, T. C., Fakurazi, S., Kaka, U., Kaka, A. & Samsudin, A. A., 2019. Effects of supplementation of rumen protected fats on rumen ecology and digestibility of nutrients in sheep. Animals, 9(7), 400.

Beitz, D. C., 2004. Lipid Metabolism. In: Dukes' Physiology of Domestic Animals. Ed: Reece, W.O., Cornell University Press, Ithaca, New York. pp. 453-471.

Bekhit, A. E. D. & Faustman, C., 2005. Metmyoglobin reducing activity. Meat Science, 71(3), 407-439.

Benchaar, C. & Greathead, H., 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Animal Feed Science and Technology, 166, 338-355.

Brendsen, N. T., Christensen, R., Bartels, E. M. & Astrup, A., 2011. Consumption of industrial and ruminant trans fatty acids and risk of coronary heart disease: A systematic review and meta-analysis of cohort studies. European Journal of Clinical Nutrition, 65(7), 773-783.

Bessa, R. J., Alves, S. P. & Santos-Silva, J., 2015. Constraints and potentials for the nutritional modulation of the fatty acid composition of ruminant meat. European Journal of Lipid Science and Technology, 117(9), 1325-1344.

Bhatt, R. S., Soni, L. K., Sahoo, A., Gadekar, Y. P. & Sarkar, S., 2020. Dietary supplementation of extruded linseed and calcium soap for augmenting meat attributes and fatty acid profile of *longissimus thoracis* muscle and adipose tissue in finisher Malpura lambs. Small Ruminant Research, 184, 106062.

Black, J. L., Davison, T. M., & Box, I., 2021. Methane emissions from ruminants in Australia: Mitigation potential and applicability of mitigation strategies, Animals, 11(4), 951.

Boughalmi, A. & Araba, A., 2016. Effect of feeding management from grass to concentrate feed on growth, carcass characteristics, meat quality and fatty acid profile of Timahdite lamb breed. Small Ruminant Research, 144, 158-163.

Brand, T. S., Van Der Westhuizen, E. J., van Der Merwe, D. A. & Hoffman, L. C., 2018. Analysis of carcass characteristics and fat deposition of Merino, South African Mutton Merino and Dorper lambs housed in a feedlot. South African Journal of Animal Science, 48(3), 477-488.

Brandt, E. J., Myerson, R., Perraillon, M. C. & Polonsky, T. S., 2017. Hospital admissions for myocardial infarction and stroke before and after the trans-fatty acid restrictions in New York. JAMA Cardiology, 2(6), 627-634.

Brask, M., Lund, P., Weisbjerg, M. R., Hellwing, A. L. F., Poulsen, M., Larsen, M. K. & Hvelplund, T., 2013. Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. Journal of Dairy Science, 96(4), 2356-2365.

Brogna, D. M., Nasri, S., Salem, H. B., Mele, M., Serra, A., Bella, M., Priolo, A., Makkar, H. P. S. & Vasta, V., 2011. Effect of dietary saponins from Quillaja saponaria L. on fatty acid composition and cholesterol content in muscle *Longissimus dorsi* of lambs. Animal, 5(7), 1124-1130.

Brouwer, I. A., Wanders, A. J. & Katan, M. B., 2010. Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans–a quantitative review. PloS one, 5(3), e9434.

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

Burns, T. A., Kadegowda, A. K. G., Duckett, S. K., Pratt, S. L. & Jenkins, T. C., 2012. Palmitoleic (16: 1 cis-9) and cis-vaccenic (18: 1 cis-11) acid alter lipogenesis in bovine adipocyte cultures. Lipids, 47(12), 1143-1153.

Busboom, J. R., Miller, G. J., Field, R. A., Crouse, J. D., Riley, M. L., Nelms, G. E. & Ferrell, C. L., 1981. Characteristics of fat from heavy ram and wether lambs. Journal of Animal Science, 52(1), 83-92.

Byelashov, O. A., Sinclair, A. J. & Kaur, G., 2015. Dietary sources, current intakes, and nutritional role of omega-3 docosapentaenoic acid. Lipid Technology, 27(4), 79-82.

Calder, P. C., 2015. Functional roles of fatty acids and their effects on human health. Journal of Parenteral and Enteral Nutrition, 39, 18S-32S.

Calkins, C. R., Dutson, T. R., Smith, G. C., Carpenter, Z. L. & Davis, G. W., 1981. Relationship of fiber type composition to marbling and tenderness of bovine muscle. Journal of Food Science, 46(3), 708-710.

Callaghan, M. J., Tomkins, N. W., Benu, I. & Parker, A. J., 2014. How feasible is it to replace urea with nitrates to mitigate greenhouse gas emissions from extensively managed beef cattle? Animal Production Science, 54(9), 1300-1304.

Calnan, H. B., Jacob, R. H., Pethick, D. W. & Gardner, G. E., 2019. Dietary vitamin E supplementation reduces lamb meat browning on display following up to 70 days of chilled storage. Livestock Science, 230, 103843.

Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L. & Ferret, A., 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science, 90(6), 2580-2595.

Campbell, M. K. & Farrell, S. O., 2012. Lipid metabolism. In: Biochemistry. 7th ed. Brooks/Cole, Cengage Learning, Belmont, CA. p. 603.

Carreño, D., Hervás, G., Toral, P. G., Belenguer, A. & Frutos, P., 2015. Ability of different types and doses of tannin extracts to modulate *in vitro* ruminal biohydrogenation in sheep. Animal Feed Science and Technology, 202, 42-51.

Carta, G., Murru, E., Banni, S. & Manca, C., 2017. Palmitic acid: Physiological role, metabolism and nutritional implications. Frontiers in Physiology, 8, 902.

Casey, N. H., Van Niekerk, W. A. & Spreeth, E. B., 1988. Fatty acid composition of subcutaneous fat of sheep grazed on eight different pastures. Meat Science, 23(1), 55-63.

Çekici, H. & Akdevelioğlu, Y., 2018. The association between trans fatty acids, infertility and fetal life: A review. Human Fertility, DOI: 10.1080/14647273.2018.1432078.

Chen, Z. Y., Ma, K. Y., Liang, Y., Peng, C. & Zuo, Y., 2011. Role and classification of cholesterol-lowering functional foods. Journal of Functional Foods, *3*(2), 61-69.

Chikwanha, O. C., Vahmani, P., Muchenje, V., Dugan, M. E. & Mapiye, C., 2018. Nutritional enhancement of sheep meat fatty acid profile for human health and wellbeing. Food Research International, 104, 25-38.

Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L. & Pariza, M. W., 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. Journal of Food Composition and Analysis, 5(3), 185-197.

Cianzio, D. S., Topel, D. G., Whitehurst, G. B., Beitz, D. C. & Self, H. L., 1985. Adipose tissue growth and cellularity: Changes in bovine adipocyte size and number. Journal of Animal Science, 60(4), 970-976.

Cieslak, A., Zmora, P., Pers-Kamczyc, E. & Szumacher-Strabel, M., 2012. Effects of tannins source (*Vaccinium vitis idaea L*.) on rumen microbial fermentation *in vivo*. Animal Feed Science and Technology, 176(1-4), 102-106.

Clapham, W. M., Foster, J. G., Neel, J. P. & Fedders, J. M., 2005. Fatty acid composition of traditional and novel forages. Journal of Agricultural and Food Chemistry, 53(26), 10068-10073.

Cloete, J. J. E., Hoffman, L. C., Cloete, S. W. P. & Fourie, J. E., 2004. A comparison between the body composition, carcass characteristics and retail cuts of South African Mutton Merino and Dormer sheep. South African Journal of Animal Science, 34(1), 44-51.

Collomb, M., Sieber, R. & Bütikofer, U., 2004. CLA isomers in milk fat from cows fed diets with high levels of unsaturated fatty acids. Lipids, 39(4), 355-364.

Costa, M., Alves, S. P., Cappucci, A., Cook, S. R., Duarte, A., Caldeira, R. M., McAllister, T. A. & Bessa, R. J., 2018. Effects of condensed and hydrolyzable tannins on rumen metabolism with emphasis

on the biohydrogenation of unsaturated fatty acids. Journal of Agricultural and Food Chemistry, 66(13), 3367-3377.

Crouse, J. D., Kemp, J. D., Fox, J. D., Ely, D. G. & Moody, W. G., 1972. Effects of castration on ovine neutral and phospholipid deposition. Journal of Animal Science, 34(3), 388-392.

DALRRD, 2022. Abstract of agricultural statistics 2022. Compiled by the directorate statistics and economics analysis. Published by the Department of agriculture, land reform and rural development, South Africa. pp. 62-63. [Online]. Available from: https://www.dalrrd.gov.za/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Abstract%202022.pdf. [Accessed 16/11/2022].

De Brito, G. F., Ponnampalam, E. N. & Hopkins, D. L., 2017. The effect of extensive feeding systems on growth rate, carcass traits, and meat quality of finishing lambs. Comprehensive Reviews in Food Science and Food Safety, 16(1), 23-38.

De Carvalho, C. C. & Caramujo, M. J., 2018. The various roles of fatty acids. Molecules, 23(10), 2583.

De Smet, S. & Vossen, E., 2016. Meat: The balance between nutrition and health. A review. Meat Science, 120, 145-156.

Della Malva, A., Albenzio, M., Annicchiarico, G., Caroprese, M., Muscio, A., Santillo, A. & Marino, R., 2016. Relationship between slaughtering age, nutritional and organoleptic properties of Altamurana lamb meat. Small Ruminant Research, 135, 39-45.

Den Hartigh, L. J., Wang, S., Goodspeed, L., Wietecha, T., Houston, B., Omer, M., Ogimoto, K., Subramanian, S., Gowda, G. A. N., O'Brian, K. D., Kaiyala, K. J., Morton, G. J. & Chait, A., 2017. Metabolically distinct weight loss by 10, 12 CLA and caloric restriction highlight the importance of subcutaneous white adipose tissue for glucose homeostasis in mice. PLoS One, 12(2), e0172912.

Denninger, T. M., Schwarm, A., Birkinshaw, A., Terranova, M., Dohme-Meier, F., Müenger, A., Eggerchwiler, L., Bopst, B/. Wegmann, S., Clauss, M. & Kreuzer, M., 2020. Immediate effect of Acacia mearnsii tannins on methane emissions and milk fatty acid profiles of dairy cows. Animal Feed Science and Technology, 261, 114388.

Deprez, S., Mila, I., Huneau, J. F., Tome, D. & Scalbert, A., 2001. Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. Antioxidants and Redox Signalling, 3(6), 957-967.

Dıaz, M. T., Velasco, S., Caneque, V., Lauzurica, S., De Huidobro, F. R., Pérez, C., González, J. & Manzanares, C., 2002. Use of concentrate or pasture for fattening lambs and its effect on carcass and meat quality. Small Ruminant Research, 43(3), 257-268.

Dıaz, M. T., Velasco, S., Pérez, C., Lauzurica, S., Huidobro, F. & Cañeque, V., 2003. Physico-chemical characteristics of carcass and meat Manchego-breed suckling lambs slaughtered at different weights. Meat Science, 65(3), 1085-1093.

Dilzer, A. & Park, Y., 2012. Implication of conjugated linoleic acid (CLA) in human health. Critical Reviews in Food Science and Nutrition, 52(6), 488-513.

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W. & Lorenzo, J. M., 2019. A comprehensive review on lipid oxidation in meat and meat products. Antioxidants, 8(10), 429.

Downs, S. M., Thow, A. M. & Leeder, S. R., 2013. The effectiveness of policies for reducing dietary trans fat: A systematic review of the evidence. Bulletin of the World Health Organization, 91, 262-269H.

Du Toit, C. J. L., Van Niekerk, W. A. & Meissner, H. H., 2013. Direct greenhouse gas emissions of the South African small stock sectors. South African Journal of Animal Science, 43(3), 340-361.

Duval, A. & Averous, L., 2016. Characterization and physicochemical properties of condensed tannins from *Acacia catechu*. Journal of Agricultural and Food Chemistry, 64(8), 1751-1760.

Dzoyem, J. P., Kuete, V. & Eloff, J. N., 2014. Biochemical parameters in toxicological studies in Africa: Significance, principle of methods, data interpretation, and use in plant screenings. In: Toxicological survey of African medicinal plants. Ed: Kuete, V., Elsevier. pp. 659-715.

Efenberger-Szmechtyk, M., Nowak, A. & Czyzowska, A., 2021. Plant extracts rich in polyphenols: Antibacterial agents and natural preservatives for meat and meat products. Critical Reviews in Food Science and Nutrition, 61(1), 149-178.

EFSA, 2017. Nitrites and Nitrates added to food. In: EFSA explains risk assessment. [Online]. Available from: https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/nitrates-nitrites-170614.pdf. [Accessed: 16/11/2022].

Enser, M., Hallett, K. G., Hewett, B., Fursey, G. A. J., Wood, J. D. & Harrington, G., 1998. Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. Meat Science, 49(3), 329-341.

EPA, 2022. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2020. U.S. Environmental Protection Agency, EPA 430-R-22-003. pp. ES-13. [Online]. Available from: https://www.epa.gov/ghgemissions/draft-inventory-us-greenhouse-gas-emissionsand-sinks-1990-2020. [Accessed: 16/11/2022].

Evans, L. M., Cowey, S. L., Siegal, G. P. & Hardy, R. W., 2009. Stearate preferentially induces apoptosis in human breast cancer cells. Nutrition and Cancer, 61(5), 746-753.

FAO, 2017. Global Livestock Environmental Assessment Model (GLEAM). [Online]. Available from: www.fao.org/gleam/en/. [Accessed: 16/11/2022].

Faustman, C. & Cassens, R. G., 1990. The biochemical basis for discoloration in fresh meat: a review. Journal of Muscle Foods, 1(3), 217-243.

FDA, 2013. A food labelling guide. US department of health and human services. Food and drug administration centre for food safety and applied nutrition. [Online]. Available from: https://www.fda.gov/files/food/published/Food-Labeling-Guide-%28PDF%29.pdf. [Accessed: 16/11/2022]

Fermor, B. F., Masters, J. R., Wood, C. B., Miller, J., Apostolov, K. & Habib, N. A., 1992. Fatty acid composition of normal and malignant cells and cytotoxicity of stearic, oleic and sterculic acids *in vitro*. European Journal of Cancer, 28(6-7), 1143-1147.

Field, C. J., Blewett, H. H., Proctor, S. & Vine, D., 2009. Human health benefits of vaccenic acid. Applied Physiology, Nutrition, and Metabolism, 34(5), 979-991.

Flores, M. & Toldrá, F., 2021. Chemistry, safety, and regulatory considerations in the use of nitrite and nitrate from natural origin in meat products-Invited review. Meat Science, 171, 108272.

Francis, G., Kerem, Z., Makkar, H. P. & Becker, K., 2002. The biological action of saponins in animal systems: A review. British journal of Nutrition, 88(6), 587-605.

Fraquelli, C., Zanzani, S. A., Gazzonis, A. L., Rizzi, R. & Manfredi, M. T., 2015. Effects of condensed tannin on natural coccidian infection in goat kids. Small Ruminant Research, 126, 19-24.

Fraser, M. D., Speijers, M. H., Theobald, V. J., Fychan, R. & Jones, R., 2004. Production performance and meat quality of grazing lambs finished on red clover, lucerne or perennial ryegrass swards. Grass and Forage Science, 59(4), 345-356.

Frutos, P., Hervás, G., Giráldez, F. & Mantecón, Á. R., 2004. Review. Tannins and ruminant nutrition. Spanish Journal of Agricultural Research, 2(2), 191-202.

Frutos, P., Hervás, G., Natalello, A., Luciano, G., Fondevila, M., Priolo, A. & Toral, P. G., 2020. Ability of tannins to modulate ruminal lipid metabolism and milk and meat fatty acid profiles. Animal Feed Science and Technology, 269, 114623.

Gadeyne, F., De Neve, N., Vlaeminck, B. & Fievez, V., 2017. State of the art in rumen lipid protection technologies and emerging interfacial protein cross-linking methods. European Journal of Lipid Science and Technology, 119(5), 1600345.

Gao, X., Wang, Z., Miao, J., Xie, L., Dai, Y., Li, X., Dai, Y., Li, X., Chen, Y., Luo, H. & Dai, R., 2014. Influence of different production strategies on the stability of color, oxygen consumption and metmyoglobin reducing activity of meat from Ningxia Tan sheep. Meat Science, 96(2), 769-774.

Garton, G. A., Hovell, F. D. & Duncan, W. R., 1972. Influence of dietary volatile fatty acids on the fatty-acid composition of lamb triglycerides, with special reference to the effect of propionate on the presence of branched-chain components. The British Journal of Nutrition, 28(3), 409-416

Gayet-Boyer, C., Tenenhaus-Aziza, F., Prunet, C., Marmonier, C., Malpuech-Brugere, C., Lamarche, B. & Chardigny, J. M., 2014. Is there a linear relationship between the dose of ruminant trans-fatty acids and cardiovascular risk markers in healthy subjects: results from a systematic review and meta-regression of randomised clinical trials. British Journal of Nutrition, 112(12), 1914-1922.

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A. & Tempio, G. 2013. Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. pp. xii, 31.

GINA, 2022. TFA country score card. Global database on the implementation of nutritional action GINA. WHO. [Online]. Available from: https://extranet.who.int/nutrition/gina/en/scorecard/TFA. [Accessed 09/11/2022].

Girard, M. & Bee, G., 2020. Invited review: Tannins as a potential alternative to antibiotics to prevent coliform diarrhea in weaned pigs. Animal, 14(1), 95-107.

Goel, G. & Makkar, H. P., 2012. Methane mitigation from ruminants using tannins and saponins. Tropical Animal Health and Production, 44(4), 729-739.

Goel, G., Puniya, A. K., Aguilar, C. N. & Singh, K., 2005. Interaction of gut microflora with tannins in feeds. Naturwissenschaften, 92(11), 497-503.

Goff, J. P., 2015. Ruminant digestive physiology and intestinal microbiology. In: Dukes' Physiology of Domestic Animals (13th ed.). Ed: Reece, W. O., John Wiley & sons, inc., New Jersey, USA., pp. 522-531.

Goodridge, J., Ingalls, J. R. & Crow, G. H., 2001. Transfer of omega-3 linolenic acid and linoleic acid to milk fat from flaxseed or Linola protected with formaldehyde. Canadian Journal of Animal Science, 81(4), 525-532.

Gravador, R. S., Moloney, A. P., Brunton, N. P., Gkarane, V., Allen, P., Fahey, A. G., Claffet, N. A., Diskin, M. G., Farmer, L. J. & Monahan, F. J., 2018. Effects of castration and slaughter age on the fatty acid composition of ovine muscle and adipose tissue from two breeds. Small Ruminant Research, 168, 94-100.

Gray, J. I. & Monahan, F. J., 1992. Measurement of lipid oxidation in meat and meat products. Trends in Food Science & Technology, 3, 315-319.

Griinari, J. M., Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela, K. V. V. & Bauman, D. E., 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ9-desaturase. The Journal of Nutrition, 130(9), 2285-2291.

Gulati, S. K., Kitessa, S. M., Ashes, J. R., Fleck, E., Byers, E. B., Byers, Y. G. & Scott, T. W., 2000. Protection of conjugated linoleic acids from ruminal hydrogenation and their incorporation into milk fat. Animal Feed Science and Technology, 86(3-4), 139-148.

Hammond, J., 1950. Measuring growth in farm animals. Proceedings of the Royal Society of London. Series B-Biological Sciences, 137(889), 452-461.

Hammond, J., 1950. Measuring growth in farm animals. Proceedings of the Royal Society of London. Series B-Biological Sciences, 137(889), 452-461.

Hansen, C. P., Berentzen, T. L., Halkjaer, J., Tjønneland, A., Sørensen, T. I. A., Overvad, K. & Jakobsen, M. U., 2012. Intake of ruminant trans fatty acids and changes in body weight and waist circumference. European Journal of Clinical Nutrition, 66(10), 1104-1109.

Harfoot, C. G., 1981. Lipid metabolism in the rumen. In: Lipid metabolism in ruminant animals Ed: Christie, W. C., Pergamon. pp. 21-55.

Harrison, F. A. & Leat, W. M. F., 1975. Digestion and absorption of lipids in non-ruminant and ruminant animals: A comparison. Proceedings of the Nutrition Society, 34(3), 203-210.

Haugebak, C. D., Hedrick, H. B. & Asplund, J. M., 1974. Relationship between extramuscular adipose tissue lipoprotein lipase activity and intramuscular lipid deposition in fattening lambs. Journal of Animal Science, 39(6), 1026-1031.

Hervás, G., Pérez, V., Giráldez, F. J., Mantecón, A. R., Almar, M. M. & Frutos, P., 2003. Intoxication of sheep with quebracho tannin extract. Journal of Comparative Pathology, 129(1), 44-54.

Hill, J., McSweeney, C., Wright, A. D. G., Hurley, G. B., Kalantar-zadeh, K., 2016. Measuring methane production in ruminants. Trends in Biotechnology, 34, 26-36.

Hocquette, J. F. & Bauchart, D., 1999. Intestinal absorption, blood transport and hepatic and muscle metabolism of fatty acids in preruminant and ruminant animals. Reproduction Nutrition Development, 39(1), 27-48.

Hocquette, J. F., Gondret, F., Baéza, E., Médale, F., Jurie, C. & Pethick, D. W., 2010. Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. Animal, 4(2), 303-319.

Hood, D. E., 1980. Factors affecting the rate of metmyoglobin accumulation in pre-packaged beef. Meat Science, 4(4), 247-265.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S. M. & Hoskin, S. O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends in Parasitology, 22(6), 253-261.

Howe, P., Buckley, J. & Meyer, B., 2007. Long-chain omega-3 fatty acids in red meat. Nutrition & Dietetics: The Journal of the Dietitians Association of Australia, 64(3), S135-S135.

HSFSA, 2016. Cardiovascular disease statistics reference document. [Online]. Available from: https://www.heartfoundation.co.za/wp-content/uploads/2017/10/CVD-Stats-Reference-Document-2016-FOR-MEDIA-1.pdf. [Accessed: 16/11/2022].

Huang, Q., Liu, X., Zhao, G., Hu, T. & Wang, Y., 2018. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Animal Nutrition, 4(2), 137-150.

Hulshof, K. F. A. M., van Erp-Baart, M. A., Anttolainen, M., Becker, W., Church, S. M., Couet, C., Hermann-Kunz, E., Kestloot, H., Leth, T., Martins, I., Moreiras, O., Moschandress, J., Pizzoferrato, L., Rimestad, A. H., Thorgeirsdottir, H., van Amalsvoort, J. M. M., Aro, A., Kafatos, A. G., Lanzmann-Petithory, D. & van Poppel, G., 1999. Intake of fatty acids in Western Europe with emphasis on trans fatty acids: The TRANSFAIR Study. European Journal of Clinical Nutrition, 53(2), 143-157.

Hunter, J. E., Zhang, J. & Kris-Etherton, P. M., 2010. Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: A systematic review. The American Journal of Clinical Nutrition, 91(1), 46-63.

Jacob, R. (2020). Implications of the variation in bloom properties of red meat: A review. Meat Science, 162, 108040.

Jakobsen, M. U., Overvad, K., Dyerberg, J. & Heitmann, B. L., 2008. Intake of ruminant trans fatty acids and risk of coronary heart disease. International Journal of Epidemiology, 37(1), 173-182.

Janssen, P. H. & Kirs, M., 2008. Structure of the archaeal community of the rumen. Applied and Environmental Microbiology, 74(12), 3619-3625.

Janssen, P. H., 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Animal Feed Science and Technology, 160(1-2), 1-22.

Jayanegara, A., Goel, G., Makkar, H. P. & Becker, K., 2015. Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population *in vitro*. Animal Feed Science and Technology, 209, 60-68.

Jeffry, L., Ong, M. Y., Nomanbhay, S., Mofijur, M., Mubashir, M. & Show, P. L., 2021. Greenhouse gases utilization: A review. Fuel, 301, 121017.

Jenkins, T. C., 1993. Lipid metabolism in the rumen. Journal of Dairy Science, 76(12), 3851-3863.

Jenkins, T. C. & Bridges Jr, W. C., 2007. Protection of fatty acids against ruminal biohydrogenation in cattle. European Journal of Lipid Science and Technology, 109(8), 778-789.

Jerónimo, E., Soldado, D., Sengo, S., Francisco, A., Fernandes, F., Portugal, A. P., Alves, S. P., Santos-Silva, J. & Bessa, R. J., 2020. Increasing the α-tocopherol content and lipid oxidative stability of meat through dietary *Cistus ladanifer L.* in lamb fed increasing levels of polyunsaturated fatty acid rich vegetable oils. Meat Science, 164, 108092.

Jo, J., Gavrilova, O., Pack, S., Jou, W., Mullen, S., Sumner, A. E., Cushman, S.W. & Periwal, V., 2009. Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. PLoS Computational Biology, 5(3), e1000324.

Johnson, K. A. & Johnson, D. E., 1995. Methane emissions from cattle. Journal of Animal Science, 73(8), 2483-2492.

Jones, W. T. & Mangan, J. L., 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia Scop.*) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. Journal of the Science of Food and Agriculture, 28(2), 126-136.

Kaçar, S., 2019. n-3 and n-6 fatty acids in fish: A focus on non-marine Species. In: Omega fatty acids in brain and neurological health. Ed: Watson, R. R. & Preedy, V. R., Academic Press. pp. 367-380.

Kamel, H. E. M., Al-Dobaib, S. N., Salem, A. Z. M., López, S. & Alaba, P. A., 2018. Influence of dietary supplementation with sunflower oil and quebracho tannins on growth performance and meat fatty acid profile of Awassi lambs. Animal Feed Science and Technology, 235, 97-104.

Kasapidou, E., Wood, J. D., Richardson, R. I., Sinclair, L. A., Wilkinson, R. G. & Enser, M., 2012. Effect of vitamin E supplementation and diet on fatty acid composition and on meat colour and lipid oxidation of lamb leg steaks displayed in modified atmosphere packs. Meat Science, 90(4), 908-916.

Kaur, N., Chugh, V. & Gupta, A. K., 2014. Essential fatty acids as functional components of foods-a review. Journal of Food Science and Technology, 51(10), 2289-2303.

Kelly, F. D., Sinclair, A. J., Mann, N. J., Turner, A. H., Abedin, L. & Li, D., 2001. A stearic acid-rich diet improves thrombogenic and atherogenic risk factor profiles in healthy males. European Journal of Clinical Nutrition, 55(2), 88-96.

Kemp, J. D., Mahyuddin, M., Ely, D. G., Fox, J. D. & Moody, W. G., 1981. Effect of feeding systems, slaughter weight and sex on organoleptic properties, and fatty acid composition of lamb. Journal of Animal Science, 51(2), 321-330.

Kempster, A. J., 1981. Fat partition and distribution in the carcasses of cattle, sheep and pigs: A review. Meat Science, 5(2), 83-98.

Kiage, J. N., Merrill, P. D., Judd, S. E., He, K., Lipworth, L., Cushman, M., Howard, V.J. & Kabagambe, E. K., 2014. Intake of trans fat and incidence of stroke in the reasons for geographic and racial differences in stroke (REGARDS) cohort. The American Journal of Clinical Nutrition, 99(5), 1071-1076.

Koleckar, V., Kubikova, K., Rehakova, Z., Kuca, K., Jun, D., Jahodar, L. & Opletal, L., 2008. Condensed and hydrolysable tannins as antioxidants influencing the health. Mini Reviews in Medicinal Chemistry, 8(5), 436-447.

Kuhnt, K., Degen, C. & Jahreis, G., 2016. Evaluation of the impact of ruminant trans fatty acids on human health: Important aspects to consider. Critical Reviews in Food Science and Nutrition, 56(12), 1964-1980.

Lacroix, E., Charest, A., Cyr, A., Baril-Gravel, L., Lebeuf, Y., Paquin, P., Chouinard, P. Y., Couture, P. & Lamarche, B., 2012. Randomized controlled study of the effect of a butter naturally enriched in trans fatty acids on blood lipids in healthy women. The American Journal of Clinical Nutrition, 95(2), 318-325.

Ladeira, M. M., Santarosa, L. C., Chizzotti, M. L., Ramos, E. M., Machado Neto, O. M., Oliveira, D. M., Carvalho, J. R. R., Lopes, L.S. & Ribeiro, J. S., 2014. Fatty acid profile, colour and lipid oxidation of meat from young bulls fed ground soybean or rumen protected fat with or without monensin. Meat Science, 96(1), 597-605.

Laliotis, G. P., Bizelis, I. & Rogdakis, E., 2010. Comparative approach of the *de novo* fatty acid synthesis (lipogenesis) between ruminant and non ruminant mammalian species: From biochemical level to the main regulatory lipogenic genes. Current Genomics, 11(3), 168-183.

Leal, L. N., Beltrán, J. A., Bellés, M., Bello, J. M., den Hartog, L. A., Hendriks, W. H. & Martín-Tereso, J., 2020. Supplementation of lamb diets with vitamin E and rosemary extracts on meat quality parameters. Journal of the Science of Food and Agriculture, 100(7), 2922-2931.

Leat, W. M. F. & Harrison, F. A., 1969. Lipid digestion in the sheep: effect of bile and pancreatic juice on the lipids of intestinal contents. Quarterly Journal of Experimental Physiology and Cognate Medical Sciences: Translation and Integration, 54(2), 187-201.

Leat, W. M. F. & Harrison, F. A., 1974. Origin and formation of lymph lipids in the sheep. Quarterly Journal of Experimental Physiology and Cognate Medical Sciences: Translation and Integration, 59(2), 131-139.

Ledward, D. A., 1992. Colour of raw and cooked meat. Special publication-Royal Society of Chemistry, 106, 128-128 (cited by Mckenna *et al*., 2005).

Lee, K., Paek, K., Lee, H. Y., Park, J. H. & Lee, Y., 2007. Antiobesity effect of trans-10, cis-12-conjugated linoleic acid-producing Lactobacillus plantarum PL62 on diet-induced obese mice. Journal of Applied Microbiology, 103(4), 1140-1146.

Lefaucheur, L., 2010. A second look into fibre typing–relation to meat quality. Meat Science, 84(2), 257-270.

Leiber, F., Willems, H., Werne, S., Ammer, S. & Kreuzer, M., 2019. Effects of vegetation type and breed on n-3 and n-6 fatty acid proportions in heart, lung and brain phospholipids of lambs. Small Ruminant Research, 171, 99-107.

Leroy, F. & Praet, I., 2015. Meat traditions. The co-evolution of humans and meat. Appetite, 90, 200-211.

Leth, T., Jensen, H. G., Mikkelsen, A. Æ. & Bysted, A., 2006. The effect of the regulation on trans fatty acid content in Danish food. Atherosclerosis Supplements, 7(2), 53-56.

Li, L., Davis, J., Nolan, J. & Hegarty, R., 2012. An initial investigation on rumen fermentation pattern and methane emission of sheep offered diets containing urea or nitrate as the nitrogen source. Animal Production Science, 52(7), 653-658.

Lima, P. D. M. T., Crouzoulon, P., Sanches, T. P., Zabré, G., Kabore, A., Niderkorn, V., Hoste, H., do Amarante, A. F. T., Costa- Júnior, L. M., Abdulla, A, L. & Louvandini, H., 2019. Effects of *Acacia mearnsii* supplementation on nutrition, parasitological, blood parameters and methane emissions in Santa Inês sheep infected with *Trichostrongylus colubriformis* and *Haemonchus contortus*. Experimental Parasitology, 207, 107777.

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Picard, B. & Bugeon, J.2, 2016. How muscle structure and composition influence meat and flesh quality. The Scientific World Journal, 2016, 3182746.

Liu, G., Ding, Z., Li, X., Chen, X., Wu, Y. & Xie, L., 2016. Relationship between polyunsaturated fatty acid levels in maternal diets and human milk in the first month post-partum. Journal of Human Nutrition and Dietetic, 29(4), 405-410.

Lock, A. L., Harvatine, K. J., Drackley, J. K. & Bauman, D. E., 2006. Concepts in fat and fatty acid digestion in ruminants. In: Proceedings of the Intermountain Nutrition Conference. pp. 85-100.

Lodish, H. F. & Rothman, J. E., 1979. The assembly of cell membranes. Scientific American, 240(1), 48-63.

Lopez, B., Lupi, T. M., León, J. M., Lopez, F., Agudo, B. & Delgado, J. V., 2018. Characterization of the commercial growth curves of Spanish Merino, Fleischschaf, and crossbred lambs in an associative economy context. Small Ruminant Research, 164, 8-14.

López-Andrés, P., Luciano, G., Vasta, V., Gibson, T. M., Biondi, L., Priolo, A. & Mueller-Harvey, I., 2013. Dietary quebracho tannins are not absorbed, but increase the antioxidant capacity of liver and plasma in sheep. British Journal of Nutrition, 110(4), 632-639.

Lopez-Huertas, E., 2010. Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. Pharmacological research, 61(3), 200-207.

Lourenço, M., Cardozo, P. W., Calsamiglia, S. & Fievez, V., 2008. Effects of saponins, quercetin, eugenol, and cinnamaldehyde on fatty acid biohydrogenation of forage polyunsaturated fatty acids in dual-flow continuous culture fermenters. Journal of Animal Science, 86(11), 3045-3053.

Lourenço, M., Ramos-Morales, E. & Wallace, R. J., 2010. The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. Animal, 4(7), 1008-1023.

Lourenço, M., Van Ranst, G., De Smet, S., Raes, K. & Fievez, V., 2007. Effect of grazing pastures with different botanical composition by lambs on rumen fatty acid metabolism and fatty acid pattern of longissimus muscle and subcutaneous fat. Animal, 1(4), 537-545.

Luciano, G., Monahan, F. J., Vasta, V., Biondi, L., Lanza, M. & Priolo, A., 2009a. Dietary tannins improve lamb meat colour stability. Meat Science, 81(1), 120-125.

Luciano, G., Monahan, F., Vasta, V., Biondi, L., Lanza, M., Pennisi, P. & Priolo, A., 2009b. Lamb meat colour stability as affected by dietary tannins. Italian Journal of Animal Science, 8(sup2), 507-509.

Luciano, G., Vasta, V., Monahan, F. J., López-Andrés, P., Biondi, L., Lanza, M. & Priolo, A., 2011. Antioxidant status, colour stability and myoglobin resistance to oxidation of *longissimus dorsi* muscle from lambs fed a tannin-containing diet. Food chemistry, 124(3), 1036-1042.

Ma, X., Jiang, Z. & Lai, C., 2016. Significance of increasing n-3 PUFA content in pork on human health. Critical Reviews in Food Science and Nutrition, 56(5), 858-870.

MacDougall, D. B.,1982. Changes in the colour and opacity of meat. Food Chemistry, 9(1-2), 75-88.

Maia, M. R., Chaudhary, L. C., Figueres, L. & Wallace, R. J., 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie Van Leeuwenhoek, 91(4), 303-314.

Makkar, H. P. S. & Becker, K., 1997. Degradation of quillaja saponins by mixed culture of rumen microbes. Letters in Applied Microbiology, 25(4), 243-245.

Makkar, H. P. S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small ruminant research, 49(3), 241-256.

Mamalakis, G., Kafatos, A., Kalogeropoulos, N., Andrikopoulos, N., Daskalopulos, G. & Kranidis, A., 2002. Prostate cancer vs hyperplasia: Relationships with prostatic and adipose tissue fatty acid composition. Prostaglandins, Leukotrienes and Essential Fatty Acids, 66(5-6), 467-477.

Mancini, R. A. & Hunt, M., 2005. Current research in meat color. Meat Science, 71(1), 100-121.

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P. W. & Cotter, P. D., 2019. The rumen microbiome: A crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. Gut Microbes, 10(2), 115-132.

McAllister, T. A., Meale, S. J., Valle, E., Guan, L. L., Zhou, M. Kelly, W. J., Henderson, G., Attwood, G.t. & Janssen, P. H. (2015). Ruminant nutrition symposium: Use of genomics and transcriptomics to identify strategies to lower ruminal methanogenesis. Journal of animal science, 93(4), 1431-1449.

McDonald, P., Edwards, R.A., Greenhalgh, J. F. D, Morgan, C. A., Sinclair, L. A., Wilkinson, R. G. Metabolism., 2011. In: Animal Nutrition. 7th ed. Pearson Educational Limited. Harlow, England. pp 192, 221.

McKenna, D. R., Mies, P. D., Baird, B. E., Pfeiffer, K. D., Ellebracht, J. W. & Savell, J. W., 2005. Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. Meat Science, 70(4), 665-682.

Mensink, R. P., de Louw, M. H. & Katan, M. B., 1991. Effects of dietary trans fatty acids on blood pressure in normotensive subjects. European Journal of Clinical Nutrition, 45, 375-382.

Micha, R. & Mozaffarian, D., 2009. Trans fatty acids: effects on metabolic syndrome, heart disease and diabetes. Nature Reviews Endocrinology, 5(6), 335-344.

Milford, A. B., Le Mouël, C., Bodirsky, B. L. & Rolinski, S., 2019. Drivers of meat consumption. Appetite, 141, 104313.

Min, B. R., Barry, T. N., Attwood, G. T. & McNabb, W. C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. Animal Feed Science and Technology, 106(1-4), 3-19.

Min, B. R., Fernandez, J. M., Barry, T. N., McNabb, W. C. & Kemp, P. D., 2001. The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in ewes during autumn. Animal Feed Science and Technology, 92(3-4), 185-202.

Min, B. R., McNabb, W. C., Barry, T. N., Kemp, P. D., Waghorn, G. C. & McDonald, M. F., 1999. The effect of condensed tannins in Lotus corniculatus upon reproductive efficiency and wool

production in sheep during late summer and autumn. The Journal of Agricultural Science, 132(3), 323-334.

Min, B. R., Pinchak, W. E., Merkel, R., Walker, S., Tomita, G. & Anderson, R. C., 2008. Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens. Scientific Research and Essay, 3(2), 066-073.

Min, B. R., Solaiman, S., Taha, E. & Lee, J., 2015. Effect of plant tannin-containing diet on fatty acid profile in meat goats., Journal of Animal Research and Nutrition. 1, 1-5.

Min, B. R., Solaiman, S., Waldrip, H. M., Parker, D., Todd, R. W. & Brauer, D., 2020. Dietary mitigation of enteric methane emissions from ruminants: A review of plant tannin mitigation options. Animal Nutrition, 6(3), 231-246.

Minihane, A. M. & Lovegrove, J. A., 2006. Health benefits of polyunsaturated fatty acids (PUFAs). In: Improving the fat content of foods. Eds. Williams, C. & Buttriss, J., Woodhead Publishing Ltd, Cambridge. pp. 107-140.

Moeletsi, M. E., Tongwane, M. I. & Tsubo, M., 2017. Enteric methane emissions estimate for livestock in South Africa for 1990–2014. Atmosphere, 8(5), 69.

Mohammadi, I., Mahdavi, A. H., Rabiee, F., Esfahani, M. H. N. & Ghaedi, K., 2020. Positive effects of conjugated linoleic acid (CLA) on the PGC1-α expression under the inflammatory conditions induced by TNF-α in the C2C12 cell line. Gene, 735, 144394.

Morgavi, D. P., Forano, E., Martin, C. & Newbold, C. J., 2010. Microbial ecosystem and methanogenesis in ruminants. Animal, 4(7), 1024-1036.

Mosley, E. E., Shafii, B., Moate, P. J. & McGuire, M. A., 2006. Cis-9, trans-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. The Journal of Nutrition, 136(3), 570-575.

Motard-Bélanger, A., Charest, A., Grenier, G., Paquin, P., Chouinard, Y., Lemieux, S., Couture, P. & Lamarche, B., 2008. Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. The American Journal of Clinical Nutrition, 87(3), 593-599.

Mozaffarian, D. & Stampfer, M. J., 2010. Removing industrial trans fat from foods. BMj, 340, c1826

Mukumbo, F.E. & Muchenje, V., 2016. Producing pork to meet modern consumer demands. SAPPO Industry-Commmissioned Review paper requested and submitted to the South African Pig Producers Association (SAPPO).

Noble, R. C., 1981. Digestion, absorption and transport of lipids in ruminant animals. In: Lipid Metabolism in Ruminant Animals. Ed: Christie, C. W., Pergamon Press Inc., New York, USA. pp. 57-93.

Nolan, J. V., Hegarty, R. S., Hegarty, J., Godwin, I. R. & Woodgate, R., 2010. Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. Animal Production Science, 50(8), 801-806.

Noone, E. J., Roche, H. M., Nugent, A. P. & Gibney, M. J., 2002. The effect of dietary supplementation using isomeric blends of conjugated linoleic acid on lipid metabolism in healthy human subjects. British Journal of Nutrition, 88(3), 243-251.

Nudda, A., Battacone, G., Boaventura Neto, O., Cannas, A., Francesconi, A. H. D., Atzori, A. S. & Pulina, G., 2014. Feeding strategies to design the fatty acid profile of sheep milk and cheese. Revista Brasileira de Zootecnia, 43, 445-456.

Nuernberg, K., Dannenberger, D., Nuernberg, G., Ender, K., Voigt, J., Scollan, N. D., Ender, K., Voigt, J., Wood, G. R. & Richardson, R. I., 2005. Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. Livestock Production Science, 94(1-2), 137-147.

OECD/FAO, 2020. Meat. In: OECD-FAO Agricultural Outlook 2020-2029. OECD Publishing, Paris. pp 162-179. [Online]. Available from: https://doi.org/10.1787/29248f46-en. [Accessed 16/11/2022]

Okeudo, N. J. & Moss, B. W., 2007. Intramuscular lipid and fatty acid profile of sheep comprising four sex-types and seven slaughter weights produced following commercial procedure. Meat Science, 76(2), 195-200.

Opio, C., Gerber, P., Mottet, A., Falcucci, A., Tempio, G., MacLeod, M., Vellinga, T., Henderson, B. & Steinfeld, H., 2013. Greenhouse gas emissions from ruminant supply chains – A global life cycle assessment. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. pp. 37

Owens, F. N., Dubeski, P. & Hanson, C. F., 1993. Factors that alter the growth and development of ruminants. Journal of Animal Science, 71(11), 3138-3150.

Palmquist, D. L., St-Pierre, N. & McClure, K. E., 2004. Tissue fatty acid profiles can be used to quantify endogenous rumenic acid synthesis in lambs. The Journal of Nutrition, 134(9), 2407-2414.

Pannier, L., Pethick, D. W., Geesink, G. H., Ball, A. J., Jacob, R. H. & Gardner, G. E., 2014. Intramuscular fat in the longissimus muscle is reduced in lambs from sires selected for leanness. Meat Science, 96(2), 1068-1075.

Park, W. J. (2018). The biochemistry and regulation of fatty acid desaturases in animals. In: Polyunsaturated fatty acid metabolism. Ed: Burdge, G. C., AOAC Press. pp. 87-100.

Parodi, P. W., 2004. Milk fat in human nutrition. Australian Journal of Dairy Technology, 59(1), 3-59.

Parvar, R., Ghoorchi, T., Kashfi, H. & Parvar, K., 2018. Effect of *Ferulago angulata* (Chavil) essential oil supplementation on lamb growth performance and meat quality characteristics. Small Ruminant Research, 167, 48-54.

Patra, A. K. & Saxena, J., 2009. The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutrition research reviews, 22(2), 204-219.

Pethick, D. W., Harper, G. S. & Oddy, V. H., 2004. Growth, development and nutritional manipulation of marbling in cattle: A review. Australian Journal of Experimental Agriculture, 44(7), 705-715.

Piñeiro-Vázquez, A. T., Canul-Solís, J. R., Alayón-Gamboa, J. A., Chay-Canul, A. J., Ayala-Burgos, A. J., Aguilar-Pérez, C. F., Solorio-Sánchez, F.J. & Ku-Vera, J. C., 2015. Potential of condensed tannins for the reduction of emissions of enteric methane and their effect on ruminant productivity. Archivos de Medicina Veterinaria, 47(3), 263-272.

Popova, T., Gonzales-Barron, U. & Cadavez, V., 2015. A meta-analysis of the effect of pasture access on the lipid content and fatty acid composition of lamb meat. Food Research International, 77, 476-483.

Prache, S., Schreurs, N. & Guillier, L., 2021. Factors affecting sheep carcass and meat quality attributes. Animal, 100330.

Precht, D. & Molkentin, J., 2000. Recent trends in the fatty acid composition of German sunflower margarines, shortenings and cooking fats with emphasis on individual C16: 1, C18: 1, C18: 2, C18: 3 and C20: 1 trans isomers. Nahrung, 44(4), 222-228.

Priolo, A., Micol, D., Agabriel, J., Prache, S. & Dransfield, E., 2002. Effect of grass or concentrate feeding systems on lamb carcass and meat quality. Meat Science, 62(2), 179-185.

Priolo, A., Bella, M., Lanza, M., Galofaro, V., Biondi, L., Barbagallo, D., Ben Salem, M. & Pennisi, P., 2005. Carcass and meat quality of lambs fed fresh sulla (*Hedysarum coronarium L.*) with or without polyethylene glycol or concentrate. Small Ruminant Research, 59(2-3), 281-288.

Priolo, Q. & Vasta, V., 2007. Effects of tannin-containing diets on small ruminant meat quality. Italian Journal of Animal Science, 6(sup1), 527-530.

Ramanathan, R., Nair, M. N., Hunt, M. C. & Suman, S. P., 2019. Mitochondrial functionality and beef colour: A review of recent research. South African Journal of Animal Science, 49(1), 9-19.

Rana, M. S., Tyagi, A., Hossain, S. A. & Tyagi, A. K., 2012. Effect of tanniniferous *Terminalia chebula* extract on rumen biohydrogenation, Δ 9-desaturase activity, CLA content and fatty acid composition in *longissimus dorsi* muscle of kids. Meat Science, 90(3), 558-563.

Ran-Ressler, R. R., Bae, S., Lawrence, P., Wang, D. H. & Brenna, J. T., 2014. Branched-chain fatty acid content of foods and estimated intake in the USA. British Journal of Nutrition, 112(4), 565-572.

Ratnayake, W. N. & Galli, C., 2009. Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism. Annals of nutrition & metabolism, 55(1/3), 8-43.

Realini, C. E., Duckett, S. K., Brito, G. W., Dalla Rizza, M. & De Mattos, D., 2004. Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. Meat Science, 66(3), 567-577.

Restrepo, B. J. & Rieger, M., 2016. Denmark's policy on artificial trans fat and cardiovascular disease. American Journal of Preventive Medicine, 50(1), 69-76.

Restrepo, B. J. & Rieger, M., 2016. Denmark's policy on artificial trans fat and cardiovascular disease. American Journal of Preventive Medicine, 50(1), 69-76.

Sabbioni, A., Beretti, V., Zambini, E. M., Superchi, P. & Ablondi, M., 2019. Allometric coefficients for physical-chemical parameters of meat in a local sheep breed. Small Ruminant Research, 174, 141-147.

Sacks, F. M., Lichtenstein, A. H., Wu, J. H., Appel, L. J., Creager, M. A., Kris-Etherton, P. M., Miller, M., Rimm, E. B., Rudel, L. L., Robinson, J, G., Stone, N, J. & Van Horn, L. V., 2017. Dietary fats and cardiovascular disease: A presidential advisory from the American Heart Association. Circulation, 136(3), e1-e23.

Safari, E., Sefidbakht, N. & Farid, A., 1988. Effect of castration and cryptorchidism on fatty acid content of ovine adipose tissue. Meat Science, 23(1), 65-69.

Sans, P. & Combris, P., 2015. World meat consumption patterns: An overview of the last fifty years (1961–2011). Meat Science, 109, 106-111.

Santos-Silva, J., Mendes, I. A., Portugal, P. V. & Bessa, R. J. B., 2004. Effect of particle size and soybean oil supplementation on growth performance, carcass and meat quality and fatty acid composition of intramuscular lipids of lambs. Livestock Production Science, 90(2-3), 79-88.

Sañudo, C., Sierra, I., Olleta, J. L., Martin, L., Campo, M. M., Santolaria, P., Santolaria, P., Wood, J. D. & Nute, G. R., 1998. Influence of weaning on carcass quality, fatty acid composition and meat quality in intensive lamb production systems. Animal Science, 66(1), 175-187.

Saric, T., Rogosic, J., Zupan, I., Beck, R., Bosnic, S., Sikic, Z., Skobic, D. & Tkalcic, S., 2015. Anthelmintic effect of three tannin-rich Mediterranean shrubs in naturally infected sheep. Small Ruminant Research, 123(1), 179-182.

Scaife, J. R., Wahle, K. W. & Garton, G. A., 1978. Utilization of methylmalonate for the synthesis of branched-chain fatty acids by preparations of chicken liver and sheep adipose tissue. Biochemical Journal, 176(3), 799-804.\

Scholtz, M. M., Jordaan, F. J., Thuli Chabalala, N., Pyoos, G. M., Joel Mamabolo, M. & Neser, F. W., 2023. A balanced perspective on the contribution of extensive ruminant production to greenhouse gas emissions in southern Africa. African Journal of Range & Forage Science, 40(1), 107-113.

Scholtz, M. M., Van Ryssen, J. V., Meissner, H. H. & Laker, M. C., 2013. A South African perspective on livestock production in relation to greenhouse gases and water usage. South African Journal of Animal Science, 43(3), 247-254.

Schönfeld, P. & Wojtczak, L., 2016. Short-and medium-chain fatty acids in energy metabolism: The cellular perspective. Journal of Lipid Research, 57(6), 943-954.

Schönfeldt, H. C. & Hall, N., 2015. Nutrient content of South African red meat and the effect of age and production system. South African Journal of Animal Science, 45(3), 313-324.

Schönfeldt, H. C., Sainsbury, J., Gibson, N. & Van Heerden, S. M., 2011. Nutrient content of uncooked and cooked meat from South African classes A2 lamb and C2 mutton. South African Journal of Animal Science, 41(2), 141-145.

Sebaaly, C., Greige-Gerges, H. & Charcosset, C., 2019. Lipid membrane models for biomembrane properties' investigation. In: Current trends and future developments on (bio-) membranes. Ed: Basile, A. & Charcosset, C., Elsevier. pp. 311-340.

Senyilmaz-Tiebe, D., Pfaff, D. H., Virtue, S., Schwarz, K. V., Fleming, T., Altamura, S., Muckenthaler, M.U., Okun, J.G., Vidal-Puig, A., Nawroth, P. & Teleman, A. A., 2018. Dietary stearic acid regulates mitochondria *in vivo* in humans. Nature Communications, 9(1), 1-10.

Shen, M. C., Zhao, X., Siegal, G. P., Desmond, R. & Hardy, R. W., 2014. Dietary stearic acid leads to a reduction of visceral adipose tissue in athymic nude mice. PLoS one, 9(9), e104083.

Shingfield, K. J., Bernard, L., Leroux, C. & Chilliard, Y., 2010. Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal, 4(7), 1140-1166.

Shingfield, K. J., Bonnet, M. & Scollan, N. D., 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal, 7(s1), 132-162.

Shisana, O., Labadarios, D., Rehle, T., Simbayi, L., Zuma, K., Dhansay, A., Reddy, P., Parker, W., Hoosain, E., Naidoo, P., Hongoro, C., Mchiza, Z., Steyn, N. P., Dwane, N., Makoae, M., Maluleke, T., Ramlagan, S., Zungu, N., Evans, M. G., Jacobs, L., Faber, M. & the SANHANES-1 Team., 2014. South African National Health and Nutrition Examination Survey (SANHANES-1). 2014 ed. HSRC Press, Cape Town, South Africa. pp. 91.

Shramko, V. S., Polonskaya, Y. V., Kashtanova, E. V., Stakhneva, E. M. & Ragino, Y. I., 2020. The short overview on the relevance of fatty acids for human cardiovascular disorders. Biomolecules, 10(8), 1127.

Simopoulos, A. P., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy, 56(8), 365-379.

Sinclair, L. A., 2007. Nutritional manipulation of the fatty acid composition of sheep meat: A review. The Journal of Agricultural Science, 145(5), 419-434.

Śliwiński, B. J., Kreuzer, M., Wettstein, H. R. & Machmüller, A., 2002. Rumen fermentation and nitrogen balance of lambs fed diets containing plant extracts rich in tannins and saponins, and associated emissions of nitrogen and methane. Archives of Animal Nutrition, 56(6), 379-392.

Smeti, S., Hajji, H., Mekki, I., Mahouachi, M. & Atti, N., 2018. Effects of dose and administration form of rosemary essential oils on meat quality and fatty acid profile of lamb. Small Ruminant Research, 158, 62-68.

Smith, S. B. & Crouse, J. D., 1984. Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. The Journal of Nutrition, 114(4), 792-800.

Smith, S. B., Lunt, D. K., Chung, K. Y., Choi, C. B., Tume, R. K. & Zembayashi, M., 2006. Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. Animal Science Journal, 77(5), 478-486.

Smith, S. B., Lunt, D. K., Smith, D. R. & Walzem, R. L., 2020. Producing high-oleic acid beef and the impact of ground beef consumption on risk factors for cardiovascular disease: A review. Meat Science, 163, 108076.

Solomon, M. B., Lynch, G. P., Ono, K. & Paroczay, E., 1990. Lipid composition of muscle and adipose tissue from crossbred ram, wether and cryptorchid lambs. Journal of Animal Science, 68(1), 137-142.

Sommerfeld, M., 1983. Trans unsaturated fatty acids in natural products and processed foods. Progress in Lipid Research, 22(3), 221-233.

Song, H. J., Grant, I., Rotondo, D., Mohede, I., Sattar, N., Heys, S. D. & Wahle, K. W. J., 2005. Effect of CLA supplementation on immune function in young healthy volunteers. European Journal of Clinical Nutrition, 59(4), 508-517.

Spruk, R. & Kovac, M., 2020. Does a ban on trans fats improve public health: Synthetic control evidence from Denmark. Swiss Journal of Economics and Statistics, 156(1), 1-32.

Steenson, S., Umpleby, A. M., Lovegrove, J. A., Jackson, K. G. & Fielding, B. A., 2017. Role of the enterocyte in fructose-induced hypertriglyceridaemi. Nutrients, 9(4), 349.

Stender, S., Astrup, A. & Dyerberg, J., 2008. Ruminant and industrially produced trans fatty acids: Health aspects. Food & Nutrition Research, 52(1), 1651.

Stender, S., Dyerberg, J., Bysted, A., Leth, T. & Astrup, A., 2006. A trans world journey. Atherosclerosis Supplements, 7(2), 47-52.

Tan, H.Y., Sieo, C.C., Abdullah, N., Liang, J.B., Huang, X.D. & Ho, Y.W., 2011. Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. Animal Feed Science and Technology, 169, 185-193.

Taormina, V. M., Unger, A. L., Schiksnis, M. R., Torres-Gonzalez, M. & Kraft, J., 2020. Branched-chain fatty acids—An underexplored class of dairy-derived fatty acids. Nutrients, 12(9), 2875.

Tebaldi, C., Ranasinghe, R., Vousdoukas, M., Rasmussen, D. J., Vega-Westhoff, B., Kirezci, E., Kopp, R.E., Sriver, R. & Mentaschi, L., 2021. Extreme sea levels at different global warming levels. Nature Climate Change, 11(9), 746-751.

Teixeira, N., Mateus, N. & de Freitas, V., 2016. Updating the research on prodelphinidins from dietary sources. Food Research International, 85, 170-181.

Toomey, S., Harhen, B., Roche, H. M., Fitzgerald, D. & Belton, O., 2006. Profound resolution of early atherosclerosis with conjugated linoleic acid. Atherosclerosis, 187(1), 40-49.

Tricon, S. & Yaqoob, P., 2006. Conjugated linoleic acid and human health: a critical evaluation of the evidence. Current Opinion in Clinical Nutrition & Metabolic Care, 9(2), 105-110.

Tshabalala, P. A., Strydom, P. E., Webb, E. C. & De Kock, H. L., 2003. Meat quality of designated South African indigenous goat and sheep breeds. Meat Science, 65(1), 563-570.

Turpeinen, A. M., Mutanen, M., Aro, A., Salminen, I., Basu, S., Palmquist, D. L. & Griinari, J. M., 2002. Bioconversion of vaccenic acid to conjugated linoleic acid in humans. The American journal of clinical nutrition, 76(3), 504-510.

Ungerfeld, E. M., 2015. Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: A meta-analysis. Frontiers in Microbiology, 6, 37. (Cited by Mohini *et al*., 2017)

Vahmani, P., Ponnampalam, E. N., Kraft, J., Mapiye, C., Bermingham, E. N., Watkins, P. J., Proctor, S.D. & Dugan, M. E., 2020. Bioactivity and health effects of ruminant meat lipids. Invited Review. Meat Science, 165, 108114.

Valenti, B., Natalello, A., Vasta, V., Campidonico, L., Roscini, V., Mattioli, S., Mattioli, S., Pauselli, M., Priolo, A., Lanza, M. & Luciano, G., 2019. Effect of different dietary tannin extracts on lamb growth performances and meat oxidative stability: Comparison between mimosa, chestnut and tara. Animal, 13(2), 435-443.

Van de Vijver, L. P. L., Kardinaal, A. F. M., Couet, C., Aro, A., Kafatos, A., Steingrimsdottir, L., Amorim cruz, J.A., Moreiras, O., Becker, W., van Amelsvoort, J. M. M., Vidal-Jessel, S., Salminen, I., Moschandreas, J., Sigfsúson, N., Martins, I., Carbajal, A., Ytterfoerd, A. & Van Poppel, G., 2000. Association between trans fatty acid intake and cardiovascular risk factors in Europe: The TRANSFAIR study. European Journal of Clinical Nutrition, 54(2), 126-135.

Van der Merwe, D. A., Brand, T. S. & Hoffman, L. C., 2019. Application of growth models to different sheep breed types in South Africa. Small Ruminant Research, 178, 70-78.

Van Heerden, S. M., Schönfeldt, H. C., Smith, M. F. & van Rensburg, D. J., 2002. Nutrient content of South African chickens. Journal of Food Composition and Analysis, 15(1), 47-64.

Van Heerden, S. M. & Smith, M. F., 2013. The nutrient composition of three cuts obtained from P-class South African pork carcasses. Food Chemistry, 140(3), 458-465.

Van Heerden, S. M., Schönfeldt, H. C., Kruger, R. & Smit, M. F., 2007. The nutrient composition of South African lamb (A2 grade). Journal of Food Composition and Analysis, 20(8), 671-680.

Van Meer, G., Voelker, D. R. & Feigenson, G. W., 2008. Membrane lipids: Where they are and how they behave. Nature Reviews Molecular cell biology, 9(2), 112-124.

Van Ranst, G., Lee, M. R. F. & Fievez, V., 2011. Red clover polyphenol oxidase and lipid metabolism. Animal, 5(4), 512-521.

Van Zijderveld, S. M., Gerrits, W. J. J., Apajalahti, J. A., Newbold, J. R., Dijkstra, J., Leng, R. A. & Perdok, H. B., 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. Journal of Dairy Science, 93(12), 5856-5866.

Vasta, V. & Priolo, A., 2006. Ruminant fat volatiles as affected by diet. A review. Meat Science, 73(2), 218-228.

Vasta, V., Mele, M., Serra, A., Scerra, M., Luciano, G., Lanza, M. & Priolo, A., 2009. Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. Journal of Animal Science, 87(8), 2674-2684.

Vasta, V., Pennisi, P., Lanza, M., Barbagallo, D., Bella, M. & Priolo, A., 2007. Intramuscular fatty acid composition of lambs given a tanniniferous diet with or without polyethylene glycol supplementation. Meat Science, 76(4), 739-745.

Vasta, V., Yáñez-Ruiz, D. R., Mele, M., Serra, A., Luciano, G., Lanza, M., Biondi, L. & Priolo, A., 2010. Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. Applied and Environmental Microbiology, 76(8), 2549-2555.

Villalobos-Villalobos, G., González-Rios, H., Núñez-González, F. A., Domínguez-Díaz, D., Peña-Ramos, E. A. & Huerta-Leidenz, N., 2014. Effects of castration and zeranol on fatty acid composition and cholesterol content of hair lamb meat. Journal of Applied Animal Research, 42(1), 65-72.

Vorster, H. H., Badham, J. B. & Venter, C. S., 2011. An introduction to the revised food-based dietary guidelines for South Africa. South African Journal of Clinical Nutrition, 26(3), S1-S164

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. Animal Feed Science and Technology, 147(1-3), 116-139.

Wallace, M. & Metallo, C. M., 2020. Tracing insights into *de novo* lipogenesis in liver and adipose tissues. Seminars in Cell & Developmental Biology, 108, 65-71.

Wang, B., Tu, Y., Zhao, S. P., Hao, Y. H., Liu, J. X., Liu, F. H., Xiong, B. H. & Jiang, L. S., 2017. Effect of tea saponins on milk performance, milk fatty acids, and immune function in dairy cow. Journal of Dairy Science, 100(10), 8043-8052.

Wang, J., Guan, Y., Wu, L., Guan, X., Cai, W., Huang, J., Wenjie, D. & Zhang, B., 2021. Changing lengths of the four seasons by global warming. Geophysical Research Letters, 48(6), e2020GL091753.

Wang, L., Manson, J. E., Forman, J. P., Gaziano, J. M., Buring, J. E. & Sesso, H. D., 2010. Dietary fatty acids and the risk of hypertension in middle-aged and older women. Hypertension, 56(4), 598-604.

Watkins, P. J., Kearney, G., Rose, G., Allen, D., Ball, A. J., Pethick, D. W. & Warner, R. D., 2014. Effect of branched-chain fatty acids, 3-methylindole and 4-methylphenol on consumer sensory scores of grilled lamb meat. Meat Science, 96(2), 1088-1094.

Watkins, P. J., Rose, G., Salvatore, L., Allen, D., Tucman, D., Warner, R. D., Dunshea, F. R. & Pethick, D. W., 2010. Age and nutrition influence the concentrations of three branched chain fatty acids in sheep fat from Australian abattoirs. Meat Science, 86(3), 594-599.

Webb, E. C. & Casey, N. H., 1995. Genetic differences in fatty acid composition of subcutaneous adipose tissue in Dorper and SA Mutton Merino wethers at different live weights. Small Ruminant Research, 18(1), 81-88.

Webb, E. C. (2021). Cis/trans-fatty acid content of red meats and the related effects on meat quality and human health. In: Meat and Nutrition. Ed. Ranabhat, C. L., Interchopen Limited. pp. 121-140.

Webb, E. C., Hassen, A., Olaniyi, M. O. & Pophiwa, P., 2022. Effect of dietary inclusion of *Azadirachta indica* and *Moringa oleifera* leaf extracts on the carcass quality and fatty acid composition of lambs fed high forage total mixed rations. Animals, 12(16), 2039.

WHO, 2004. Discussion paper on definition of trans fatty acids. Codex committee on nutrition and foods for special dietary uses. pp. 2. [Online]. Available from: www.codexalimentarious.net. [Accessed: 15/11/2022].

WHO, 2018. WHO plans to eliminate industrially-produced trans-fatty acids from global food supply. [Online]. Available from: www.who.int/news/item/14-05-2018-who-plam-to-eliminate-industrially-produced-rans-fatty-acids-from-global-food-supply. [Accessed: 14/05/2018].

WHO, 2021. World health statistics 2021: Monitoring health for the SDGs, sustainable development goals. World Health Organization. pp 30. [Online]. Available from: https://apps.who.int/iris/handle/10665/342703. [Accessed: 16.11.2022].

Wiens, J. J., 2016. Climate-related local extinctions are already widespread among plant and animal species. PLoS Biology, 14(12), e2001104.

Williams, C. M. & Burdge, G., 2006. Long-chain n− 3 PUFA: plant v. marine sources. Proceedings of the Nutrition Society, 65(1), 42-50.

Williams, P., 2007. Nutritional composition of red meat. Nutrition & Dietetics, 64, S113-S119.

Wonghirundecha, S. & Sumpavapol, P., 2012. Antibacterial activity of selected plant by-products against foodborne pathogenic bacteria. In: International Conference on Nutrition and Food Sciences, 39 (11), 116-120).

World health statistics 2021: monitoring health for the SDGs, sustainable development goals. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO

Wood, J. D., 1984. Fat deposition and the quality of fat tissue in meat animals. In: Fats in Animal Nutrition. Ed: Wiseman, J., Anchor Brendon Ltd, Essex, Great Britain. pp. 407-435.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S.I. & Whittington, F. M., 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Science, 78(4), 343-358.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Whittington, F. M. & Richardson, R. I., 2003. Effects of diets on fatty acids and meat quality. Options Méditerranéennes, Series A, 67, 133-141.

Wood, J. D., Enser, M., Fisher, A., Nute, G. R., Richardson, R. I. & Sheard, P. R., 1999. Manipulating meat quality and composition. Proceedings of the Nutrition Society, 58(2), 363-370.

Wood, J. D., Enser, M., Richardson, R. I. & Whittington, F. M., 2007. Fatty acids in meat and meat products. In: Fatty acids in foods and their health implications. Ed: Chow, C. K., CRC Press. pp. 101-122.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P.R. & Enser, M., 2004. Effects of fatty acids on meat quality: A review. Meat science, 66(1), 21-32.

Woods, V. B. & Fearon, A. M., 2009. Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: A review. Livestock Science, 126(1-3), 1-20.

Woodside, J. V., McKinley, M. C. & Young, I. S., 2008. Saturated and trans fatty acids and coronary heart disease. Current Atherosclerosis Reports, 10(6), 460-466.

Wu, Z., Ohajuruka, O. A. & Palmquist, D. L., 1991. Ruminal synthesis, biohydrogenation, and digestibility of fatty acids by dairy cows. Journal of Dairy Science, 74(9), 3025-3034.

Young, O. A., Lane, G. A., Priolo, A. & Fraser, K., 2003. Pastoral and species flavour in lambs raised on pasture, lucerne or maize. Journal of the Science of Food and Agriculture, 83(2), 93-104.

Zandalinas, S. I., Fritschi, F. B. & Mittler, R., 2021. Global warming, climate change, and environmental pollution: Recipe for a multifactorial stress combination disaster. Trends in Plant Science, 26(6), 588-599.

Zhang, Z., Gillespie, C. & Yang, Q., 2017. Plasma trans-fatty acid concentrations continue to be associated with metabolic syndrome among US adults after reductions in trans-fatty acid intake. Nutrition Research, 43, 51-59.

Zhou, X. R., Sun, C. H., Liu, J. R. & Zhao, D., 2008. Dietary conjugated linoleic acid increases PPARγ gene expression in adipose tissue of obese rat, and improves insulin resistance. Growth Hormone & IGF Research, 18(5), 361-368.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Experimental design

The experiment was conducted at the Hatfield Experimental farm of the University of Pretoria with the approval of the Animal Ethics Committee: approval number EC061-14. The research was an extension of Festus Adejoro's PhD-study, titled "The effect of tannin and tannin utilisation on growth performance, nutrient digestibility and *in vivo* methane production" (Adejoro, 2019). A total of 40 Dohne Merino lambs (16 ewe lambs and 24 ram lambs) were randomly selected from the sheep flock of the University of Pretoria. The number of animals were limited due to ethical considerations relating to the number and size of pens at the research facility. Once the growth performance study was completed, the lambs were slaughtered at different weights for the current study.

In accordance with the randomised compete block design set by Adejoro (2019), 40 healthy four-month-old lambs were weighed and blocked according their weight (a total of five blocks). The average starting weight was 34.7kg (Adejoro, 2019). Ewe lambs were blocked into two weight categories and ram lambs were blocked into three weight categories. Two lambs within a block were randomly assigned to a pen. A pen from each block was assigned to one of four treatment groups; Urea-based total mixed ration (TMR), Urea-based total mixed ration with tannin, cacium nitrate (CaN) based TMR and CaN-based total mixed ration with tannin. There were therefore 5 pens per treatment group. The pens were 8x4m^2 in a well-ventilated building with open sides.

At the end of the production trial (60 days) two rams were randomly removed from each of the four treatment groups. Therefore, there were 4 rams and 4 ewes within each treatment group. Within each treatment, four lambs (two rams and two ewes) were slaughtered heavy and four lambs (two rams and two ewes) were slaughtered light. Therefore, eight of the 16 rams in the trial were slaughtered when they reached 50kg≤2n≤55kg (light weight category) and eight were slaughtered when they reached 55.1kg>2n>60kg (heavy weight category). Ewe lambs also went through a second round of selection so that eight were slaughtered between 45>2n>48kg (light weight category) and eight between 48.1>2n>51kg (heavy weight category). The purpose of the selection criteria was to get a variation of A2 and A3 carcasses, representative of different carcass fat contents. The animals were weighed weekly and slaughtered bi-weekly depending on when they reached their target slaughter weight.

3.2 Diet

All animals were supplied with an *ad libitum* total mixed ration (TMR) and water. The ration was formulated to contain a roughage to concentrate ratio of 43: 57%. The concentrate with urea or calcium nitrate was supplied by AFGRI (Pty) Ltd and the *Eragrostis curvula*, milled lucerne (*Medicago sativa*) and *Acacia mearnsii* tannin extract (UCL Company (Pty) Ltd. Dalton, South Africa) were added using a vertical mixer on the Hatfield Experimental Farm at the University of Pretoria. Kynoch (BV) calcium nitrate (a fertilizer grade calcium nitrate) from Agrimark (Pty) Ltd. was supplied to AFGRI (Pty) Ltd. Treatment one contained a TMR with urea as the non-protein nitrogen (NPN) source, treatment two contained urea and tannin supplementation, treatment three contained calcium nitrate (Protea chemicals (Pty) Ltd. Germiston, South Africa) as an NPN source, and treatment four contained calcium nitrate and tannin. The feed was mixed once a week to ensure it was fresh.

The *Acacia mearnsii* extract was included at 42g/kg feed DM. The extract contained a total phenol concentration of 65g/100g, total tannin concentration of 58.5g/100g (tannic acid equivalent) and condensed tannin concentration of 30.5g/100g (leucocyanidin equivalent) (Adejoro et al., 2020). This equated to a total of 17g CT/ kg feed as a leucocyanidin equivalent (Adejoro et al., 2020). The urea and calcium nitrate were provided above recommended concentrations to compensate for the tannin bound protein. The urea-based diet contained 10g/kg feed grade urea and the calcium nitrate-based diet contained 32g/kg calcium nitrate salt. To allow for the rumen microbial population to adapt to the new ration, the lamb grower pellets were replaced with their respective total mixed rations (TMR) over a three-week period. The proportion of TMR fed was increased by 30, 60 and 100% from week one to week three respectively, and the experimental animals remained on the diet until slaughter. Ingredients of experimental diet are provided in Table 3.2.1 (Adejoro, 2017).

Table 3.2.1: Ingredients of experimental diet (Adejoro, 2019)

	Urea-based diet		Calcium nitrate-based diet	
Ingredients (%)	No tannin	Tannin	No tannin	Tannin
Sunflower oilcake	16.8	16.1	17.1	16.4
Fine milled maize	27.6	26.4	28.2	27.0
Wheat bran	4.9	4.7	3.4	3.3
Molasses	5.9	5.7	5.1	4.9
Lucerne meal	19.70	18.9	19.0	18.2
Eragrostis hay	23.2	22.2	23.1	22.1
Coarse salt	0.5	0.5	0.5	0.5
Premix[1]	0.4	0.4	0.4	0.4
Urea	1.0	1.0	0	0
Nitrate[2]	0	0	3.2	3.1
Tannin extract	0	4.2	0	4.2

[1]Supplied as g/kg of the following: vit A 18,000 iu; vit D, 3920 iu; vit E, 2,45 iu; Zn, 5.0 mg; Mn, 4.1 mg; Cu, 0.5 mg; Se, 0.02mg; Mg, 28 mg; Ci, 0,2 mg. [2]25Ca(NO3)2.NH4.NO3.10H20; 155 g/kg N, 75% NO3 in DM. [3]Contains 350 g/kg condensed tannin (leucocyanidin equivalent). Adejoro (2019)

3.3 Animal care

The lambs were vaccinated with Pasteurella and Coglavax, dewormed with Zolvix, shorn and hooves trimmed before commencement of the trial. The pens and water troughs were cleaned every morning and the animals were fed twice daily. The pens could split in half in the event of a medical emergency. The lambs remained healthy during the duration of the trial. However, if there was a bump to a horn they were treated and separated for a day or two while the wound healed. When the slaughtering process began, sheep were regrouped with individuals in the same treatment and sex to prevent the stress of being in a pen alone.

3.4 Sample collection, storage and preparation:

Blood samples were taken from the jugular vein by jugular venopuncture on day 61 on feed from the 32 sheep used in the current study. Lambs were slaughtered at Klipeiland abattoir in Bronkhorspruit, South Africa, when they reached their slaughter weight category. The carcasses were electrically stunned, bled and chilled overnight. Tags with the animal's number on them were attached to the calcaneal tendon with a cable tie, after they had been skinned and before the head was removed.

Table 3.4.1: Summary of sample collection and analyses that were done on each sample

3-rib cut (RHS)			**SCF (LHS)**	**Perirenal fat**	**Rumen fluid**	**Blood**
Body composition			LCFA	LCFA	LCFA	Haematocrit Blood serum
Longissimus dorsi muscle			**SCF**			
pH, temperature, EMA			colour			
Colour						
DM & Ash		LCFA & EE				

3.4.1 Haematological and serum biochemical parameters

Two 5 mL blood samples were taken from the jugular vein on day 60 before the slaughtering process began and stored in an icebox. A haematocrit sample was taken to analyse packed cell volume, and a serum sample to analyse albumin, cholesterol and blood urea nitrogen (BUN). The haematocrit samples were collected in BD vacutainer tubes, and blood serum was collected in vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA). The samples were transported to the Clinical Pathology laboratory at the Faculty of Veterinary Science, University of Pretoria, South Africa for analysis the same morning.

3.4.2 Peri-renal fat

On the day of slaughter 5g of peri-renal fat (PRF) was collected from each lamb carcass and placed in zip lock bags. They were transported on ice and frozen at -20°C until analysis. The samples were cut up finely in a 4°C cold room before analysis.

3.4.3 Subcutaneous fat

At 24 hours after slaughter a 5g subcutaneous fat sample was dissected from each lamb carcass using an adaption of Casey *et al*. (1988). The sample was removed from the left side of each carcass over the 11th rib 25mm from the midline. A 25mm square was carefully removed using a clean scalpel blade and tweezers. They were placed in labelled zip lock bags and frozen at -20°C until analysis. The samples were cut up finely in a 4°C cold room before analysis.

3.4.4 Three-rib-cut sample

A three-rib-cut sample (8th to 10th ribs) was dissected from the right side of each carcass according to Casey *et al*. (1988). Its ventral cut was determined by a line drawn from the middle of the first rib to the cranial point of the symphysis of the pelvis. Samples were transported on ice to the University of Pretoria. Carcass pH, temperature, and eye muscle area were measured, after which the longissimus dorsi muscle was carefully dissected, weighed (for body composition) and used for colour analysis. During colour analysis (day one to six) the *longissimus dorsi* muscle was stored at 4°C. Once colour analysis was complete, it was stored at -20°C for proximate and long chain fatty acid (LCFA) analysis. Once all the lambs had been slaughtered, the *longissimus dorsi* muscle was defrosted at 4°C for 24 hours or until there were no more ice crystals. It was roughly cut and then homogenised for eight seconds using an IKA A10 analytical grinder/mill fitted with a removable IKA M21 spare cutter and a water-cooling system. The water from inside the bags was added to the homogenate. The grinder and blade were cleaned with ethanol and distilled water and then dried between samples. Five grams (5g) of *longissimus dorsi muscle* was removed for dry matter (DM) and ash and stored at -20°C. Twenty grams (20g) were used for LCFA analysis and the remainder was used for ether extract (EE). The ether extract sample was freeze dried on tin foil trays for seven days, and the intramuscular LCFA sample was freeze dried until the sample reached 1°C to prevent changes to the fatty acid profile. The condenser was set at -40°C and the shelf temperature was increased daily to allow for primary and secondary drying. The shelf temperatures were increased daily as follows: -40°C, -30, -20, -10, 0, 10 and out on the seventh day. Two digital thermometers were inserted into a sample on the top and bottom shelf to determine when the LCFA samples reached 1°C. Batch one of the long chain fatty acids samples came out on day three and the second batch came out on day two. The EE and LCFA samples were then crushed and milled to a fine powder for four seconds with an IKA A10 Analytical grinder/mill fitted with a removable IKA M23 star shaped cutting blade and a water-cooling system. The grinder was cleaned with ethanol and distilled water and dried between samples. The LCFA samples were stored in sterilised bottles at -20°C until analysis and the ether extract samples were stored at 4°C until analysis.

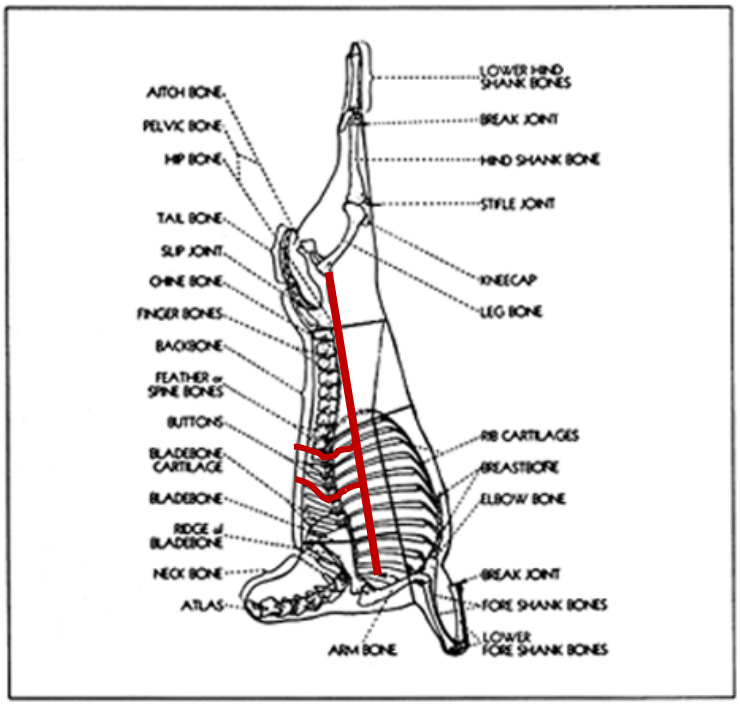

Figure 3.4.1: Three rib cut in colour. Adapted from: https://www.fao.org/3/t0279e/T0279E108.gif

3.3.4 Rumen fluid

Rumen content was mixed in a bucket and filtered through one layer of cheese cloth (Adejoro, 2017). The collected contents (100mL) were stored in 150mL sterilised sample bottles at -20°C until analysis. Before analysis, the rumen fluid samples were defrosted at 4°C. A 10 mL sample of rumen fluid was filtered through 2 layers of cheese cloth and 7mL was pipetted into a falcon centrifuge tube. The sample was centrifuged at 3500 rpm for 10 minutes according to Wales & Doyle (2003) and used immediately for LCFA analysis.

3.5 Sample analysis

3.5.1 Carcass pH and Temperature

Carcass pH and temperature were taken by inserting a probe into the middle of the *longissimus dorsi* muscle 24 hours after slaughter. The carcass pH was measured using a Hanna Instruments portable HI 8424 pH/mV/ °C meter. The pH meter was calibrated using pH 4 and pH 7 standards.

3.5.2 Colour

Lightness (L*), redness (a*), yellowness (b*) Chroma (C*) and hue angle (h*) were measured using a Minolta Chroma meter CR-400 at 24hrs after slaughter (day 1), and again 6 days later. On day one, the day after slaughter, a 2cm incision was made from the end of the *longissimus dorsi* muscle, bloomed for 20 minutes, and an average of two measurements were taken. This was repeated 6 days later. The

colorimeter was cleaned with ethanol and distilled water between each sample and calibrated using a white tile each day (Y=85.7, x=0.3166, y=0.3242). A 25mm^2 subcutaneous fat sample was dissected from the middle of the 8th rib of each three-rib-cut sample. Colour readings were also taken for subcutaneous fat on day six.

3.5.3 Body composition

The three-rib-cut samples were carefully and meticulously dissected using scalpel blades and tweezers into bone, muscle and fat according to Casey *et al*. (1988). Between each sample gloves and blades were changed, and the surface area and tweezers were cleaned with ethanol and distilled water. Each portion was weighed and represented as a percent of the total rib cut weight. The *longissimus dorsi* muscle and subcutaneous fat were weighed when dissected for colour analysis.

3.5.4 Back fat depth

Fat thickness was measured using a modification of Cloete *et al*. (2012) 25mm from the midline of each carcass at the position of the 8th and 11th ribs using a Mastercraft 0-200mm digital Vernier calliper manufactured by Massmart Holdings (Pty) Ltd, Sunninghill, Sandton.

3.5.5 Eye muscle area

Eye muscle area (EMA) was measured by tracing the area of the eye muscle onto a clear plastic sleeve with a permanent marker. Its length and breadth were measured using a Vernier calliper (mm). The area was estimated as an oval and therefore the equation of an oval was used and converted to cm^2.

$$EMA = \frac{[(0.5 \times \text{vertical measurement}) \times (0.5 \times \text{horizontal measurement})]}{10 \qquad\qquad 10} \times PI \qquad (1)$$

3.5.6 Dry matter and ash

The 5g ground *longissimus dorsi* sample from each carcass was thawed at 4°C, and the percent of dry matter and ash was determined using the Association of Official Analytical Chemistits, AOAC (1990) method. Samples were done in duplicate and the average weight was recorded for each animal. Meat samples of 2.5g were weighed into pre- weighed crucibles. The samples were then baked for 24h in a preheated 105°C Prolab oven. They were cooled in a desiccator for 30 minutes and weighed back. The samples were then put into an ashing oven at 600°C for 6 hours until reaching a constant weight. The ashing oven was switched off and the samples were taken out the following day. They were cooled in

a desiccator for 30 minutes and the weights were recorded. The formulas for percent dry matter (DM) and ash were as follows:

$$\%DM = \frac{\text{Mass of dried sample}}{\text{Mass of wet sample}} \times 100 \qquad \%Ash = \frac{\text{Mass of ash}}{\text{Mass of dry sample}} \times 100 \qquad (2)$$

3.5.7 Ether extract by Soxtec extraction

Ether extract was analysed by Soxtec extraction using the AOAC (2000) official method of analysis 920.39. The temperature on the Soxtec 2043 was set to 90°C with a water flow of 2L/minute. The programme was set to boil for 2 hours, rinse for 3 hours and dry for 20 minutes. Whatman cellulose extraction thimbles were prepared by blowing particles out of them using high pressure. Labelled aluminium collection cups and glass beads were cleaned with petroleum ether and warm soapy water. They were rinsed with water and dried for 30 minutes at 70°C, and cooled in a desiccator for 10 minutes. Three glass beads were placed into each collection cup and a total weight was recorded. A 1g sample of milled *longissimus dorsi* was weighed into the centre of a Whatman number one filter paper. The filter paper was folded three times on each open side and folded in on itself to fit into the thimbles. The cups and thimbles were inserted into the machine and the program was started. Once complete, the cups were placed in an oven set at 100°C for 30 minutes to evaporate any remaining ether. The samples were cooled in a desiccator for at least 10 minutes and weighed back. Samples were done in duplicate and the average fat% weights were recorded per animal. The formula was as follows:

$$\%Fat = \frac{(\text{Mass of cup + Beads + Fat}) - (\text{Mass of cups + beads})}{\text{Weight of sample}} \times 100 \qquad (3)$$

3.5.8 Medium and long chain fatty acids

3.5.8.1 Sample preparation

Fatty acid methyl esters (FAME) of medium and long chain fatty acids were identified using gas chromatography. Fatty acids were extracted by acid hydrolysis using an adaption of the AOAC (2000) official method of analysis 996.06. Acid catalysed methylation was used due to its ability to methylate all common lipid classes (Aldai *et al*., 2012). The AOAC (2000) 996.06 official method of analysis has three stages to preparing the samples for gas chromatography: digestion, extraction and methylation. Fatty acids in animal tissue need to be released from their alcohol, ester or amide bonds to become free fatty acids (FFA) available for methylation (Aldai *et al*., 2005). Pyrogallic acid was used to protect the sample fat from oxidation. After digestion fat was extracted into ether, dried, and trans-esterified

to methyl esters using 2% Sulphuric acid in methanol. Methylation catalysts, often present in methanol, can be in the form of acids or alkalis. The most common acids are hydrochloric acid (HCl), boron fluoride alcoholate (BF3) and sulphuric acid (H_2SO_4). Sulphuric acid is often used in a range of between 1 and 2% and was officially recognised by the Official Analytical chemists as far back as 1965 (Liu, 1994). Sulphuric acid acts similarly to HCl MeOH and is not as toxic as BF_3 (Liu, 1994). Variability in extraction and variations in the analytical instrument over time, as well as solvent evaporation, has the potential to effect areas and retention times of FAMEs. To correct for this, an internal standard was added to the sample and its concentration was incorporated into the gravimetric calculation. Pentadecanoic acid (C15:0) was used as an internal standard because its presence was limited and stable and the retention time was central to the FAMEs under observation. The area, when added to the sample in the same concentration also needed to be consistent. The methylated fatty acids were run on a Shimadzu 2010 Tracera fitted with a Restek 2560 capilliary column and captured by Shimadzu LabSolutions lite software. Fatty acids of known concentrations were run to determine the retention times of fatty acids of interest. Fatty acid methyl esters (FAMES) were measured as a molar percent (w/w%) and gravimetrically (mg/g sample). All glassware was washed with warm soapy water, ethanol, and dried in a drying oven before use. Sample preparation based on the AOAC (2000) 996.06 official method of analysis is described on detail below.

a) Sample digestion

Samples were digested using the AOAC (2000) official method of analysis 996.06. 100mg of finely chopped fat was weighed into a 30mL test tube with three boiling beads and 100mg pyrogallic acid (Sigma Aldrich, Missouri, United States). 2mL pentadecanoic acid (Sigma Aldrich, Missouri, United States) in hexane (1mg/mL) (Sigma Aldrich, Missouri, United States) and 2mL 95% (AR) ethanol (EthanolSA (Pty) Lt. Pretoria, South Africa) were added to the test tube and gently shaken to make sure the sample was dispersed within the mixture. Ten millileters (10mL) of 32% HCl was added, and the test tube was gently shaken until the acid had dispersed. A tefflon lined screw cap was then tightly screwed onto each test tube, placed in a rack, and heated in a Haake SWB25 shaking water bath at 75°C with a 25n agitation for 40 minutes. A Heidolph REAX top vortex on high speed was used every 10 minutes to make sure all the sample was in the solution. After 40 minutes the digested samples were removed, and the test tubes were dried and placed under a fume hood until they reached room temperature.

*Intramuscular fat and rumen fluid:

Due to there being less fat in intramuscular fat than SCG and PRF, 1g finely milled intramuscular fat sample was added to the test tube. If the sample stuck to the bottom of the test tube a glass rod was

used to mix the sample after the ethanol was added. The sample was vortexed for one minute after the HCl acid was added and if it was clumped a glass rod was used to help disperse the sample into the solution. The rest of the digestion procedure remained the same as above.

Before sample digestion, the rumen fluid was defrosted at 4°C and filtered through 2 layers of cheese cloth. 8mL of rumen fluid was pippeted into centrifuge tubes. It was centrifuged at 3500 rpm for 10 minutes according to Wales & Doyle, 2003. A 2mL sample of centrifuged rumen fluid was immediately pippeted into the 30mL test tube and the rest of the digestion procedure remained the same.

b) Extraction

Samples were extracted using an adaption of the AOAC (2000) official method of analysis 996.06. The fat was extracted using 20mL Diethyl ether (Sigma Aldrich, Missouri, United States) and Petroleum ether (Sigma Aldrich, Missouri, United States) in equal quantities respectively. The diethyl ether was added until it reached the neck of the test tube. It was gently shaken for 5 minutes and poured into a 150mL beaker. The remainder of the diethyl ether was used to flush the test tube. Petroleum ether (Sigma Aldrich, Missouri, United States) was added to the beaker and mixed on a Heidolph ROTAMAX 120 for 5 minutes at a speed of 4. Glass rods were also used to mix the solution while on the Rotamix. The samples were left over night. The bottom purple layer was aspirated and discarded. The beakers were left under a fume hood until the fat was dry and the ether had evaporated.

*Intramuscular fat and rumen fluid:

The contents were poured into a glass centrifuge tube and centrifuged at 600rpm on a Backman Coultier Allegra X-12 for 5 minutes according to AOAC (2000) 996.06 method of analysis. The clear ether top layer was aspirated into a 150mL beaker and left to dry under a fume hood overnight according to AOAC (2000) 996.06. The subcutaneous fat separated more easily and therefore did not need to be centrifuged for it to separate.

c) Methylation

Samples were methylated using an adaption of the AOAC (2000) official method of analysis 996.06. 3mL chloroform (Sigma Aldrich, Missouri, United States) was added to the beaker and swirled until all the fat up the side of the flask had dissolved into solution. 3mL Diethyl ether was added and gently mixed. The solution was aspirated into a 10mL test tube as quickly as possible. The solution was evaporated to dryness under a low nitrogen stream until only the fat remained, but not over dried so as to lose the more volatile fatty acids. 2mL 2% sulphuric acid (Sigma Aldrich, Missouri, United States) in methanol (Sigma Aldrich, Missouri, United States) and 1mL toluene (Sigma Aldrich, Missouri, United States) were added and mixed gently to make sure all the sample was in solution. The test tube was

sealed with a Teflon lined screw cap and heated at 100°C for 45 minutes. The samples were then cooled under a fume hood. Once they reached room temperature the reaction was stopped with 5mL deionised water. 1mL hexane was added to each sample. The caps were put back on the test tubes and vortexed for 1 minute. They were left to allow for the none-polar layer to separate above the polar layer. 1g anhydrous sodium sulphate (Na_2SO_4) (Sigma Aldrich, Missouri, United States) was added and gently mixed to quench any water from the none-polar layer. The layers were allowed to separate once again. The top non-polar layer was aspirated into a 2mL amber glass vial with a Teflon lined screw cap and wrapped with aluminium foil. The samples were labelled and stored at -20°C until analysis.

*Intramuscular fat and rumen fluid:

The vortex was not used but the samples were rather gently mixed without it. This is because they struggled to separate.

3.5.8.2 Medium and long chain sample analysis

a) Gas chromatography

Samples were analysed using an adaption of the AOAC (2000) official method of analysis 996.06. A 1ul sample was injected into a Shimadzu 2010 Tracerra gas chromatography machine fitted with a flame ionization detector (FID) and Restek 2560 capillary column (100m, 0.25mm ID, 0.20μm). The auto-sampler used was an AOC-20i. The method parameters were developed with the help of Shimadzu South Africa (Pty) Ltd. technitions to create the best quality and most repeatable peaks on the specific GC available for the analysis and tailored to the concentration of fat in the sample. The split ratio was according to Delmonte *et al*. (2007) but changed for rumen fluid due to its low concentration of fat in comparison to the other samples.

The method parameters put into Labsolutions Lite software were as follows:

Detector gas:	H_2: 40 mL/minute
	Air: 400mL/minute
	Make up flow (He): 30mL/minute
Carrier gas (He):	22cm/s
Injection volume:	1μL
Split:	1:100 (Subcutaneous, Peri-renal, Intramuscular fat)

1:1 (Rumen fluid fat)

Pressure: 300.9kPa

Total flow: 138.3 mL/minute (Subcutaneous, Peri-renal, Intramuscular)

5.7mL/minute (Rumen fluid)

Purge flow: 3 mL/minute

Stabilisation time: 5 minutes

Total run time: 55.97 minutes

Temperature parameters:

- Starting temperature of 125°C with a hold time of 5 minutes
- Ramp 4°C/minute to 226.3°C and hold for 0 minutes
- Ramp 1.5°C/ minute to 226.3°C and hold for 3 minutes
- Ramp 2°C/minute to 240°C and hold for 10 minutes

Injector temperature: 260°C

Detector temperature (FID): 250°C

Rinsing formalities: Before each sample was injected the needle was flushed 3 times with hexane and once with the sample to prevent ghost peaks.

b) Calibration

Fatty acid methyl esters (FAMEs) were identified by comparison with the retention times of FAME standards. Standards were diluted with hexane to better fit the sample chromatogram concentrations. A Supelco 37 component FAME mix (Sigma Aldrich, Laramie, Wyoming) was initially run at concentrations of 10, 25, 50 75 and 100% in hexane to identify the peaks of the samples at specific retention times. Other methylated standards were also used to identify additional fatty acids of interest. C18:1 cis trans mix (Restek, Bellefinte, PA) and conjugated linoleic acid (Sigma Aldrich, Saint Louis, MO) diluted with hexane and spiked with methylated pentadecanoic acid (Sigma Aldrich, Saint Louis, MO) were prepared as a single standard. Rumenic acid (Laraoda, Solna, Sweden) diluted with methylated pentadecanoic acid in hexane was also run as an additional standard.

Unidentified peaks greater than or equal to 0.1% of the molar percent were lumped together as total unidentified peaks. These were most likely the cis and trans counterparts of those in the standards.

The retention time order for the conjugated linoleic acid (CLA) fatty acids in the mix was not defined between all CLA isomers. Therefore, the elution order was determined from Sehat *et al*. (1998) who also used a 100m x 0.25mm i.d. x 0.2um column. It is also well documented that rumenic acid is the largest contributing CLA in ruminant fat (Reas *et al*., 2004; Martins *et al*., 2007).

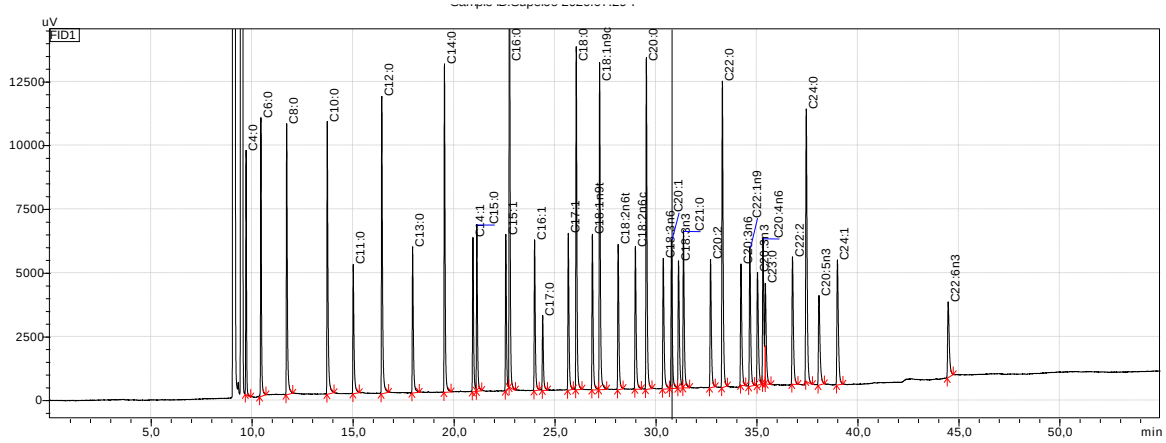

Figure 3.5.1: Chromatogram representing the FAME 37 mix

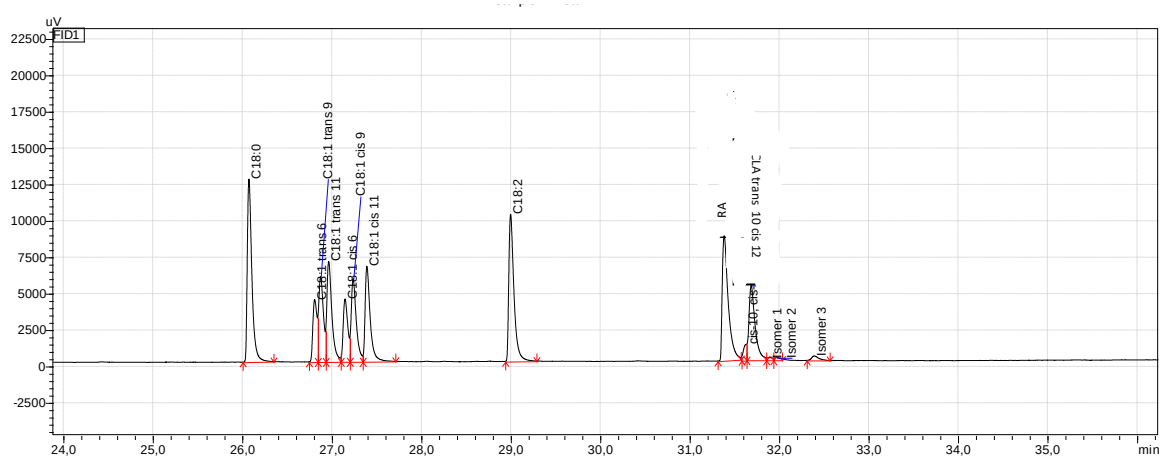

Figure 3.5.2: Chromatogram representing the C18:1 cis/trans mix and CLA mix

Table 3.5.1: LCFA names, abbreviations and retention times of fatty acids on a Shimadzu 2010 Tracerra GC fitted with a Restek 2560 capilliary column

Shorthand	*Trivial names*	*Abbreviations*	*Retention time*
C4:0	Butyric acid		9.731
C6:0	Caprioc acid		10.464
C8:0	Caprylic		11.74
C10:0	Capric acid		13.75
C11:0	Undecanoic acid		15.037
C12:0	Lauric acid		16.45
C13:0	Tridecanoic acid		17.978

C14:0	Myristic acid		19.553
C14:1	Myristoleic acid		20.973
C15:0	Pentadecanoic acid		21.158
C15:1	Pentadecenoic acid		22.591
C16:0	Palmitic acid		22.772
C16:1	Palmitoleic acid		24.018
C17:0	Heptadecanoic acid		24.419
C17:1	Heptadecenoic acid		25.686
C18:0	Stearic acid	SA	26.078
C18:1n6t	Petroselaidic acid		26.812
C18:1n9t	Eleidic acid		26.878
C18:1n11t	Trans Vaccenic acid	Trans VA	26.974
C18:1n6c	Petroselinoic acid		27.153
C18:1n9c	Oleic acid		27.237
C18:1n11c	Cis Vaccenic acid	Cis VA	27.395
C18:2n6t	Linoleleidic acid		28.155
C18:2n6c	Linoleic acid	LA	29.008
C20:0	Arachidonic acid		29.55
C18:3n6	Gamma linolenic acid		30.388
C20:1	Eicosenoic acid		30.795
C18:3n3	Linolenic acid	LNA	31.145
C21:0/CLA cis-9, trans-11/trans-9, cis-12	Heneicosanoic acid RA		31.389 31.379
CLA cis-10, cis-12			31.692
CLA trans-10, cis-12			31.675
CLA isomer 1			31.891
CLA isomer 2			31.968
CLA isomer 3			32.373
C20:2	Eicosadienoic acid	EDA	32.73

C22:0	Behenic acid		33.316
C20:3n6	Cis-8,11,14- Eicosatrienoic acid	ETA	34.236
C22:1n9	Erucic acid		34.676
C20:3n3	Cis-11,14,17-Eicosatrienoic acid		35.056
C23:0	Tricosanoic acid		35.327
C20:4n6	Arachidonic acid	AA	35.443
C22:2	Docosadienoic acid		36.788
C24:0	Lignoceric acid		37.47
C20:5n3	Eicosapentaenoic acid	EPA	38.098
C24:1	Nervonic acid		39.013
C22:6n3	Docosahexaenoic acid	DHA	44.496

Calibration parameters:

Width: 3 sec

Slope: 1000 µV/minute

Drift: 0 mV/minute

T.DBL: 10000 minimum

Minimum area/ Height 1000 counts (but manually integrated smaller peaks of interest)

Max peak: 6 counts relative to main peak

Identification:

Window: 5%

Default 0.01 Minutes

ID: Absolute retention time

Peak selection: All peaks

Component type: Target

c) Quality control

Between batches the GC's liner and septa were changed, and the machine was baked. Three blank hexanes were run through the machine to ensure the column was clean (no ghost peaks, high noise or exaggerated base line rises). Supelco was repeated at a known concentration to prime the GC and ensure repeatability of both area and retention time. When the redisual square difference (RSD) was below four the sample batch was started. A batch containing 32 samples was run from beginning to end over a 42-hour period. After every five samples a hexane was injected to clean the column and check for ghost peaks. After every ten samples two Supelco's were injected consecutively to ensure repeatability. In the event of a baseline shift, provided it did not affect the area, the closest Supelco could be used to identify the new retention times.

d) Calculations

Fatty acids were expressed as a percent of total fatty acids (w/w %) as well as gravimetric concentrations (mg/g of sample) according to Webb *et el* (1998).

Molar percent of fatty acid:

$$w/w\% = \frac{\text{Area of fatty acid peak}}{\text{Total area of all peaks}} \times 100 \quad (4)$$

Gravimetric fatty acid content (mg/g): (5)

$$\text{Concentration (mg/ul)} = \frac{\text{Area of fatty acid peak}}{\text{Area internal standard}}$$

$$\text{Gravimetric (mg/g)} = \frac{\text{1000mg/ul internal standard}}{\text{Sample weight (mg)}} \times \text{concentration (mg/ul)}$$

- Sums and ratios

Sums and ratios were calculated in IMF, SCF and PRF for SFA, MUFA, PUFA, UFA, trans/cis ratio, CLA, PUFA/SFA, MUFA/SFA, SFA/UFA, n-3 FAs, n-6 FAs, n-6/n-3 ratio, cis FA and trans FAs. In rumen fluid, ratios relating to the C18:2n6c and C18:3n3 biohydrogenation pathways were also analysed; RA/LA, VA/RA, SA/VA, VA/CLA, CLA/LA, VA/LNA and SA/OA.

Table 3.5.2: Ratios analysed with their abbreviations

Sums and rations	***Abbreviation***
Saturated fatty acid/unsaturated fatty acid ratio	SFA/UFA
Monounsaturated fatty acid/saturated fatty acid ratio	MUFA/SFA
Polyunsaturated fatty acid/ saturated fatty acid ratio	PUFA/SFA
[CLA cis-9, trans-11 & trans-9 cis-12]/C18:1n6c	RA/LA
C18:1n11t/[CLA cis-9, trans-11 & trans-9 cis-12]	VA/RA
C18:0/C18:1n11t	SA/VA
C18:1n11t/CLA	VA/CLA
CLA/C18:2n6c	CLA/LA
C18:1n11t/C18:3n3	VA/LNA
C18:0/ C18:1n9c	SA/OA

Table 3.5.3: Treatment interactions and their abbreviations

Interaction	***Abbreviation***
Treatment and sex	Treat*MF
Treatment and weight	Treat*HL
Acacia tannin and sex	T*MF
NPN source and sex	N*MF
Acacia tannin and weight	T*HL
NPN source and weight	N*HL
Sex and weight	MF*HL
Non protein nitrogen source (Urea vs Calcium nitrate)	NPN source

- Health indices

Indices were used to give an indication of the effect treatment, sex and weight had on *de novo* fatty acid synthesis enzymes. To determine the nutritional quality of the lipid fraction, health indices were used to give an overall impression of the healthfulness of the fatty acid profile. This is because not all saturated fatty acids are harmful, and PUFAs vary in their degree of health potential. Indices of atherogenicity (AI) and thrombogenicity (TI) were calculated according to Ulbrich & Southgate (1991) and the desirable fatty acids (DFA) were calculated according to Rhee (1992).

Hypocholesterolaemic /hypercholesterolaemic fatty acid ratio (h/H) was calculated according to Pilarczyk *et al*. (2015) based on the calculations by Ulbrich & Southgate (1991).

$$\text{Atherogenic index (AI)} = \frac{\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}}{\Sigma\text{MUFA} + \Sigma\text{n-3 PUFA} + \Sigma\text{n-6 PUFA}} \quad (6)$$

$$\text{Thrombogenic index (TI)} = \frac{\text{C14:0} + \text{C16:0} + \text{C18:0}}{[(0.5 \times \text{MUFA}) + (0.5 \times \Sigma\text{n-6}) + (3 \times \Sigma\text{n-3}) + (\Sigma\text{n-3 PUFA}/\ \Sigma\text{n-6 PUFA})]} \quad (7)$$

$$\text{Desirable fatty acids (DFA)} = \text{MUFA} + \text{PUFA} + \text{C18:0} \quad (8)$$

$$\text{Hypocholesterolaemic fatty acids (h)} = \Sigma\text{C18:1} + \Sigma\text{PUFA} \quad (9)$$

$$\text{Hypercholesterolaemic fatty acids (H)} = \text{C14:0} + \text{C16:0}$$

$$\text{h/H} = \frac{\Sigma\text{C18:1} + \Sigma\text{PUFA}}{\text{C14:0} + \text{C16:0}} \quad (10)$$

- Enzyme indices:

Elongase was calculated according to Facciologo *et al*. (2018), overall delta-9 desaturase was calculated according to Noci *et al*. (2007), delta 9 desaturase C14, C16 and C18 were calculated as stipulated by Junior *et al*. (2019) and delta 9 desaturase RA was calculated according to (Garnsworthy *et al*., 2010). All equation were turned into a percent for uniformity.

$$\text{Elongase} = \frac{\text{C18:0} + \text{C18:1n9c}}{[\text{C16:0} + \text{C16:1} + \text{C18:0} + \text{C18:1n9c}]} \times 100 \quad (11)$$

$$\text{Delta 9 desaturase} = \frac{\text{C14:1n9c} + \text{C16:1n9c} + \text{C18:1n9c}}{\text{C14:0} + \text{C14:1n9c} + \text{C16:0} + \text{C16:1n9c} + \text{C18:0} + \text{C18:1n9c}} \times 100 \quad (12)$$

$$\text{Delta 9 desaturase C14} = \frac{\text{C14:1}}{[\text{C14:0} + \text{C14:1}]} \times 100 \quad (13)$$

$$\text{Delta 9 desaturase C16} = \frac{\text{C16:1}}{[\text{C16:0} + \text{C16:1}]} \times 100 \quad (14)$$

$$\text{Delta 9 desaturase C18} = \frac{\text{C18:1n9c}}{[\text{C18:0} + \text{C18:1n9c}]} \times 100 \quad (15)$$

$$\text{Delta 9 desaturase RA} = \frac{\text{CLA cis 9, trans 1}}{[\text{C18:1n11t} + \text{CLA cis 9, trans 11}]} \times 100 \quad (16)$$

Table 3.5.4: Indices and their abbreviations

Indices	*Abbreviation*
Elongase	Elongase
Delta 9 desaturase (C14+C16+C18)	Delta 9 desaturase (C14+C16+C18)
Delta 9 desaturase C14	Delta 9 desaturase C14
Delta 9 desaturase C16	Delta 9 desaturase C16
Delta 9 desaturase C18	Delta 9 desaturase C18
Delta 9 desaturase CLA cis9-trans-11/trans-9, cis-12	Delta 9 desaturase RA
Atherogenic index	AI
Thrombogenic index	TI
Desirable fatty acid	DFA
Hypocholesterolemic index	h
Hypercholesterolemic index	H
h/H ratio	h/H

3.5.8.3 Statistical analysis

Statistical analyses were done for treatments (calcium nitrate-based TMR; calcium nitrate-based TMR and tannin; urea-based diet; urea-based TMR and tannin), sex (ram vs ewe lambs) and weight (heavy vs light) and the interactions between them. Data was tested for normality and analysed by means of the Analysis of Variance using the Generalised Linear Models procedure of IBM SPSS version 28.0.1.0. Significance was determined where $P \leq 0.05$ (significant), $P \leq 0.01$ (highly significant), $P \leq 0.005$ (very highly significant), $P \leq 0.001$ (extremely significant) and $0.05 \leq P \leq 0.1$ (tendency). Differences between treatment means were tested at $P \leq 0.05$.

a) Blood

Data obtained was analysed using the general linear model (GLM) procedures of IBM SPSS statistics software version 28.0.1.0 (142) (IBM Corp., Armonk, NY., USA). Descriptive statistics were used to establish the mean and standard deviation (SD), and the Tukey HSD test was used to determine significant difference between treatment and sex (ram vs ewe lamb).

b) Carcass

Data obtained was analysed using the general linear model (GLM) procedures of IBM SPSS statistics software for windows version 28.0.1.0 (142) (IBM Corp., Armonk, NY., USA). Descriptive statistics were used to establish the mean and standard deviation (SD). Differences within the carcass parameters between treatments, sexes and weights were determined using a multivariate analysis of variance (MANOVA). The data was not balanced. Therefore, post hoc analysis was done using Bonferroni's range test to test for significance between treatments where $P \leq 0.05$. Row means with different superscripts differed significantly ($P \leq 0.05$). A two tailed t-test was used to determine any associations between variables.

c) Proximate

Data obtained was analysed using the general linear model (GLM) procedures of IBM SPSS statistics software for windows version 28.0.1.0 (142) (IBM Corp., Armonk, NY., USA). Descriptive statistics were used to establish the mean and standard deviation (SD). Differences between treatments, sex and weight were determined within the proximate parameters using a multivariate analysis of variance (MANOVA). Post hoc analysis was done using Bonferonni's range test to determine significance between treatments. Row means with different superscripts differed significantly ($P \leq 0.05$). Bonferroni was used because the data was not balanced.

d) Long chain fatty acid analysis

Data obtained for LCFA was analysed using the general linear model (GLM) procedures of IBM SPSS statistics software for windows version 28.0.1.0 (142) (IBM Corp., Armonk, NY., USA). Descriptive statistics were used to establish the mean and standard deviation (SD) of the fatty acids within each treatment, sex and weight category. It was also used to interpret interactions between factors. Differences were determined using a multivariate analysis of variance (MANOVA). A post Hoc test using Bonferonni's range test determined significance at $P \leq 0.05$ between treatments. Row means with different superscripts differed significantly ($P \leq 0.05$).

e) Colour

Colour data obtained was analysed using the general linear model (GLM) procedures of IBM SPSS windows version 28.0.1.0 (142) (IBM Corp., Armonk, NY., USA). Descriptive statistics were used to establish the mean and standard deviation (SD) of lightness (L*), redness (a*), yellowness (b*), chroma (C) and hue angle (h) between treatment, weight, sex and day. Within each treatment, sex and weight category, significance was determined using a multivariate analysis of variance (MANOVA). A post Hoc test using Bonferonni's range test determined significance at $P \leq 0.05$ between treatments. Row means with different superscripts differed significantly ($P \leq 0.05$).

f) Principal component analysis

The data was summarised using principal component analysis (PCA) using PAST software.

3.5.9 References

Adejoro, F. A., 2019. The use of condensed tannins and nitrate to reduce enteric methane emission and enhance utilization of high-forage diets in sheep. pHd (Agric) dissertation, University of Pretoria, South Africa. pp. 71.

Adejoro, F. A., Hassen, A., Akanmu, A. M. & Morgavi, D. P., 2020. Replacing urea with nitrate as a non-protein nitrogen source increases lambs' growth and reduces methane production, whereas acacia tannin has no effect. Animal Feed Science and Technology, 259, 114360.

Aldai, N., Kramer, J. K. G., Cruz-Hernandez, C., Santercole, V., Delmonte, P., Mossoba, M. M. & Dugan, M. E. R. 2012. In: Fats and fatty acid metabolism in poultry nutrition and health. Eds: Cherian, G. & Pouelsami, R., Context products Ltd. Leicestershire, United Kindom. pp. 258.

Aldai, N., Murray, B. E., Nájera, A. I., Troy, D. J. & Osoro, K., 2005. Derivatization of fatty acids and its application for conjugated linoleic acid studies in ruminant meat lipids. Journal of the Science of Food and Agriculture, 85(7), 1073-1083.

AOAC, 2000. Official method of analysis 920.39. Ed: 17th. Association of official analytical chemists. Washington, D.C.

AOAC, 2000. Official method of analysis 996.06. Ed: 17th. Association of official analytical chemists. Washington, D.C.

AOAC, 1990. Official methods of analysis. Association of official analytical chemists. Washington, D.C.

Casey, N. H., Van Niekerk, W. A. & Spreeth, E. B., 1988. Fatty acid composition of subcutaneous fat of sheep grazed on eight different pastures. Meat Science, 23(1), 55-63.

Cloete, J. J. E., Hoffman, L. C. & Cloete, S. W. P., 2012. A comparison between slaughter traits and meat quality of various sheep breeds: Wool, dual-purpose and mutton, Meat Science, 91(3), 318-324.

Delmonte, P. & Rader, J. I., 2007. Evaluation of gas chromatographic methods for the determination of trans fat. Analytical and bioanalytical chemistry, 389, 77-85.

FAO image. Available from: https://www.fao.org/3/t0279e/T0279E108.gif. [Accessed 18.11.2022].

Garnsworthy, P. C., Feng, S., Lock, A. L. & Royal, M. D., 2010. Heritability of milk fatty acid composition and stearoyl-CoA desaturase indices in dairy cows. Journal of Dairy Science, 93(4), 1743-1748.

Junior, F. M. V., Martins, C. F., Feijó, G. L. D., Teixeira, A., Leonardo, A. P., de Almeida Ricardo, H., Fernanded, A. R. M. & Reis, F. A., 2019. Evaluation of genotype on fatty acid profile and sensory of meat of indigenous Pantaneiro sheep and Texel or Santa Inês crossbred finished on feedlot. Small Ruminant Research, 173. 17-22.

Liu, K. S., 1994. Preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. Journal of the American Oil Chemists' Society, 71(11), 1179-1187.

Martins, S. V., Lopes, P. A., Alfaia, C. M., Ribeiro, V. S., Guerreiro, T. V., Fontes, C. M., Castro, M. F., Soveral. G. & Prates, J. A., 2007. Contents of conjugated linoleic acid isomers in ruminant-derived foods and estimation of their contribution to daily intake in Portugal. British Journal of Nutrition, 98(6), 1206-1213.

Noci, F., French, P., Monahan, F. J. & Moloney, A. P., 2007. The fatty acid composition of muscle fat and subcutaneous adipose tissue of grazing heifers supplemented with plant oil-enriched concentrates. Journal of Animal Science, 85(4), 1062-1073.

Pilarczyk, R., Wójcik, J., Sablik, P. & Czerniak, P., 2015. Fatty acid profile and health lipid indices in the raw milk of Simmental and Holstein-Friesian cows from an organic farm. South African Journal of Animal Science, 45(1), 30-38.

Raes, K., De Smet, S. & Demeyer, D., 2004. Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. Animal Feed Science and Technology, *113*(1-4), 199-221.

Rhee, K. S., 1992. Fatty acids in meat and meat products. In: Fatty acids in foods and their health benefits. Ed: Chow, C. K., CRC Press, New York, USA. pp. 65-99.

Sehat, N., Kramer, J. K., Mossoba, M. M., Yurawecz, M. P., Roach, J. A., Eulitz, K., Morehouse, K. M. & Ku, Y., 1998. Identification of conjugated linoleic acid isomers in cheese by gas chromatography,

silver ion high performance liquid chromatography and mass spectral reconstructed ion profiles. Comparison of chromatographic elution sequences. Lipids, 33(10), 963-971.

Ulbrich, T. L. V. & Southgate, D. A. T., 1991. Coronary heart disease; seven dietary factors, 338 (8773). 985-992.

Wales, W. J. & Doyle, P. T., 2003. Effect of grain and straw supplementation on marginal milk-production responses and rumen fermentation of cows grazing highly digestible subterranean clover pasture. Australian Journal of Experimental Agriculture, 43(5), 467-474.

Webb, E. C., De Smet, S., Van Nevel, C., Martens, B. & Demeyer, D. I., 1998. Effect of anatomical location on the composition of fatty acids in double-muscled Belgian Blue cows. *Meat science*, *50*(1), 45-53.

CHAPTER 4

4 RESULTS AND DISCUSSION

4.1 The effect of treatment, sex and weight on carcass characteristics

As indicated in Table 4.1.1, lambs were between 34 and 35 kg when first introduced to the experimental ration and slaughtered as A2 and A3 carcasses. Ewe lambs slaughtered between 45 and 48kg were considered light and ewe lambs slaughtered between 48.1 and 51kg were heavy. Ram lambs between 50 and 55kg were categorized as light, and rams between 55.1 and 60kg were heavy. There was a large variation in slaughter date with the exception of ewe lambs in the calcium nitrate-based total mixed ration (TMR) without tannin.

Table 4.1.1: Average live weights (kg ±SD) and number of days lambs spent in the trial to reach their target slaughter weights

			Urea		Calcium nitrate	
			None	Tannin	None	Tannin
		# sheep	8	8	8	8
Starting weight (kg)	Female	4	34.7±2.84	34.4±1.70	34.4±2.31	34.5±2.46
	Male	4	37.4±13.77	35.8±6.25	34.9±6.59	36.6±5.85
Final weight (kg)	Female	4	48.6±1.17	48.2±2.73	49.7±1.48	48.3±2.07
	Male	4	54.8±2.69	55.0±3.93	56.3±3.90	56.2±3.23
Days in trial	Female	4	83.0±13.50	121±7.00	76.0±0.00	107±17.60
	Male	4	72±13.80	100±21.00	72±21.00	79±21.00

The means and standard deviation (SD) on the effect of tannin and calcium nitrate on selected carcass characteristics are shown in Table 4.1.2 and interactions are presented in Table 4.1.4. The effect of sex on the carcass characteristics are presented in Table 4.1.3 and correlations between carcass parameters are presented in Table 4.1.5. These tables are at the end of this section. Table 4.1.2 shows that tannin significantly affected cold carcass weight (CCW) ($P<0.001$), dressing percent (D%) ($P<0.001$), temperature of the carcass ($P<0.05$) and days on feed ($P<0.005$). Tannin decreased CCW significantly in the calcium nitrate-based diet ($P<0.05$). Tannin decreased D% in both the urea and calcium nitrate-based diets ($P<0.05$). Although calcium nitrate decreased the D% ($P<0.05$), it was not significantly reduced in the diets containing and not containing tannin ($P>0.05$). As presented in bold in Table 4.1.4, tannin supplementation affected the temperature of ram and ewe lamb *longissimus dorsi* (LD) differently, as did calcium nitrate on the SCF depth over the 11th rib ($P<0.05$).

Table 4.1.3 indicated that sex affected body composition ($P<0.001$), live weight (LW; $P<0.001$), cold carcass weight (CCW; $P<0.005$), dressing percent (D%; $P<0.005$), SCF depth (8th and 11$^{th;}$;$P<0.010$ and

P=0.001 respectively), eye muscle area (EMA; P<0.005) and days in trial (DIT; P<0.05) to reach slaughter weight. Therefore, only carcass pH and temperature were not affected (P>0.05). As expected, due to the experimental design the mean slaughter weight of ram lambs was 14.12% heavier (P<0.001) than that of ewes. This resulted in a 7.23% heavier carcass weight in rams (P<0.05) and a 6.44% higher dressing percent in ewes (P<0.05). All significant carcass parameters had a higher mean value for rams with the exception of fat percent, subcutaneous fat depth and dressing percent which was higher in ewe lambs (P<0.05). This is consistent with ewes maturing earlier than rams. The subcutaneous fat depth of ewes was 30.91% thicker (P<0.05) over the 8th rib and 49.03% thicker over the 13th rib (P<0.05). However, eye muscle area, which is positively correlated with liveweight and carcass weight (Table 4.1.5), was 29.81% higher in rams than ewe lambs (P<0.05). The weight category significantly affected the CCW, SCF depth and EMA (P<0.05) (Table 4.1.3).

4.1.1 Days in trial

Lambs fed tannin took longer to reach their slaughter weight (P<0.005, Table 4.1.2). Thus, lambs on the urea-based diet with tannin took longer to reach their slaughter weight than lambs on the calcium nitrate-based diet without tannin (P<0.05). This discrepancy led to a 36 day difference, which would be over a month of additional cost to the farmer to reach an A2/A3 carcass. However, the SDs were above 13 days in all treatments. Ewe lambs took an average of 20% longer to reach their targeted slaughter weight (Table 4.1.3). Although there was a visible increase in the number of days the ewes were on the trial rashion to reach their target slaughter weight when tannin was fed (Table 4.1.1), the interaction between sex and tannin was insignificant due to the large standard deviation in number of days (Table 4.1.4). However, ewes supplemented with tannin took 42.98% longer to reach their slaughter weight in comparison to ewes who were not fed tannin (Table 4.1.1).

4.1.2 Carcass pH

Carcass pH is an important parameter because of its effect on shelf life, colour and quality of meat (García *et al*., 2019). The pH value 24 hours post slaughter regardless of treatment was 5.6 (Table 4.1.2). This is within the desirable carcass pH range stipulated by the South African Meat Safety Act no.40 0f 2000 where red meat should be below 6.3. Devine *et al*. (1993) identified a reduced consumer acceptability in meat where values rose above 5.8. This range also indicated that the lambs were not stressed before slaughter. When sheep are stressed, they use more glycogen for glycolysis, which slows the rate of decrease in carcass pH post-slaughter (Bond *et al*., 2004). The supplementation of tannin and the replacement of urea with calcium nitrate did not affect the ultimate carcass pH of the *longissimus dorsi* 24 hours after slaughter. This agrees with previous reports on the effect of tannin

supplementation (Priolo *et al*., 2002; Priolo *et al*., 2005; Vasta *et al*., 2007; Francisco *et al*., 2015; Garcia *et al*., 2019). It also confirms the report by Hegarty *et al*. (2016) that the replacement of urea with calcium nitrate does not affect meat pH. In agreement with previous studies (Sañudo *et al*., 1998; Diaz *et al*., 2003; Tejeda *et al*., 2008) sheep sex did not influence pH 24 hours post slaughter (Table 4.1.3). Table 4.1.3 also indicates that weight did not have an effect on pH (P>0.05).

4.1.3 Temperature

The length of time lambs were fed (days in trial) to reach their target weight was positively correlated to the temperature of the carcass (P<0.001; 0.883; Table 4.1.5). Meat tenderness is affected by temperature and ultimate carcass pH (Yu *et al*., 1986). If muscle temperature is high, post-mortem proteins degrade at a faster rate, thus increasing meat tenderness (Yu *et al*., 1986). Temperature was also found to be lower where EMA was greater (P<0.010; -0.462). This was possibly due to the length of time it would take to cool a thicker piece of meat. As indicated in Table 4.1.2, lambs supplemented with tannin reached ambient temperature more quickly than unsupplemented lambs. The lambs on the tannin supplemented urea and calcium nitrate-based diets increased the *longissimus dorsi* temperature by 23.76% and 12.92%, respectively. Therefore, it increased from ±17°C to ±20°C/21°C. On further examination, the Bonferroni statistical test did not find these results to show any significance between treatments. The temperature of the *longissimus dorsi* is most likely as a result of traveling (samples were kept on ice in a cooler), the speed at which it warmed up to laboratory conditions, and the thickness of the rib cut. Sex and weight did not have an effect on temperature (Table 4.1.3).

4.1.4 Body composition

4.1.4.1 Influence of treatment on bone: muscle: fat ratio

Tissue composition estimated by the three- rib-cut (8^{th} to 11^{th} rib) indicated that nitrate source and tannin had no effect on the bone: muscle: fat ratio (P > 0.05) (Table 4.1.2). The absence of the effect of long-term tannin supplementation on carcass composition of lambs is consistent with Rojas-Román *et al*. (2017), who supplemented 6g tannin per kg corn- based finishing diet. Although it was not significant (P > 0.05), lambs on the urea-based diet without tannin were 15.89% fatter (34%±8.99) and 7.78% leaner (51.7%±7.7) than the lambs on the same diet supplemented with tannin (29.3%±6.24 and 55.8%±5.63, respectively). The insignificance was possibly due to the high variation within treatments. There was no interaction between treatment and sex (P > 0.05). In a study conducted by Guerreiro *et al*. (2020), slower development was identified in lambs supplemented with 2.5% condensed tannin (CT) as indicated by lighter carcasses, higher bone% and lower muscle/bone ratio.

Bone develops earlier than muscle which accelerates earlier than fat. In the current study the average body weight of lambs at 60 days on feed tended to be lighter for the lambs fed on the TMR with tannin (P<0.096) (Adejoro, 2020). This also resulted in them reaching their target slaughter weight slightly later. Therefore, tannin may have influenced the three-rib cut composition if the lambs were slaughtered at the same time as opposed to a pre-determined weight.

No literature can be found on the effect of calcium nitrate and urea on bone, muscle and fat composition. In the current study, replacing urea with calcium nitrate for the purpose of reducing methane emissions does not have any negative effects on the 3-rib-cut sample (P>0.05).

4.1.4.2 Influence of Sex on bone: muscle: fat ratio

Ram and ewe lambs differed in composition regardless of the treatment (P<0.001; Table 4.1.3). Rams had a higher percent of bone (15.95% increase) and muscle (56.75% increase), and a lower percent of fat (27.23% decrease) than ewes. This is because as the amount of fat increased in the carcass, the percent of bone and muscle decreased (Table 4.1.5). This trend is similar to previous reports on the impact of sex on body composition (Thompson *et al*., 1985; Johnson *et al*., 1995; Van der Merwe *et al*., 2020). The same pattern applied to Van der Merwe *et al*. (2020) with higher muscle and bone in rams (44.8% and 21.6%, respectively) and lower fat (33.1%) in comparison to ewes with lower bone and muscle (19.4% and 42.9%, respectively) and higher fat (37.3%). However, differences in magnitude between the studies may be as a result of the weight at which they were slaughtered.

The current study showed that liveweight is highly correlated to changes in the body composition ratio (Table 4.1.5); liveweight was positively correlated with an increase in muscle (P<0.008;0.468) and bone percent (P<0.001; 0.607) and negatively correlated to the percent of fat (P<0.002; -0.544). This may be an indication that lambs, particularly rams, were still in a phase of accelerated growth in bone and muscle when they were slaughtered. McClelland *et al*. (1976) proposed that differences in composition of ewes and rams at the same weight was a result of differences in their state of maturity. This may be explained by differences in their growth curves and mature weight between the sexes (McClelland *et al*., 1976). Although ewes were slaughtered lighter (between 45 and 51kg) they contained more fat than rams (slaughtered between 50 and 60kg). Thus, the rate of change in the ratio differed between sexes due to the ewes' more advanced position on the growth curve and closeness to their mature weight when slaughtered.

4.1.4.3 Influence of slaughter group on bone: muscle: fat ratio

The two-tailed t-test showed that liveweight was positively correlated to the percent of bone and muscle, and negatively correlated to the percent of fat (P<0.001, P<0.010, P<0.005 respectively; Table

4.1.5). This contrasted with Webb & Casey (1995a) who associated an increase in weight with a decrease in the ratio of muscle and bone and an increase in fat. Brand *et al*. (2018) showed a similar pattern with increased time in the feedlot. Younger animals are associated with a higher percent of bone and muscle, which is diluted by fat when it reaches sexual maturity. The animals in the current study, regardless of weight category were already at a more mature weight. Brand et al. (2018) observed sheep until they reached 100 days in the feedlot. Between 84 and 105 days the ratios also became less predictable.

In contrast to the t-test, MANOVA, indicated in Table 4.1.3, reported that the weight at which sheep were slaughtered did not affect the composition of the three-rib-cut body composition ratio (P>0.05). This is possibly because they were all A2 and A3 carcasses at slaughter and the slaughter weights SD was 1.17 for ewes and 3.19 for rams (Table 4.1.3). Therefore, this test suggests that the heavy and light weight groups were in the same state of maturity at slaughter.

4.1.5 Subcutaneous fat depth

As indicated in Table 4.1.5, the subcutaneous back fat depth was highly correlated to the percent of fat in the carcass (P<0.001; 0.662). Therefore, a higher bone and muscle percent resulted in a thinner layer of subcutaneous fat (Table 4.1.5). As liveweight increased the SCF depth over the 11th rib decreased (P<0.05; -0.402). Replacing urea with calcium nitrate in the TMR with or without the supplementation of tannin also had no effect on the fat depth over the 8th rib (P>0.05). The fat ranged from 3.35mm±0.806 to 4.15mm±0.877 in Table 4.1.2. The absence of the effect of tannin or nitrate source on fat depth was also reported by Krueger *et al*. (2010), Hegarty *et al*. (2016) and Rojas-Roman *et al*. (2017).

Subcutaneous fat depth was considerably thicker over the 8th rib in ewes than rams (P<0.013; Table 4.1.3). This was predicted following reports by Diaz *et al*. (2003) and Cloete *et al*. (2012). Cloete *et al*. (2012) observed a SCF depth of 0.95mm in 60kg rams and 3.29mm in 50kg ewes over the 13th rib. The current study's ewes contained 4.22mm±1.091 fat and rams contained 3.23mm±0.989 fat over the 8th rib. Although it was not originally in the study, the thicker subcutaneous fat over the 11th rib was also measured to amplify the difference (P<0.001); rams measured 4.17mm±0.190 and ewes measured 6.22mm±1.952. The thickness of the subcutaneous fat over the 8th and 11th ribs were highly correlated (P<0.552; 0.552; Table 4.1.5). Subcutaneous fat is the first to develop, therefore it confirms the theory that ewes began depositing subcutaneous fat earlier than the rams. Whether the sheep were slaughtered heavy or light, no differences were identified between the two weight groups possibly because the majority were all A2 carcasses at slaughter with a few A3.

4.1.6 Eye muscle area

According to Table 4.1.5 eye muscle area (EMA) increased with liveweight (P<0.001; 0.689) and cold carcass weight (P<0.001; 0.576) and was negatively correlated to the length of time it took for lambs to reach slaughter weight (P<0.002; -0.537). The percent of bone, which increase was also associated with an increase in liveweight, increased with EMA (P=0.058; 0.345).

Non-protein nitrogen source and/or tannin supplementation did not to affect the EMA (P>0.05; Table 4.1.2). This is possibly due to liveweight at slaughter being similar across all treatments (P>0.05). The absence in the effect of the non-protein nitrogen (NPN) source is consistent with Hegarty *et al*. (2016) and Rojas-Román *et al*. (2017) who found no significant difference with tannin supplementation. However, the amount of tannin administered to the diet may have been below the optimal value needed for negative change. Costa *et al*. (2021) observed a significant linear decrease in EMA from 15 to 11.9cm^2 as they increased supplementation of *Acacia mearnsii* from 0, 20, 40, 60 and 80g/kg DM.

The EMA increased by 22.40% in the heavier weight group (P<0.05; Table 4.1.3). This increase was consistent with the correlation between liveweight and EMA (Table 4.1.5). The increased liveweight in rams also lead to a greater EMA (P<0.005; Table 4.1.3). Rams had an area of 19.61cm^2±4.794 in comparison to ewes who averaged 15.10cm^2±2.760 (Table 4.1.3). This finding agrees with Cloete *et al*. (2004) where ram and ewe SA Mutton Merino and Dorper sheep displayed differences in EMA (P<0.01). In comparison to the current study, rams had an area of 15.8cm^2 and ewes had an area of 12.3cm^2.

4.1.7 Cold carcass weight and dressing percent

As the percent of fat increased in the three-rib-cut, the dressing percent (D%) also increased (P<0.001; -0.545; Table 4.1.5). Therefore, a lower dressing percent was associated with a higher muscle (P<0.005; -0.545) and bone percent (P<0.001; -0.548). Therefore, dressing percent was also positively correlated with fat depth at the 11th rib (P<0.002; 0.535) and cold carcass weight (CCW; P<0.021; 0.414). As shown in Table 4.1.2, the lower dressing percent and cold carcass weight were associated with tannin supplementation (P<0.001). The lower dressing percent and cold carcass weight as a result of tannin supplementation was also observed by Seoni *et al*. (2018) and Chikwana *et al*. (2019).

In agreement with reports from Cloete *et al*. (2004) and Johnson *et al*. (2005), the effect of sex on these parameters was also observed (P<0.05; Table 4.1.3). With an increased amount of fat in ewe lambs came a 6.44% increased dressing percent. There was also a 7.23% increase in CCW. The CCW was also 10.57% greater in heavier carcasses (P<0.001; Table 4.1.3).

4.1.8 Proximate analysis

The effect of calcium nitrate and tannin on moisture, ash and ether extract (EE; percent of lipid present in the *Longissimus dorsi*) is presented in Table 4.1.6. The effect of sex (ewe vs ram lambs) on the proximate analysis are depicted in Table 4.1.7. Interactions between factors treatment, weight and sex are presented in Table 4.1.8.

As depicted in Table 4.1.6, moisture (dry matter, DM) and ash were not affected by either NPN source or tannin supplementation (P>0.05). However, there was a tendency for tannin to reduce the amount of moisture in meat (P=0.076). Tannin also increased the concentration of lipid in the L*ongissimus dorsi* (P>0.05). Ewes and rams differed significantly in all parameters except for ash. There was no change in moisture, ash and EE when lambs were slaughtered at a heavier weight (P>0.05).

Louvandini *et al*. (2014) also experienced no effect of *Acacia mearnsii* tannin, supplemented to Santa Inez lambs on *Andropogon gayanus* pasture, on moisture and ash. This was also in agreement with Vasta *et al*. (2007). South African Mutton Merinos (SA Mutton Merino) and USDA Tables indicate that ash content increases with age (Schönfeldt *et al*., 2011). Therefore, although the mean average slaughter date varied between the treatments and between sex, the standard deviation within treatments and sex was high, and therefore did not affect ash content (P>0.05). The current study looked at a combination of A2 and A3 carcasses whereas Schönfeldt *et al*. (2011) compared A2 (lamb) and C2 (mutton) carcasses. These differences in carcasses are possibly also due to variations in carcass classification.

There were differences in moisture and EE between ewes and rams (P<0.005; Table 4.1.7). Kemp *et al*. (1976) reported that ewe carcasses contained more fat and less moisture than wethers. This was also reflected in the current study where EE in the *Longissimus dorsi* of ewes was 31.17% higher (P<0.001) and moisture was 3.1% lower (P<0.05) than rams. These results are similar to Hoffman *et al*. (2003) where the moisture content in the *M. semimembranosus* was 74.93% in rams but only 70.97% for ewes, and lipid content was 6.67% in rams in comparison to 9.52% in ewes. The results are consistent with reports by Kasap *et al*. (2018) that lambs with a higher fat content (i.e. the ewes) tend to have a lower moisture content. Intramuscular fat is deposited at an accelerated rate during the last phase of growth. Therefore, as lambs become heavier in light of their proximity to their mature size, ether extract in meat increases at the expense of protein and moisture (Kemp *et al*., 1976).

The effect of treatments on proximate analysis is reported in Table 4.1.6. The replacement of the urea NPN source with calcium nitrate had no effect on the lipid content (EE) in meat (P>0.05). Overall, the supplementation of *Acacia mearnsii* tannin increased the concentration of lipid (P<0.05). However, within the individual urea and nitrate-based diets, the EE level's increase was not significant with the

inclusion of tannin (P>0.05). However, the urea-based TMR with the inclusion of tannin had the highest lipid percent (16.43%), in comparision to the much lower nitrate-based diet without tannin (11.72%; P<0.05). The urea-based diet without tannin and calcium nitrate-based diet with tannin did not differ between treatments (P>0.05). In contrast to the results of this study, Louvandini *et al*. (2014) reported a drop in EE (P=0.05) in 25kg lambs supplemented with tannin. Costa *et al*. (2021) determined that *Acacia Mearnsii* can reduce lipid in meat when supplied at and above 20g/kg DM. Therefore, reduced lipid deposition in muscle may be dose dependant. The higher quality ration may also have offset the antinutritional qualities of the tannin and therefore had less of an effect on fat deposition in muscle.

Weight had no effect on the moisture, ash or ether extract percent (Table 4.1.7). Table 4.1.8 indicated no interactions between the factors (treatment, weight and sex of the lambs (ewe vs ram lambs).

Table 4.1.2: Means (± SD) depicting the effects of condensed tannin and NPN source on selected carcass characteristics and body composition of Merino lambs

	Urea		Calcium nitrate		P-value			
	None	Tannin	None	Tannin	Treat	T	N	T*N
Days in trial	77 ^{b}±13.8	110^{a}±23.7	74^{b}±14.0	93^{ab}±23.4	**0.01**	**0.003**	0.167	0.514
Bone %	14.1±2.13	14.6±1.33	14.5±2.02	15.5±2.15	0.257	0.137	0.253	0.514
Muscle %	51.7±7.66	55.8±5.63	52.7±5.29	51.4±6.42	0.281	0.493	0.379	0.12
Fat %	34.0±8.98	29.3±6.24	32.8±6.19	32.8±8.03	0.318	0.233	0.563	0.2
LW	51.7±3.84	51.5±4.79	53.0±4.40	52.2±4.91	0.51	0.622	0.16	0.777
CCW%	24.1^{a}±1.81	22.2^{ab}±1.99	24.1^{a}±1.66	21.6^{b}±2.01	**0.006**	**0.001**	0.407	0.744
D%	46.7^{a}±2.57	43.2^{bc}±1.82	45.5^{ab}±1.82	41.5^{c}±2.91	**0.001**	**<0.001**	**0.054**	0.861
SCF 8TH	3.63±1.42	3.77±1.40	3.35±0.81	4.15±0.88	0.5	0.217	0.794	0.391
SCF 11TH	5.4±2.39	4.6±1.74	5.6±1.24	5.2±1.95	0.529	0.283	0.524	0.455
EMA	17.92±4.186	16.39±4.550	19.17±5.472	15.94±3.616	0.35	**0.092**	0.8	0.553
Carcass pH	5.6±0.12	5.6±0.10	5.6±0.05	5.6±0.08	0.882	0.674	0.619	0.664
Temp	17.6±2.07	21.8±4.33	17.9±2.66	20.2±4.31	**0.076**	**0.016**	0.574	0.434

T: effect of tannin supplementation, N: effect of nitrogen source, T*N: interaction between tannin and nitrogen source, LW: live weight, CCW: cold carcass weight (kg), D%: dressing percent, SCF: subcutaneous fat depth at the 8th and 11th ribs (mm), EMA: eye muscle area, temp: temperature (°C).
Means within a row not bearing a common superscript differ (P<0.05)

Table 4.1.3: The effect of lamb sex and weight on selected carcass characteristics and body composition

	Sex		Weight category		P-value		
	Female	Male	Heavy	Light	FM	HL	MF*HL
Bone %	13.4±1.20	16.0±1.61	14.7±2.20	14.6±1.60	**<0.001**	0.520	0.620
Muscle %	38.0±5.00	56.8±4.91	51.6±5.88	54.4±6.52	**<0.001**	0.184	0.404
Fat %	37.2±5.89	27.2±4.70	33.426.86	30.9±7.78	**<0.001**	0.314	0.447
LW	48.7±1.86	55.5±3.19	53.8±4.47	50.1±3.32	**<0.001**	**<0.001**	**0.034**
CCW%	22.2±1.71	23.8±2.19	24.1±1.75	21.8±1.79	**0.003**	**<0.001**	0.227
D%	45.6±2.68	42.8±2.74	44.9±2.87	43.5±3.09	**0.002**	0.165	0.921
SCF 8TH	4.22±1.091	3.23±0.989	4.06±1.091	3.35±1.114	**0.007**	**0.049**	0.970
SCF 11TH	6.22±1.952	4.17±0.190	5.79±2.083	4.51±1.214	**0.001**	**0.021**	**0.015**
EMA	15.10±2.760	19.61±4.793	18.98±4.837	15.51±3.280	**0.003**	**0.014**	0.293
Ph	5.6±0.07	5.6±0.11	5.6±0.09	5.6±0.09	0.565	0.933	0.592
Temp	20.4±3.62	18.4±3.77	18.6±3.72	20.1±3.77	0.138	0.375	0.463
Days in trial	96±21.2	80±23.3	84±22.5	93±24.4	**0.041**	0.349	0.624

F: ewe lambs, M: ram lambs, FM: effect of sex, HL: effect of weight, MF*HL: interaction between sex and weight, LW: live weight, CCW: cold carcass weight (kg), D%: dressing percent, SCF: subcutaneous fat depth at the 8^{th} and 11^{th} ribs (mm), EMA: eye muscle area.
Means within a row not bearing a common superscript differ (P<0.05)

Table 4.1.4: The interaction between treatment, condensed tannin, and calcium nitrate on sex and weight

	P-value					
	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL
Bone %	0.602	0.357	0.535	0.548	0.106	0.267
Muscle%	0.452	0.960	0.296	0.823	0.926	0.825
Fat %	0.308	0.892	0.226	0.923	0.583	0.976
LW	0.763	0.812	0.607	0.371	0.677	0.652
CCW	0.931	0.999	0.964	0.937	0.997	0.905
D (%)	0.748	0.941	0.898	0.544	0.879	0.646
SCF 8TH	0.316	0.439	0.510	0.089	0.580	0.154
SCF2 11TH	0.502	0.563	0.739	**0.015**	0.553	0.242
EMA	0.947	0.395	0.961	0.561	0.679	0.610
Ph	0.218	0.676	0.515	0.080	0.250	0.995
Temp	0.192	0.702	**0.046**	0.719	0.608	0.896
Days in trial	0.684	0.990	0.265	0.624	1.000	0.812

LW: live weight, CCW: cold carcass weight, D%: dressing percent, SCF subcutaneous fat depth at the 8th and 11th rib (mm), EMA: Eye muscle area, Treat*MF: interaction between treatment and sex, Treat*HL: interaction between treatment and weight, T*MF: interaction between tannin supplementation and sex, N*MF: interaction between NPN source ad sex, T*HL: interaction between tannin and weight category, N*HL: interaction between NPN source and weight category.

Table 4.1.5: Correlation between carcass characteristics, body composition and days in trial of Merino lambs

	Muscle %		Bone %		Fat %		LW		CCW		D%	
	Corr	P-value	Corr	P-value	Corr	P-value	Corr	P-value	Corr	P-value	Corr	P-value
Muscle %	1											
Bone%	0.527	**0.002**	1									
Fat %	-0.976	**<0.001**	-0.692	**<0.001**	1							
LW	0.468	**0.008**	0.607	**<0.001**	-0.544	**0.002**	1					
CCW	0.093	0.619	0.207	0.264	-0.126	0.501	0.768	**<0.001**	1			
D%	-0.545	**0.002**	-0.548	**0.001**	0.6	**<0.001**	-0.263	0.152	0.414	0.021	1	
SCF 8TH	-0.668	**<0.001**	-0.475	**0.007**	0.662	**<0.001**	-0.223	0.228	-0.005	0.979	0.331	**0.069**
SCF 11TH	-0.699	**<0.001**	-0.672	**<0.001**	0.754	**<0.001**	-0.402	**0.025**	-0.024	0.899	0.535	**0.002**
EMA	0.216	0.244	0.345	**0.058**	-0.26	0.158	0.689	**<0.001**	0.576	**0.001**	-0.112	0.549
Carcass pH	-0.204	0.27	0.038	0.838	0.168	0.367	0.012	0.951	-0.081	0.666	-0.143	0.442
DIT	0.068	0.717	-0.159	0.392	-0.044	0.814	-0.342	0.059	-0.382	**0.034**	-0.09	0.628
Temp	0.127	0.497	-0.113	0.545	-0.102	0.586	-0.251	0.172	-0.277	0.131	-0.072	0.702

*Significant correlations are in bold.

Table 4.1.5 continued												
	SCF 8TH		SCF 11TH		EMA		Carcass pH		DIT		TEMP	
	Corr	P-value	Corr	P-value	Corr	P-value	Corr	P-value	Corr	P-value	Corr	P-value
Muscle %												
Bone %												
Fat %												
LW												
CCW												
D%												
SCF 8TH	1											
SCF 11TH	0.552	**0.002**	1									
EMA	-0.112	0.549	-0.037	0.843	1							
carcass pH	-0.143	0.079	-0.022	0.906	-0.048	0.799	1					
DIT	-0.09	0.628	-0.042	0.823	-0.537	**0.002**	-0.048	0.799	1			
Temp	-0.087	0.64	-0.032	0.864	-0.462	**0.009**	-0.198	0.285	0.883	**<0.001**	1	

LW: live weight, CCW,:cold carcass weight (kg), D%: dressing percent, SCF: subcutaneous fat depth over the 8th and 11th ribs (mm), EMA: eye muscle area, temp: temperature (°C); DIT: days in trial.

Table 4.1.6: The effect of condensed tannin and NPN source on the proximate composition of the *Longissimus dorsi*

	Urea diet		Nitrate diet		P-value			
	None	Tannin	None	Tannin	Treat	T	N	T*N
Replication	8	8	8	8				
Moisture%	71.99±2.130	70.35±2.250	72.10±1.864	71.03±2.010	0.293	0.076	0.567	0.702
Ash%	1.14±0.049	1.14±0.063	1.12±0.049	1.14±0.063	0.983	0.836	0.791	0.756
EE%	13.19^{ab}±4.191	16.43^{a}±3.728	11.72^{b}±4.580	14.49^{ab}±3.377	**0.037**	**0.011**	0.113	0.868

T: Tannin supplementation, N: NPN source, T*N: interaction between tannin and NPN source.

Table 4.1.7: The effect of sex and slaughter weight on the on the proximate composition of the *Longissimus dorsi*

	Sex		Weight		P-value		
	F	M	H	L	MF	HL	MF*HL
Replication	16	16	17	15			
Moisture%	70.27±1.501	72.47±2.060	71.12±2.144	71.65±2.078	**0.009**	0.451	0.853
Ash%	1.14±0.051	1.14±0.063	1.13±0.064	1.13±0.042	0.388	0.964	0.656
EE%	16.53±3.251	11.38±3.386	14.62±4.088	13.21±4.308	**<0.001**	0.258	0.465

F: female, M: male; H: heavy, L: light, MF: sex, HL: weight, MF*HL: interaction between weight and sex.

Table 4.1.8: Proximate interactions between treatment, weight and sex

	P-Value					
	Treat*MF	Treat*HL	T*HL	T*MF	N*HL	N*MF
Moisture%	0.769	0.781	0.839	0.914	0.328	0.701
Ash%	0.374	0.999	0.934	0.171	0.944	0.586
EE%	0.776	0.158	0.124	0.452	0.126	0.701

Treat*MF: Treatment and sex interaction, Treat*HL: Treatment and weight interaction, T*HL, tannin and weight interaction, T*MF: tannin and sex interaction, N*HL: None protein nitrogen source and weight interaction, N*MF: None protein nitrogen source and sex interaction.

4.1.9 Conclusion

The largest disadvantage of the *Acacia mearnsii* tannin treatment is the increased length of time it would take for lambs to reach their target slaughter weight and cold carcass weight. It also lowered the dressing percent and increased the amount of lipid (EE) in the *longissimus dorsi* muscle due to the higher temperature of the *longissimus dorsi* muscle which in turn could potentially increase meat tenderness. It did not affect any of the other carcass characteristics (body composition, fat depth, eye muscle area or pH). Calcium nitrate could be used without affecting any of the carcass characteristics or proximate analysis with the exception of a decrease in dressing percent.

At the weight and age the lambs were slaughtered, sex had a major affect on carcass characteristics. Ewes cost more as they took longer to reach their target slaughter weights and were already depositing subcutaneous and intramuscular fat (EE) when they were slaughtered. Rams were higher in muscle, bone and moisture and ewe body composition was higher in fat and lower in muscle and bone. Therefore, to be of equal mature size to rams, ewes could have been slaughtered at an even lighter weight category. Cold carcass weight and eye muscle area were higher in rams, and due to ewes' higher ratio of fatty acid, dressing percent was greater in ewes.

The weight of lambs at slaughter was predetermined and lambs slaughtered at a heavier weight took longer to reach their target weight. The heavier lambs had a higher CCW but D% was not affected. Although it was not yet evident in the body composition, the heavier lambs had started depositing fat in their subcutaneous fat. However, the final deposition site, IMF (EE), was not yet different between the weight categories. Ash and moisture did not differ either. Due to their live weight difference, eye muscle area was also greater in heavier lambs. Therefore, the higher carcass weight is advantageous provided it offsets the cost of the feed.

4.2 The effect of treatment, sex and weight on blood parameters

The results pertaining to selected blood parameters are presented in Table 4.2.1 for treatment and Table 4.2.2 for sex. There was no interaction between treatment and sex or between NPN source and tannin supplementation.

The haematocrit (also called packed cell volume (PCV) or erythrocyte volume fraction (EVF)) is the percent of red blood cells in blood (Etim *et al*., 2014). It was within the normal reference range specified by the University of Pretoria Diagnostic laboratory and SA Mutton Merino sheep studied by Akanmu *et al*. (2020). It therefore indicated adequate haematocrit for the transportation of oxygen and nutrients to tissues regardless of treatment or sex (Etim *et al*., 2014). It did not differ with NPN source or tannin supplementation (P>0.05). It also did not differ between sex (P>0.05).

Albumin is a major protein produced by the liver and is an indication of health, disease (Rothschild, 1977, Don & Kayson, 2004) and protein status of an animal (Mapiye *et al*., 2010). In the current study albumin was higher (35 to 36%) in comparison to the reference range (28 to 34%) but still within the range specified by Akanmu *et al*. (2020) for SA Mutton Merino sheep (35.06 to 37.58%). The differences between the studies may have to do with the length of time the sheep were on the feed. Therefore, the results indicated that the sheep were healthy and had reached their NPN and crude protein requirements. There was no interaction between the tannin and NPN source (Table 4.2.1). Sheep on a calcium nitrate based TMR had more albumin (P<0.05; Table 4.2.1). Kaysen *et al*. (1989) reported an increase in albumin synthesis when high protein diets were fed to rats. In accordance with this, when sheep were deprived of protein, Sykes (1978) reported reduced albumin concentrations. Therefore, the sheep in the current study must have absorbed more protein from the calcium nitrate-based diets. Higher albumin concentrations are consistent with the finding that there was more BUN in the calcium nitrate-based diets (P<0.05). Confirming the reports by Buccioni *et al*. (2017) and Torres *et al*. (2022), tannin had no effect on albumin concentrations (P>0.05). In contrast to Van Zyl (1967) rams did not have more albumin than ewes (P>0.05; Table 4.2.2).

The blood urea nitrogen concentration (BUN) is a common analysis used to determine the general protein status of livestock (Hammond, 1983). The BUN level was higher than the reference concentration for all the treatments (Table 4.2.1 and Table 4.2.2). This is probably due to the concentration of NPN added to the diet in combination with the crude protein already in the TMR. Nitrate and urea are converted to ammonia in the rumen which may have resulted in an increase in its absorption through the rumen wall and intestine and into the portal blood (Lewis *et al*., 1957; Latham *et al*., 2016). There is also evidence that nitrate and nitrite can also rapidly absorb through the rumen wall and into the blood stream if it is present in high amounts (Villar *et al*., 2021). On slow

introduction (as was done in the current study) the activity of microbes that reduce nitrate to ammonia are increased, thereby reducing the concern for toxicity (Lee and Beauchemin, 2014). Ruminants also adapt by improving the access of oxygen through increased haemoglobin, red blood cells and blood volume, thereby reducing the risk of methemoglobinemia (Latham *et al*., 2016). The current study was unable to analyse the methaemaglobin in blood to verify this. If the energy levels differed between the diets, a higher energy level may have also resulted in a lower BUN concentration (Kirkpatrick et al 1965).

There was no interaction between nitrogen source and tannin inclusion for the BUN concentration (Table 4.2.1). Table 4.2.1 indicated that CaN inclusion tended to have more BUN than the urea-based diets (P=0.066). Therefore, the calcium nitrate based TMR tended to provide more nitrogen. As a whole tannin did not affect BUN levels (P>0.05). However, the calcium nitrate diet without tannin had the highest BUN concentration and the urea diet with tannin had the lowest concentration (P<0.05). Therefore, when tannin was added to the calcium nitrate-based diet the value reduced enough that it did not differ between the treatments (P>0.05). Tannin has protein binding properties (Jayanegara *et al*., 2018; Yusiati *et al*., 2018; Zeller, 2019). Therefore, it showed an inclination to counteract the high ammonia concentration. BUN concentrations did not differ between the ewes and rams (Table 4.2.1). However, Mukhoty *et al*. (1969) reported that the state of maturity affects the BUN concentration and that the point at which BUN increased more rapidly was earlier in females than males.

The effect of treatment on cholesterol was reported in Table 4.2.1. The cholesterol concentration was similar to SA Mutton Merino rams reported by Akanmu *et al*. (2020) which ranged between 1.42 and 1.53mmol/L. In general, the animals fed calcium nitrate as a NPN source had more cholesterol (P<0.05). A study on the dose effect of encapsulated nitrate on feedlot finishing bulls hypothesised that a linear increase in cholesterol with increasing dose may have been as a result of reduced thyroid activity (Araujo *et al*., 2021). The Urea-based TMR supplemented with tannin had the lowest cholesterol concentration, which was lower than the calcium nitrate-based diet without the added tannin (P<0.05). Therefore, although tannin did not significantly reduce the cholesterol concentration (P=0.089), the dosage may have been enough to reduce the calcium nitrate based TMR to a level similar to all the diets (P>0.05). Zimmer and Cordesse (1995) reported a small but significant reduction in cholesterol when tannin was fed to sheep. According to Silanikove *et al*. (2006) tannin has the ability to reduce protein degradability and trap fat and cholesterol in fibres. A deficiency in vitamin B12 and a disruption in microbial vitamin B12 synthesis can also cause a decrease in haemaglobin (Priolo & Vasta, 2007).

There was a trend in ewes having 16.16% more cholesterol than rams (P=0.102; Table 4.2.2). A difference was also reported by Ban-Tokuda *et al*. (2007) in Brahman cattle. According to a study on rats conducted by Lee *et al*. (2008), the differences between males and females becomes significant upon reaching sexual maturity as a result of testosterone supressing plasma cholesterol levels. SA Mutton Merino rams reach a mature weight around 120kg (Van der Merwe *et al*., 2019). Therefore, some of them would not have reached 40% to 50% their mature weight at the time of collection.

Conclusion:

Haematocrit and albumin were normal, indicating no deleterious effects on lamb health when calcium nitrate and *Acacia mearnsii* tannin were fed at the current dose. The BUN level was higher than the reference range but due to a slow introduction of nitrate to the diet, microbes were able to adapt and no morbidity or mortality was recorded. Cholesterol was at an acceptable range for SA Mutton Merinos. BUN, cholesterol and albumin increased with calcium nitrate supplementation, but tannin had no effect on any of the parameters. Sex and weight had no effect on blood parameters.

Table 4.2.1: Means (±SD) depicting the effect of condensed and calcium nitrate on the selected blood and serum parameters

			Urea diet		Nitrate diet		P-VALUE		
	Ref. range	Average	None	Tannin	None	Tannin	T	N	T*N
# Lambs		29	7	8	8	8			
Haematocrit (L/L)	0.22-0.44	0.344±0.026	0.336±0.030	0.346±0.029	0.35±0.028	0.344±0.019	0.851	0.537	0.417
BUN (mmol/L)	2.7-6.6	9.266±1.213	8.943±0.637ab	8.643±1.116a	10.271±1.546b	9.213±0.958ab	0.112	0.03	0.336
Cholesterol (mmol/L)	NR	1.314±0.249	1.329±0.269ab	1.100±0.141a	1.443±0.223b	1.375±0.244ab	**0.089**	**0.029**	0.346
Albumin (g/L)	28-34	36.693±1.979	36.043±1.545	35.729±1.975	37.643±2.317	37.275±1.741	0.635	0.036	0.97

T: Tannin, N: NPN source, T*N: interaction between tannin and NPN source

Table 4.2.2: Means (±SD) depicting the effect of sex on selected blood and serum parameters

	Ref. range	Female	Male	Treat	Sex	Sex*Treat
# Lambs		13	16			
Haematocrit L/L	0.22-0.44	0.343±0.016	0.344±0.032	0.791	0.726	0.45
BUN (mmol/L)	2.7-6.6	9.462±1.439	9.106±1.016	0.116	0.555	0.103
Cholesterol (mmol/L)	NR	1.423±0.283	1.225±0.181	0.085	0.102	0.675
Albumin (g/L)	28-34	36.762±2.481	36.638±1.514	0.157	0.697	0.597

Reference ranges from Ondersterpoort

4.3 The effect of treatment, sex and weight on fatty acid composition

The fatty acids identified in intramuscular fat (IMF), subcutaneous fat (SCF), perirenal fat (PRF) and rumen fluid are presented in Table 4.3.2 to 4.3.5. The fatty acid section will begin looking at the effect of tannin (T) and none protein nitrogen source (N) and their interaction (T*N) on the fatty acid composition of rumen fluid (w/w%), with the purpose of getting an idea of the effect of treatment on rumen microbial activity, and therefore the fatty acids available for absorption. Sex and weight were not used as factors in the statistical analysis. The molar percent of fatty acid (w/w%) has also been referred to as 'percent' and 'percent of detected fatty acids'. The gravimetric concentration (mg/g) has also been referred to as 'concentration'. Significant differences in the table will be highlighted in bold.

The percent (w/w%) of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and major fatty acids (C16:0, C18:0 and C18:1n9c) will then be compared between fat depots; intramuscular fat (IMF), subcutaneous fat (SCF) and perirenal fat (PRF) and with literature. The purpose of this is to get a feeling of the general fatty acid profile in Merino lambs before studying the effect of treatment, sex and weight on the fatty acid profile of the different fatty acid depot sites. Following this, the fatty acid profile will be discussed simultaneously as a percent of detected lipid (w/w%) and gravimetrically (mg/g). First the effect of the combination of tannin/no tannin and urea/calcium nitrate will be discussed, followed by the effect of sex and weight.

Sex and weight will be discussed to confirm the basic observations are in line with other literature and support the treatment results. Within the factors of treatment, sex and weight the fatty acids will be discussed in the order of the least stable to most stable fatty acid depot sites; IMF, SCF and then PRF. The SFA, MUFA, PUFA and unsaturated fatty acids (UFA) are first discussed to give an overall impression of the fatty acid profile. This is followed by an in-depth discussion of the significant individual fatty acids and/or fats of interest, sums, ratios and enzyme indices relative to the SFA, MUFA and PUFA categories.

Interactions between treatment and sex or weight will be acknowledged where the interaction is significant ($P<0.05$). Interactions tables between all factors are in the Appendix. The significant interactions are in bold where $P<0.05$. Bar graphs will be used as a visual aid to suggest why the interaction is significant for fatty acids of interest and with large numerical differences. Standard error on the bar graphs are only used to help with visual interpretation.

There is a common misconception that red meat only contains harmful fatty acids like saturated fat, which it does not (Webb, 2021). Some SFAs and trans fatty acids do not pose as much of a health risk

as others and therefore need further exploration. For example, C12:0, C14:0 and C16:0 fatty acids are harmful as they are hypercholesteroleamic. However, C18:0 decreases low density lipoprotein (LDL) cholesterol and/or has a neutral effect (Grundy, 1997; Hunter *et al*., 2010). Therefore, selected indices adopted from other authors have been reported on to give a broader picture as to how healthy the fat is, and not just how saturated or trans it is as a whole. The degree to which treatment, sex and weight were affected was dependent on the fatty acid depot and method of interpretation (as a percent of total fatty acid or as a concentration).

4.3.1 The effect of calcium nitrate and condensed tannin on the fatty acid profile of rumen fluid

The effect of tannin and calcium nitrate on rumen fluid is shown in Table 4.3.1. The fatty acids absorbed by the ruminant differ from the fatty acid in the feed. This is due to hydrolysis of acyl lipids to free fatty acids followed by the biosynthesis of microbial fatty acids in the rumen (Harfoot, 1981). The linoleic and linolenic acid (present in high amounts in feed) is lost through biohydrogenation to intermediate fatty acids and stearic acid, the end-product of C18 biohydrogenation (Harfoot, 1981; Honkanen *et al*., 2012). Studies have shown that inclusion of tannin has the potential to alter rumen biohydrogenation *in vivo* (Alves *et al*., 2017) and *in vitro (*Buccioni *et al*., 2011). The fatty acid composition of the outflow is dependent on whether it affects the earlier (Kronberg *et al*., 2007; Minieri *et al*., 2014) or later (Khiaosa-Ard *et al*., 2009; Vasta *et al*., 2010) step in biohydrogenation. To my knowledge minimal research has been done to identify the effect of calcium nitrate on biohydrogenation. Although the fatty acid profile of the feed was not analysed, the rumen profile was used to get an idea of the microbial activity in the rumen fluid to the fatty acid profile of lipid. Table 4.3.1 shows that biohydrogenation intermediate ratios of the C18 pathway were not significant and therefore suggest that *Acacia mearnsii* tannin, at the current dose, did not influence the biohydrogenation pattern (P>0.05). Calcium nitrate decreased the C18:0/C18:1n11t (SA/VA) ratio (P<0.01). This would suggest decreased biohydrogenation at the final step of the biohydrogenation pathway as a result of decreased microbial activity.

a) The effect of calcium nitrate and condensed tannin on the SFA profile of rumen fluid

The total saturated fatty acid (SFA) content tended to be lower in the rumen fluid of sheep consuming tannin in their diet (P=0.073), indicating that tannin showed potential to reduce complete biohydrogenation. However, the standard deviation (SD) within the treatments varied between approximately 8 to 11%. Alves *et al*. (2017) also reported reduced SFA in lambs fed a diet containing tannin and oil. Using calcium nitrate as an NPN source did not change the percent of SFA in rumen fluid (P>0.05). Individual SFAs were also affected by treatment.

The product of the final step of C18 FA biohydrogenation, stearic acid (C18:0), was the major fatty acid contributing approximately 35 to 40% of rumen fluid fatty acid (approximately 60% of SFA) and was unaffected by tannin and NPN source. In contrast to the current study, Buccioni *et al*. (2017) demonstrated in dairy ewes that Quebrecho (a condensed tannin) was able to reduce C18:0. Khiaos-Ard *et al*. (2009) incubated rumen fluid with Acaica mearnsii (CT) and came to the same conclusion. In agreement with the current study, Vasta *et al.* (2010) reported no significant decrease in C18:0 when added to a concentrate-based diet. In the current study, tannin supplementation reduced the second largest contributing fatty acid, C16:0 ($P<0.005$). It also decreased C12 ($P<0.05$), C13 ($P<0.001$), C14 ($P<0.005$), C17 ($P<0.005$), C20 ($P<0.05$), C23 ($P<0.005$) and C24 ($P<0.05$) SFAs in rumen fluid. Calcium nitrate only increased C12 and C13 SFAs ($P<0.05$).

Tannin decreased the percent of C12:0 and calcium nitrate increased it. Therefore, the urea-based diet with tannin was lower than calcium nitrate-based diet without tannin ($P<0.05$). Tannin decreased C14:0 in the urea-based diet ($P<0.05$) and although it decreased in the calcium nitrate-based diet it was not significant ($P>0.05$). Odd chain fatty acids (C13:0, C17:0 and C23:0) were reduced through tannin supplementation ($P<0.001$, $P<0.005$ and $P<0.005$, respectively). Tannin and calcium nitrate both decreased the concentration of C13:0 ($P<0.05$). Therefore, the urea-based diet with tannin was lower in C13:0 than the urea-based diet without tannin ($P<0.05$). The calcium nitrate-based diet without tannin was also higher than the calcium nitrate-based diet with tannin ($P<0.05$). The urea-based diet without tannin was higher in C13:0 than all the other treatments ($P<0.05$). Although the urea-based diet with tannin was higher than the calcium nitrate-based diet with tannin, it was not enough to be significant ($P>0.05$). The C17:0 interacted with both tannin and NPN source ($P<0.005$). Therefore, tannin only reduced its percent in lambs on the urea-based diet ($P<0.05$). The urea-based diet without tannin was higher than all the treatments ($P<0.05$). This may be as a result of increased rumen microbial activity as results suggest it was not hindered by calcium nitrate nor tannin. Tannin decreased the overall percent of C23:0 ($P<0.005$). However, the Bonferroni test did not detect the decrease as significant within the urea or calcium nitrate-based diet ($P>0.05$).

The C15:0 and C17:0 odd chain fatty acids are synthesized through the elongation of propionate and valerate by rumen microbes (Vlaeminck *et al*., 2006a). According to Adejoro *et al*. (2020), propionate increased with tannin supplementation. Therefore, it would suggest that microbial elongation was inhibited, particularly in the urea-based diet with tannin. This is consistent with Lee *et al*. (2017) who suggested a decrease in C17:0 in IMF was a result of reduced activity of rumen microbes. The current

study did not differentiate between branched and unbranched fatty acids. Vlaeminck *et al*. (2006b) reported that changes in OBCFAs in milk were likely a result of the OBCFA composition of the microbial population.

b) The effect of calcium nitrate and condensed tannin on the MUFA profile of rumen fluid

Tannin tended to reduce MUFA in rumen fluid (P=0.075), and calcium nitrate tended to increase it (P=0.062). Although Vasta *et al*. (2009) found no significant difference in total MUFA in rumen fluid of sheep fed herbage diet with tannin (approximately 11 to 12% MUFA), there was a significant effect from tannin when added to a concentrate-based diet (approximately 19 to 33% MUFA). A later report by this author identified no effect on the MUFA percent when Quebracho tannin was added to a concentrate diet (Vasta *et al*., 2010). Therefore, the composition of the diet may influence the effect of tannin.

Approximately 97% of MUFAs in the current study came from C18:1 fatty acids. Making up approximately half of these fatty acids was oleic acid (C18:1n9c). Tannin decreased the percent of C18:1n9c (P<0.005), due to a large decrease in the calcium nitrate-based diet (P<0.05). Although it decreased, it was not significantly lower in the urea-based diet (P>0.05). The biohydrogenation effect of tannin (regardless of NPN source), represented by the C18:0/ C18:1n9c ratio (SA/OA), was not affected by tannin or NPN source (P>0.05). This suggests that the microbes responsible for the biohydrogenation of oleic acid (C18:1n9c) to stearic acid (C18:0) were unaffected by tannin. C24:1 also tended to be lower in the tannin supplemented diet (P=0.064).

There may be another reason for a decrease in C18:1n9c. *In vitro* reports have demonstrated that oleic acid (C18:1n9c) can also isomerise to other members of the C18:1 family (Buccioni *et al*., 2006), particularly trans isomers according to Mosley *et al*. (2002). However, only a limited number of C18 MUFAs were analysed in this study, and therefore a further investigation would be required to determine if tannin could increase and/or affect other oleic acid isomers. *Fusocillus babrahamensis* P2/2 and *Fusocillus* T344 hydrogenate oleic acid to stearic acid in the rumen (*Kemp et al*., 1975). Oleic acid is not always hydrogenated directly to stearic acid because *F. babrahamensis* P2/2 can also hydrate oleic acid to hydroxystearic acid, and *Fusocillus* T344 can also convert oleic acid to vaccenic acid (C18:1n11t) (Kemp *et al*., 1975). However, vaccenic acid was not affected.

Linoleic acid (C18:2n6c) and α-linolenic acid (C18:3n3) are first isomerised at the cis double bond to form conjugated C18:2 and C18:3 fatty acids, which then undergo hydrogenation to vaccenic acid

(C18:1n11t), and is further reduced to stearic acid (C18:0; Shingfield *et al*., 2010). Although stearic acid is the major fatty acid in the rumen, biohydrogenation of vaccenic acid to stearic acid is considered the rate limiting step (Shingfield *et al*., 2010; Meynadier *et al*., 2018). Therefore, an increase in PUFA and MUFA intermediates at the expense of SFA is determined by the step in which biohydrogenation is inhibited. The product of the second step of C18 FA biohydrogenation of linoleic acid (C18:2n6c) and third step in α-linolenic acid (C18:3n3) biohydrogenation, vaccenic acid (C18:1t11), is the major trans monoene of ruminant fat and an important substrate for delta 9 desaturase activity needed for the synthesis of rumenic acid (CLA cis-9, tans-11) in ruminant fat (Bessa *et al*., 2015). Based on the RA/LA, VA/RA, VA/CLA, SA/VA, CLA/LA and VA/LNA ratios not being affected by tannin ($P>0.05$), neither the first nor second step in biohydrogenation was affected by the supplementation of tannin at the current dose.

Other studies have successfully used tannin to change microbial activity. An *in vitro* study by Durmic *et al*. (2008) reported that the minimum concentration of *Acacia mearnsii* needed to inhibit *Cl. Proteoclasticum,* prominent in the last step in biohydrogenation, was a lot lower (<1mg/ml) in comparison to *B. fibrisolvens* (10mg/ml). This would suggest an accumulation of vaccenic acid, as demonstrated *in vitro* with *Acacia mearnsii* (7.9% DM) by Khiosa-Ard *et al*. (2009). This agreed with Ishlak *et al*. (2015) who sequenced Butyrivibrio *spp* into stearic acid producing and vaccenic acid producing bacteria. They reported that Quebracho tannin was able to significantly increase the DNA abundance of *Butyrivibrio* vaccenic acid producing bacteria from 0.02 to 0.04 but had no effect on Butyrivibrio stearic acid producing bacteria. The condensed tannin was unable to decrease B. Proteoclasticum (phyolgenically related to Fusocillus). However, the quebracho tannin did not increase the vaccenic acid, possibly as a result of the production of unknown biohydrogenation intermediates.

Calcium nitrate did not affect C18:1n9c or the SA/OA ratio in rumen fluid ($P>0.05$) but it did increase C18:1n9t ($P<0.005$) and C18:1n11t fatty acids ($P<0.05$). Due to its large contribution to the trans fatty acid percent in rumen fluid, it increased the total percent of trans fatty acids and the overall trans/cis ratio in rumen fluid ($P<0.05$). Increased C18:1n11t also decreased the SA/VA ratio ($P<0.010$). This would suggest an accumulation of vaccenic acid as a result of a reduction in microbial activity at the final step of the biohydrogenation pathway. Rumen microbes responsible for biohydrogenation are affected when the ruminal activities and dynamics change as a result of hydrogen availability and the microbial populations involved with different metabolic processes (Lourenço *et al*., 2010). According to Adejoro *et al*. (2020), Merino lambs on a calcium nitrate-based total mixed ration (TMR) had 20%

less methane emissions than those on the urea-based diet. The relationship between methanogenesis and biohydrogenation has not been widely studied *in vivo*. Although nitrate can be used as an alternative hydrogen sink to reduce methanogenic activity, Yang *et al*. (2019) did not find an overall effect on biohydrogenation in bovine ruminal digesta. Guo *et al*. (2021) found differences in methanogenic bacteria but also found no differences in bacteria, archaea, protozoa, fungi and *Butyrivibrio spp.* associated with biohydrogenation in water buffalo. Perhaps this is because although biohydrogenation competes for hydrogen, only 1-2% of metabolic hydrogen is used for its reaction (Nagaraja *et al*., 1997).

c) *The effect of calcium nitrate and condensed tannin on the PUFA profile of rumen fluid*

Polyunsaturated fatty acid (PUFA) remained consistent at approximately 3% regardless of tannin or calcium nitrate supplementation (P>0.05). This agrees with Vasta *et al*. (2010) who reported no significant difference in total PUFAs, C18:2n6c (the major PUFA), or total CLAs. However, in the current study the total unsaturated fatty acid UFA (MUFA plus PUFA) percent was lower in the tannin rich feed (P<0.05).

As expected, the C18:2n6c percent was higher than C18:3n3 due to the concentrate in the ration. This is because C18:3n3, present in glycolipids and phospholipids, are present in higher quantities in grass and forage, but C18:2n6c, present predominantly in triglycerides, is higher in the fat of concentrate rations (Lourenço *et al*., 2010). Neither tannin nor calcium nitrate influenced C18:2n6c or C18:2n6t fatty acids (P>0.05). The null effect of tannin may have been dose dependent. Carreño *et al*. (2015) reported that at their highest level of tannin supplementation *in vitro* (80g/kg DM), C18:3n3 no longer differed from the control. The initial isomerisation product from C18:2n6c biohydrogenation, CLA trans-10, cis-12, decreased with tannin supplementation (P<0.05), and calcium nitrate tended to increase it (P=0.086). Although there was a decrease within the urea and calcium nitrate-based diets, the Bonferroni test did not report it as significant (P>0.05). The urea-based diet with tannin had the highest CLA trans-10, cis-12 percent (0.202%±0.111) and the calcium nitrate-based diet without tannin had the lowest (0.099%±0.032; P<0.05). Isomer 3 also tended to be lower in the rumen fluid of the lambs on the tannin containing diet (P=0.09). Total CLA and CLA cis-9, trans-11 + trans-9, cis-12 was unaffected by supplementation (P>0.05).

Tannin did not increase the isomerization of C18:2n6c to CLA cis-9, trans-11 + trans-9, cis-12 or CLA observed through the CLA cis-9, trans-11 + trans-9, cis-12/ C18:2n6c (RA/LA) ratio and the CLA/C18:2n6c (CLA/LA) ratio (P>0.05). The biohydrogenation of CLA cis-9, trans-11 + trans-9 to

C18:1n11t, identified through the C18:1n11t/CLA cis-9, trans-11+trans-9, cis-12 (VA/RA) ratio did not change (P>0.05). Neither did the C18:1n11t/CLA ratio (VA/CLA; P>0.05). In contrast to the current study, Vasta *et al*. (2009) identified higher amounts of CLA cis-9, trans-11 when tannin was supplemented, indicating differences in the microbes the tannin targeted. However, the total percent of CLA did not differ. The discrepancies between studies may be as a result of the type of tannin and its dose supplied to the diet. For example, Carreno *et al*. (2015) reported that the type of tannin (Quebracho, grape, Oak and chestnut) and dose can affect CLA trans-10, trans-11 and cis-9, trans-11 fatty acids *in vitro*.

Calcium nitrate supplementation did not influence total CLA (P>0.05) but CLA cis-9, trans-11 (rumenic acid) + trans-9, cis-12 tended to be lower (P=0.096). The RA/LA and CLA/LA ratios indicated no increase in isomerization in the calcium nitrate-based diet (P>0.05). The VA/RA ratio did not differ for the rumen fluid of sheep fed calcium nitrate (P>0.05). VA/CLA also did not differ with NPN source (P>0.05). The ratio would suggest that biohydrogenation was not affected at that point. Therefore, the only biohydrogenation ratio to be affected was the SA/VA ratio (P<0.01). In an *in vitro* experiment on buffalo Guo *et al*. (2021) increased sodium nitrate concentrations in increments from 0 to 3mg/mL and also identified an increase in C18:1n11t concentration but with no effect on CLA cis-9, trans-11 or C18:0. *Clostrium* and *Butyrivibrio* were unaffected by 2% calcium nitrate in an *in vitro* study conducted by Zhao *et al*. (2015). Many bacteria that can convert conjugated linoleic acid (CLA) to C18:1n11t, are not able to convert C18:1n11t to C18:0. Li *et al*. (2012) demonstrated that certain bacteria within the *Firmucutes* and *Proteobacteria* families (such as *B. proteoclasticus*) were able to biohydrogenate C18:1n11t to C18:0. C18:1n11t and CLA can also be incorporated into protozoa (Devillard *et el*., 2006). Rumen fluid fatty acid data suggests microbes were affected at the last step of biohydrogenation.

The C18:1n11t/C18:3n3 ratio (VA/LNA) also tended to increase in the calcium nitrate-based diet (P=0.057). This is because C18:1n11t increased (P<0.05) at a greater rate than C18:3n3 (P<0.05) when calcium nitrate was used as an NPN source. This ratio suggests a decrease in microbial activity. There was also an interaction in the ratio where tannin increased the percent in the urea-based diet but decreased it in the calcium nitrate-based diet (P=0.054). This result must be interpreted with caution as a result of an extreme C18:3n3 outlier in the calcium nitrate-based diet supplemented with tannin. Sakurama *et al*. (2014) reported the ability of *Clostridium bifermentans* JCM 1386 to saturate Arachidonic acid (C20:4n6) to cis-5, cis-8, trans –13 eicosatrienoic acid (ETA). The current study did not detect arachidonic acid in the rumen fluid. Tannin and calcium nitrate decreased C20:3n3 (P<0.05).

Table 4.3.1: Means (± SD) depicting the effect of condensed tannin and NPN source on the medium and long chain fatty acid profile in rumen fluid expressed as a percent of detected fatty acid (w/w%)

(w/w%)	Urea		Calcium nitrate		P-value		
	None	Tannin	None	Tannin	T	N	T*N
Replicates (n)	8	8	8	8			
C12:0	**0.905^{ab}±0.238**	**0.547^{b}±0.234**	**1.175^{a}±0.406**	**0.908^{ab}±0.449**	**0.016**	**0.015**	0.71
C13:0	**0.186^{a}±0.041**	**0.103^{bc}±0.027**	**0.141^{b}±0.031**	**0.094^{c}±0.019**	**<0.001**	**0.018**	0.104
C14:0	**1.793^{a}±0.386**	**1.197^{b}±0.262**	**1.711^{ab}±0.494**	**1.469^{ab}±0.332**	**0.004**	0.484	0.196
C16:0	17.654±2.087	15.372±0.934	17.121±2.473	15.486±1.349	**0.005**	0.746	0.618
C17:0	**0.755^{a}±0.111**	**0.520^{b}±0.075**	**0.600^{b}±0.124**	**0.600^{b}±0.100**	**0.003**	0.314	**0.004**
C17:1	0.048±0.026	0.071±0.054	0.077±0.073	0.046±0.022	0.817	0.889	0.127
C18:0	38.664±10.001	35.118±11.580	39.832±9.303	36.421±7.538	0.32	0.722	0.984
C18:1n9t	0.198±0.056	0.199±0.054	0.292±0.078	0.254±0.079	0.448	**0.004**	0.425
C18:1t11	2.388±0.847	2.052±0.829	3.020±0.840	3.147±1.386	0.77	**0.022**	0.52
C18:1n6c	1.999±0.211	2.181±0.395	1.906±1.519	2.095±0.182	0.15	0.482	0.977
C18:1n9c	**5.016^{ab}±0.766**	**4.519^{ab}±0.518**	**5.300^{a}±0.664**	**4.239^{b}±0.577**	**0.002**	0.992	0.221
C18:1n11c	0.558±0.166	0.505±0.139	0.511±0.158	0.490±0.113	0.474	0.546	0.764
C18:2n6t	0.060±0.032	0.056±0.063	0.051±0.018	0.054±0.036	0.95	0.695	0.817
C18:2n6c	1.662±0.777	1.913±0.332	1.886±0.950	1.570±0.415	0.892	0.802	0.24
C20:0	0.857±0.171	0.656±0.140	0.832±0.143	0.761±0.142	**0.016**	0.452	0.229
C20:1	0.039±0.016	0.100±0.124	0.045±0.009	0.037±0.010	0.238	0.211	0.132
C18:3n3	0.314±0.102	0.335±0.131	0.387±0.192	0.213±0.120	0.136	0.629	0.059
CLA cis-9, trans-11/trans-9, cis-12	0.560±0.350	0.417±0.296	0.585±0.310	0.544±0.450	0.47	0.551	0.69

Table 4.3.1 continued…

CLA Trans-10, cis-12	**0.202^{a}±0.111**	**0.136^{ab}±0.079**	**0.148^{ab}±0.043**	**0.099^{b}±0.032**	**0.033**	**0.086**	0.754
CLA isomer 3	0.212±0.086	0.199±0.120	0.248±0.060	0.151±0.079	**0.09**	0.868	0.193
C22:0	0.424± 0.103	0.341±0.078	0.430±0.103	0.448±0.123	0.378	0.135	0.178
C20:3n6	0.267±0.117	0.203±0.072	0.212±0.146	0.231±0.40	0.538	0.71	0.254
C20:3n3	**0.257^{ab}±0.028**	**0.207^{b}±0.022**	**0.299^{a}±0.097**	**0.254^{ab}±0.035**	**0.02**	**0.027**	0.905
C23:0	**0.094^{ab}±0.028**	**0.070^{b}±0.015**	**0.103^{a}±0.026**	**0.077^{ab}±0.020**	**0.004**	0.326	0.89
C24:0	0.530±0.099	0.433±0.060	0.547±0.135	0.498±0.095	**0.048**	0.261	0.505
C24:1	0.078±0.019	0.053±0.017	0.074±0.051	0.057±0.079	**0.064**	0.991	0.721
UIP	24.280±8.846	32.499±11.786	22.465±11.026	29.759±9.295	**0.042**	0.537	0.9
Sums and ratios							
SFA	61.863±9.274	54.356±11.650	62.492±10.803	56.761±8.102	**0.073**	0.673	0.804
MUFA	10.324±0.631	9.680±1.038	11.226±1.087	10.365±1.630	**0.075**	**0.062**	0.792
PUFA	3.524±0.823	3.465±0.641	3.817±0.661	3.115±0.596	0.123	0.89	0.202
USF	13.857±1.047	13.141±1.218	15.043±1.296	13.480±1.837	**0.027**	0.131	0.391
SFA / UFA	4.509±0.898	4.164±0.967	4.179±0.829	4.233±0.551	0.623	0.66	0.501
MUFA/SFA	0.171±0.030	0.183±0.034	0.184±0.031	0.184±0.022	0.549	0.526	0.535
PUFA/MUFA	0.343±0.081	0.361±0.075	0.342±0.069	0.306±0.073	0.737	0.301	0.313
CLA	0.974±0.420	0.752±0.392	0.982±0.353	0.973±0.507	0.179	0.87	0.91
n-3 PUFA	0.5710.089	0.542±0.120	0.686±0.273	0467±0.133	**0.048**	0.73	0.122
n-6 PUFA	1.990±0.832	2.172±0.361	2.149±0.979	1.855±0.392	0.822	0.75	0.341
n6/n3 ratio	3.578±1.843	4.089±0.715	3.673±2.185	4.336±1.803	0.344	0.782	0.902
Cis FA	10.237±1.641	10.086±1.121	10.697±1.372	9.232±0.595	**0.077**	0.657	0.146
Trans FA	3.621±0.865	3.059±0.515	4.346±1.115	4.248±1.700	0.418	**0.024**	0.567

Table 4.3.1 continued…

Trans/Cis ratio	0.372±0.143	0.307±0.063	0.419±0.143	0.462±0.182	0.818	**0.05**	0.284
Ratios relating to biohydrogen patterns of C18:2n6c and C18:3n3 fatty acids							
RA/LA	16.575±6.365	17.643±7.768	13.170±5.091	20.211±7.898	0.107	0.864	0.229
VA/RA	0.115±0.065	0.084±0.074	0.176±0.114	0.126±0.084	0.196	0.103	0.940
SA/VA	16.756±3.254	17.881±4.244	13.464±2.113	13.375±5.407	0.713	**0.009**	0.667
VA/CLA	2.945±1.566	5.083±5.910	3.344±1.411	4.904±3.193	0.149	0.931	0.359
CLA/LA	0.741±0.480	0.406±0.226	0.775±0.644	0.581±0.502	0.136	0.549	0.686
VA/LNA	8.845±5.156	6.836±3.095	8.715±3.011	24.270±23.739	0.132	**0.057**	**0.054**
SA/OA	8.086±3.095	8.070±3.626	7.638±2.230	8.849±2.863	0.577	0.877	0.567

*Significant differences are highlighted in bold.

4.3.2 Major fatty acids present in IMF, SCF and PRF of Merino lambs

The fatty acids identified in IMF, SCF, PRF and rumen fluid are depicted in Table 4.3.2 to 4.3.5 and their chromatograms are presented in Figure 4.3.3 to 4.3.6. As shown in Figure 4.3.1, SFA represents the biggest proportion of total fat, closely followed by MUFA, with the lowest proportion corresponding to PUFA. Saturated fatty acid and MUFA differed between the fatty acid depots ($P<0.001$). Intramuscular PUFA differed from SCF and PRF, but PRF and SCF did not differ from each other ($P>0.05$).

As indicated in Figure 4.3.1, IMF contained the least SFA, SCF contained 10.98% more, and PRF had 32.03% more SFA than IMF. Monounsaturated fat was highest in IMF, SCF was 11% lower and PRF had 30.25% less MUFA than IMF. Polyunsaturated fat was highest in IMF and did not differ between SCF and PRF. Mapiye *et al*. (2015) also reported more SFA, less MUFA and similar PUFA percents when PRF was compared to SCF in beef steers. They attributed the differences to increased delta 9 desaturase activity in external fatty acid depot sites such as SCF, leading to reduced SFA in comparison to internal fatty acid depots like PRF. Lee *et al*. (2011) identified a correlation between stearoyl-coenzyme A desaturase (SCD) mRNA expression and adipose site, with SCF showing greater expression than PRF. This agrees with Archibeque *et al*. (2005) who demonstrated that SCD activity in interfascicular fat was twice that of SC adipose tissue on a per gram of tissue basis. Lourenço *et al*. (2007) studied the effect of sheep reared on three different pastures (until 28kg) on SC and IM fatty acid profiles. Saturated fatty acid was higher in SCF and lower in PUFA than IMF. Unlike the current study, IMF was not higher in MUFA.

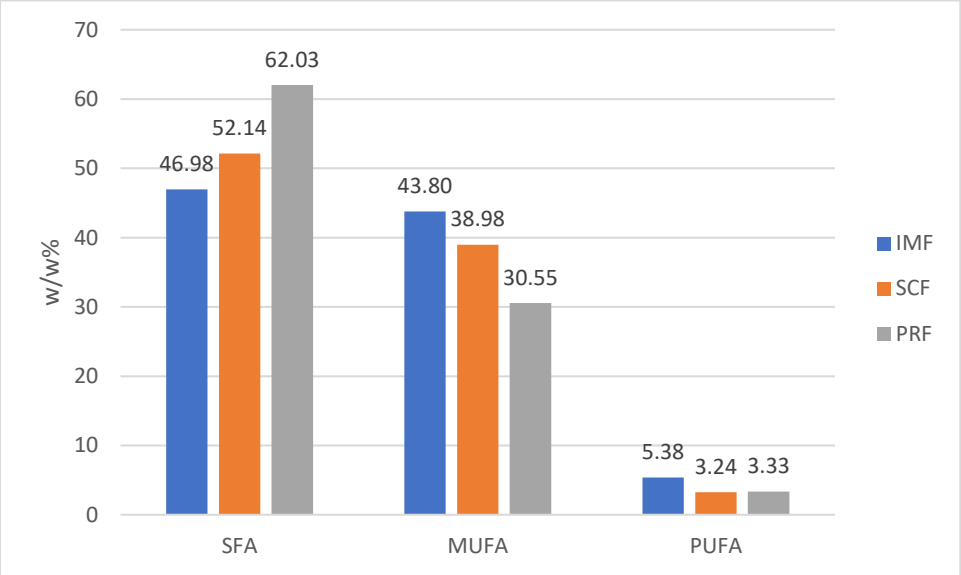

Figure 4.3.1: A comparison of SFA, MUFA and PUFA between IMF, SCF and PRF (w/w%)

The most abundant fatty acids in IMF, SCF and PRF are shown in Figure 4.3.2, and they all differed from each other (P<0.001). Intramuscular fat and SCF were highest in oleic acid (C18:1n9c), palmitic acid (C16:0) and stearic acid (C18:0), respectively. However, as indicated in Figure 4.3.2, perirenal fat (PRF) was highest for stearic acid (C18:0), followed by oleic acid (C18:1n9c) and then palmitic acid (C16:0).

Regardless of the treatments, the major IMF fatty acids were palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1n9c). These fatty acids, SFA and MUFA were within the range of other studies on SA Mutton Merino IMF (Cloete *et al*., 2004; Tejeda *et al*., 2008 and Booyens *et al*., 2012). Within these studies SFA and MUFA varied between approximately 43 to 50%, and 37 to 42%, respectively. The current study IMF had a very low percent of PUFA, largely as a result of smaller amounts of linoleic acid (LA) and linolenic acid (LNA) present in IMF. The major PUFA values in the current study were closer to Hoffman *et al*. (2003) who identified between 5.914 and 7.924% PUFA, 3.735 to 4.356% LA and between 1.310 and 1.277% LNA. The closest LNA value to the current study was that of Booyens *et al*. (2012) of 0.61 in SA Mutton Merino lambs.

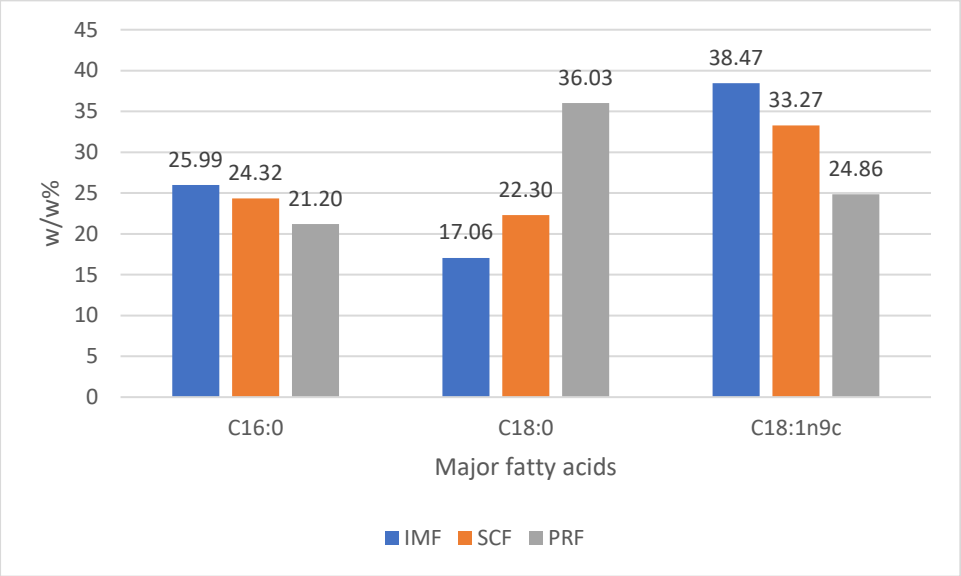

Figure 4.3.2: A comparison of C16:0, C18:0 and C18:1n9c between IMF, SCF and PRF (w/w%)

The main fatty acids in SCF were oleic acid (C18:1n9c), palmitic acid (C16:0) and stearic acid (C18:0). These major fatty acids, SFA and MUFA were similar to SA Mutton Merino wethers slaughtered at 37 and 43 kg by Webb & Casey (1995). However, C18:2, C18:3 and PUFA were lower in the current study. This range was similar to Casey *et al*. (1988) who grazed SA Mutton Merino on 8 different pastures. In agreement with the current study, Webb *et al*. (2022) reported that PUFA made up approximately

2.8% of total fatty acids in 60 to 65kg SA Mutton Merino ram lambs fed a high forage total mixed ration (TMR). The UFA percent was in the range of Webb *et al*. (1994).

Visceral fat in the form of perirenal fat (PRF) was analysed to understand the effect of calcium nitrate and tannin on the fatty acid metabolism of a third and internal fatty acid depot site. Approximately 82% of total fatty acids in PRF were made up of stearic acid (C18:0), oleic acid (C18:1n9c) and palmitic acid (C16:0). The order of magnitude of these fatty acids was unusual. Although C16:0 was generally the lowest of these fatty acids, in contrast to reports from other studies, C18:0 was present as a greater proportion than C18:1n9c (Banskalieva, 2000; Bas *et al*., 2000; Castro *et al*., 2005; Guler *et al*., 2011; Kafle *et al*.,2021). For example, Guler *et al*. (2011) reported C18:1n9c to be between approximately 24 to 31%, C18:0 to be between 23 And 27% and C16:0 to be between 19 and 22%. The percent of these major fatty acids in the current study lay within the general range of these five studies, except for C18:0 which was higher. In these studies, C18:1n9c (sometimes presented as total C18:1) ranged between approximately 24 to 38%, C18:0 ranged between approximately 17 and 32%, and C16:0 ranged between approximately 19 and 25%. However, Roberts (1966) identified a higher percent of C18:1 than C18:0 when cattle were fed a hay-based diet. Therefore, other factors may have influenced its magnitude of representation.

The lower PUFA proportion, particularly in IMF, may be as a result of differences in weight, fat proportion, age, sex and diet. Tejeda *et al*. (2008) reported a decrease in PUFA when SA Mutton Merino ewes (but not rams) were slaughtered at 29kg as opposed to 24kg. Polyunsaturated fatty acid typically decreases with age as a result of the majority of it present in the phospholipid fraction (Link *et al*., 1970). Phospholipids do not increase as rapidly as triacylglycerol (TAG) because they need to maintain a consistent membrane function (De Smet *el al*., 2004). Link *et al*. (1970) reported a concomitant increase in the neutral lipid (NL) fraction with increasing age of steers. De Smet *et al*. (2004) reported an increase in MUFA and SFA at the expense of PUFA with increased fatness. However, Cloete *et al.* (2004) slaughtered lambs at similar weights to the current study and analysed their intramuscular fatty acid composition. They predominantly grazed kikuyu and were slaughtered at about 45 kg for SA Mutton Merino and Dorper ewes and about 65kg for SA Mutton Merino and Dorper rams. The current study slaughtered ewes fed a TMR between 45 and 50kg, and rams between 50 and 60kg. Although the weights were within a similar range, the current study still had a much lower PUFA percent. This was likely as a result of differences in feed and management practices. Queiroz *et al*. (2021) reported that lambs fed a higher concentrate diet had lower PUFA and n-3 fatty acid percent than lambs fed a higher roughage-based diet.

As shown in Table 2.8.1 (literature review) and demonstrated by Sinclair *et al*. (1982), ruminant fat particularly from beef, sheep and goat is typically higher in saturated fat than monogastric animals. In comparison to Table 2.8.1 (literature review) this remains true in the current study. Reducing the quantity of SFA and increasing PUFA in ruminant meat is advantageous, particularly if it is able to simultaneously reduce methane emissions. Tannin has been reported to do this (Hess *et al*., 2006; Morales & Ungerfeld, 2015). Although nitrate has been proven to reduce methane emissions (Hulshof *et al*., 2012; Li *et al*., 2012), little research has gone into its ability to improve the healthfulness of the fatty acid profile.

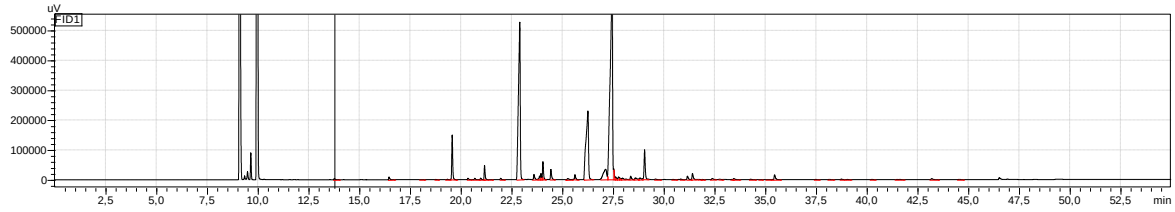

Figure 4.3.3: Intramuscular fat chromatogram of Dohne Merino sheep

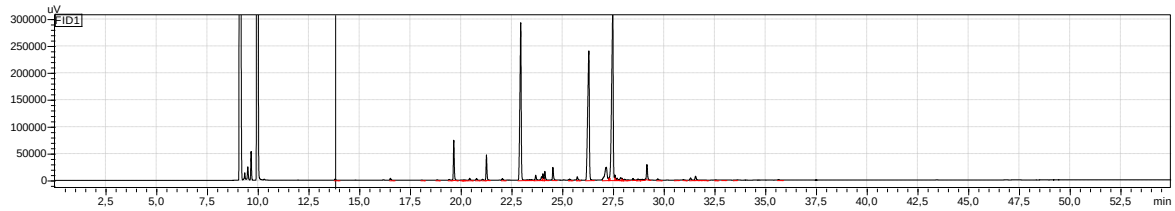

Figure 4.3.4: Subcutaneous fat chromatogram of Dohne Merino sheep

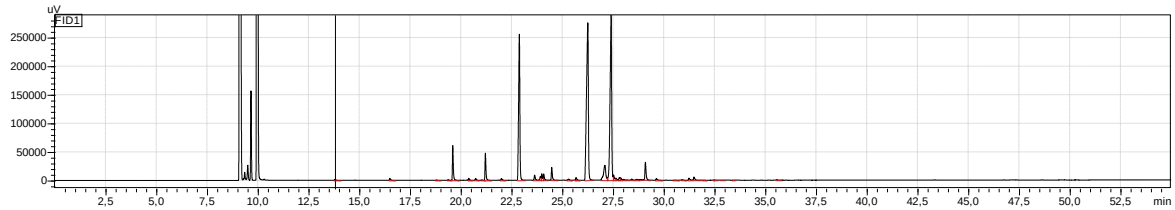

Figure 4.3.5: Perirenal fat chromatogram of Dohne Merino sheep

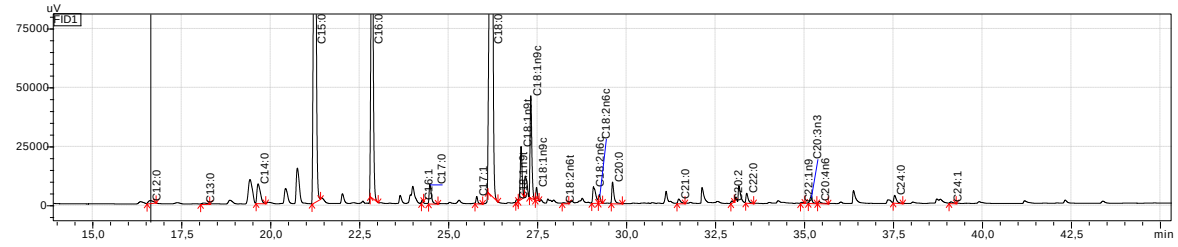

Figure 4.3.6: Rumen fluid chromatogram of Dohne Merino sheep

Table 4.3.2: Fatty acids identified in intramuscular fat

SFA	MUFA	PUFA
C10:0	C14:1	C18:3n6
C12:0	C16:1	C18:3n3
C14:0	C18:1nt11	CLA cis-9, trans-11/ trans-9, cis-12
C16:0	C18:1n9c	CLA cis-10, cis-12
C17:0	C18:1n11c	CLA isomer 1
C18:0	C20:1	CLA isomer 3
C20:0	C22:1n9	C20:2
C22:0	C24:1	C20:3n6
C23:0		C20:4n6
C24:0		C20:5n3
		C22:6n3
		C18:2n6t
		C18:2n6c

Table 4.3.3: Fatty acids identified in subcutaneous fat

SFA	MUFA	PUFA
C10:0	C14:1	C18:3n6
C12:0	C16:1	C18:3n3
C13:0	C18:1nt11	CLA cis-9, trans-11/ trans-9, cis-12
C14:0	C18:1n9c	CLA isomer 1
C16:0	C18:1n11c	CLA isomer 3
C17:0	C20:1	C20:2
C18:0		C20:2
C20:0		C20:4n6
C22:0		C18:2n6t
		C18:2n6c

Table 4.3.4: Fatty acids identified in perirenal fat

SFA	MUFA	PUFA
C10:0	C14:1	C18:3n6
C12:0	C16:1	C18:3n3
C14:0	C18:1nt11	CLA cis-9, trans-11/ trans-9, cis-12
C16:0	C18:1n9c	CLA isomer 1
C17:0	C18:1n11c	CLA isomer 3
C18:0	C20:1	C20:4n6
C20:0		C18:2n6t
C22:0		C18:2n6c

Table 4.3.5: Fatty acids identified in rumen fluid

SFA	MUFA	PUFA
C10:0	C14:1	C18:3n6
C12:0	C16:1	C18:3n3
C14:0	C18:1nt11	CLA cis-9, trans-11/ trans-9, cis-12
C16:0	C18:1n9c	CLA isomer 1
C17:0	C18:1n11c	CLA isomer 3
C18:0	C20:1	C20:4n6
C20:0		C18:2n6t
C22:0		C18:2n6c

4.3.3 The effect of calcium nitrate and condensed tannin on internal and external fatty acid depots

4.3.3.1 The effect of condensed tannin and calcium nitrate supplementation on intramuscular fatty acids

The effect of tannin and non-protein nitrogen (NPN) source on the intramuscular fatty acid profile of freeze-dried *longissimus dorsi* (LD) was shown as a percent of identified fatty acid (w/w%), as well as sums and ratios of groups of fatty acids in Table 4.3.6. The gravimetric (mg of FA/g of freeze-dried LD) of these fatty acids, sums and ratios are presented in Table 4.3.7. Enzyme indices are presented in Table 4.3.8 (from w/w%) and 4.3.9 (from mg/g), and are discussed within the SFA, MUFA and/or PUFA section where it is applicable to explain the fatty acid composition as affected by treatment. The effect

of tannin and calcium nitrate on the health indices of fats are also presented in Table 4.3.8 (from w/w%) and 4.3.9 (from mg/g).

Tannin had no effect on the overall percent (w/w%; Table 4.3.6) or concentration (mg/g; Table 4.3.7) of SFA, MUFA, PUFA or UFA ($P<0.05$). The only exception was the PUFA concentration, which tended to increase in IMF ($P=0.09$). Calcium nitrate decreased the percent of SFA ($P=0.001$), increased MUFA ($P<0.005$) and UFA ($P<0.001$) and had no effect on PUFA ($P>0.05$). There was no effect on the concentration of MUFA, PUFA or UFA ($P>0.05$), but it tended to decrease SFA ($P=0.09$). Regardless of whether there was an overall significance in each of these categories (as a percent or concentration), there were individual SFAs, MUFAs and PUFAs that showed significance in response to calcium nitrate and tannin within these categories.

a) The effect of condensed tannin and calcium nitrate on the SFA profile of IMF

Table 4.3.6 indicates the percent of total and individual SFAs (w/w%). Table 4.3.7 shows the total and individual concentration (mg/g *Longissimus dorsi*) of SFAs. The supplementation of tannin did not reduce the percent or concentration of SFA regardless of it tending to be lower in rumen fluid, as indicated in 4.3.1 ($P>0.05$). Lambs fed calcium nitrate in the TMR had a lower percent (Table 4.3.6) and concentration (Table 4.3.7) than those fed urea ($P=0.001$ and $P=0.09$, respectively). Although both decreased, this was because SFA decreased in the diet containing tannin ($P<0.05$; Table 4.3.6 and Table 4.3.7).

Typically, higher SFA is largely a result of microbial biohydrogenation changing the composition of fat to a more saturated state before it is absorbed and deposited in tissue (Demeyer & Doreau, 1999; Jenkins *et al*., 2008). In the current study, tannin tended to reduce saturation in rumen fluid, while calcium nitrate had no effect on complete biohydrogenation. Therefore, either the concentration of fatty acid was too low in rumen fluid to give an accurate picture of microbial activity or other endogenous factors played a role. The lower SFA is advantageous as it decreases the risk of coronary heart disease (Mozaffarian *et al*., 2010). Therefore, the increased interest in calcium nitrate to reduce methane emissions shows the additional potential to reduce SFAs, thereby improving the health value of ruminant meat, particularly in IMF. However, 17g *Acacia Mearnsii* condesed tannin (CT) per kg feed was not enough to inhibit the microorganisms from completely biohydrogenating linoleic acid (C18:2n6c) and α-linolenic acid (C18:3n3) enough to improve the IMF fatty acid profile.

The lack in effect of tannin on the percent and gravimetric total SFA was due to no changes in the main contributing C16:0 and C18:0 fatty acids upon supplementation ($P>0.05$; Table 4.3.6 and Table 4.3.7). Therefore, the decrease in C16:0 in rumen fluid (Table 4.3.1) was not enough to reduce C16:0 in IMF. In contrast to this study, Vasta *et al*. (2009) reported a decrease in SFAs when Quebracho tannin was

supplemented at ±40g/kg of DM to either herbage or concentrate based diet. Although some studies have not identified differences in total SFAs, many have reported a decrease in C18:0 (Vasta *et al*., 2009; Kamel *et al*., 2018; Guerreiro *et al*., 2020; Costa *et al*., 2021). The reason for no effect of tannin on C18:0 in the current study may be as a result of the high roughage content in the TMR. Vasta *et al*. (2009) only identified differences in a concentrate diet. A feed and tannin reaction were also recorded *in vitro* by Vasta *et al*. (2008).

Tannin increased the small concentrations of C13:0 and C17:0 odd chain fatty acids (P<0.05; Table 4.3.7) and tended to increase its percent (P=0.061 and P=0.069, respectively; Table 4.3.6). Odd chain fatty acids are almost exclusive to ruminant fat and have been used as biomarkers to determine ruminant fat intake by humans (Vlaeminck *et al*., 2006b). In contrast, Fernandes *et al*. (2021) reported a decrease in C17:0, C18:0 and SFA in lambs fed *mimosa tanuiflora* hay rich in condensed tannin. Lee *et al*. (2017) suggested that lower C17:0 was as a result of decreased bacterial activity in the rumen. In contrast to the IMF in the current study, the rumen fluid decreased C13:0 and C17:0 fatty acids. Therefore, the discrepancy between the rumen fluid C17:0 and IMF is curious, and possibly as a result of the negligible percent found in the rumen fluid (between 0.520%±0.075 to 0.755%±0.111; Table 4.3.1), escape from absorption, and very low concentration in meat (0.510mg/g±0.177 to 0.630mg/g±0.158; Table 4.3.7).

Calcium nitrate did not affect C16:0 and C18:0 fatty acids in rumen fluid (P>0.05; Table 4.3.1). Therefore, there was another reason for a tendency for reduced C16:0 (percent: P=0.06) and C18:0 fatty acids (percent: P<0.05; concentration: P<0.05) in IMF. This is shown in Table 4.3.6 and 4.3.7, respectively. The lack in effect of calcium nitrate on rumen fluid agrees with Adejoro (2020) who reported no increase in the molar proportion of acetate, the *de novo* fatty acid precursor of palmitic acid, nor the acetate: propionate ratio when a nitrate-based TMR was fed to Merino lambs. The decrease in C16:0 may be explained by a change in enzymatic activity brought on by its further elongation and desaturation in tissue (Shingfield *et al*., 2013). The enzyme indices are reported in table 4.3.8 and 4.3.9. Lambs fed calcium nitrate tended to have a higher elongase index (P=0.100). However, this was not the reason as calcium nitrate also decreased C18:0. Although the delta 9 desaturase activity was higher in the calcium nitrate-based diet (P<0.001), its activity on C16:0 was not (P>0.05). The decrease in C18:0 was likely a result of increased delta 9 desaturase activity on C18:0 (P<0.01). Although delta 9 desaturase C18 index increased when calcium nitrate was supplemented, it only increased when tannin was included in the diet (P<0.05).

A decrease in C18:0 in the calcium nitrate-based diet does not provide any health benefit or detriment. Extensive research shows that C18:0 is a neutral fatty acid because it does not increase serum

cholesterol concentrations, is therefore less harmful to human health (Grundy, 1994), and its availability improves the desirable fatty acid (DFA) equation (Rhee *et al*., 1992). However, palmitic acid increases low density lipoprotein cholesterol (Grundy, 1994). Therefore, a tendency to reduce its percent in intramuscular fat using calcium nitrate is promising.

Very long chain fatty acids (VLCFA), including and above C20:0, are associated with improved cardiovascular health (Liu *et al*., 2020). Although the percent and concentration of C20:0 was lower in meat of lambs consuming calcium nitrate ($P<0.05$; Table 4.3.6 and Table 4.3.7), the overall C20:0 was very small in meat (between 0.08 and 0.09%, and between 0.05 and 0.06mg/g DM) and was, therefore, unlikely to have had a negative effect when nitrate was used as an NPN source. C20:0 typically arises in meat from the elongation of C18:0 (Liu *et al*., 2020). However, the lower C20:0 concentration may also have been a result of less C20:0 in rumen fluid, as a result of changes in microbial activity ($P<0.05$; Table 4.1).

b) The effect of condensed tannin and calcium nitrate on the MUFA profile of IMF

Total monounsaturated fatty acid and individual monounsaturated fatty acids are reported in Table 4.3.6 (w/w%) and 4.3.7 (mg/g Longissimus dorsi). Tannin had no effect on total MUFA in IMF ($P>0.05$). Although it was not affected as a concentration, it had a higher w/w% in the calcium nitrate-based diet ($P<0.005$). The diet containing tannin was most responsive, with increased MUFA when calcium nitrate was used as a NPN source. A contributing factor for this may have been a tendency for rumen fluid to have a higher MUFA percent in the calcium nitrate-based diet ($P<0.062$; Table 4.3.1). Therefore, the overall UFA w/w% was also higher in the calcium nitrate-based TMR ($P<0.001$), particularly when the diet contained tannin ($P<0.05$).

The majority of MUFAs came from C18:1 fatty acids, particularly oleic acid (C18:1n9c), which also contributed the largest proportion of cis fatty acid in IMF. As indicated in equations (1, 2,3 and 4) in the method, C18:1n9c is a desirable fatty acid for the health-conscious consumer as it is hypocholesterolaemic and decreases the atherogenic (AI) and thrombogenic (TI) indices. Neither tannin nor NPN source changed the concentration of C18:1n9c ($P>0.05$; Table 4.3.7). However, as a percent of detected lipid (w/w%), it was higher ($P<0.05$; Table 4.3.6). The major contributing factor was that the urea-based diet with tannin had a higher percent of C18:1n9c than the calcium nitrate-based diet without tannin ($P<0.05$). A higher C18:1n9c percent led to a higher percent (w/w%) of total cis fatty acids in IMF of lambs fed the calcium nitrate-based diet ($P<0.05$). This was driven by a greater cis percent in calcium nitrate-based diet without tannin than the urea-based diet with tannin ($P<0.05$).

The amount of oleic acid available to ruminant tissue is dependent on the amount lost through hydrogenation to stearic acid in the rumen, and the amount present in tissue is also dependent on *de novo* fatty acid synthesis (Smith *et al*., 2006). Palmitic acid (C16:0), C18:0 and C18:1n9c are the major end products of *de novo* fatty acid biosynthesis (Natali *et al*., 2007). The endogenous increase in MUFAs (for example palmitoleic and oleic fatty acids from palmitic and stearic fatty acids, respectively) and CLA are possible through the endogenous biosynthesis of fatty acids through delta 9 desaturase activity in ruminant adipose tissue (Enoch *et al*., 1976; ; Tanaka *et al*., 2005; Smith *et al*., 2006). In the current study, neither C18:1n9c nor its substrate, C18:0, differed in the rumen fluid of lambs fed the calcium nitrate-based diet (Table 4.3.1). Therefore, the results would suggest that the increase in percent was not as a result of reduction in microbial activity. Therefore, the higher percent of C18:1n9c in IMF of lambs fed the calcium nitrate-based diet was likely due to improved delta 9 desaturase activity on C18:0 (Table 4.3.8; $P<0.01$). The delta 9 desaturase activity on C18:0 was also higher when calculated from the concentration (Table 4.3.9). Whether calculated from the percent or concentration, diets containing and not containing tannin increased when calcium nitrate was used as an NPN source. Although only those fed tannin had a higher delta 9 desaturase activity on C18:0. Marra *et al*. (2000) reported that calcium deprivation decreased delta 9 desaturase by 45 to 55% in the liver microsomes of rats. Therefore, calcium may act on desaturase indirectly, through its effect on phospholipase A2 and membrane (Marra *et al*., 2000. In addition, Marra *et al*. (2002) suggested that calcium had a positive effect on the transcription rate on desaturase mRNA. Therefore, the increased availability of calcium may have been a reason for the increased desaturase activity in IMF.

Although tannin decreased the percent of oleic acid (C18:1n9c) in rumen fluid (Table 4.3.1), particularly in the calcium nitrate-based diet, it was not enough to reduce the percent in IMF ($P>0.05$; Table 4.3.6). It also was not counteracted by depressed delta 9 desaturase activity in tissue ($P>0.05$; Table 4.3.8 and Tabl 4.3.9). Literature varies in its response to tannin. Gurrier *et al*., 2020 reported tannin to decrease oleic acid *in vitro*, and Gesteira *et al*. (2018) identified a decrease in oleic acid in the meat of Nellore steers fed 0, 20, 30 and 50g condensed tannin extract per kg feed DM. Kamel *et al*. (2018), who investigated the effect of 3 levels of Quebracho tannin on Awassi lamb IMF and SCF (0, 20 and 40g/kg DM of the diet), found an increase between 0 and 20g/kg DM but not between 20 and 40g/kg DM.

Non-proetin nitrogen source did not change the percent (Table 4.3.6) or concentration (Table 4.3.7) of vaccenic acid (C18:1n11t; $P<0.05$). Therefore, the increase in C18:1n11t in rumen fluid (Table 4.3.1) was not enough to elevate C18:1n11t in IMF or its conversion to CLA cis-9, trans-11 in the *longissimus dorsi* ($P>0.05$). The supplementation of tannin increased the percent and concentration of C18:1n11t

in IMF, regardless of it not having an effect at a biohydrogenation level (P<0.01). Higher C18:1n11t led to more trans fatty acids in the *longissimus dorsi* of the lambs fed tannin (P<0.01).

Based on the rumenic acid desaturase index there was no evidence supporting the ability of tannin and or calcium nitrate to increase the activity of the enzyme on vaccenic acid (P>0.05; Table 4.3.8 and Table 4.3.9). This contrasts Vasta *et al*. (2007), who reported a higher CLA/vaccenic acid (CLA/VA) ratio in the IMF of Comisana ramb lambs when carob pulp, naturally high in tannin, was added to a concentrate-based diet (45% carob as is or 2.7% CT). It also contrasts Rana *et al*. (2012) who determined that tannin from tanniniferous *Terminalia chebula* was able to increase CLA in kids through a 47% increase in desaturase activity in the *longissimus dorsi*.

Trace amounts of C24:1 (present in concentrations less than 0.02mg/g DM) was the only MUFA that showed an interaction (and only as a concentration) between tannin and NPN source (P<0.05; Table 4.3.9). These interactions may be influenced by the small concentration detected in IMF.

c) The effect of condensed tannin and calcium nitrate on the PUFA profile of IMF

Total polyunsaturated fatty acid and individual PUFAs are reported in Table 4.3.6 (percent) and 4.3.7 (concentration). Polyunsaturated fatty acid did not differ in IMF for any of the treatments (P>0.05). This is likely a result of it not affecting the fatty acids in rumen fluid (Table 4.3.1). This is consistent with Jeronimo *et al*. (2012) who reported that *cistus ladanifer* (high in CT) also had no effect on PUFA. However, this is in contrast to Vasta *et al.* (2007) and Costa *et al*. (2021) who reported an improved PUFA as a result of its effect on microbial activity. Linoleic acid (C18:2n6c) was the largest contributing PUFA and varied between 2.720%±0.378 to 3.616%±1.208 (Table 4.3.6). This is consistent with Demirel *et al*. (2006) who reported considerably more C18:2n6c than C18:3n3 in lamb IMF on a concentrate-based diet.

Ruminant meat is a natural source of CLA (Mulvihill, 2001). It is typically higher in ruminant as opposed to monogastric fat (Bessa *et al*., 2015). SA Mutton Merinos have previously been reported to have CLA contents ranging between 0.51 to 0.75% of IMF (De Klerk, 2016). The current results fitted within the top end of this range. The total amount of CLA in the *Longissimus dorsi* did not differ with tannin supplementation or NPN source (P>0.05; Table 4.3.6 and 4.3.7).

Rumenic acid (the major contributing CLA) is the main product of linoleic acid isomerization by microbes in the rumen (Jenkins *et al*., 2008). In addition, in adipose tissue, rumenic acid is synthesized endogenously by delta 9 desaturase activity (Bauman *et al*., 1999). Therefore, an increase in rumenic acid (CLA cis-9, trans-11) in meat is dependent on the amount of vaccenic acid (C18:1n11t) and rumenic acid in the rumen, the conversion of vaccenic acid to rumenic acid by delta 9 desaturase in

tissue, and the availability of triacylglycerol (TAG) for rumenic acid deposition (Bessa *et al*., 2015). The NPN source did not change the percent (Table 4.3.6) or concentration (Table 4.3.7) of vaccenic acid (C18:1n11t) or CLA cis-9, trans-11 (rumenic acid) + trans-9, cis-12 in IMF (P>0.05). Therefore, the increase in C18:1n11t in rumen fluid was not enough to increase C18:1n11t or CLA cis-9, trans-11 in the *longissimus dorsi* IMF (LD; P>0.05). Although C18:1n11t was higher in the IMF of lambs fed tannin, there was not an increase in CLA cis-9, trans-11 + trans-9, cis-12 (P>0.05).

However, the concentration of another CLA intermediate, CLA cis-10, cis-12, decreased when urea was replaced by calcium nitrate as an NPN source (P<0.05; Table 4.3.7). This fatty acid also had a very high tendency to be greater when tannin was supplemented (P=0.055). Therefore, CLA cis-10, cis-12 was higher in the urea-based diet with tannin (0.005mg/g±0.001) than the calcium nitrate-based diet without tannin (0.003mg/g±0.001; P<0.05). The concentration was very small for any major health benefits.

A lack of change to the linoleic acid/CLA ratio (LA/CLA) in rumen fluid indicated no changes towards the isomerization of linoleic acid by the microbial population in response to tannin and NPN source. This was confirmed by no changes in C18:2n6c as a percent of total lipid in IMF (P>0.05; Table 4.3.6). As a concentration, there was more C18:2n6c in the IMF of lambs on the tannin-based diet (P<0.005; Table 4.3.7). Kafle *et al*. (2021) and Bhatt *et al*. (2020) also identified no effect of tannin on the amount of CLA present in goat and sheep IMF respectively. In contrast to our study, Rana *et al*. (2012) reported an increase in CLA content as a result of a 47% increase in delta 9 desaturase activity when kids were fed increasing levels of tanniniferous *Terminalia chebula* on a concentrate diet (fed tannin at 0, 1.06 and 3.18g/kg body weight).

The total amount of omega 3 fatty acids in IMF were small and unaffected by tannin or NPN source (P>0.05). This was largely due to no changes in α-linolenic acid (C18:3n3), its major constituent (P>0.05). Priolo *et al*. (2005) identified a significant increase in omega 3 fatty acids in lambs fed fresh Sulla containing condensed tannin. However, their control group was fed concentrate and hay, a diet typically lower in linolenic acid in comparison to forages. In comparison, the lambs in the current study were fed the same basal TMR. Therefore, the amount of α-linolenic in the LD did not differ. It was low and varied between 0.151±0.060mg/g DM to 0.194±0.055mg/g DM. Thus, 1mg per 100g freeze-dried LD had much less than 300mg/100g required by the European union for it to be classified as a certified source of α-linolenic acid (Vahmani *et al*., 2015).

Total Omega 3 fatty acids were not affected by treatment, but individual n-3 fatty acids were (P>0.05). α-linolenic acid (C18:3n3) from the diet is elongated and desaturated to longer chain n-3 fatty acids such as eicosapentaenoic acid (EPA; C20:5n3), docosapentaenoic acid (DPA; C22:5n3) and

docosahexaenoic acid (DHA; C22:6n3) (Vahmani *et al*., 2020). eicosapentaenoic acid (EPA) and DHA (DPA not present in the standard mix) were lower in the LD as a percent and concentration when tannin was supplemented to the TMR ($P<0.01$ and $P<0.001$, respectively). The major reason for this was that the calcium nitrate-based diet without tannin had a higher concentration (Table 4.3.7) of EPA (0.027mg/g±0.016) than the urea-based diet with tannin (0.052mg/g±0.020; $P<0.05$). In contrast to the current results, Costa *et al*. (2021) reported a significant increase in EPA for 60 and 80g CT/kg DM.

Tannin decreased the percent and concentration of DHA (C22:6n3) in the calcium nitrate-based diet ($P<0.05$). This was because DHA decreased from 0.083%±0.038 (0.046mg/g±0.019) to 0.039%±0.009 (0.024mg/g ±0.005) when tannin was added to a calcium nitrate-based diet (Table 4.3.6 and Table 4.3.7, respectively). Although it decreased, the effect of tannin was not significant in the urea-based diet ($P>0.05$). There was a tendency for the calcium nitrate-based diet to have a higher percent ($P=0.092$) and concentration ($P=0.083$) of DHA than the urea-based diet. Although present in low amounts in meat and eggs, it is advantageous for brain development and functioning, and reduced risk of hypertension, arthritis, atherosclerosis, depression, late onset diabetes, myocardial infarction and some cancers (Horrocks & Yeo, 1999). Regardless of the negative effect of tannin, the EPA and DHA combined value was below 40g per 100g and was therefore less than the requirement by the European union to be a good source of Omega 3 fatty acid (Vahmani *et al*., 2015). These values were low due to the limited supply of α-linolenic acid in the diet and the high amount required to counteract the low conversion of α-linolenic acid to EPA and DHA in tissue (Vahmani *et al*., 2020).

The total amount of omega 6 fatty acid (n-6 fatty acid) was affected by both tannin and calcium nitrate. Calcium nitrate increased the omega 6 concentration ($P<0.05$; Table 4.3.7), with a tendency to increase its percent ($P=0.079$; Table 4.3.6). Tannin tended to decrease its percent ($P=0.092$) but had no effect on its concentration ($P>0.05$). Although literature varies in its response, Priolo *et al*. (2005) and Vasta *et al*. (2007) also reported a decrease in omega 6 fatty acids in IMF when lambs were fed tannin. There were several n-6 fatty acids identified in IMF: C18:2n6t, C18:2n6c, C20:3n6 and C20:4n6. The major n-6 fatty acid derived from feed was linoleic acid (C18:2n6c), and it made up between 0.748%±0.405 to 1.275%±0.464 of IMF (Table 4.3.6).

As a percent of detected lipid, C18:2n6c was not affected by calcium nitrate or tannin ($P>0.05$; Table 4.3.6). As a concentration, calcium nitrate was also not affected ($P>0.05$; Table 4.3.7). Tannin increased the concentration of C18:2n6c ($P<0.005$; Table 4.3.7). This was because of a difference between the urea-based diet without tannin and calcium nitrate-based diet with tannin ($P<0.05$). This would suggest changes in delta 9 desaturase activity between these treatments as no changes in rumen fluid through biohydrogenation were identified.

Arachidonic acid (C20:4n6) tended to increase as a percent of detected lipid in the IMF of lambs fed the calcium nitrate-based diet (P=0.073; Table 4.3.7). It also tended to decrease as a percent when tannin was supplemented to the diet (P=0.075; Table 4.3.6). This is the major C20 fatty acid brought about by the elongation and desaturation of C18:2n6c in adipose tissue (Webb & O'Neill, 2008). As a concentration, it also tended to increase in the tannin diet (P=0.079) and increase in the calcium nitrate-based diet (P<0.05). The difference between the treatments lay between the urea-based diet with tannin and the calcium nitrate-based diet without tannin (P<0.05). Of the individual n-6 fatty acids affected by tannin, only the concentrations of C18:2n6t was higher in IMF when tannin was present in the diet (P<0.05; Table 4.3.7).

d) The effect of condensed tannin and calcium nitrate in the ratios of fatty acids in IMF

The fatty acid ratios from the percent are shown in Table 4.3.6 and from the concentration are presented in Table 4.3.7. The MUFA/SFA percent and concentration ratio was significantly higher in the calcium nitrate-based diet (P<0.001). As a percent this was because of an increased C18:1n9c percent, and a decrease in the major contributing C16:0 and C18:0 saturated fatty acids (Table 4.3.6). The significantly reduced C18:0 concentration had an effect on the MUFA/SFA concentration ratio (Table 4.3.7). The MUFA/SFA ratio for both the percent and concentration showed the greatest improvement (increase) when calcium nitrate was supplied to a tannin- based diet (P<0.05). Table 4.3.6 and Table 4.3.7 also indicate that the calcium nitrate-based diet with and without tannin was also greater than the urea-based diet with tannin (P<0.05).

Because of the large contribution of MUFA to UFA, the SFA/UFA ratio was also better in the calcium nitrate-based diet (P=0.001; Table 4.3.6 and Table 4.3.7). This was because of an improvement when calcium nitrate was added to a tannin-based diet (P<0.05; Table 4.3.6 and Table 4.3.7). The calcium nitrate-based diet with and without tannin was also lower than the urea-based diet with tannin (P<0.05 Table 4.3.6 and Table 4.3.7). The change in ratios was not as a result of changes to biohydrogenation patterns, as they did not differ in rumen fluid (P>0.05; Table 4.3.1). The increase in the ratio was likely as a result of an increase in delta 9 desaturase activity in these treatments (P<0.05), particularly C18 delta 9 activity (P<0.01; Table 4.3.8 and Table 4.3.9). These ratios were not affected by tannin (P>0.05; Table 4.3.6 and Table 4.3.7).

The PUFA/ SFA ratio was unaffected by tannin or calcium nitrate (P>0.05; Table 4.3.6 and Table 4.3.7). The recommended dietary PUFA/SFA ratio, according to Enser *et al*. (1998), should be above 0.45. There was a tendency for calcium nitrate to give a slightly better ratio (Percent: P=0.068;

Concentration: P=0.087). The lambs in the current study, regardless of treatment, had a substantially lower ratio than the recommended value (between 0.102±0.030 and 0.129±0.035; Table 4.3.7). This is unsurprising as it is well documented that ruminants typically have a lower PUFA/SFA ratio as a result of biohydrogenation in the rumen in comparison to monogastric animals (Wood *et al*., 2008). Costa *et al*. (2021) reported that *Acacia mearnsii* significantly increased the ratio in a dose dependent manor. The ratio increased from 0.19g/100g FAME to 0.20g/100g FAME for inclusion levels of 0.20 and 40g/kg DM CT. However, it increased to 0.27g/100g FAME when supplied to the rams' diet at 60 and 80g/kg DM. Therefore, an improved PUFA/SFA ratio may have needed a higher concentration of *Acacia mearns*ii CT for a reduced complete biohydrogenation, or improved delta 9 desaturase activity.

Neither calcium nitrate or tannin effected the n-6/n-3 ratio and it ranged between 2.099±0.419 and 2.763±0.914 (P>0.05; Table 4.3.7). According to Ma *et al*. (2006) the desirable ratio should be below four. Therefore, it was well within the desirable range. This was expected, as ruminants are known to have a better n6/n3 ratio than other meat sources (Enser *et al*., 1998). Although literature varied, Vasta *et al*. (2007) also reported no changes to the ratio when tannin was supplemented.

As a percent of detected lipid, lambs fed calcium nitrate had more cis fatty acids (P<0.010; table 4.3.6), and those fed tannin had a higher percent and concentration of trans fatty acids (P<0.05; Table 4.3.6 and Table 4.3.7). The major contributors of this were C18:1n9c, C18:1n11t and C18:2n6c. This resulted in a higher trans:cis ratio in the tannin supplemented lambs (P<0.05; Table 4.3.6 and Table 4.3.7). A higher trans/cis ratio does not necessarily mean the increased trans fatty acid in the tannin supplemented group made the *longissimus dorsi* less healthy. Although trans fatty acids from partially hydrogenated vegetable oil is considered unhealthy, until recently, less has been known about ruminant trans-fat (Song *et al*., 2019). Recent research indicates there is less inflammation, endoplasmic reticulum stress and cholesterol synthesis than industrial trans fatty acids (Oteng & Kersten, 2020). Studies have shown that vaccenic acid, which is the major contributor to trans fatty acids in ruminant fat, has the potential to improve insulin sensitivity, suppress intestinal inflammation, is hypolipidemic and anticarcinogenic (Wang *et al*., 2008; Jacome-Sosa *et al*., 2016; Wang *et al*., 2016; Song *et al*., 2019). Vaccenic acid is also the precursor to rumenic acid, not only in ruminants but also human adipose tissue (Turpeinen *et al*., 2002). It is well documented to have many health benefits and has been put into the category of 'generally recognized as safe' by the United States of America since 2008 (Kim *et al*., 2016).

e) The effect of condensed tannin and calcium nitrate on the health indices of IMF

Indices were used and presented in Table 4.3.8 and Table 4.3.9 to give a more detailed overview of the health value of the fatty acid profile. The tables indicated that tannin did not have an effect on the

atherogenic index (AI), thrombogenic index (TI), desirable fatty acids (DFAs), total major hypocholesterolaemic fatty acids (h), hypocholesterolaemic fatty acids (H) or the h/H ratio. In contrast to this study, Costa *et al*. (2021) identified an increase in both AI and TI when *Acacia mearnsii* condensed tannin (CT) was increased in the diet. However, a neutral effect on AI and TI was also observed by Majewska & Kowalik (2020), when supplementing lambs with Lingonberry leaves and Oak bark rich in tannin.

The calcium nitrate-based diet reduced the AI and TI indices ($P<0.01$, $P<0.001$ respectively; Table 4.3.8 and Table 4.3.9). When calculated from the percent (Table 4.3.8), TI was higher in the urea-based diet with tannin than the calcium nitrate-based diets (with and without tannin; $P<0.05$). Calcium nitrate improved the hypocholesterolemic fatty acid (h)/ hypercholesterolemic fatty acid (H) index ratio (h/H) from the percent and concentration ($P<0.05$). This was due to a higher percent of oleic acid (c18:1n9c; Table 4.3.6 and 4.3.7), increasing C18:1 fatty acids in the h index ($P<0.05$), and a lower percent of C16:0 (Table 4.3.6) that elevated the H index ($P<0.05$). Therefore, there were more fatty acids that reduced cholesterol in the calcium nitrate-based diet (with and without tannin) than the urea-based diet with tannin ($P<0.05$; Table 4.3.8). The urea-based diet without tannin did not differ from any of the treatments ($P>0.05$; Table 4.3.8). Only the DFA percent tended to be higher in the calcium nitrate-based diet ($P=0.075$; Table 4.3.8). DFA did not change as a concentration ($P>0.05$; Table 2.3.9). Thus, when tannin was included in the diet, the calcium nitrate-based diet was better.

Table 4.3.6: Means (± SD) depicting the effect of condensed tannin and NPN source on the medium and long chain fatty acid profile of the longissimus dorsi between the 8th and 10th ribs expressed as a percent of detected fatty acids (w/w%)

(w/w%)	Urea		CaN		P-value		
	None	Tannin	None	Tannin	T	N	T*N
Replicates (n)	8	8	8	8			
Fatty acids							
C10:0	0.111±0.015	0.111±0.017	0.102±0.029	0.111±0.014	0.47	0.46	0.48
C12:0	0.124±0.028	0.145±0.044	0.103±0.034	0.121±0.054	0.184	0.124	0.958
C13:0	0.011±0.003	0.013±0.004	0.008±0.003	0.011±0.004	**0.061**	**0.095**	0.917
C14:0	2.670±0.467	2.876±0.565	2.368±0.522	2.692±0.683	0.169	0.201	0.707
C14:1	0.075±0.017	0.081±0.022	0.058±0.015	0.079±0.032	0.341	0.644	0.809
C16:0	26.672±2.368	26.500±1.696	25.400±2.810	25.384±0.959	0.986	**0.06**	0.777
C16:1	1.406±0.189	1.423±0.167	1.435±0.194	1.400±0.235	0.981	0.952	0.795
C17:0	0.821±0.179	0.922±0.104	0.808±0.082	0.896±0.082	**0.069**	0.734	0.844
C18:0	17.553±1.340	17.686±1.305	16.733±1.373	16.259±1.100	0.63	**0.023**	0.434
C18:1t11	2.368±0.254	2.983±0.509	2.428±0.367	3.115±0923	**0.014**	0.601	0.963
C18:1n9c	**38.421^{ab}±1.505**	**36.969^{b}±1.403**	**39.574^{a}±1.303**	**38.914^{ab}±2.141**	0.11	**0.023**	0.496
C18:1n11c	0.976±0.133	0.993±0.107	1.037±0.152	0.939±0.311	0.492	0.868	0.361
C18:2n6t	0.026±0.008	0.034±0.008	0.027±0.007	0.033±0.009	**0.051**	0.633	0.471
C18:2n6c	2.72±0.378	3.171±0.906	3.246±1.122	3.616±1.208	0.245	0.182	0.919
C20:0	0.085±0.012	0.089±0.008	0.082±0.013	0.079±0.009	0.789	**0.027**	0.195
C18:3n6	0.034±0.007	0.035±0.008	0.040±0.006	0.036±0.007	0.436	0.118	0.249
C20:1	0.056±0.009	0.055±0.009	0.058±0.011	0.054±0.007	0.273	0.989	0.628
C18:3n3	0.251±0.101	0.278±0.094	0.308±0.102	0.304±0.044	0.773	0.179	0.573

Table 4.3.6 continued…

CLA cis-9, trans-11/ trans-9, cis- 12	0.458±0.150	0.505±0.215	0.506±0.177	0.480±0.136	0.919	0.675	0.439
CLA cis-10, cis-12	0.006±0.002	0.007±0.002	0.005±0.002	0.006±0.003	0.216	0.229	0.82
CLA trans-10, cis-12	0.013±0.010	0.014±0.013	0.013±0.010	0.010±0.004	0.551	0.796	0.348
CLA isomer 1	0.008±0.002	0.007±0.003	0.008±0.003	0.007±0.004	0.407	0.893	0.869
CLA isomer 3	0.188±0.092	0.205±0.124	0.158±0.069	0.127±0.040	0.662	0.173	0.347
C20:2	0.052±0.010	0.045±0.007	0.052±0.007	0.043±0.009	**0.012**	0.698	0.689
C22:0	0.028±0.007	0.033±0.011	0.034±0.013	0.030±0.011	0.855	0.558	0.08
C20:3n6	0.090±0.047	0.069±0.037	0.100±0.040	0.100±0.068	0.437	0.188	0.61
C22:1n9	0.096±0.225	0.014±0.006	0.018±0.008	0.030±0.048	0.414	0.473	0.273
C23:0	0.024±0.005	0.024±0.006	0.029±0.012	0.025±0.008	0.272	0.185	0.351
C20:4n6	0.909±0.443	0.644±0.361	1.136±0.419	0.910±0.427	**0.075**	**0.073**	0.973
C24:0	0.040±0.012	0.035±0.013	0.046±0.017	0.038±0.014	0.101	0.241	0.66
C20:5n3	0.076±0.054	0.044±0.036	0.100±0.061	0.055±0.018	**0.01**	0.191	0.598
C24:1	0.027±0.011	0.027±0.013	0.034±0.014	0.026±0.009	**0.069**	0.136	0.092
C22:6n3	**0.065^{ab}±0.040**	**0.030^{b}±0.018**	**0.083^{a}±0.038**	**0.039^{b}±0.009**	**<0.001**	**0.092**	0.414
UIP	3.538±0.446	3.933±0.487	3.854±0.895	4.033±1.511	0.427	0.46	0.667
Sums and ratios							
SFA	**48.140^{ab}±1.784**	**48.433^{a}±2.186**	**45.713^{b}±2.602**	**45.644^{b}±0.447**	0.777	**0.001**	0.876
MUFA	**43.425^{ab}±1.531**	**42.544^{b}±1.205**	**44.654^{a}±1.183**	**44.557^{a}±1.393**	0.329	**0.004**	0.429
PUFA	4.897±1.341	5.089±1.341	5.089±1.359	5.766±1.601	0.935	0.116	0.766
USF	**48.322 ^{ab} ±1.644**	**47.633^{b}±2.090**	**50.433 ^{a}±1.873**	**50.323 ^{a} ±1.177**	0.463	**0.001**	0.689
SFA / UFA	**0.998 ^{ab} ±0.071**	**1.020 ^{a} ±0.087**	**0.909 ^{b} ±0.083**	**0.907 ^{b} ±0.017**	0.607	**0.001**	0.702

Table 4.3.6 continued…

MUFA/SFA	**0.904^{ab}±0.058**	**0.881^{b}±0.062**	**0.980^{a}±0.069**	**0.976^{a}±0.028**	0.468	**<0.001**	0.698
PUFA/SFA	0.103±0.030	0.106±0.035	0.129±0.046	0.126±0.035	0.978	**0.068**	0.734
CLA	0.673±0.228	0.739±0.329	0.690±0.234	0.630±0.172	0.808	0.844	0.367
n-3 PUFA	0.393±0.176	0.352±0.145	0.491±0.190	0.398±0.060	0.155	0.131	0.515
n-6 PUFA	1.033±0.496	0.749±0.405	1.275±0.464	1.046±0.494	**0.092**	**0.079**	0.948
n-6/n-3 ratio	2.763±0.914	2.099±0.419	2.729±0.713	2.603±1.004	0.23	0.483	0.354
Cis FA	**45.255^{ab}±1.790**	**43.878^{b}±2.510**	**47.288^{a}±1.828**	**46.544^{ab}±1.503**	0.145	**0.004**	0.646
Trans FA	3.067±0.246	3.756±0.831	3.145±0.527	3.778±0.852	**0.035**	0.701	0.764
Trans/cis ratio	0.068±0.008	0.086±0.023	0.067±0.012	0.082±0.020	**0.036**	0.775	0.676

Table 4.3.7: Means (± SD) depicting the effect of condensed tannin and NPN source on the medium and long chain fatty acid profile of the longissimus dorsi between the 8th and 10th ribs expressed gravimetrically (mg of fatty acid per g of longissimus dorsi on a DM basis)

(mg/g)	Urea		Calcium nitrate		P-value		
	None	Tannin	None	Tannin	T	N	T*N
Replicates (n)	8	8	8	8			
Fatty acids							
C10:0	0.070±0.025	0.076±0.022	0.062±0.028	0.071±0.019	0.28	0.319	0.748
C12:0	0.078±0.029	0.102±0.041	0.064±0.037	0.082±0.055	0.121	0.196	0.923
C13:0	0.007±0.003	0.009±0.004	0.005±0.003	0.007±0.004	**0.05**	0.128	0.93
C14:0	1.725±0.719	1.990±0.625	1.464±0.708	1.789±0.814	0.151	0.244	0.79
C14:1	0.048±0.021	0.056±0.021	0.044±0.024	0.054±0.032	0.248	0.571	0.796
C16:0	16.866±5.522	17.981±3.532	15.369±5.692	16.233±3.560	0.307	0.129	0.928
C16:1	0.896±0.323	0.965±0.201	0.868±0.344	0.905±0.278	0.407	0.473	0.985
C17:0	0.510±0.177	0.630±0.158	0.467±0.100	0.574±0.140	**0.034**	0.319	0.933
C18:0	10.877±2.547	11.966±2.276	9.793±2.541	10.324±1.962	0.219	**0.053**	0.763
C18:1t11	1.498±0.496	2.041±0.578	1.428±0.457	1.975±0.615	**0.008**	0.716	0.998
C18:1n9c	24.075±6.418	24.931±4.025	23.379±6.688	25.007±6.175	0.341	0.717	0.681
C18:1n11c	0.595±0.106	0.663±0.078	0.593±0.103	0.583±0.190	0.487	0.317	0.312
C18:2n6t	0.017±0.008	0.023±0.007	0.016±0.007	0.021±0.006	**0.045**	0.748	0.583
C18:2n6c	**1.616^{b}±0.281**	**2.066^{ab}±0.237**	**1.784^{ab}±0.272**	**2.207^{a}±0.451**	**0.004**	0.313	0.992
C20:0	0.052±0.011	0.060±0.010	0.047±0.010	0.050±0.011	0.116	**0.046**	0.572
C18:3n6	0.021±0.003	0.023±0.004	0.023±0.005	0.023±0.005	0.427	0.453	0.257
C20:1	0.034±0.006	0.037±0.006	0.033±0.006	0.034±0.009	0.377	0.358	0.901
C18:3n3	0.151±0.060	0.184±0.052	0.175±0.060	0.194±0.055	0.252	0.457	0.761

Table 4.3.7 continued…

CLA cis-9, trans-11/ trans-9, cis-12	0.279±0.095	0.352±0.183	0.311±0.175	0.317±0.143	0.574	0.914	0.483
CLA cis-10, cis 12	**0.004^{ab}±0.002**	**0.005^{a}±0.001**	**0.003^{b}±0.001**	**0.004^{ab}±0.002**	**0.055**	**0.037**	0.917
CLA trans-10, cis-12	0.007±0.005	0.010±0.010	0.008±0.008	0.007±0.004	0.859	0.794	0.28
CLA isomer 1	0.005±0.002	0.005±0.003	0.005±0.002	0.005±0.003	0.953	0.824	0.894
CLA isomer 3	0.115±0.054	0.142±0.093	0.098±0.058	0.085±0.037	0.897	0.145	0.299
C20:2	0.031±0.003	0.030±0.005	0.030±0.009	0.027±0.007	0.396	0.325	0.664
C22:0	0.017±0.003	0.022±0.005	0.019±0.004	0.018±0.003	0.285	0.574	0.06
C20:3n6	0.050±0.014	0.044±0.016	0.053±0.011	0.057±0.024	0.7	0.147	0.53
C22:1n9	0.062±0.147	0.009±0.002	0.009±0.002	0.016±0.020	0.396	0.414	0.288
C23:0	0.015±0.002	0.016±0.003	0.016±0.004	0.015±0.002	0.886	0.535	0.274
C20:4n6	**0.511^{ab}±0.132**	**0.408^{b}±0.144**	**0.622^{a}±0.098**	**0.541^{ab}±0.147**	**0.079**	**0.026**	0.903
C24:0	0.023±0.002	0.022±0.004	0.025±0.004	0.023±0.004	0.186	0.366	0.625
C20:5n3	**0.042^{ab}±0.023**	**0.027^{b}±0.016**	**0.052^{a}±0.020**	**0.034^{ab}±0.008**	**0.01**	0.144	0.716
C24:1	0.015±0.002	0.017±0.006	0.018±0.004	0.016±0.003	0.290	0.184	**0.017**
C22:6n3	**0.037^{ab}±0.017**	**0.019^{b}±0.008**	**0.046^{a}±0.019**	**0.024^{b}±0.005**	**<0.001**	**0.083**	0.444
UIP	2.189±0.527	2.687±0.628	2.192±0.519	2.665±1.353	**0.055**	0.967	0.961
Sums and ratios							
SFA	30.240±8.778	32.873±6.353	27.331±9.005	29.186±6.369	0.218	**0.09**	0.978
MUFA	27.223±7.321	28.718±4.729	26.374±7.532	28.589±6.736	0.229	0.663	0.718
PUFA	2.893±0.405	3.341±0.470	3.229±0.525	3.549±0.586	**0.09**	0.202	0.74
USF	30.116±7.339	32.059±4.767	29.602±7.772	32.138±6.869	0.174	1.790	0.757
SFA / UFA	**0.998^{ab}±0.071**	**1.020^{a}±0.087**	**0.909^{b}±0.083**	**0.907^{b}±0.017**	0.607	**0.001**	0.701

Table 4.3.7 continued…

MUFA/SFA	**0.904^{ab}±0.058**	**0.881^{b}±0.062**	**0.980^{a}±0.069**	**0.976^{a}±0.028**	0.467	**<0.001**	0.698
PUFA/SFA	0.103±0.030	0.106±0.035	0.129±0.046	0.126±0.035	0.976	0.068	0.737
CLA	0.411±0.137	0.513±0.273	0.425±0.234	0.417±0.182	0.651	0.706	0.397
n-3 PUFA	0.230±0.089	0.230±0.070	0.274±0.088	0.252±0.062	0.672	0.262	0.675
n-6 PUFA	0.583±0.146	0.475±0.160	0.698±0.108	0.621±0.165	0.108	**0.03**	0.881
n6/n3 ratio	2.763±0.914	2.099±0.419	2.730±0.713	2.603±1.004	0.23	0.484	0.354
Cis FA	28.222±6.846	29.498±4.165	27.777±7.168	29.747±6.404	0.255	0.822	0.706
Trans FA	2.412±0.702	2.530±0.550	1.862±0.773	2.289±0.661	**0.027**	0.69	0.78
Trans/cis ratio	0.068±0.008	0.086±0.023	0.067±0.012	0.082±0.020	**0.035**	0.773	0.677
Total FA	60.356±16.066	64.933±10.931	56.933±16.631	61.324±13.266	**0.191**	0.285	0.895

Table 4.3.8: Means (± SD) depicting the effect of condensed tannin and NPN source on the enzyme and health indices of the longissimus dorsi between the 8th and 10th ribs from the percent of detected fatty acids (w/w%)

	Urea		Calcium nitrate		P-value		
	None	Tannin	None	Tannin	T	N	T*N
Replicates (n)	8	8	8	8			
Enzyme indices							
Elongase	0.666±0.027	0.662±0.019	0.678±0.028	0.673±0.012	0.463	**0.100**	0.872
Delta 9 desaturase (C14+C16+C18)	**45.976^{ab}±1.641**	**44.994^{b}±1.864**	**48.032^{a}±1.720**	**47.642^{a}±1.372**	0.246	**<0.001**	0.649
Delta 9 desaturase C14	2.698±0.287	2.714±0.371	2.838±0.328	2.771±0.523	0.978	0.588	0.853
Delta 9 desaturase C16	4.997±0.377	5.099±0.527	5.364±0.583	5.206±0.664	0.907	0.280	0.554
Delta 9 desaturase C18	**68.654^{ab}±1.832**	**67.649^{b}±2.055**	**70.305^{ab}±1.848**	**70.502^{a}±2.287**	0.632	**0.006**	0.351
Delta 9 desaturase RA	16.206±5.151	13.957±3.146	16.888±3.606	14.081±4.664	0.108	0.679	0.733
Health indices							
AI	0.838±0.104	0.876±0.099	0.756±0.114	0.788±0.060	0.224	**0.012**	0.974
TI	**1.975^{ab}±0.151**	**2.035^{a}±0.175**	**1.797^{b}±0.161**	**1.816^{b}±0.039**	0.358	**<0.001**	0.752
DFA	65.875±2.481	65.320±2.023	67.116±2.472	66.581±1.920	0.373	**0.075**	0.888
h	**46.662^{ab}±1.728**	**46.034^{b}±2.117**	**48.819^{a}±1.908**	**48.733^{a}±1.179**	0.501	**<0.001**	0.720
H	29.343±2.706	29.375±2.055	27.768±3.217	28.075±1.273	0.700	**0.052**	0.732
h/H	1.606±0.203	1.578±0.186	1.788±0.299	1.739±0.100	0.485	**0.015**	0.780

Table 4.3.9: Means (± SD) depicting the effect of condensed tannin and NPN source on the enzymes and health indices of the longissimus dorsi between the 8th and 10th ribs from the gravimetric concentration (mg of fatty acid per g of longissimus dorsi on a DM basis)

	Urea		Calcium nitrate		P-value		
	None	Tannin	None	Tannin	T	N	T*N
Replicates (n)	8	8	8	8			
Enzyme indices							
Elongase	0.666±0.027	0.662±0.019	0.678±0.028	0.673±0.012	0.463	0.100	0.873
Delta 9 desaturase (C14+C16+C18)	**45.976^{ab}±1.641**	**44.993^{b}±1.854**	**48.032^{a}±1.710**	**47.642^{a}±1.372**	0.246	**<0.001**	0.649
Delta 9 desaturase C14	2.698±0.287	2.714±0.371	2.838±0.328	2.771±0.523	0.932	0.587	0.851
Delta 9 desaturase C16	4.997±0.377	5.099±0.527	5.364±0.583	5.206±0.664	0.907	0.280	0.554
Delta 9 desaturase C18	**68.654^{ab}±1.832**	**67.649^{b}±2.055**	**70.305^{ab}±1.848**	**70.502^{a}±2.287**	0.632	**0.006**	0.351
Delta 9 desaturase RA	16.206±5.151	13.957±3.146	16.888±3.606	14.081±4.664	0.108	0.679	0.733
Health indices							
AI	0.838±0.104	0.876±0.099	0.756±0.114	0.788±0.060	0.223	**0.012**	0.974
TI	**1.954^{ab}±0.157**	**2.015^{a}±0.177**	**1.777^{b}±0.169**	**1.799^{b}±0.038**	0.341	**<0.001**	0.782
DFA	40.993±9.871	40.026±6.839	39.395±10.240	42.462±8.772	0.177	0.436	0.888
h	29.060±7.056	30.976±4.579	28.630±7.411	31.113±6.602	0.168	0.821	0.76
H	18.591±6.224	19.97-±4.124	16.834±6.364	18.022±4.329	0.269	0.137	0.904
h/H	1.607±0.203	1.578±0.186	1.788±0.299	1.740±0.100	0.485	0.015	0.781

4.3.3.2 The effect of condensed tannin and calcium nitrate supplementation on subcutaneous fatty acids

The effect of tannin and NPN source on the subcutaneous fatty acid profile, sums of fatty acids and ratios are presented in Table 4.3.10 and 4.3.11 as a percent of identified fatty acids (w/w%) and gravimetrically (mg/g of subcutaneous fat), respectively. Enzyme and health indices are reported in Table 4.3.12 (from w/w%) and Table 4.3.13 (from mg/g of SCF). Enzymes are discussed within the SFA, MUFA and PUFA sections where applicable to the fatty acid results and discussion.

Calcium nitrate did not alter the fatty acid profile of subcutaneous fat (P>0.05; Table 4.3.10 and Table 4.3.11). The only exception was the concentration of C20:2 that increased in the calcium nitrate-based diet (P<0.05; Table 4.3.11). Tannin had no effect on the percent of SFA, MUFA or UFAs (P>0.05), but increased PUFA as a percent (P<0.05; Table 4.3.10). Gravimetrically, as indicated in Table 4.3.11, tannin tended to decrease SFA (P=0.07), MUFA (P>0.005) and therefore reduce total UFA (P<0.01). An explanation for this could be a less fatty carcass, resulting in subcutaneous fat with less fat and more water where lambs were fed tannin (P<0.01).

Guerreiro *et al*. (2020) studied three levels of a *cistus ladanifer* extract with a lucerne and soybean oil diet. In agreement with the current study, the CT level tended to increase PUFA. However, it was also reported that there was a significant increase SFA and decrease MUFA. The fatty acids have been discussed in more detail below with emphasis on tannin as a result of no changes in response to NPN source.

a) The effect of condensed tannin and calcium nitrate on the SFA profile of SCF

The percent of fatty acids are depicted in Table 4.3.10 and the concentration is depicted in Table 4.3.11. The calcium nitrate-based diet did not affect the SFA percent or concentration in SCF (P>0.05). Tannin did not lower SFA as a percent but tended to decrease it as a concentration (P=0.07). When tannin was included in the TMR, the decrease in concentration of SFA in SCF was mostly due to lower palmitic acid (C16:0; P<0.01). Stearic acid (C18:0) also decreased as a concentration, but the standard deviation was large and therefore did not decrease significantly (P>0.05; Table 4.3.11). As shown in Table 4.3.10, the molar percent tended to be lower in C16:0 (P=0.082) but there was no decrease in C18:0 (P>0.05).

Lambs on the urea-based diet with tannin had a higher C16:0 concentration than the lambs on the calcium nitrate-based diet without tannin (P<0.05; Table 4.3.11). A decrease in C16:0 was likely a result of a decrease in C16:0 in rumen fluid when tannin was included in the diet (Table 4.3.1). Studies vary in their SC FA response to tannin. In contrast to this study, Min *et al* (2015) and Bhatt *et al*. (2020) identified significant increases in C16:0 in sheep and goats consuming tannin, respectively. Other

studies reported no response to C16:0 (Lee *et al*., 2017; Guerriero *et al*., 2020). Unlike the current study that had no effect, Min *et al*. (2015) and Guerriero *et al*. (2020) reported an increase in C18:0 when tannin was supplemented.

Present as a much smaller percent in Table 4.3.10, C13:0 and C20:0 tended to increase in the tannin supplemented diet (P=0.079 and P=0.093, respectively). There was also a tendency for the concentration of C13:0 to increase (P=0.091) and C10:0 to be lower in tannin supplemented lambs (P<0.05; Table 4.3.11). This was contrary to rumen fluid that decreased in C13:0 and C20:0 fatty acids (Table 4.3.1). In agreement with the results, Guerriero *et al*. (2020) also reported a significant increase in C20:0.

b) The effect of condensed tannin and calcium nitrate on the MUFA profile of SCF

The percent of fatty acids are depicted in Table 4.3.10 and the concentration is depicted in Table 4.3.11. Tannin decreased the concentration of MUFA (P<0.005) but did not decrease it significantly as a percent of detected fatty acids (P>0.05). Therefore, there was also a decrease in the concentration of total UFA when tannin was supplemented to the diet (P<0.01). The decreased concentration of MUFA may be as a result of a tendency for its lower percent of detected fatty acids in rumen fluid (P=0.075; Table 4.3.1). This was confirmed by no changes in delta 9 desaturation activity (Table 4.3.12), that desaturates C18:1n9c to C18:0.

As the major MUFA in SCF, oleic acid (C18:1n9c) tended to be lower as a percent (P=0.104; Table 4.3.10) and lower as a concentration (P<0.005; Table 4.3.11). This too was reciprocated in rumen fluid (Table 4.3.1). Although tannin decreased the concentration of oleic acid in both the urea and calcium nitrate-based diets, the decrease was only significant in the calcium nitrate-based diet (P<0.05; Table 4.3.11). The urea-based diet lay within these values. This meant that the concentration of cis fatty acids also decreased when tannin was supplemented (P<0.005; Table 4.3.11). This resulted in the same pattern of significance as oleic acid between treatment groups.

Vaccenic acid (C18:1n11t), the major trans fatty acid in SCF, did not differ when tannin was supplemented to the diet (P>0.05; Table 4.3.10 and Table 4.3.11). However, Table 4.3.12 and Table 4.3.13 indicated that delta 9 desaturase (converting vaccenic acid to rumenic acid) had a tendency towards an increased activity (w/w%: P<0.09; mg/g SCF: P=0.078). Cis vaccenic acid (C18:1n11c) only increased as a molar percent in tannin supplemented diets (P<0.010; Table 4.3.10). A decrease in MUFA was also reported by Guerreiro *et al*. (2020), mainly a result of a significant decrease in oleic acid (C18:1c9) from 289 mg/g to 267mg/g and a decrease in palmitoleic acid (C16:1) from 9.26 to 6.39mg/g when CT extract was increased from 0 g/kg to 41g/kg basal diet in.

c) The effect of condensed tannin and calcium nitrate on the PUFA profile of SCF

Polyunsaturated fatty acids are shown as a percent in Table 4.3.10 and as a concentration in Table 4.3.11. The PUFA percent of detected fatty acids (w/w%) was elevated in tannin supplemented lamb SCF ($P<0.05$). It was not higher as a concentration ($P>0.05$). The NPN source did not affect the percent (w/w%) or concentration (mg/g SCF) of PUFA in SCF.

Of the omega 3 fatty acids, only C18:3n3 was detected and there was an interaction between tannin and NPN source (w/w%: $P<0.006$; mg/g SCF: $P<0.005$). Linolenic acid (C18:3n3) in SCF only increased when tannin was added to the urea-based diet. Table 4.3.1 indicated that C18:3n3 did not undergo greater biohydrogenation in the rumen fluid ($P>0.05$). Therefore, there was another reason for its increase.

Omega 6 fatty acids C18:2n6t, C18:2n6c, C18:3n6 and C18:4n6 were identified in the SCF of lambs. C18:2n6c, the major PUFA in SCF, increased as a percent of detected fat in tannin supplemented lambs, and tended to increase as a concentration ($P<0.05$ and $P=0.089$, respectively). In contrast to this, C18:2n6t was lower (w/w%: $P=0.082$; mg/g: $P<0.01$). There was an interaction between tannin supplementation and NPN source for the percent of C20:4n6 in lamb SCF ($P<0.05$). When tannin was supplied to the urea-based diet it increased. However, it decreased when tannin was supplied to the calcium nitrate-based diet. The C18:3n6 percent and concntration was unaffected by treatment ($P>0.05$).

The total n-3 and n-6 PUFAs were affected by tannin as a percent of fatty acids ($P<0.05$). Although the n-3 fatty acids in SCF were higher than IMF, it was still not as high as 300mg/100g (Vahmani *et al*., 2015). In addition, the less desirable n-6 fatty acids were also higher. Therefore, the n-6/n-3 ratio in SCF did not improve with supplementation of tannin and neither did calcium nitrate ($P>0.05$). At between 5.7 and 6.9 it was higher than the dietary recommended ratio of less than 4 (WHO, 2003). It was, therefore, less desirable than the IMF which lay below 5.

According to Guerreiro *et al*. (2020) tannin supplementation increased C20:4n6, C20:5n3 and C18:3n3 PUFAs in SCF. Of these fatty acids only C18:3n3 was increased as a percent of detected lipid (w/w%) in the current study ($P<0.05$). Linoleic acid (C18:2n6) and C18:3n3 in the current study were substantially lower than Guerreiro *et al*. (2020). This is consistent with lower values in the IMF. In contrast to Guerreiro *et al*. (2020), Min *et al*. (2015) also reported a significant increase in C18:2n6 cis and trans fatty acids, as well as C18:3n3 and C20:4n6 fatty acids when goats fed a concentrate-based diet were supplemented with tannin from pine bark.

As a percent in Table 4.3.10 and as a concentration in Table 4.3.11, total CLA was not affected by tannin or NPN source (P>0.05) However, trace amounts of CLA cis-9, trans-11 (RA) + trans-9, cis-12 tended to have a higher percent in the tannin supplemented diet (P=0.07). As a concentration it was not significant (P>0.05). Tannin decreased the percent and concentration of CLA isomer 1 in the calcium nitrate-based diet (P<0.05). Tannin decreased it in the calcium nitrate-based diet (P<0.05). The urea-based values lay within these results.

d) The effect of condensed tannin and calcium nitrate on the ratios of fatty acid in SCF

The ratios of subcutaneous fatty acids from the percent (w/w%) are presented in Table 4.3.10 and from the concentration (mg/g) are presented in Table 4.3.11. Neither tannin nor calcium nitrate affected SFA/UFA, MUFA/SFA, n-6/n-3, or trans/cis ratios from the percent or concentration (P>0.05). However, the PUFA/SFA ratio improved (increased) as a percent and concentration when tannin was supplemented in the diet (P<0.05). The highest result from Table 4.3.10 was 0.077%±0.012 in the urea based diet with tannin, which is still well below 0.45.

e) The effect of condensed tannin and calcium nitrate on enzyme and health indices of SCF

The effect of tannin and calcium nitrate on health indices are shown in Table 4.3.12 (calculated from the percent of fatty acids) and 4.3.13 (calculated from the concentration of fatty acids). The indices suggest that delta-9 desaturase activity was not affected by tannin or NPN source (P>0.05; Table 4.3.12 and Table 4.3.13), except for a decrease in the desaturation of C18:1n11t to CLA cis-9, trans-11 (shown by the delta 9 desaturase index for rumenic acid) in lambs fed the calcium nitrate-based diet (w/w%t: P<0.05; mg/g: P=0.078). There were no changes to the percent or concentration of C18:1n11t or CLA cis-9, trans-11 (P>0.05; Table 4.3.10 and Table 4.3.11). There was also no change in elongase activity amongst the different treatments (P>0.05; Table 4.3.12 and Table 4.3.13). Therefore, the decrease in C16:0 upon tannin supplementation was not because of changes in enzymatic activity.

Neither tannin nor calcium nitrate improved the atherogenic index (AI), thrombogenic index (TI), desirable fatty acid index (DFA), hypocholesterolemic fatty acid index (h), hypocholesterolemic fatty acid index (H) or h/H ratio from the percent of detected fatty acids (P>0.05; table 4.3.12). The AI, TI and h/H ratio also did not change from the concentration when tannin was supplemented (P>0.05; Table 4.3.13). However, the DFA index from the percent in SCF dropped when tannin was supplied to the ration (P<0.05). This was likely a result of a decrease in C18:1n9c reducing the MUFA concentration, and although C18:0 did not decrease significantly, tannin reduced it by 4.17% in the urea-based diet and 3.28% in the calcium nitrate-based diet (Table 4.3.11). Although the undesirable cholesterolaemic 'H' fatty acids decreased as a concentration in the SCF of lambs fed tannin, so did

the more desirable ‘h’ fatty acids (table 4.3.13). This was due to a lower concentration of C18:1n9c and C16:0 (Table 4.3.11). Therefore, the h/H ratio from the concentration was unaffected (P>0.05). Calcium nitrate had more effect on the IMF than SCF from a global health perspective. Although tannin reduced the H and DFA indicis as a concentration, none of the other indicis were negatively or positively affected.

Table 4.3.10: Means (± SD) depicting the effect of condensed tannin and NPN source on the medium and long chain fatty acid profile of subcutaneous fat expressed as a percent of detected fatty acids (w/w%)

(w/w%)	Urea		Calcium nitrate		P-value		
	None	Tannin	None	Tannin	T	N	T*N
Replicates (n)	8	8	8	8			
Fatty acids							
C10:0	0.149±0.019	0.14±0.0152	0.153±0.0249	0.136±0.030	0.153	0.936	0.719
C12:0	0.148±0.0473	0.160±0.059	0.153±0.076	0.126±0.051	0.898	0.399	0.5
C13:0	0.022±0.003	0.031±0.019	0.018±0.005	0.026±0.020	**0.079**	0.287	0.982
C14:0	3.338±0.651	3.362±0.700	3.522±0.701	3.253±0.751	0.809	0.924	0.736
C14:1	0.086±0.030	0.089±0.017	0.084±0.024	0.078±0.032	0.887	0.35	0.833
C16:0	24.510±1.026	23.220±1.943	25.225±1.700	23.971±1.996	0.082	0.335	0.84
C16:1	1.068±0.281	1.009±0.152	1.088±0.136	0.929±0.160	0.174	0.464	0.625
C17:0	1.803±0.340	1.811±0.583	1.500±0.118	1.983±0.784	0.171	0.725	0.186
C18:0	22.104±4.290	22.203±3.417	22.163±1.599	22.747±3.990	0.812	0.715	0.885
C18:1t11	3.384±0.370	3.836±0.549	3.654±0.743	4.768±2.181	0.13	0.182	0.543
C18:1n9c	34.176±3.837	33.076±2.684	34.042±1.189	31.799±2.733	0.104	0.482	0.563
C18:1n11c	0.576±0.122	0.676±0.061	0.580±0.077	0.692±0.142	**0.006**	0.56	0.923
C18:2n6t	0.049±0.012	0.045±0.012	0.049±0.020	0.041±0.016	**0.082**	0.826	0.293
C18:2n6c	1.677±0.120	2.526±0.269	1.753±0.282	2.163±1.386	**0.023**	0.584	0.384
C20:0	0.123±0.224	0.143±0.027	0.121±0.022	0.135±0.032	0.093	0.607	0.761
C18:3n6	0.022±0.001	0.028±0.003	0.026±0.006	0.026±0.007	0.211	0.458	0.119
C20:1	0.053±0.004	0.065±0.011	0.063±0.026	0.062±0.015	0.399	0.509	0.258
C18:3n3	**0.290^{b}±0.034**	**0.403^{a}±0.059**	**0.321^{ab}±0.056**	**0.312^{b}±0.073**	**0.024**	0.174	**0.006**

Table 4.3.10 continued…

CLA cis-9, trans-11/ trans-9, cis-12	0.611±0.144	0.617±0.112	0.591±0.189	0.549±0.080	0.604	0.493	0.533
CLA Trans-10, cis-12	0.010±0.009	0.015±0.009	0.011±0.006	0.018±0.011	**0.07**	0.527	0.661
CLA isomer 1	0.013^{ab}±0.004	0.009^{ab}±0.004	0.015^{a}±0.007	0.008^{b}±0.003	**0.001**	0.557	0.149
CLA isomer 3	0.072±0.042	0.090±0.045	0.070±0.028	0.095±0.033	0.121	0.949	0.737
C20:2	0.027±0.003	0.029±0.004	0.029±0.011	0.031±0.008	0.596	0.445	0.997
C22:0	0.013±0.005	0.011±0.003	0.010±0.005	0.112±0.004	0.973	0.492	0.551
C20:4n6	0.075±0.008	0.097±0.023	0.080±0.012	0.072±0.027	0.341	0.186	**0.036**
UIP	5.60±1.126	6.309±2.568	4.678±0.573	5.967±3.540	0.189	0.407	0.718
Sums and ratios							
SFA	52.209±5.193	51.081±4.981	52.865±1.579	52.388±5.355	0.618	0.535	0.775
MUFA	39.344±4.214	38.751±2.633	39.510±1.220	38.328±1.996	0.322	0.921	0.719
PUFA	2.848±0.302	3.859±0.320	2.946±0.476	3.317±1.455	**0.028**	0.474	0.24
USF	42.192±4.441	42.61±2.869	42.457±1.341	41.645±2.738	0.797	0.763	0.496
SFA / UFA	1.259±0.238	1.211±0.205	1.247±0.075	1.268±0.196	0.862	0.739	0.512
MUFA/SFA	0.768±0.169	0.769±0.126	0.748±0.043	0.742±0.122	0.915	0.604	0.893
PUFA/SFA	0.055±0.011	0.077±0.012	0.056±0.010	0.065±0.033	**0.035**	0.435	0.325
CLA	0.707±0.161	0.731±0.145	0.689±0.198	0.671±0.099	0.936	0.59	0.627
Σ n-3 PUFA	**0.290^{b}±0.034**	**0.403^{a}±0.059**	**0.321^{ab}±0.056**	**0.312^{b}±0.073**	**0.024**	0.174	**0.006**
Σ n-6 PUFA	1.774±0.201	2.651±0.274	1.858±0.281	2.261±1.393	**0.023**	0.567	0.351
n6/n3 ratio	6.157±0.814	6.633±0.687	5.899±1.012	6.945±2.778	0.166	0.988	0.579
Cis	38.051±4.300	37.998±3.028	38.065±1.154	36.164±2.353	0.329	0.363	0.355
Trans	4.141±0.494	4.612±0.625	4.392±0.927	5.481±2.156	0.153	0.222	0.607

Table 4.3.10 continued…

Trans Cis ratio	0.110±0.017	0.122±0.021	0.116±0.025	0.153±0.0612	0.121	0.194	0.458

Table 4.3.11: Means (± SD) depicting the effect of condensed tannin and NPN source on the medium and long chain fatty acid profile of subcutaneous fat expressed gravimetrically (mg of fatty acid per g of subcutaneous fat)

(mg/g)	Urea		Calcium nitrate		P-value		
	None	Tannin	None	Tannin	T	N	N*T
Replicates (n)	8	8	8	8			
Fatty acids							
C10:0	0.541±0.073	0.484±0.084	0.579±0.066	0.468±0.106	**0.018**	0.788	0.432
C12:0	0.540±0.190	0.565±0.239	0.568±0.298	0.447±0.215	0.709	0.474	0.508
C13:0	0.081±0.011	0.102±0.043	0.066±0.020	0.083±0.044	**0.091**	0.129	0.972
C14:0	12.184±2.797	11.764±3.269	13.137±2.848	11.429±3.535	0.401	0.918	0.665
C14:1	0.311±0.092	0.304±0.044	0.310±0.098	0.263±0.092	0.599	0.361	0.748
C16:0	**89.108^{ab}±7.867**	**80.389^{b}±13.470**	**94.53^{a}3±7.102**	**83.096^{ab}±13.999**	**0.011**	0.275	0.736
C16:1	3.859±0.930	3.470±0.615	4.095±0.615	3.224±0.771	**0.04**	0.805	0.488
C17:0	6.536±1.196	0.065±1.132	5.694±0.375	6.577±1.429	0.653	0.734	0.104
C18:0	80.632±17.620	77.266±17.801	82.373±7.251	79.674±20.086	0.421	0.553	0.958
C18:1t11	12.295±1.496	13.325±2.812	13.958±3.011	16.557±7.853	0.351	0.129	0.737
C18:1n9c	**123.763^{ab}±10.768**	**113.230^{ab}±8.799**	**127.721^{a}±10.736**	**109.353^{b}±14.040**	**0.001**	0.914	0.273
C18:1n11c	2.082±0.379	2.326±0.322	2.191±0.345	2.377±0.534	0.137	0.285	0.526
C18:2n6t	0.178±0.043	0.156±0.044	0.195±0.086	0.140±0.053	**0.014**	0.469	0.135
C18:2n6c	6.073±0.610	8.660±1.043	6.760±0.841	7.380±4.897	**0.089**	0.766	0.258
C20:0	0.448±0.090	0.498±0.125	0.442±0.085	0.473±0.138	0.331	0.702	0.803

Table 4.3.11 continued…

C18:3n6	0.081±0.006	0.096±0.018	0.100±0.026	0.091±0.029	0.849	0.271	0.079
C20:1	0.193±0.009	0.221±0.030	0.275±0.205	0.212±0.052	0.614	0.32	0.223
C18:3n3	1.055±0.127	1.382±0.209	1.311±0.402	1.078±0.287	0.684	0.883	**0.005**
CLA cis-9, trans-11/trans-9, cis-12	2.215±0.499	2.111±0.383	2.193±0.836	1.873±0.176	0.221	0.653	0.465
CLA Trans-10, cis-12	0.037±0.031	0.052±0.029	0.045±0.023	0.062±0.035	0.151	0.452	0.907
CLA isomer 1	**0.049^{ab}±0.016**	**0.033^{b}±0.014**	**0.063^{a}±0.034**	**0.027^{b}±0.007**	**<0.001**	0.308	0.075
CLA isomer 3	0.260±0.147	0.312±0.152	0.252±0.112	0.321±0.094	0.219	0.966	0.816
C20:2	0.099±0.008	0.100±0.019	0.133±0.050	0.108±0.032	0.165	**0.029**	0.138
C22:0	0.043±0.016	0.040±0.014	0.036±0.013	0.043±0.015	0.662	0.754	0.361
C20:4n6	0.273±0.033	0.340±0.104	0.273±0.098	0.253±0.107	0.504	0.186	0.114
UIP	20.216±3.418	20.945±5.426	17.004±2.391	19.410±7.324	0.411	0.194	0.709
Sums and ratios							
SFA	190.113±25.056	117.174±32.590	197.428±10.564	182.291±35.217	**0.07**	0.395	0.862
MUFA	142.502±11.695	132.876±11.644	148.551±12.151	131.986±15.602	**0.004**	0.453	0.339
PUFA	10.319±0.885	13.242±1.386	11.324±1.654	11.333±5.109	0.179	0.724	0.126
USF	152.821±12.224	146.118±12.830	159.875±12.762	143.319±16.990	**0.011**	0.524	0.211
SFA / UFA	1.259±0.238	1.211±0.205	1.239±0.072	1.268±0.196	0.92	0.793	0.464
MUFA/SFA	0.768±0.169	0.769±0.126	0.752±0.041	0.742±0.122	0.879	0.634	0.858
PUFA/SFA	0.055±0.011	0.077±0.012	0.058±0.009	0.065±0.033	**0.039**	0.499	0.257
CLA	2.561±0.544	2.508±0.511	2.552±0.877	2.283±1.121	0.386	0.735	0.522
n-3 PUFA	1.055±0.127	1.382±0.209	1.311±0.402	1.078±0.287	0.684	0.883	**0.005**
n-6 PUFA	6.426±0.616	9.096±1.132	7.133±0.861	7.724±4.922	0.089	0.742	0.237

Table 4.3.11 continued…

n6/n3 ratio	6.157±0.814	6.633±0.687	5.763±1.288	6.945±2.778	0.148	0.915	0.514
Cis FA	**137.787^{ab}±11.983**	**130.130^{ab}±10.642**	**143.170^{a}±11.233**	**124.338^{b}±13.769**	**0.003**	0.959	0.141
Trans FA	15.034±1.875	15.988±3.158	16.706±3.758	18.981±7.831	0.456	0.16	0.827
Trans/cis ratio	0.110±0.017	0.122±0.021	0.117±0.025	0.153±0.062	0.127	0.178	0.48
Total FA	342.934±16.848	323.292±41.150	357.303±21.549	325.610±49.779	**0.010**	0.323	0.457

Table 4.3.12: Means (± SD) depicting the effect of condensed tannin and NPN source on the enzyme and health indices of subcutaneous fat from the percent of detected fatty acids (w/w%)

	Urea		Calcium nitrate		P-value		
	None	Tannin	None	Tannin	T	N	T*N
Replicates	8	8	8	8			
Enzyme indices							
Elongase	0.687±0.013	0.695±0.019	0.681±0.019	0.687±0.014	0.369	0.317	0.656
Delta 9 desaturase (C14+C16+C18)	41.485±5.293	41.324±4.688	40.890±1.282	39.785±4.539	0.638	0.464	0.721
Delta 9 desaturase C14	2.556±0.853	2.766±1.140	2.299±0.232	2.512±1.623	0.517	0.447	0.928
Delta 9 desaturase C16	4.184±1.140	4.195±0.733	4.136±0.450	3.721±0.473	0.521	0.203	0.492
Delta 9 desaturase C18	60.781±7.224	59.907±5.553	60.591±2.076	58.452±6.040	0.439	0.614	0.766
Delta 9 desaturase RA	15.185±2.306	13.966±2.566	13.752±2.558	11.583±4.225	0.123	**0.090**	0.647
Health indices							
AI	0.931±0.162	0.890±0.166	0.949±0.109	0.915±0.159	0.591	0.815	0.796
TI	0.628±0.049	0.612±0.027	0.628±0.031	0.613±0.035	0.288	0.925	0.848
DFA	64.296±1.377	64.813±1.797	64.620±1.925	64.392±2.857	0.998	0.876	0.402
h	40.985±4.184	41.447±2.736	41.222±1.334	40.576±2.756	0.859	0.798	0.507
H	27.848±1.583	26.582±2.508	28.746±2.057	27.224±2.658	0.154	0.475	0.958
h/H	1.480±0.108	1.579±0.248	1.443±0.149	1.511±0.251	0.349	0.588	0.718

Table 4.3.13: Means (± SD) depicting the effect of condensed tannin and NPN source on the enzyme and health indices of subcutaneous fat from the gravimetric concentration (mg of fatty acid per g of subcutaneous fat)

	Urea		Calcium nitrate		P-value		
	None	Tannin	None	Tannin	T	N	N*T
Replicates (n)	8	8	8	8			
Enzyme indices							
Elongase	0.687±0.013	0.695±0.019	0.680±0.019	0.687±0.014	0.345	0.297	0.723
Delta 9 desaturase (C14+C16+C18)	41.485±5.293	41.324±4.688	40.969±1.239	39.785±4.539	0.618	0.481	0.700
Delta 9 desaturase C14	2.611±0.896	2.871±1.165	2.313±0.253	2.562±1.684	0.505	0.429	0.888
Delta 9 desaturase C16	4.184±1.140	4.195±0.733	4.142±0.442	3.721±0.473	0.511	0.206	0.482
Delta 9 desaturase C18	60.781±7.224	59.907±5.553	60.781±1.980	58.452±6.040	0.412	0.653	0.728
Delta 9 desaturase RA	15.185±2.306	13.966±2.566	13.386±3.361	11.583±4.255	0.186	**0.078**	0.785
Health indices							
AI	0.931±0.162	0.890±0.166	0.944±0.109	0.915±0.159	0.619	0.850	0.758
TI	2.365±0.446	2.245±0.404	2.326±0.141	2.368±0.399	0.776	0.756	0.441
DFA	233.452±9.177	223.384±26.534	242.249±17.993	222.993±33.418	**0.016**	0.372	0.340
h	148.458±11.426	142.123±12.255	155.195±12.458	139.619±16.455	**0.012**	0.497	0.21
H	101.292±9.410	92.153±16.496	107.670±8.778	94.526±17.349	**0.021**	0.357	0.706
h/H	1.480±0.208	1.579±0.248	1.448±0.148	1.511±0.251	0.361	0.606	0.693

4.3.3.3 The effect of condensed tannin and calcium nitrate supplementation on perirenal fatty acids

The effect of tannin and calcium nitrate supplementation on the PR fatty acid profile was reported as a percent of identified fatty acids (w/w%), including the sums and ratios of these fatty acids, in Table 4.3.14. Its gravimetric (mg/g PRF) counterpart is presented in Table 4.3.15. The enzyme and health indices are presented in Table 4.3.16 (w/w%) and 4.3.17 (mg/g PRF). The enzymes indices are discussed within the SFA, MUFA or PUFA categories to explore the reasons for differences within the fatty acid profile because of dietary treatment.

The NPN source did not affect the percent (w/w%) of total SFA, MUFA, PUFA or UFA in PRF ($P>0.05$; Table 4.3.14). Calcium nitrate increased the concentration of MUFA, UFA ($P<0.01$) and tended to increase the PUFA concentration ($P=0.091$; Table 4.2.15). The NPN source also did not alter the concentration of SFA ($P>0.05$; Table 4.3.15).

Tannin did not alter the percent and concentration of SFA, MUFA or UFA ($P>0.05$; Table 4.3.14 and Table 4.3.15). However, it did affect the percent and concentration of PUFA ($P<0.005$; Table 4.3.14 and Table 4.3.15, respectively). Tannin increased PUFA as a percent and concentration (w/w%: $P<0.05$; mg/g: $P<0.005$). There was an interaction between tannin and NPN source for the concentration of PUFA ($P<0.05$; Table 4.3.15) which meant that the increase only had significance in the urea-based diet ($P<0.05$; Table 4.3.15). Therefore, the supplementation of tannin was beneficial for a healthier fatty acid profile when lambs were on a urea-based diet. Although there was no interaction as a percent, tannin also increased PUFA in the urea-based diet ($P<0.05$, Table 4.3.14). The calcium nitrate-based diet had a higher concentration of UFA than the urea-based diet ($P<0.010$; Table 4.3.15). The urea-based diet without tannin was the lowest and the calcium nitrate-based diet with tannin had the highest concentration ($P<0.05$).

There are not many studies that have looked at the effect of tannin or calcium nitrate on its effect on the fatty acid composition of PRF, particularly in sheep. Kafle *et al*. (2021) reported changes to the perirenal fatty acid profile when tannin rich peanut skins were fed to goats. However, when 50% and 75% tannin rich peanut skins were fed, the MUFA concentration decreased significantly in PRF. This is contrary to our results where there was no significant difference (although the direction of change was increased upon tannin supplementation). The PUFA decreased when 25% and 75% peanut skins were supplemented to the diet. This is in contrast to the current study where tannin increased the PUFA percent and concentration. The results are discussed in more detail below.

a) The effect of condensed tannin and calcium nitrate on the SFA profile of PRF

The percent (w/w%) of SFAs in PRF are presented in Table 4.3.14 and the concentration of fatty acids (mg/g) are presented in Table 4.3.15. The major SFAs in PRF, C16:0 and C18:0, were unaffected by tannin and calcium nitrate (P>0.05). As a percent of detected fatty acid, only C20:0 decreased in the calcium nitrate-based diet (P<0.05). There was no effect of tannin or NPN source on the individual SFA concentrations in PRF. Tannin decreased the percent of C10:0 and increased the percent of C17:0 (P<0.05). An increase in C17:0 is related to feed and biohydrogenation. Biohydrogenation can increase propionic acid synthesis, which in turn is a precursor for odd chain fatty acid synthesis (Marmer *et al*., 1985; Beriain *et al*., 2000; Fievez *et al*., 2012). Therefore, an increase in C17:0 in IMF and PRF may be an indication of how tannin is able to alter biohydrogenation.

b) The effect of condensed tannin and calcium nitrate on the MUFA profile of PRF

The percent (w/w%) of MUFAs in PRF are presented in Table 4.3.14 and the concentration of fatty acids (mg/g) are presented in Table 4.3.15. Non-protein nitrogen affected C18:1n9c and C18:1n11c as a concentration and C18:1n11t as a percent and concentration. Tannin influenced the percent and concentration of C18:1n11t and C18:1n11c fatty acids in PRF.

The largest represented MUFA and cis fatty acid, C18:1n9c, was not affected by tannin and/or calcium nitrate as a percent of total detected fatty acids in PRF (P>0.05; Table 4.3.14). However, its concentration tended to be higher in the calcium nitrate-based diet (P=0.064; Table 4.3.15). This contributed to more total cis fatty acids (P<0.05; Table 4.3.15).

The major trans MUFA in PRF, vaccenic acid (C18:1n11t), increased as a percent and concentration in lamb fed the calcium nitrate-based diet (P<0.05; Table 4.3.14 and Table 4.3.15, respectively). As presented in Table 4.3.14 (percent) and 4.3.15 (concentration), this inevitably concluded with more total trans fatty acid (percent: P<0.05; concentration: P<0.01). When the TMR contained tannin, C18:1n11t tended to increase both as a percent and as a concentration (P=0.068 and P=0.08, respectively). This contributed to a higher trans fatty acid percent (P<0.05) and concentration (P<0.01) in the PRF of tannin supplemented lambs (Table 4.3.14 and Table 4.3.15, respectively). Total trans fatty acids are presented in Table 4.3.14 (percent) and 4.3.15 (concentration). Total trans fatty acids in the urea-based diet without tannin (4.061%±0.515 and 14.987mg/g±2.109) was higher than the calcium nitrate-based diet with tannin (5.831%±1.918 and 22.641mg/g±7.803; P<0.05). The total concentration of trans fatty acids increased to a greater extent than the total cis fatty acids, lending to a tendency for any increased trans/cis ratio (P=0.064; Table 4.3.15). This was not observed as a percent of detected lipid (P>0.05; Table 4.3.14).

The percent of C18:1n11c increased in PRF when tannin was supplemented to the TMR (P<0.05). Not only did tannin significantly raise its concentration (P<0.05), but the calcium nitrate-based diet tended to increase it (P=0.059). This meant that there was a greater C18:1n11c concentration in the calcium nitrate-based diet with tannin (2.775mg/g±0.807) than the urea-based diet without tannin (2.082±0.143; P<0.05). Due to a tendency for a higher concentration of C18:1n9c (P=0.064) and C18:1n11c (P=0.059) fatty acids, the calcium nitrate-based diet had an increased total cis fatty acid concentration (P<0.05; Table 4.3.15).

There were some similarities between PRF, IMF and SCF. Vaccenic acid (C18:1n11t), and total trans fatty acids (TFA) also increased as a concentration with tannin supplementation (Table 4.3.15, Table 4.3.7 and Table 4.3.11, respectively). The percent of C18:1n11c to increase in SCF with tannin supplementation (Table 4.3.10) also resulted in its increase in PRF (Table 4.3.14). Although it was only evident as a tendency in the concentration in PRF (Table 4.3.15), IMF C18:1n9c also increased as a percent in the calcium nitrate-based diet (Table 4.3.6).

c) The effect of condensed tannin and calcium nitrate on the PUFA profile of PRF

The percent (w/w%) of PUFAs in PRF are presented in Table 4.3.14 and the concentration of fatty acids (mg/g) are presented in Table 4.3.15. Non-protein nitrogen (NPN) source affected C18:2n6t, CLA isomer 1 and 3 as a percent of identified fatty acids, and all CLAs as a concentration. Tannin changed the percent and concentration of C18:3n6, C20:4n6, C18:2n6c, C18:3n3 and therefore total n-3 and n-6 fatty acids.

There was an interaction between tannin and NPN source for the C18:3n3 percent (P<0.005) and concentration (P<0.015). It increased from 0.263%±0.040 (0.971mg/g±0.151) to 0.396%±0.054 (1.433mg/g±0.221) when lambs on the urea-based diet were fed tannin (P<0.05). Tannin had no effect on the calcium nitrate-based diet (P>0.05). The calcium nitrate-based diet (with and without tannin) had a lower percent of C18:3n3 than the urea-based diet with tannin (P<0.05). As C18:3n3 in rumen fluid is not affected (Table 4.3.1), it suggests that the C18:3n3 pathway may have been least affected in the urea-based diet with tannin. However, no other n-3 fatty acids were identified in PRF. As a concentration, both calcium nitrate-based diets did not differ from the urea-based diets regardless of the addition of tannin (P>0.05).

Tannin only increased the percent of omega 6 fatty acids (n-6) in the urea-based diet (P<0.05). It increased 46.75% from 1.983%±0.303 to 2.910%±0.543 (Table 4.3.14). The increase was not substantial in the calcium nitrate-based diet (P>0.05). The calcium nitrate-based diets (with and without tannin) were significantly lower than the urea-based diet with tannin, and the same as the urea-based diet without tannin (P>0.05; table 4.3.14). Of the individual n-6 fatty acids presented as a

percent in Table 4.3.14 and as a concentration in Table 4.3.15, tannin increased C18:2n6c, C18:3n6 and C20:4n6 in lamb PRF as a percent of detected lipid ($P<0.005$, $P<0.05$ and $P<0.010$ respectively) and as a concentration ($P<0.010$, $P<0.05$ and $P<0.05$, respectively).

There was a 32.5% increase in trace percent of C20:4n6 when tannin was added to the urea-based diet (0.040%±0.009 vs 0.053%±0.017; Table 4.3.14). The increase was not significant in the calcium nitrate-based diet ($P<0.05$). Calcium nitrate did not have an overall effect on the percent or concentration of n-6 fatty acids ($P>0.05$), although it did increase the percent ($P<0.05$) and concentration ($P<0.01$) of C18:2n6t. The n-6/n-3 ratio did not differ between treatments ($P>0.05$; Table 4.3.14 and table 4.3.15).

Tannin had no effect on CLA percent or concentration ($P>0.05$; Tble 4.3.14 and Table 4.3.15, respectively). This agrees with Staerfl *et al*. (2011) who identified no changes in rumenic acid (CLA cis-9, trans-11) in fattening bulls fed tannin. In comparison to this, Kafle *et al*. (2021) reported a decrease in CLA in perirenal fat when a 25% peanut skin diet containing 3.9% CT was fed to kiko cross-bred male goats.

In the current study, calcium nitrate was effective in increasing the total concentration of CLA ($P<0.05$; Table 4.3.15) but not as a percent ($P>0.05$; Table 4.3.14). The increased concentration is advantageous because CLAs have reportedly shown potential to reduce hypertension, are antitumor, antiatherogenic, antidiabetic and have anti-obese properties (Nagao *et al*., 2005). Calcium nitrate increased the total CLA concentration ($P<0.05$) through a higher concentration of trans-10, cis-12 CLA ($P<0.05$), CLA isomers 1 and 3 ($P<0.01$) and a tendency for an increased CLA cis-9, trans-11 + trans-9, cis-12 ($P=0.079$). The percent of CLA isomer 1 and 3 was also higher in the perirenal fat of lambs on the calcium nitrate-based diet ($P<0.01$ and $P<0.05$, respectively).

In a study conducted by Staerfl *et al*. (2011) bulls supplemented with tannin on maize only differed in their C16:0 acid concentration. However, when supplemented on a grass-based diet, total PUFA, n-3 fatty acids, C15:1, C17:0, C18:3n6, C18:3n3, C20:4n3 and C20:5n3 decreased upon tannin supplementation, and C16:0 and C17:1n7 increased upon tannin supplementation. Animal species, basal diet, dosage and type of tannin may have resulted in differences in the effect of tannin on PRF in the current study; based on its increase in PUFAs, C18:2n6c, C18:3n3 and some intermediate fatty acids. There may also have been differences in the targeted biohydrogenation site in the case of C18:1nt11, which was higher in the current study but lower in previous studies.

d) The effect of condensed tannin and calcium nitrate on the ratios of fatty acids in PRF

The fatty acid ratios from the percent (w/w%) of fatty acids detected in PRF are presented in Table 4.3.14 and ratios from the concentration of fatty acids (mg/g) are presented in Table 4.3.15. The PUFA/SFA ratio only differed between treatments as a concentration ($P<0.05$) but not as a percent of detected lipid ($P>0.05$). The tannin and NPN source interacted ($P<0.05$), thus only when tannin was fed in the urea-based diet was the ratio improved ($P<0.005$; Table 4.3.15). 0.137mg/g±0.019 was still lower than the recommended dietary allowance of 0.45 specified by Enser *et al*. (1998).

The trans/cis ratio from the concentration tended to be higher ($P=0.064$) in the calcium nitrate-based diet due to the higher trans fatty acid profile ($P=0.01$; Table 4.3.15). However, its major constituent, C18:1n11t (as previously discussed) is not a health concern.

e) The effect of condensed tannin and calcium nitrate on the enzymes and health indices of PRF

As demonstrated in Table 4.3.16 (from the percent of fatty acids) and 4.3.17 (from the concentration of fatty acids), enzymes indices were not affected by the tannin or NPN source when calculated from the percent of detected fatty acids or as a concentration ($P>0.05$).

The health indices were not affected when calculated as a percent of detected fatty acids ($P>0.05$). The desirable fatty acid index (DFA) and hypochelsterololemic (h) indices were affected by the calcium nitrate-based diet when calculated from the concentration ($P=0.015$ and $P<0.01$, respectively). Calcium nitrate improved DFA because of a higher UFA (concentration) in lamb PRF and a small but insignificant increase in C18:0 (Table 4.3.15). The h indices was best in the calcium nitrate-based diet ($P<0.01$) because of a higher C18:1 and PUFA concentration (presented in Table 4.3.15). Because tannin increased h, the calcium nitrate-based diet with tannin had a higher index than the urea-based diet without tannin ($P<0.05$)

Table 4.3.14: Means (± SD) depicting the effect of condensed tannin and NPN source on the medium and long chain fatty acid profile of perirenal fat expressed as a percent of detected fatty acids (w/w%)

(w/w%)	Urea		CaN		P-value		
	None	Tannin	None	Tannin	T	N	T*N
Replicates (n)	8	8	8	8			
Fatty acids							
C10:0	0.123±0.016	0.109±0.016	0.116±0.023	0.108±0.016	**0.097**	0.364	0.429
C12:0	0.121±0.034	0.129±0.046	0.116±0.053	0.115±0.047	0.793	0.56	0.831
C14:0	2.908±0.353	2.748±0.497	2.797±0.635	2.749±0.528	0.665	0.623	0.62
C14:1	0.032±0.012	0.034±0.015	0.033±0.013	0.029±0.012	0.947	0.614	0.535
C16:0	22.084±2.286	20.331±1.187	21.370±2.460	21.018±1.257	0.136	0.794	0.167
C16:1	0.602±0.102	0.636±0.122	0.657±0.080	0.619±0.111	0.906	0.589	0.32
C17:0	1.514±0.080	1.552±0.100	1.460±0.106	1.591±0.112	**0.06**	0.951	0.304
C18:0	37.744±2.917	35.737±3.446	35.278±2.958	35.351±3.035	0.412	0.2	0.339
C18:1n11t	**3.577^{b}±0.459**	**4.235^{ab}±0.596**	**4.279^{ab}±0.614**	**5.264^{a}±1.919**	**0.068**	**0.035**	0.767
C18:1n9c	23.847±3.231	25.226±1.973	25.839±2.958	24.525±2.463	0.951	0.488	0.187
C18:1n11c	0.566±0.053	0.666±0.056	0.605±0.082	0.711±0.202	**0.029**	0.224	0.894
C18:2n6t	0.023±0.008	0.019±0.007	0.026±0.007	0.028±0.008	0.449	**0.032**	0.502
C18:2n6c	**1.898^{b}±0.315**	**2.810^{a}±0.548**	**2.149^{ab}±0.244**	**2.660^{ab}±1.104**	**0.004**	0.782	0.327
C20:0	0.226±0.032	0.231±0.017	0.198±0.030	0.209±0.035	0.445	**0.029**	0.715
C18:3n6	0.022±0.004	0.028±0.003	0.022±0.005	0.027±0.007	**0.032**	0.82	0.475
C20:1	0.049±0.008	0.058±0.007	0.053±0.008	0.052±0.017	0.193	0.835	0.13
C18:3n3	**0.263^{b}±0.040**	**0.396^{a}±0.054**	**0.305^{b}±0.031**	**0.322^{b}±0.037**	**<0.001**	0.31	**0.002**
CLA cis-9, trans-11/	0.390±0.075	0.465±0.095	0.469±0.099	0.448±0.057	0.592	0.294	0.133

trans-9, cis-12

Table 4.2.14 continued…

CLA Trans-10, cis-12	0.007±0.002	0.008±0.002	0.010±0.003	0.008±0.003	0.664	0.126	0.072
CLA isomer 1	0.005±0.002	0.004±0.003	0.008±0.003	0.009±0.006	0.992	**0.01**	0.543
CLA isomer 3	0.060±0.013	0.067±0.013	0.067±0.013	0.081±0.029	0.884	**0.02**	0.193
C20:2	0.021±0.004	0.022±0.003	0.022±0.005	0.021±0.006	0.751	0.859	0.368
C22:0	0.024±0.005	0.023±0.005	0.021±0.005	0.021±0.008	0.752	0.139	0.84
C20:4n6	**0.040^{b}±0.009**	**0.053^{a}±0.017**	**0.041^{ab}±0.017**	**0.046^{ab}±0.017**	**0.009**	0.358	0.229
UIP	3.854±0.465	4.409±0.355	4.044±0.657	3.995±0.249	0.224	0.672	0.063
Sums and ratios							
SFA	65.085±3.789	61.326±2.875	61.826±3.299	61.609±3.913	0.162	0.183	0.124
MUFA	28.673±3.377	30.857±2.392	31.465±3.125	31.2±3.059	0.481	0.143	0.235
PUFA	**2.389^{b}±0.273**	**3.408^{a}±0.555**	**2.664^{ab}±0.253**	**3.195^{ab}±1.078**	**0.003**	0.845	0.255
USF	31.062±3.478	34.265±2.644	34.130±3.153	34.395±3.890	0.205	0.156	0.187
SFA / UFA	2.132±0.377	1.805±0.232	1.831±0.243	1.820±0.291	0.158	0.145	0.114
MUFA/SFA	0.444±0.076	0.506±0.062	0.513±0.083	0.511±0.087	0.371	0.163	0.225
PUFA/SFA	0.037±0.005	0.056±0.011	0.043±0.005	0.053±0.023	**0.006**	0.649	0.273
CLA	0.461±0.088	0.545±0.105	0.568±0.117	0.539±0.055	0.652	0.144	0.115
n-3 PUFA	**0.263^{b}±0.040**	**0.396^{a}±0.054**	**0.305^{b}±0.031**	**0.322^{b}±0.037**	**<0.001**	0.31	**0.002**
n-6 PUFA	**1.983^{b}±0.303**	**2.910^{a}±0.543**	**2.239^{b}±0.232**	**2.760^{b}±1.024**	**0.004**	0.772	0.327
n6/n3 ratio	7.573±0.877	7.421±1.313	7.392±0.837	8.422±2.023	0.41	0.402	0.267
Cis FA	27.340±3.491	29.932±2.371	29.726±3.053	29.012±2.953	0.473	0.456	0.132
Trans FA	**4.061^{b}±0.515**	**4.799^{ab}±0.666**	**4.873^{ab}±0.709**	**5.831^{a}±1.918**	**0.069**	**0.029**	0.893
Trans/cis ratio	0.150±0.025	0.161±0.021	0.165±0.028	0.202±0.061	0.149	**0.064**	0.421

Table 4.3.15: Means (± SD) depicting the effect of condensed tannin and NPN source on the medium and long chain fatty acid profile of perirenal fat expressed gravimetrically (mg of fatty acid per g of perirenal fat)

(mg/g)	Urea		Calcium nitrate		P-value		
	None	Tannin	None	Tannin	T	N	N*T
Replicates (n)	8	8	8	8			
Fatty acids							
C10:0	0.452±0.056	0.40±0.073	0.445 ±0.103	0.419±0.054	0.118	0.911	0.33
C12:0	0.445±0.119	0.462±0.150	0.443±0.205	0.443±0.169	0.839	0.818	0.932
C14:0	10.711±1.294	9.897±1.627	10.667±2.483	10.623±1.931	0.619	0.726	0.386
C14:1	0.117±0.044	0.123±0.050	0.127±0.048	0.112±0.045	0.891	0.881	0.611
C16:0	81.389±8.844	73.615±7.690	81.553±11.143	81.435±6.450	0.23	0.223	0.1
C16:1	2.212±0.333	2.292±0.397	2.501±0.322	2.386±0.369	0.862	0.137	0.429
C17:0	5.587±0.446	5.607±0.458	5.552±0.281	6.169±0.576	0.112	0.161	0.137
C18:0	139.256±13.619	129.553±17.915	134.415±12.488	137.065±14.642	0.581	0.87	0.248
C18:1n11t	**13.201^{a}±1.899**	**15.206^{ab}±1.358**	**16.276^{ab}±2.182**	**20.445^{a}±7.780**	**0.08**	**0.015**	0.557
C18:1n9c	87.731±10.883	91.034±6.723	98.468±12.237	94.807±7.917	0.925	**0.064**	0.342
C18:1n11c	**2.082^{b}±0.143**	**2.403^{ab}±0.135**	**2.302^{ab}±0.298**	**2.755^{a}±0.807**	**0.033**	**0.059**	0.802
C18:2n6t	0.086±0.030	0.070±0.022	0.098±0.026	0.107±0.031	0.479	**0.013**	0.363
C18:2n6c	7.000±1.206	10.136±1.946	8.146±0.993	10.326±4.106	**0.006**	0.424	0.562
C20:0	0.834±0.117	0.834±0.073	0.755±0.120	0.806±0.122	0.477	0.187	0.487
C18:3n6	0.079±0.016	0.101±0.015	0.086±0.019	0.104±0.029	**0.046**	0.45	0.67
C20:1	0.179±0.027	0.210±0.021	0.201±0.035	0.203±0.066	0.225	0.532	0.232
C18:3n3	**0.971^{b}±0.151**	**1.433^{a}±0.221**	**1.160^{ab}±0.129**	**1.248^{ab}±0.158**	**0.001**	0.98	**0.015**

Table 4.3.15 continued…

CLA cis-9, trans-11/ trans-9, cis-12	1.436±0.278	1.667±0.282	1.780±0.347	1.732±0.223	0.591	**0.079**	0.197
CLA Trans-10, cis-12	0.025±0.007	0.029±0.008	0.027±0.012	0.030±0.011	0.622	**0.042**	**0.086**
CLA isomer 1	0.019±0.009	0.015±0.009	0.029±0.012	0.036±0.023	0.942	**0.007**	0.441
CLA isomer 3	0.219±0.046	0.242±0.038	0.312±0.116	0.291±0.082	0.864	**0.006**	0.292
C20:2	0.076±0.015	0.082±0.014	0.084±0.022	0.083±0.023	0.76	0.424	0.521
C22:0	0.089±0.018	0.083±0.017	0.078±0.020	0.079±0.030	0.718	0.33	0.665
C20:4n6	0.147±0.036	0.192±0.067	0.157±0.062	0.178±0.062	**0.008**	0.748	0.357
UIP	**14.180^{b}±1.573**	**15.884^{a}±0.694**	**16.023^{a}±1.056**	**15.457^{ab}±0.810**	0.143	**0.066**	**0.006**
Sums and ratios							
SFA	238.762±18.771	220.466±22.667	233.909±18.016	237.040±20.164	0.386	0.526	0.111
MUFA	105.522±11.442	111.268±6.700	119.876±12.810	120.707±11.381	0.476	**0.008**	0.497
PUFA	**24.245^{b}±2.313**	**29.893^{a}±2.591**	**27.911^{ab}±1.513**	**29.609^{a}±4.463**	**0.002**	**0.091**	**0.053**
USF	**129.767^{b}±13.183**	**141.161^{ab}±7.929**	**147.786^{ab}±13.227**	**150.316^{a}±15.055**	0.165	**0.007**	0.3
SFA / UFA	1.868±0.331	1.568±0.203	1.560±0.201	1.660±0.241	0.14	0.143	0.082
MUFA/SFA	0.447±0.078	0.510±0.063	0.517±0.084	0.515±0.087	0.370	0.162	0.219
PUFA/SFA	0.102^{b}±0.015	0.137^{a}±0.019	0.120^{ab}±0.011	0.126^{ab}±0.027	0.005	0.484	0.024
CLA	1.699±0.325	1.953±0.303	2.157±0.422	2.090±0.226	0.655	**0.028**	0.176
n-3 PUFA	**0.971^{b}±0.151**	**1.433^{a}±0.221**	**1.160^{ab}±0.129**	**1.248^{ab}±0.158**	**0.001**	0.98	0.015
n-6 PUFA	7.313±1.168	10.498±1.939	8.487±0.954	10.715±4.150	**0.006**	0.412	0.565
n6/n3 ratio	7.573±0.877	7.422±1.313	7.352±0.802	8.422±2.023	0.39	0.427	0.253

Table 4.3.15 continued…

Cis FA	100.697±11.725	108.090±8.000	113.305±12.701	112.277±10.330	0.473	**0.048**	0.286
Trans FA	**14.987^{b}±2.109**	**17.228^{ab}±1.454**	**18.521^{ab}±2.503**	**22.641^{a}±7.803**	**0.081**	**0.011**	0.641
Trans/cis ratio	0.150±0.025	0.160±0.020	0.165±0.028	0.201±0.061	0.149	**0.064**	0.423
Total FA	328.529±11.874	361.627±22.553	381.695±17.522	387.355±15.785	0.970	0.010	0.281

Table 4.3.16: Means (± SD) depicting the effect of condensed tannin and NPN source on the enzyme and health indices of perirenal fat from the percent of detected fatty acids (w/w%)

	Urea	CaN	Urea	CaN	P-value		
	None	Tannin	None	Tannin	T	N	T*N
Replicates (n)	8	8	8	8			
Enzyme indices							
Elongase	0.731±0.025	0.744±0.015	0.735±0.029	0.735±0.012	0.454	0.844	0.217
Delta 9 desaturase (C14+C16+C18)	28.090±3.953	30.593±2.713	30.865±3.569	29.895±3.157	0.638	0.360	0.155
Delta 9 desaturase C14	1.077±0.389	1.193±0.363	1.146±0.280	1.012±0.255	0.946	0.632	0.308
Delta 9 desaturase C16	2.682±0.548	3.032±0.510	3.009±0.458	2.865±0.521	0.680	0.542	0.135
Delta 9 desaturase C18	38.687±4.861	41.463±4.168	42.263±4.378	40.990±4.144	0.713	0.330	0.215
Delta 9 desaturase RA	9.832±1.512	9.899±1.667	9.839±0.127	8.502±2.385	0.352	0.383	0.308
Health indices							
AI	1.113±0.215	0.923±0.069	0.973±0.199	0.951±0.154	0.102	0.217	0.089
TI	3.931±0.714	3.254±0.400	3.252±0.433	3.465±0.576	0.106	0.155	0.082
DFA	68.806±2.167	70.003±1.263	69.408±2.727	69.746±1.624	0.328	0.650	0.400
h	30.379±3.390	33.536±2.533	33.387±3.099	33.695±3.833	0.194	0.151	0.190
H	24.992±2.503	23.079±1.417	24.168±2.977	23.766±1.592	0.155	0.723	0.181
h/H	1.233±0.227	1.457±0.127	1.408±0.273	1.430±0.252	0.165	0.246	0.139

Table 4.3.17: Means (± SD) depicting the effect of condensed tannin and NPN source on the enzyme and health indices of perirenal fat from the gravimetric concentration (mg of fatty acid per g of PRF)

	Urea		CaN		P-value		
	None	Tannin	None	Tannin	T	N	N*T
Replicates (n)	8	8	8	8			
Enzyme indices							
Elongase	0.731±0.025	0.744±0.015	0.735±0.029	0.735±0.012	0.454	0.844	0.217
Delta 9 desaturase (C14+C16+C18)	28.090±3.959	30.593±2.713	30.865±3.569	29.895±3.157	0.638	0.360	0.155
Delta 9 desaturase C14	1.077±0.389	1.193±0.363	1.146±0.280	1.012±0.255	0.945	0.631	0.308
Delta 9 desaturase C16	2.682±0.548	3.032±0.510	3.009±0.458	2.865±0.521	0.680	0.542	0.135
Delta 9 desaturase C18	38.687±4.861	41.467±4.168	42.263±4.378	40.990±4.144	0.713	0.330	0.215
Delta 9 desaturase RA	9.832±1.512	9.900±1.667	9.839±1.270	8.502±2.385	0.352	0.383	0.308
Health indices							
AI	1.113±0.215	1.923±0.069	0.973±0.199	0.951±0.154	0.101	0.218	0.089
TI	3.955±0.720	3.273±0.408	3.340±0.534	3.485±0.583	0.148	0.148	0.113
DFA	269.023±12.413	270.714±16.316	282.202±14.033	287.380±13.128	0.537	**0.015**	0.751
h	127.259b±12.929	138.536ab±7.628	144.957ab±12.984	147.615a±14.937	0.158	**0.007**	0.304
H	92.100±9.644	83.513±8.243	92.220±13.133	92.059±7.451	0.225	0.231	0.090
h/H	1.404±0.257	1.669±0.151	1.604±0.297	1.618±0.261	0.142	0.267	0.092

4.3.4 The effect of sex on the fatty acid profile of lamb

Figure 4.3.7 shows a chromatogram from two subcutaneous fat samples. The pink line represents the ewe, and the black line represents a ram. Sex altered the fatty acid composition of fat, and therefore the nutritional status of lamb. The response to the fatty acid composition varied between fat depots. SFA was lower in ewes than rams in IMF (percent: P=0.087 and concentration: P<0.001; Table 4.3.18) and SCF (percent: P<0.010 and concentration: P<0.001; Table 4.3.20) but had no effect on PRF (P>0.05 Table 4.3.22). Perirenal fat (PRF) had a higher PUFA percent and concentration (P<0.05; Table 4.3.22) but only the percent was affected in IMF (Table 4.3.18) and SCF (Table 4.3.20) (P<0.005 and P=0.059, respectively). The total MUFAs had a more variable response, with the concentration increasing in ewe IMF (Table 4.3.18) and SCF (Table 4.3.20) (P<0.001 and P=0.064, respectively), but decreasing as a percent in SCF (P<0.05; Table 4.3.20). Perirenal fat SFA and MUFA was not affected by sex (P>0.05; Table 4.3.22).

The potential of rams to decrease SFA and MUFA while increasing PUFA and total UFA is in contrast to Sañudo *et al*. (1998) and Diaz *et al*. (2003) who reported higher SFA in rams as a result of later sexual maturity, as described by Webb *et al*. (1994). The difference between studies may be a result of ewes having a higher percent of fat and ether extract (EE) than rams. Neutral lipid, which contains SFA and MUFA, increases with fatness, whereas phospholipid, which predominantly contains PUFA, remains relatively unchanged in comparison (Wood *et al*., 2008). In agreement with the current study, MUFA was also higher in ewes in the study conducted by Diaz *et al*. (2003), and PUFA was also reported to be higher in studies conducted by Tejeda *et al*. (2008) and Facciolongo *et al*. (2018). An increase in unsaturated fatty acids causes an increase in melting point and decrease in the softness of carcass fat at a given temperature (Elliot *et al*., 1969). Therefore, an increase in PUFA in rams has the potential to decrease its melting point, thereby improving the softness of fat and acceptability of the meat. However, too much PUFA can also result in oxidative deterioration of lipid, decreasing the shelf life of meat and increasing carcinogenic free radicals (Ahmed *et al*., 2015). The PUFA was low in the current study and therefore unlikely to cause a problem.

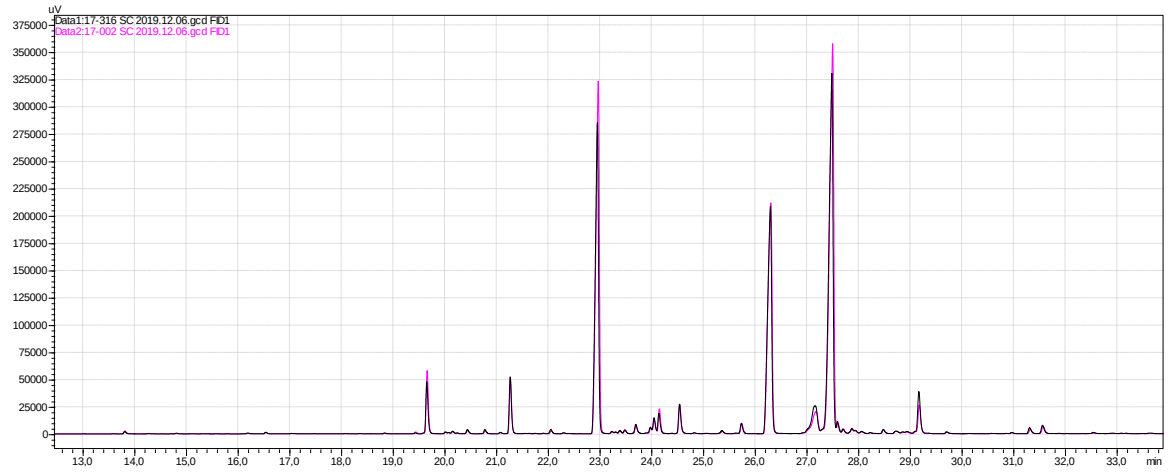

Figure 4.3.7: A chromatogram representing the effect of sex on fatty acid composition

4.3.4.1 The effect of sex on the IM fatty acid profile of lamb

The effect of sex on the fatty acid composition of the LD as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g DM LD), and their sums and ratios, are shown in Table 4.3.18. Their enzyme and health indices are presented in 4.3.19. The enzyme indices are used to describe the changes in fatty acids due to sex within the SFA, MUFA and PUFA sections.

a) The effect of sex on the SFA profile of IMF

The effect of sex on the saturated fatty acid composition of the LD as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g DM LD) are presented in Table 4.3.18. The concentration of C10:0, C12:0, C13:0, C14:0, C16:0, C17:0, C18:0 and C20:0 were affected by sex ($P<0.05$). C23:0 also had a tended to be affected ($P=0.093$). The percent of C14:0, C16:0, C18:0, C20:0, C22:0, C23:0 and C24:0 were also affected by lamb sex ($P<0.05$).

Ewes had more SFA than rams and a concentration ($P<0.001$) but only tended to be higher as a percent ($P=0.087$). Ewes increased the percent of SFA by 2.76% and concentration by 43.62%. This was because of a greater concentration of C14:0 and C16:0 fatty acids in ewes (w/w%: $P<0.005$; mg/g: $P<0.001$ and w/w%: $P<0.05$; mg/g: $P<0.001$, respectively). Myristic acid (C14:0) increased the percent and concentration by 28.53% and 77.39%, respectively.Palmitic acid (C16:0) increased by 6.57% (as a percent of detected lipid) and 48.53% (as a concentration). In agreement with the current study, C14:0 and C16:0 fatty acids were also reported to be higher in ewes by Okeudo *et al*. (2007) and Tejeda *et al.* (2008). These fatty acids are hyperlipidemic, and therefore increase serum cholesterol concentrations (Solomon *et al*., 1990).

Although there was a 32.68% increase in C18:0 in ewe IMF ($P<0.05$), as a percent of detected lipid it decreased by 5.55% ($P<0.05$). The impact of sex on C18:0 also varied considerably between studies;

Kemp *et al*. (1981) reported higher C18:0 in ewe IMF, whereas Okeudo *et al*. (2007) reported it to be higher in rams (intact and vasectomized). A reason for more C18:0 (as mg/g DM LD) in ewes may be explained by a 2.36% decreased elongase activity (P<0.05; Table 4.3.19). The higher IMF content in ewes, and therefore increased neutral lipid concentrations, can also explain the higher proportions of the predominant C14:0, C16:0 and C18:0 SFAs in the LD. C18:0 is hypolipidemic in comparison to the C14:0 and C16:0 fatty acids (Solomon *et al*., 1990). It is a neutral fatty acid because it is not very digestible and can easily desaturate to C18:1n9c, which reduces cholesterol and low-density lipoproteins (LDL; Solomon *et al*., 1990). Therefore, the increased concentration of C18:0 in ewe IMF was not a health concern.

Saturated fatty acid is present in much smaller concentrations in comparison to the above, were also altered by sex. Fatty acids such as C10:0, C12:0, C13:0 and C17:0 increased in concentration in ewes (P=0.01; P<0.001; P=0.01; P<0.01, respectively). Lauric acid (C12:0) also tended to be higher as a percent in ewes (P=0.082). However, the percent of trace amounts of the longer chain fatty acids such as C20:0, C22:0, C23:0 and C24:0 were higher in rams (P<0.001; P<0.001; P<0.05 and P<0.05, respectively), as was a tendency for the concentration of C23:0 (P=0.093). Arachidonic acid (C20:0) differed in its direction of significance depending on the method of interpretation. Rams had a higher percent of C20:0 in LD lipid (P<0.001), but as a concentration it was lower (P<0.05). Of these less prolific fatty acids, and in agreement with the current study, Diaz *et al* (2003) also reported higher C17:0 in ewes. In contrast to the current study, Tejeda *et al* (2008) identified increased C17:0 in rams and higher C24:0 in ewes.

b) The effect of sex on the MUFA profile of IMF

The effect of sex on the monounsaturated fatty acid composition of the LD as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g DM LD) are presented in Table 4.3.18. The percent of C14:1 (P<0.05), 16:1 (P<0.05), C20:1 (P<0.005), C24:1 (P<0.001) and C18:1n11c (P<0.05) were affected by sex. The concentration of C14:1 (P<0.005), 16:1 (P<0.001), C20:1 (P<0.05), C24:1 (P<0.05), C18:1n11c (P<0.01), C18:1n11t (P<0.05) and C18:1n9c (P<0.001) affected by the sex of the lambs. Although the total percent of MUFA was not affected by sex (P>0.05), the concentration was larger in ewes (P<0.001). This resulted in a 42.29% increased concentration of MUFA in ewe LD, due to 43.04% increase in C18:1n9c (P<0.001) and 33.11% increase in C18:1n11t (P<0.05). Cis vaccenic acid (C18:1n11c) increased by 17.77% in rams as a percent of detected lipid (P<0.05). In contrast, it decreased by 16.16% as concentration in rams (P<0.013).

Kemp *et al*. (1981) did not report differences in C18:1n9c between ewe and wether IMF. However, it was reported to be higher in SCF and PRF. In contrast to the current study, no differences in C18:1n11t

were recorded by Malau-Aduli *et al*. (2015) and Facciolongo *et al*. (2018). According to Solomon *et al*. (1990), differences in fatty acid composition between rams, wethers and cryptorchids was likely a consequence of variations in the fat to lean ratio as a direct result of differences in hormonal balance on growth, which affected fat deposition, muscle development and differences in physiological maturity. This may suggest that there were also differences between ewes and rams for this reason in the current study. This agrees with Diaz *et al*. (2003), where ewe lambs tended to put on fat at an earlier age, and because of a slower growth rate, reached slaughter weight later than ram lambs. De Smet *et al*. (2004) went one step further to suggest that differences in sex hormones could alter the activity of enzymes such as delta- 9 desaturase. Other authors have also detected changes in enzyme activity (Facciolongo *et al*., 2018; Junior *et al*., 2019). In the current study there was no change in delta 9 desaturase activity for the desaturation of C14:0, C16:0 and CLA cis-9, trans-11 fatty acids ($P>0.05$; Table 4.3.19), but there was an increase in C18 desaturation in ewes ($P<0.05$; Table 4.3.19). It was not enough to suggest an elevated overall delta 9 desaturase activity in ewes ($P>0.05$; Table 4.3.19).

The selectivity of the enzyme to the fatty acids may explain why there was no difference in the delta 9 desaturase activity of rumenic acid. Therefore, there may be another reason for higher C18:1n11t in ewes. Literature varies in reported response of delta 9 desaturase activity to sex. In agreement with the current study, Facciolongo *et al*. (2018) also identified an increase in C18 delta 9 desaturase activity. Unlike the current study, C16 delta 9 desaturase was also significantly affected. In comparison, Junior *et al*. (2019) did not find an increase in delta 9 desaturase activity for C18 and C16 fatty acids, but they did report a higher C14 delta 9 desaturase activity.

Of the less prominent fatty acids, ewes were higher in the percent and concentration of C14:1 ($P<0.05$ and $P<0.005$, respectively) and C16:1 fatty acid ($P<0.05$ and $P<0.001$, respectively). This was not as a result of increased C14 or C16 delta 9 desaturase activity ($P>0.05$; Table 4.3.19). It was most likely because there was more C14:0 and C16:0 available for desaturation. Ram IMF had a greater percent of the longer chain C20:1 ($P<0.005$) and C24:1 fatty acids (w/w%: $P<0.001$; mg/g: $P<0.05$, respectively). Unlike the percent, the concentration of C20:1 was lower in ram IMF ($P<0.05$). Rams had a higher percent and concentration of C18:0 and concentration of C18:1n9c, and a significantly lower percent and concentration of C16:0 and C16:1. This would explain the differences in elongase activity between ewe and ram lambs ($P=0.05$; Table 4.3.19).

The large discrepancy in the MUFA concentration between ewe and ram lambs was a large factor in the higher total cis and total trans fatty acids in ewes ($P<0.001$ and $P<0.01$ respectively; Table 4.2.18). This is because of the large contribution of the major fatty acids C18:1n9c and C18:1n11t. Regardless of the significant changes, the trans/cis ratio remained unaltered ($P>0.05$; Table 4.3.18).

c) The effect of sex on the PUFA profile of IMF

The effect of sex on the polyunsaturated fatty acid composition of the LD as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g DM LD) are presented in Table 4.3.18. The percent of detected fatty acids showed more changes in the fatty acid profile due to the sex of lambs than the concentrations did. The percent of detected fatty acid showed changes in total PUFA ($P<0.005$), total n-3 ($P<0.05$) and n-6 fatty acids ($P<0.005$). This was because of differences in C18:2n6c ($P<0.005$), C18:3n6 ($P=0.057$), C20:4n6 ($P<0.005$), C18:3n3 ($P<0.05$), C22:6n3 ($P<0.05$), C20:5n3 ($P<0.01$) and C20:2 fatty acids ($P<0.01$). The concentration detected differences in C20:2, CLA cis-9, trans-11 + trans-9, cis-12 as well as CLA cis-10, cis-12, CLA isomer 1 ($P<0.05$) and a tendency for CLA isomer 3 to differ between sexes ($P=0.086$). The differences in CLA isomers between sexes decreased the total fatty acid CLA concentration in rams ($P<0.05$).

The total PUFA was present as a much smaller proportion than SFA and MUFA and did not differ between ewes and rams as a concentration ($P>0.05$). As a percent of detected lipid, the proportion of PUFA increased by 41.23% in rams ($P<0.005$). The total UFA concentration, as the sum of MUFA and PUFA, tended to be higher in rams (2.6% increase; $P=0.057$). However, UFA had a 37.26% increased concentration in ewe LD ($P<0.001$). This was as a result of a much higher MUFA concentration due to elevated C18:1n9c levels.

Rams had a 39.30% increased percent of Omega 3 fatty acids ($P<0.05$) because of a 27.09% increase in C18:3n3, a 57.14% increase in C22:6n3, and a 91.49% increase in C20:5n3. The omega 3 fatty acids did not reciprocate their significance as a concentration ($P>0.05$). The largest contributor to n-3 fatty acids, C18:3n3, was also reportedly higher in studies conducted by Cloete *et al*. (2004) and Junior *et al*. (2019). While Okeudo *et al* (2007) recorded a higher total C18:3 in rams, Diaz *et al* (2003) reported the opposite to be true.

The total omega 6 fatty acid concentration did not differ between ewe and ram lambs ($P>0.05$). However, it was 69.51% higher as a percent of detected lipid in ram lambs due to a 46.88% increase in C18:2n6c, an 84.13% increase in C20:3n6, and a 70.68% increase in C20:4n6 fatty acids. Linolenic acid (C18:3n6) also tended to be higher in ram lambs ($P=0.057$) but concentration increased by 25% in ewes ($P<0.005$).

The 41.83% increase in the percent of PUFA in rams was largely due to a 46.88% increase in C18:2n6c ($P<0.005$). In agreement with this, Tejeda *et al*. (2008) and Diaz *et al*. (2003) also reported increased PUFA and C18:2n6 in ram intramuscular fat. Linoleic acid (C18:2n6c) cannot be synthesized by animals and must be supplied in the diet for the synthesis of arachidonic acid (C20:4n6) and eicosanoids (Mattos *et al*., 2000). Safari *et al*. (1988) suspected that hormones were responsible for the deposition

and mobilization of lipids, and therefore C18:2n6c, which differed between castrates and intact rams. As an animal matures, the phospholipid percent is diluted by an increase in triglycerides upon fat deposition (De Smet *et al*., 2004). Linoleic acid is preferentially deposited in phospholipid (De Smet *et al*., 2004). This may explain the increased C18:2n6c percent in ram lambs, which were leaner than ewe lambs (P<0.001).

Although it has not been widely published as a fat commonly affected by sex, in the current study, CLA increased from 0.358mg/g±0.219 in rams to 0.525mg/g±0.159 in ewes. This was largely in response to a 49.01% increase in CLA cis-9, trans-11+ trans-9, cis-12, a 33.33% increase in CLA cis-10, cis-12, and a 50% increase in CLA isomer 1 in ewe IMF (P<0.05). There was also a tendency for an increase in CLA isomer 3 (P=0.086).

Eicosadienoic acid (C20:2) accumulated to a greater extent in ewe lamb LD as a concentration (0.027mg/g±0.006 vs 0.032mg/g±0.005; P<0.05) but decreased as a percent of detected lipid (0.044%±0.006 vs 0.052%±0.010; P<0.008). Although Junior *et al*. (2019) detected C20:2 in their IMF, unlike the current study there was no difference between sexes.

d) The effect of sex on the ratio of fatty acid in IMF

The effect of sex on ratios of fatty acids in the LD from the percent of detected fatty acids (w/w%) and gravimetrically (mg/g DM LD) are presented in Table 4.3.18. The PUFA/SFA ratio was better in ram than ewe lambs (P<0.05). Polyunsaturated fatty acid is preferentially deposited in phospholipid, of which the latter is diluted with increasing triglyceride upon fatty acid deposition. The former is also diluted with increased *de novo* synthesis of SFA and MUFA in ewes (De Smet *et al*., 2004). Although the rams' ratio was within the range of 0.112 to 0.154 in IMF, as specified by Hoffman *et al*. (2003) for SA Mutton Merinos, it was slightly lower for ewes in the current study (0.094±0.016). The rams' PUFA/SFA ratio also corresponded with Geay *et al*. (2001) for bovine and lamb meat. The reason for the lower ratio in ewe meat was most likely from a lower percent of PUFA as a result of of a more mature carcass at time of slaughter. Therefore, ram meat is possibly healthier for human consumption, although it is still below the recommended health value of 0.45 specified by Webb *et al*. (2005). The SFA/UFA was also better (lower) in rams (P<0.07)

Facciolongo *et al*. (2018) also reported an increase in total Omega 6 fatty acid in rams, thereby improving the n-6/n-3 ratio. The ratio was not improved in the current study as both n-3 and n-6 fatty acids increased in rams to a similar degree. The trans/cis ratio was also unaffected (P>0.05). The ratio was 2.362 (±0.815) in ewes and 2.735 (±2.735) in rams. This is well below the recommended dietary allowance of less than four recommended by WHO (2003).

e) The effect of sex on the health indices of IMF

The effect of sex on the health indices of IMF are presented on Table 4.3.19. All the health indices except for the thrombogenic index (TI) were affected by sex. The atherogenic index (AI) was higher (poorer) in ewes ($P<0.01$) and the h/H index was better in rams ($P<0.05$). This was because there was more C14:0 and C16:0 fatty acids in ewes (as a percent and concentration; Table 4.3.18), and less PUFA (as a percent; Table 4.3.18). In light of this, there was a higher percent of hypocholesterolemic fatty acids (h) in ram IMF ($P<0.05$). However, the concentration improved in the ewe IMF ($P<0.001$). There were cumulatively less hyperatherogenic fatty acids (H) in ram IMF ($P<0.01$). This indicated a benefit to eating ram lamb meat.

The only index in favour (higher) of ewe meat (and only as a concentration) was the desirable fatty acids (DFA) index (percent: $P<0.01$; concentration: $P<0.001$). As a percent it was lower in ewes and higher in rams ($P<0.01$) This was largely as a result of a 43.04% increase in C18:1n9c and 32.68% increase in C18:0 in rams. This index indicated that between 65 and 68% of fat in IMF lipid is suitable for human consumption. Therefore, PUFA/SFA, AI, h/H and H indicated that ram lamb meat in the current study was better for the health-conscious consumer regardless of whether the percent of the detected lipid or the concentration of fatty acid within the IMF were analysed.

Table 4.3.18: Means (± SD) depicting the medium and long chain fatty acid profile of the *longissimus dorsi* between the 8th and 10th ribs for the main effect of sex expressed as a percent of detected fatty acids (%) and gravimetrically (mg of fatty acid per g of *longissimus dorsi* on a *DM basis)*

(w/w%)	w/w%		mg/g		P-values	
	Female	Male	Female	Male	w/w%	mg/g
Replicates (n)	16	16	16	16		
Fatty acids						
C10:0	0.109±0.017	0.109±0.021	0.081±0.021	0.058±0.019	0.875	**0.010**
C12:0	0.137±0.043	0.110±0.038	0.103±0.041	0.060±0.030	**0.082**	**0.007**
C13:0	0.012±0.004	0.010±0.003	0.009±0.004	0.005±0.003	0.172	**0.012**
C14:0	2.982±0.443	2.320±0.486	2.228±0.553	1.256±0.482	**0.004**	**<0.001**
C14:1	0.086±0.0.019	0.066±0.022	0.065±0.022	0.036±0.018	**0.041**	**0.004**
C16:0	26.815±1.770	25.162±2.062	19.856±3.089	13.368±3.322	**0.028**	**<0.001**
C16:1	1.500±0.128	1.332±0.205	1.109±0.172	0.709±0.215	**0.039**	**<0.001**
C17:0	0.840±0.146	0.883±0.095	0.622±0.141	0.468±0.126	0.411	**0.008**
C18:0	16.571±0.927	17.544±1.566	12.249±1.693	9.232±1.978	**0.045**	**0.001**
C18:1t11	2.671±0.416	2.776±0.815	1.982±0.438	1.489±0.620	0.729	**0.015**
C18:1n9c	38.796±1.603	38.142±2.017	28.660±3.670	20.036±3.641	0.318	**<0.001**
C18:1n11c	0.906±0.206	1.067±0.127	0.662±0.132	0.555±0.092	**0.045**	**0.013**
C18:2n6t	0.031±0.009	0.029±0.008	0.023±0.007	0.015±0.005	0.191	**0.001**
C18:2n6c	2.583±0.450	3.794±1.067	1.889±0.303	1.947±0.462	**0.003**	0.593
C20:0	0.077±0.008	0.090±0.009	0.057±0.011	0.047±0.009	**<0.001**	**0.019**
C18:3n6	0.034±0.005	0.039±0.008	0.025±0.004	0.020±0.003	**0.057**	**0.004**
C20:1	0.051±0.004	0.060±0.010	0.038±0.006	0.031±0.007	**0.002**	**0.021**

Table 4.3.18 continued…

C18:3n3	0.251±0.068	0.319±0.093	0.187±0.063	0.165±0.048	**0.051**	0.347
CLA cis-9, trans-11/ trans-9, cis-12	0.503±0.149	0.471±0.183	0.377±0.135	0.253±0.137	0.472	**0.033**
CLA cis-10, cis-12	0.006±0.002	0.006±0.002	0.004±0.001	0.003±0.002	0.833	**0.022**
CLA Trans-10, cis-12	0.013±0.008	0.012±0.011	0.010±0.005	0.007±0.008	0.567	0.185
CLA isomer 1	0.008±0.002	0.007±0.004	0.006±0.002	0.004±0.002	0.474	**0.019**
CLA isomer 3	0.174±0.047	0.166±0.117	0.128±0.035	0.092±0.081	0.637	**0.086**
C20:2	0.044±0.006	0.052±0.010	0.032±0.005	0.027±0.006	**0.008**	**0.025**
C22:0	0.024±0.006	0.038±0.010	0.018±0.003	0.020±0.005	**<0.001**	0.259
C20:3n6	0.063±0.017	0.116±0.055	0.045±0.010	0.057±0.020	**0.005**	0.104
C22:1n9	0.051±0.160	0.028±0.033	0.034±0.104	0.013±0.014	0.574	0.442
C23:0	0.022±0.005	0.029±0.009	0.016±0.002	0.015±0.003	**0.033**	**0.093**
C20:4n6	0.665±0.189	1.135±0.481	0.482±0.113	0.559±0.170	**0.004**	0.181
C24:0	0.031±0.006	0.048±0.015	0.023±0.003	0.024±0.004	**0.002**	0.547
C20:5n3	0.047±0.018	0.090±0.059	0.034±0.013	0.043±0.024	**0.008**	0.18
C24:1	0.021±0.007	0.036±0.011	0.015±0.004	0.018±0.003	**<0.001**	**0.018**
C22:6n3	0.042±0.024	0.066±0.041	0.031±0.017	0.032±0.018	**0.038**	0.992
UIP	3.832±1.114	3.847±0.689	2.833±0.889	2.034±0.538	0.961	**0.004**
Sums and ratios						
SFA	47.622±1.909	46.344±2.471	35.262±5.150	24.553±5.697	**0.087**	**<0.001**
MUFA	44.083±1.415	43.507±1.662	32.565±4.092	22.887±4.293	0.253	**<0.001**
PUFA	4.463±0.653	6.303±1.529	3.278±0.487	3.227±0.592	**0.002**	0.786
USF	48.546±1.696	49.809±2.256	35.843±4.358	26.114±4.431	**0.057**	**<0.001**

Table 4.3.18 continued…

SFA / UFA	0.983±0.071	0.934±0.092	0.983±0.071	0.934±0.092	**0.069**	**0.070**
MUFA/SFA	0.928±0.060	0.943±0.070	0.928±0.060	0.943±0.079	0.521	0.521
PUFA/SFA	0.094±0.016	0.138±0.039	0.094±0.016	0.138±0.039	**0.004**	**0.004**
CLA	0.704±0.178	0.662±0.291	0.525±0.159	0.358±0.219	0.487	**0.034**
n-3 PUFA	0.341±0.092	0.475±0.174	0.252±0.079	0.241±0.075	**0.020**	0.672
n-6 PUFA	0.761±0.207	1.290±0.536	0.551±0.122	0.637±0.187	**0.004**	0.194
n6/n3 ratio	2.362±0.814	2.736±0.770	2.362±0.815	2.735±0.770	0.212	0.212
Cis FA	45.140±1.832	46.343±2.553	33.338±3.970	24.284±3.882	**0.098**	**<0.001**
Trans FA	3.406±0.493	3.467±0.901	2.530±5.530	1.862±0.773	0.977	**0.012**
Trans/cis ratio	0.076±0.012	0.076±0.023	0.076±0.012	0.075±0.023	0.851	0.848
Total FA	-	-	71.105±9.173	50.668±9.943	-	**<0.001**

Table 4.3.19: Means (± SD) for the enzyme and health indices of the *longissimus dorsi* between the 8th and 10th ribs for the main effect of sex from the percent of identified fatty acids (w/w%) and from the gravimetric concentration (mg of fatty acid per g of *longissimus dorsi* on a DM basis)

	w/w%		mg/g		P-values	
	Female	Male	Female	Male	**w/w%**	mg/g
Replicates (n)	16	16	16	16		
Enzyme indices						
Elongase	0.662±0.018	0.678±0.024	0.662±0.018	0.678±0.024	**0.050**	**0.050**
Delta 9 desaturase (C14+C16+C18)	46.555±1.750	46.767±2.295	46.558±1.750	46.767±2.295	0.752	0.752
Delta 9 desaturase C14	2.803±0.297	2.708±0.440	2.803±0.297	2.708±0.440	0.601	0.601
Delta 9 desaturase C16	5.302±0.380	5.031±0.645	5.302±0.380	5.031±0.645	0.218	0.218
Delta 9 desaturase C18	70.059±1.713	68.497±2.514	70.058±1.713	68.497±2.514	**0.046**	0.046
Delta 9 desaturase RA	15.814±3.719	14.752±4.726	15.814±4.718	14.752±4.725	0.425	0.425
Health indices						
AI	0.862±0.077	0.766±0.105	0.862±0.077	0.766±0.105	**0.010**	**0.010**
TI	1.943±0.141	1.868±0.192	1.930±0.141	1.842±0.195	0.165	0.107
DFA	65.117±1.553	67.354±2.306	48.092±5.817	35.346±6.159	**0.009**	**<0.001**
h	46.837±1.641	48.288±2.295	34.582±4.216	25.307±4.238	**0.032**	**<0.001**
H	29.798±1.827	27.843±2.430	22.084±3.524	14.625±3.779	**0.009**	**<0.001**
h/H	1.579±0.137	1.777±0.242	1.580±0.137	1.777±0.242	**0.011**	**0.011**

f) Interaction between tannin and sex in IMF

The gravimetric interaction between tannin and sex for intramuscular fat are presented in Appendix Tables 7.1.1 and their enzyme and health indices in Table 7.1.2. The molar interactions are presented in Table 7.1.7 and 7.1.8, respectively. There was an interaction between tannin and sex as a molar percent (w/w%) for C16:0 and C20:5n3 fatty acids ($P<0.05$). As shown in Figure 4.3.8, tannin reduced the difference in C16:0 between sexes. In the diet without tannin, C16:0 was higher in ewes. Vasta *et al*. (2007) also reported increased C16:0 in ram lambs given a tanniniferous diet containing carob pulp. A decrease of C16:0 in ewes is advantageous as this fat increases low density lipoprotein (LDL) cholesterol levels associated with increased risk of disease such as myocardial infarcation and atherosclerotic cardiovascular disease (Nestel *et al*., 1994; Mortensen & Noordestgaard, 2020). However, the increase was not enough to affect the overall health indices ($P>0.05$; Table 7.1.8).

As indicated in Figure 4.3.9, C20:5n3 was lower in lambs supplemented with tannin ($P=0.01$). However, rams had a much higher percent of C20:5n3 when there was no tannin added to the diet. Further analysis would be required to determine whether this difference was a result of an interaction between sex, tannin and enzymes within the n-3 fatty acid pathway.

There was an interaction between tannin and sex for the concentration (mg/g) of CLA isomer 3 ($P<0.05$; Figure 4.3.10). Tannin reduced the difference between the sexes. However, when tannin was not added to the diet, rams had a lower concentration of CLA isomer 3 then ewes.

There was an interaction between tannin and sex for elongase activity in the longissimus dorsi ($P<0.05$; Table 7.1.2 and 7.1.8). The amount was small and not biologically significant. Therefore, due to the differences not being numerically large it was not reported on a bar chart. The C16:0 interaction (Table 7.1.7) correlates with the elongase index (Table 7.1.8).

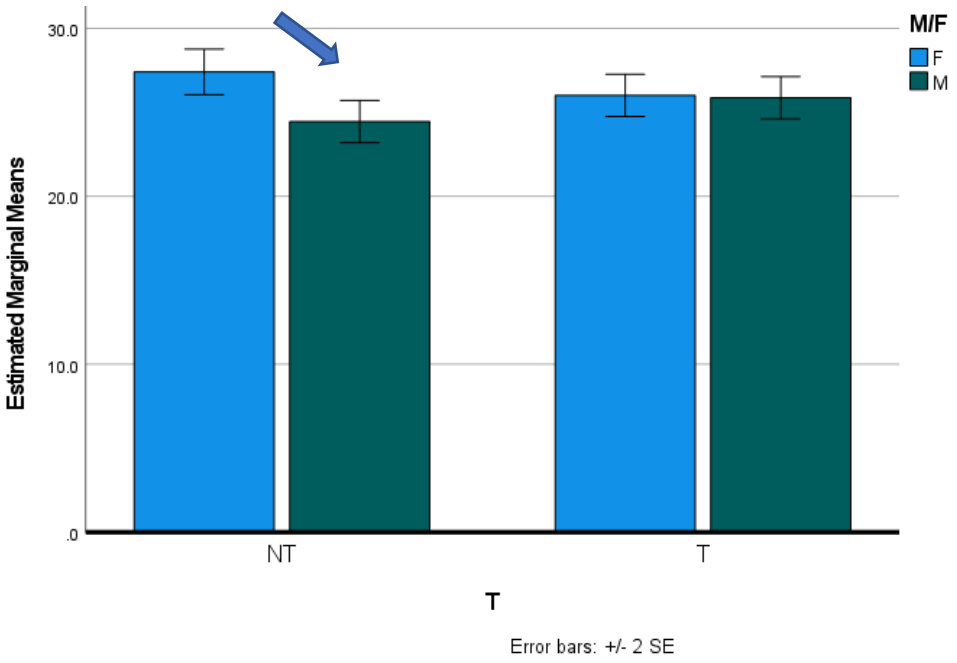

Figure 4.3.8: Estimated marginal means (w/w%) and standard error of C16:0 in rams and ewes supplemented with and without condensed tannin

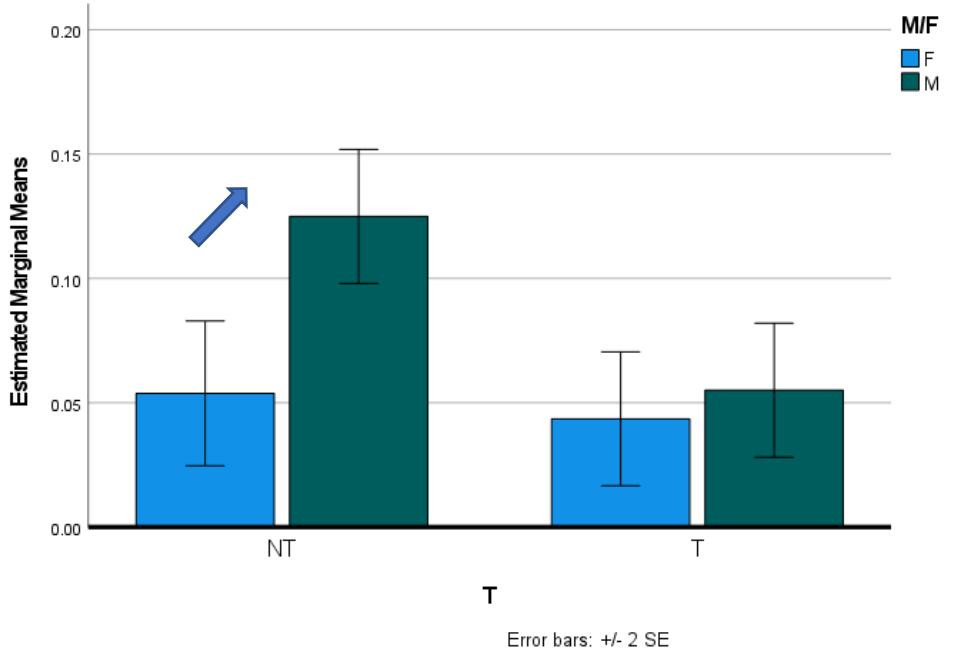

Figure 4.3.9: Estimated marginal means and standard error of C20:5n3 in rams and ewes supplemented with and without condensed tannin

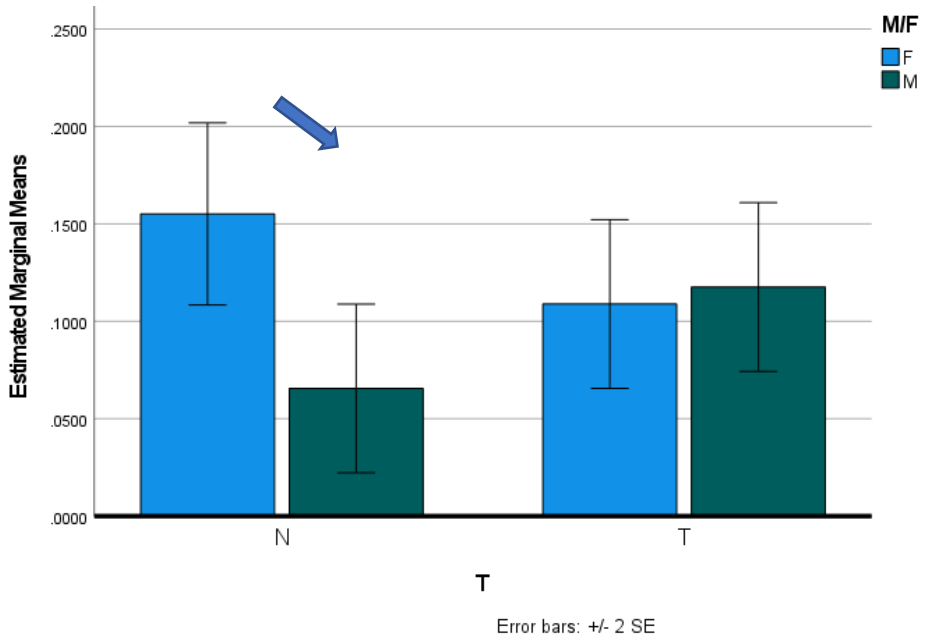

Figure 4.3.10: Estimated marginal means (mg/g) and standard error of CLA isomer 3 in rams and ewes supplemented with and without condensed tannin

g) *Interaction between NPN source and sex in IMF*

The gravimetric interaction between the NPN source and sex for intramuscular fat are presented in Appendix Tables 7.1.1 and their enzyme and health indices in Table 7.1.2. The molar interactions are presented in Table 7.1.7 and 7.1.8, respectively. The CLA trans-10, cis-12 interacted as a percent (P<0.05) and C20:2 interacted as a concentration (<0.05). However, the numerical difference between them was small and therefore had little biological value.

4.3.4.2 The effect of sex on the SC fatty acid profile of lamb

The effect of sex on the fatty acid composition of SCF and their sums and ratios as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g SCF) are shown in Table 4.3.20. The enzyme and health indices are presented in Table 4.3.21.

Sex influenced the fatty acid composition of SCF. However, the most abundant fatty acids (C18:0, C16:0 and C18:1n9c, respectively) remained in the same order of magnitude regardless of sex. The effect of sex on Dohné Merino sheep SCF is lacking. Its effect on SA Mutton Merino sheep and sheep in general is also dated and limited. Apart from the slightly lower PUFA, C18:2 and C18:3 percent in the current study, SC fatty acids were in a similar range to SA Mutton Merino wethers on medium and high energy diets reported by Webb *et al*. (1994). Like the IMF in the current study, the SCF had a higher percent and concentration of SFA (P<0.05) and a lower percent of UFA in ewe SCF (P<0.05). the SFA increased by 9.20% as percent of detected fatty acids and by 21.13% as a concentration. UFA

increased by 6.61% in rams. Kemp *et al*. (1981) also reported higher unsaturation in the SCF of rams. Previous studies have shown that ewes mature earlier than rams (Arnold & Meyer, 1988). This has been associated with differences in their fatty acid composition when slaughtered at similar weights (Sañudo *et al*., 1998). In light of this, studies have shown a correlation between later maturity and increased SFA and lower UFA in subcutaneous fat (Webb *et al*., 1994).

a) The effect of sex on the SFA profile of SCF

The effect of sex on the SFA composition of SCF as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g DM LD) are presented in Table 4.3.20. The concentration of SFA in SCF was higher in ewes as a result of increased C18:0 (P<0.001), C12:0 (P<0.01), C14:0 (P<0.005) and C16:0 fatty acids (P<0.005). They increased by 27.69%, 60.44%, 37.52% and 15.47%, respectively. The percent of C17:0 also tended to be higher in rams (P=0.071). Ewes increased the percent of C18:0 (P<0.01), C12:0 (P<0.05) and C14:0 (P<0.05) by 15.38%, 44.17%, 24.12% and 4.08% as a percent of detected fatty acids, respectively. This contrasted with Sañudo *et al*. (1998) who reported that total SFA, C12:0, C14:0 and C16:0 fatty acids were higher in ram SCF, and C18:0 did not differ between sex when slaughtered at 22kg. The results agreed with Bas *et al*. (2007) who also reported an increase in C16:0 and C18:0 fatty acids in ewe lambs. Increased C18:0 is associated with increased melting point and increased firmness of carcass fat (Wood *et al*., 2003). Busboom *et al*. (1981) also associated C18:0 with whiter fat due to its higher melting point.

b) The effect of sex on the MUFA profile of SCF

The effect of sex on the MUFA composition of SCF as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g DM LD) are presented in Table 4.3.20. Rams increased MUFA as the percent of total lipid by 5.59% (P<0.05). In contrast, MUFA tended to be higher as a concentration in ewes (P=0.064). As a percent, rams tended to increase C18:1n9c by 5.26% (P=0.100), C18:1n11c by 14.26% (P<0.05) and C20:1 by 24.07% (P<0.05). In contrast to this, ewes only tended to increase the concentration of C18:1n9c by 6.19% (P=0.065). This tended to increase the concentration of total MUFA in ewes by 5.61% (P=0.064). There was a high standard deviation (between 11 and 15) within the ewe and ram categories. Kemp *et al*. (1981), Sañudo *et al*. (1998) and Bas *et al*. (2007) also reported higher C18:1n9c in SCF of ewes, although it was not enough to increase MUFA in the latter.

c) The effect of sex on the PUFA profile of SCF

The effect of sex on the PUFA composition of SCF as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g DM LD) are presented in Table 4.3.20. Although there was a tendency for a 19.68% improved (increased) percent of PUFA (P=0.059), the increase was not significant as a concentration (P>0.05). They also had a 6.61% higher UFA percent (P<0.01). The effect of sex affected

the percent of C18:2n6c; CLA trans-10, cis-12; CLA isomer 3 and C20:4n6 (P=0.059; P<0.05; P<0.05; P<0.05, respectively). As a concentration only CLA trans-10 cis-12 and C20:4n6 was affected (P<0.05 and P<0.005).

Omega 6 fatty acids in rams tended to increase by 26.27% as a percent of detected fatty acids (P=0.069), influenced by a tendency for its major fatty acid, C18:2n6c, to be higher (P=0.059). C20:4n6c, present in small amounts, increased in ewes as a percent of detected lipid (19.29% increase) and as a concentration (41.10% increase; P<0.05 and P<0.005, respectively). The other product of the n-6 pathway, C18:3n6 was identified in SCF but did not differ significantly between sexes (P>0.05). Arachidonoc acid (C20:4n6) is an important fatty acid only acquired from animals and has a great rate of turn over as a result of its high demand. It is an important source of pro-inflammatory eucasonoids and prostanoids used in physiological and biological processes (Le *et al*., 2009). Of the omega 3 fatty acids, only C18:3n3 was identified and it did not differ between sex.

Another group of PUFAs with health benefits, CLA, did not differ as a sum of its isomers (P>0.05). However, individually there were some significant effects. CLA trans-10, cis-12 increased in rams by 80% (percent) and 35.14% (concentration; P<0.05). CLA isomer 3 also increased by 55.78% as percent of detected lipid (P<0.05). The CLA cis-9, trans-11 and trans-9, cis-12 fatty acids did not differ (P>0.05). No other PUFAs were affected. The concentration of the UIPs were higher in rams (P<0.05).

The higher concentrations of fatty acids in ewes were likely due to a greater concentration of lipid in 1g of subcutaneous fat (P<0.001). Diaz *et al*. (2003) reported significantly more PUFA in the SCF of ewe lambs slaughtered between 10 and 14kg. This was because of elevated C18:2 and C18:3 fatty acids. This may have been due to the age and weight of lambs at slaughter and position on the growth curve. There were no significant differences in PUFA between sexes reported by Kemp *et al*. (1981) and Sañudo *et al*. (1998).

d) The effect of sex on the ratio of fatty acids on SCF

The effect of sex on ratios of fatty acids in the SCF from the percent of detected fatty acids (w/w%) and gravimetrically (mg/g DM LD) are presented in Table 4.3.20. As C18:1n9c is the major cis fatty acid in SCF, there was an overall 6.62% increase in total cis fatty acids as a proportion of total lipid in ram SCF (P<0.05). Although not significant, the concentration of cis fatty acid was higher in ewes (P>0.05). Therefore, total amount of trans fatty acid as well as the trans/cis ratio were unaffected (P>0.05). The PUFA/SFA ratio did not improve in rams (0.073mg/g±0.022 vs 0.054mg/g ± 0.012; P<0.05). This was a fraction of the desirable ratio of above 0.45 as specified by Webb (2005). The n-6/n-3 ratio was unaffected and desirable (below 4), regardless of the sex. The MUFA/SFA ratio was higher in rams than ewes (P<0.01), suggesting higher delta 9 desaturase activity. This may explain why there was

more C18:0 in ewes and more C18:1n9c in rams. In agreement with this, the overall delta 9 desaturase activity was higher in rams (P<0.05; table 4.3.21). Both C14 and C18 delta 9 desaturase activity was higher (P<0.05), and C16 delta 9 desaturase tended to be more active too (percent: P=0.067; concentration: P=0.065; table 4.3.21). In contrast to this study Bas *et al*. (2007) reported a decrease in desaturase activity on C14:0, C16:0, C18:0 and CLA fatty acids.

There was an interaction between sex and weight in sheep for C18:2n11c (P<0.05). However, there was also an interaction between tannin, calcium nitrate and sex (P<0.05; Available on request). There was also an interaction between tannin, sex and weight for C18:2n6t (P<0.05; Available on request). Therefore, the interpretation of these fatty acid needs to be treated with caution.

e) The effect of sex on the health indices of SCF

The effect of sex on the health indices of SCF are presented on Table 4.3.21. The health indices were affected by the sex of the lamb. The atherogenic index (AI) was lowered as a percent and concentration in ram SCF (P<0.01). The thrombogenic index (TI) was lower as a percent of total lipid in ewes (P=0.069), but higher when calculated from the concentration (P<0.005). Ewes tended to increase the hypercholesterolemic (H) index as a percent (P=0.081) and concentration (P<0.005), while rams improved the hypocholesterolemic (h) index as a percent (P<0.05). However, as a concentration ewe lambs tended to have a higher h index as well (P=0.097). Regardless of this, the h/H ratio from the percent and concentration improved in rams because the major fatty acid that reduced cholesterol (C18:1n9c) increased more than those that increased cholesterol (C14:0 and C16:0; Table 4.3.20). Therefore, the h/H ratio was improved in rams regardless of the method of interpretation (P<0.05). The desirable fatty acid index (DFA) was better in ewes, but only when calculated from the concentration (P<0.001). Although it was higher as a percent it was not significant (P>0.05). Therefore, most (but not all) of the health indices for SCF are still in favor of ram lamb SCF. The higher AI in ewes agrees with Sabbioni *et al*. (2019) who reported a higher index in ewes when sheep were slaughtered until 50kg body weight.

Table 4.3.20: Means (± SD) depicting the medium and long chain fatty acid profile of subcutaneous fat for the main effect of sex expressed as a percent of detected fatty acid (w/w%) and gravimetrically (mg of fatty acid per g of subcutaneous fat sample)

	w/w%		mg/g		P-values	
	Female	Male	Female	Male	w/w%	mg/g
Replicates (n)	16	16	16	16		
Fatty acids						
C10:0	0.139±0.019	0.150±0.025	0.522±0.074	0.514±0.109	0.135	0.864
C12:0	0.173±0.064	0.120±0.035	0.653±0.249	0.407±0.132	**0.039**	**0.014**
C13:0	0.020±0.005	0.028±0.019	0.077±0.018	0.089±0.044	0.103	0.252
C14:0	3.731±0.616	3.006±0.527	14.044±2.460	10.212±2.283	**0.017**	**0.002**
C14:1	0.080±0.022	0.088±0.027	0.303±0.088	0.291±0.079	0.294	0.919
C16:0	24.716±1.276	23.747±2.117	93.012±6.552	80.551±12.645	0.189	**0.003**
C16:1	0.991±0.175	1.056±0.209	3.737±0.715	3.586±0.868	0.216	0.724
C17:0	1.609±0.259	1.939±0.667	6.036±0.873	6.400±1.316	**0.071**	0.39
C18:0	23.897±2.310	20.712±3.472	89.714±7.248	70.258±16.075	**0.007**	**<0.001**
C18:1t11	3.788±0.615	4.033±1.688	14.235±2.261	13.833±6.150	0.669	0.715
C18:1n9c	32.421±2.329	34.126±3.055	122.076±11.336	114.957±14.144	0.100	**0.065**
C18:1n11c	0.589±1.101	0.673±0.114	2.214±0.370	2.273±0.439	**0.029**	0.875
C18:2n6t	0.450±0.019	0.047±0.009	0.170±0.075	0.165±0.043	0.905	0.358
C18:2n6c	1.775±0.561	2.284±0.884	6.646±2.056	7.791±3.036	**0.059**	0.219
C20:0	0.131±0.029	0.130±0.025	0.492±0.105	0.438±0.108	0.943	0.185
C18:3n6	0.025±0.004	0.026±0.006	0.095±0.016	0.089±0.027	0.84	0.366
C20:1	0.054±0.008	0.067±0.019	0.204±0.032	0.247±0.146	**0.04**	0.297
C18:3n3	0.314±0.052	0.350±0.081	1.179±1.195	1.234±0.382	0.108	0.63

4.3.20 continued…

CLA cis-9, trans-11/ trans-9, cis-12	0.588±0.115	0.596±0.153	2.219±0.484	1.977±0.547	0.997	0.174
CLA Trans-10, cis-12	0.010±0.006	0.018±0.010	0.037±0.020	0.050±0.032	**0.019**	**0.033**
CLA isomer 1	0.012±0.005	0.011±0.006	0.044±0.020	0.041±0.028	0.595	0.415
CLA isomer 3	0.067±0.028	0.097±0.040	0.249±0.100	0.323±0.141	**0.032**	0.138
C20:2	0.029±0.007	0.029±0.008	0.108±0.025	0.112±0.038	0.950	0.871
C22:0	0.117±0.004	0.012±0.004	0.044±0.015	0.037±0.014	0.724	0.257
C20:4n6	0.088±0.023	0.074±0.015	0.333±0.088	0.236±0.070	**0.037**	**0.003**
UIP	4.697±0.475	6.580±2.888	17.444±1.943	21.344±6.301	**0.018**	**0.033**
Sums and ratios						
SFA	54.428±2.436	49.843±4.741	204.596±9.054	168.907±28.010	**0.006**	**<0.001**
MUFA	37.923±2.227	40.043±2.683	142.770±11.606	135.187±15.674	**0.035**	**0.064**
PUFA	2.952±0.636	3.533±0.967	11.079±2.337	12.030±3.341	**0.059**	0.395
USF	40.875±2.253	43.576±2.950	153.849±11.688	147.217±16.831	**0.014**	0.108
SFA / UFA	1.339±0.136	1.154±0.176	1.339±0.136	1.150±0.173	**0.006**	**0.005**
MUFA/SFA	0.700±0.070	0.815±0.130	0.700±0.070	0.816±0.129	**0.013**	**0.012**
PUFA/SFA	0.054±0.012	0.072±0.023	0.054±0.012	0.073±0.022	**0.016**	**0.010**
CLA	0.676±0.114	0.723±0.178	2.584±0.478	2.403±0.643	0.508	0.425
n-3 PUFA	0.314±0.052	0.350±0.081	1.179±0.195	1.233±0.382	0.108	0.63
n-6 PUFA	1.888±0.577	2.384±0.890	7.074±2.114	8.116±3.071	**0.069**	0.27
n6/n3 ratio	6.005±1.483	6.812±1.554	6.005±1.483	6.744±1.688	0.143	0.193
Cis FA	36.366±2.402	38.773±2.942	136.896±11.681	130.816±14.875	**0.025**	0.113
Trans FA	4.509±0.687	4.804±1.701	16.953±2.596	16.401±6.252	0.627	0.648

4.3.20 continued…

Trans/cis ratio	0.125±0.024	0.125±0.050	0.125±0.024	0.126±0.050	0.950	0.984
Total FA	-	-	358.445±12.387	316.124±38.904	-	**<0.001**

Table 4.3.21: Means (± SD) for the enzyme and health indices of subcutaneous fat for the main effect of sex from the percent of total lipid (%) and from the gravimetric concentration (mg of fatty acid per g of subcutaneous fat sample)

	%		mg/g		P-values	
	Female	Male	Female	Male	%	mg/g
Replicates (n)	16	16	16	16		
Enzyme indices						
Elongase	0.687±0.015	0.689±0.018	0.687±0.015	0.688±0.018	0.886	0.951
Delta 9 desaturase (C14+C16+C18)	39.015±2.731	42.727±4.454	39.015±2.731	42.767±4.431	**0.017**	**0.016**
Delta 9 desaturase C14	2.092±0.360	2.975±1.309	2.126±0.374	3.026±1.364	**0.024**	**0.026**
Delta 9 desaturase C16	3.854±0.640	4.264±0.800	3.854±0.640	4.267±0.798	**0.067**	**0.065**
Delta 9 desaturase C18	57.571±3.930	62.295±5.676	57.571±3.390	62.390±5.630	**0.016**	**0.014**
Delta 9 desaturase RA	13.515±2.108	13.729±4.008	13.515±2.108	13.545±4.279	0.850	0.988
Health indices						
AI	0.997±0.115	0.846±0.134	0.997±0.115	0.843±0.132	**0.012**	**0.010**
TI	0.610±0.029	0.631±0.039	2.517±0.254	2.134±0.340	**0.069**	**0.004**
DFA	64.772±1.438	64.288±2.413	243.564±8.595	217.475±27.176	0.310	**<0.001**
h	39.750±2.143	42.365±2.832	149.604±11.147	143.093±14.044	**0.014**	**0.097**
H	28.447±1.598	26.753±2.580	107.057±7.968	90.763±14.772	**0.081**	**0.002**
h/H	1.403±0.127	1.503±0.213	1.403±0.127	1.606±0.236	**0.022**	**0.019**

f) Interaction between tannin and sex in SCF

The gravimetric interaction between tannin and sex for subcutaneous fat are presented in Appendix Tables 7.1.3 and their enzyme and health indices in Table 7.1.4. The molar interactions are presented in Table 7.1.9 and 7.1.10 respectively. There was an interaction between tannin and sex in SCF for C18:2n6t as a percent (w/w%) and concentration (mg/g; P<0.05). As illustrated in Figure 4.3.11, when there was no tannin in the diet, ewes had visibly more C18:2n6t, but rams had more when lambs were supplemented with tannin. This is because tannin reduced C18:2n6t in ewes. Therefore, tannin may have reduced the variation between elongase and desaturase enzymes within the n-3 series. The w/w% showed a similar trend as a mg/g and has therefore not been depicted in a Figure.

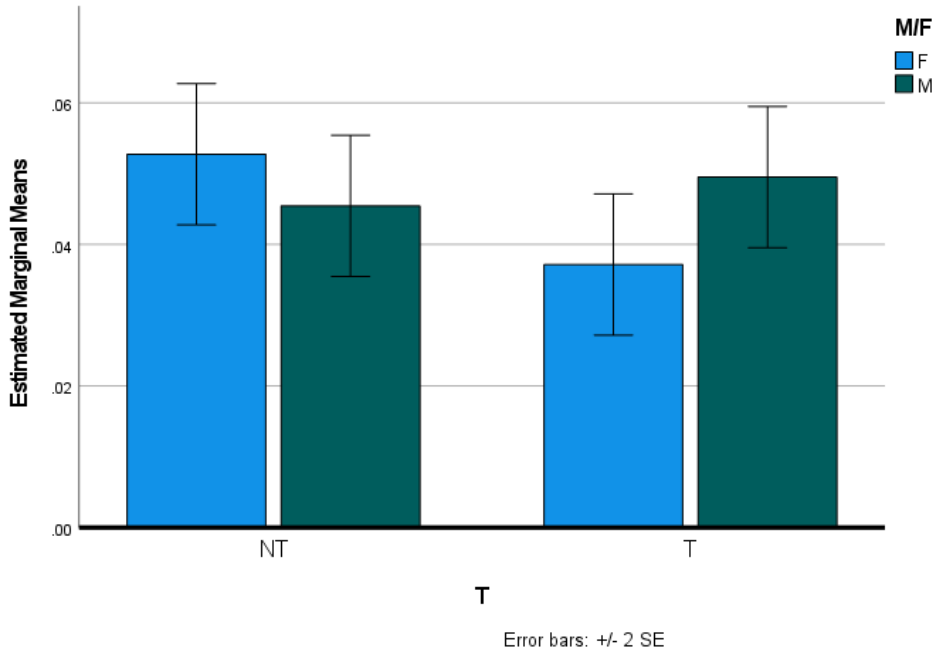

Figure 4.3.11: Estimated marginal means (w/w%) and standard error of C18.2n6t in rams and ewes supplemented with and without Acacia tannin

g) Interaction between NPN source and sex in SCF

The gravimetric interaction between NPN source and sex for subcutaneous fat are presented in Appendix Tables 7.1.3 and their enzyme and health indices in Table 7.1.4. The molar interactions are presented in Table 7.1.9 and 7.1.10 respectively. Calcium nitrate had no overall effect on SCF except for a higher concentration of C20:2 (P<0.05; Table 4.3.11). However, there were interactions as a percent of detected lipid and concentration for individual MUFAs; C16:1 and C18:1n9c (P<0.05). Therefore, an interaction was also identified for the percent and concentration of total cis fatty acids and MUFA (P<0.05). The percent of UFA had an interaction with sex (P<0.05) but only a tendency as a concentration (P=0.059). Due to these changes the SFA/UFA and MUFA/SFA ratio differed as a percent, and the SFA/ UFA ratio differed as a concentration (P<0.05). This was due to its major

contribution of C18:1n9c (P<0.05). It has been reported previously that ewes have a higher C16:1 and C18:1n9c content than rams (Horcada *et al*., 1998 & Diaz *et al*., 2003). However, as shown in Figure 4.3.12 and 4.3.13, C16:1 and C18:1n9c fatty acids were only higher in ewes than rams on the calcium nitrate-based diet. This variability between sexes in the calcium nitrate-based diet could not be explained by delta 9 desaturase activity due to its limited variability (Figure 4.3.14). The higher delta 9 desaturase activity on C18:0 in rams on the urea-based diet (Table 7.1.4) caused variability in C18:1n9c between the sexes (Table 7.1.3). An increased delta 9 desaturase activity on C16:0 (Table 7.1.4) in rams on the urea-based diet presented some variability in C16:1 between the sexes (Table 7.1.3; Figure 4.3.14).

C18:1n9c is known for its beneficial effect on combatting cancer, auto-immune and inflammatory diseases (Sales-Campos *et al*., 2013). It also has a higher melting point, and if kept on meat could potentially increase its juiciness and flavor (Smith, 2016). Therefore, calcium nitrate in the diet of ewes potentially resulted in more of these properties than rams.

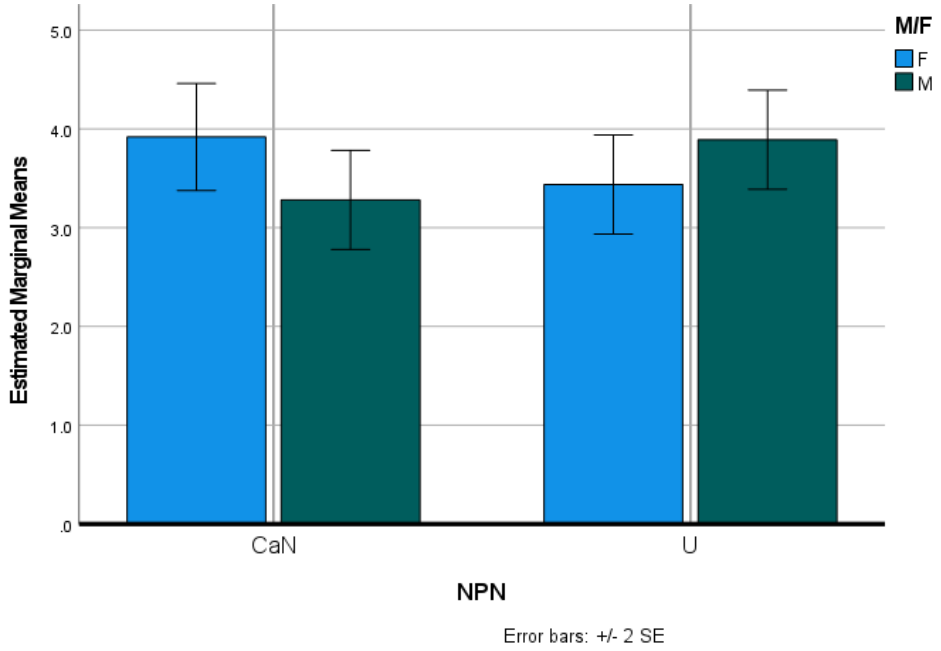

Figure 4.3.12: Estimated marginal means (mg/g) and standard error of C16:1 in rams and ewes fed calcium nitrate or urea as a NPN source

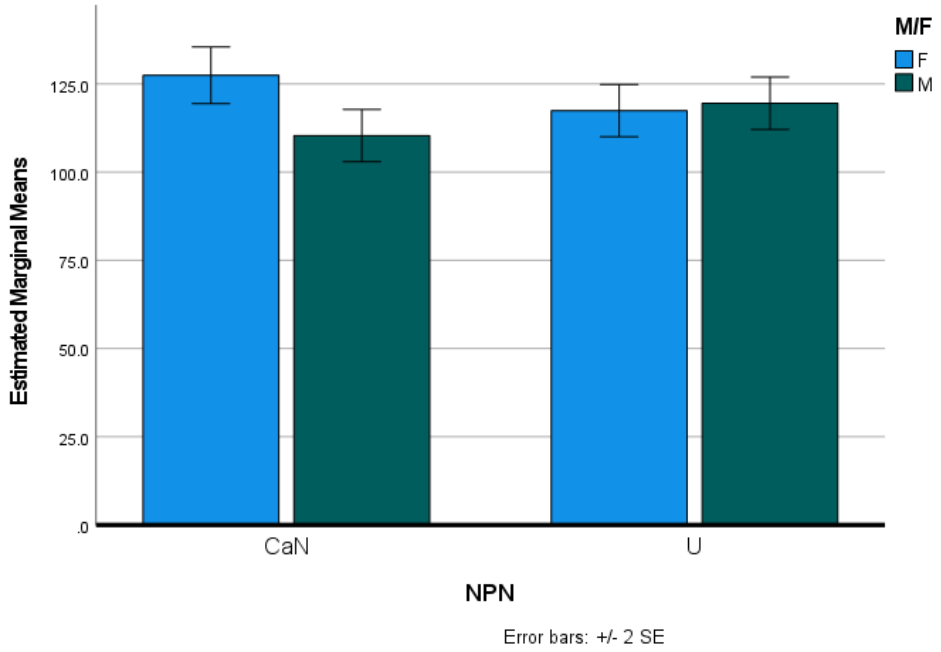

Figure 4.3.13: Estimated marginal means (mg/g) and standard error of C18:1n9c in rams and ewes fed calcium nitrate or urea as a NPN source

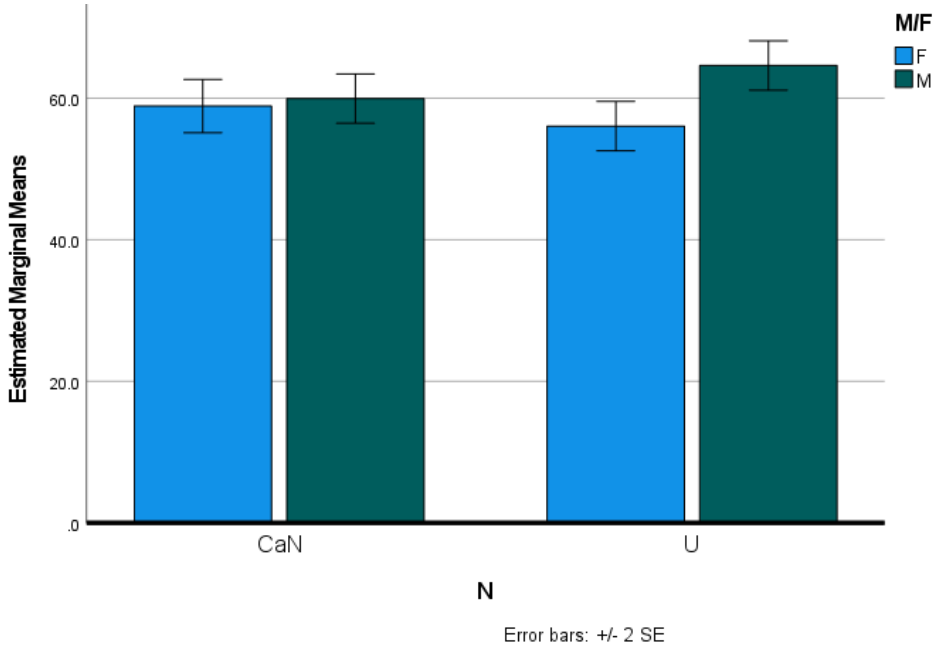

Figure 4.3.14: Estimated marginal means and standard error of C16 delta 9 desaturase activity in rams and ewes fed calcium nitrate or urea as a NPN source

4.3.4.3 The effect of sex on the PR fatty acid profile of lamb

The effect of sex on the fatty acid composition of PRF as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g PRF), with their sums and ratios, are shown in Table 4.3.22. Their enzyme and health indices are presented in 4.3.23. The enzyme indices are used to describe the changes in fatty acids due to sex within the SFA, MUFA and PUFA sections.

Research on the effect of sex on lamb PRF is scarce due to greater demand from a consumer's perspective on the IM fatty acid profile. Kemp *et al*. (1981) found that although sex effected IMF and SCF, PRF was unaffected. Although the current study indicated no changes to total percent and concentration of SFA, MUFA or UFAs in PRF ($P>0.05$), rams increased PUFA by 19.14% (percent) and 7.85% (concentration) ($P<0.05$). There were also differences in individual fatty acids, enzymes, ratios and indices between ewes and rams.

a) The effect of sex on the SFA profile of PRF

The effect of sex on the SFA composition of PRF as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g PRF) are presented in Table 4.3.22. The undesirable C14:0 and C16:0 fatty acids increased in ewe PRF by 18.96% (20.03% from the concentration) and 7.89% (8.96% of the concentration), respectively. The longer chain C20:0 and C22:0 fatty acids increased by 11.76% (10.84% of the concentration) and 25% (7.85% of the concentration) in ram PRF ($P<0.05$). The lower C16:0 percent and concentration in rams may have been a result of increased elongase activity ($P<0.05$; Table 4.3.23). This may also explain the increased C20:0 and C22:0 fatty acids in rams as these fatty acids may also arise endogenously from the elongation of C18:0 (Sampath & Ntambi, 2005). However, C18:0 was not affected by this enzyme. Behenic acid (C22:0), present in higher amounts in rams, is also a cholesterol raising fatty acid (Cater & Denke, 2001). In a cohort study designed by Lemaitre *et al*. (2018) it was reported that C24:0 and C20:0 lowered the risk of heart failure, but C22:0 did not. As already discussed, it has long been knowledge that C14:0 and C16:0 fatty acids are associated with increased cholesterol levels, atherogenic index (AI) and thrombogenic index (TI; Zock *et al*., 1994 & Chen & Liu, 2020).

b) The effect of sex on the MUFA profile of PRF

The effect of sex on the MUFA composition of PRF as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g PRF) are presented in Table 4.3.22. Only C18:1n11c tended to differ between ewes and rams (percent: $P=0.063$; concentration: $P=0.07$). As a result, it increased by 14.65% and 14.62% in rams as a percent of detected lipid and as a concentration, respectively.

c) The effect of sex on the PUFA profile of PRF

The effect of sex on the PUFA composition of PRF as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g PRF) are presented in Table 4.3.22. The percent and concentration of PUFA increased from 2.660%±0.401 (26.923mg/g ±2.845) in ewes to 3.169%±0.897 (29.036±4.00) in rams (P<0.05). This was because of an overall increase in n-6 fatty acids (percent: P<0.05; concentration: P=0.058), due to a 24.70% (23.50% of concentration) increase in the major Omega-6 fatty acid, C18:2n6c (P<0.05). The percent of other Omega-6 fatty acids in the current study either had no effect (C18:3n6), decreased by 10.52% (C18:2n6t) or increased by 72.73% (C20:4n6) (P>0.05, P<0.05 and P<0.001, respectively). Ewes also increased the concentration of C18:2n6t (P<0.05) and decreased C18:2n6c (P<0.05).

Arachidonic acid (C20:4n6) and C18:3n6 are products of the n-6 fatty acid pathway (Ratnayake *et al*., 2009). Linoleic acid (C18:2n6c) is desaturated by delta 6 desaturase to C18:3n6, which is then elongated to C20:3n6 and desaturated by delta 5 desaturase to C20:4n6 (Ratnayake *et al*., 2009). Therefore, the results suggest that the desaturase activity (and possibly elongase activity) in the n-6 pathway was greater in ewes. The only Omega-3 fatty acid detected in PRF, C18:3n3, tended to be higher in rams (P=0.092). The concentration of CLA trans-10, cis-12 also tended to be higher in rams (P=0.079). This CLA isomer, formerly associated with milk fat depression in cows and body fat accretion in growing mice (Bauman *et al*., 1999), also decreases lipogenesis in human adipocytes (Obsen *et al*., 2012). None of the other CLA isomers were affected (P>0.05). The CLA trans-10, cis-12 percent and concentration results need to be interpreted with caution as there was an interaction between tannin, NPN source, sex and weight (P<0.05; data available on request).

d) The effect of sex on the fatty acid ratios in PRF

The effect of sex on ratios of fatty acids in the PRF from the percent of detected fatty acids (w/w%) and gravimetrically (mg/g PRF) are presented in Table 4.3.22. Only the PUFA/SFA ratio increased by 13.16% (23.81% from the concentration) in rams (P<0.05). The ratio was still below the recommended value of 0.45 (Enser *et al*., 1998).

e) The effect of sex on the health indices of PRF

The effect of sex on the health indices of PRF are presented on Table 4.3.23. The thrombogenic index (TI), hypercholesterolemic index (H), hypocholesterolemic index (h) and h/H ratio were unaffected by sex (P>0.05). The atherogenic index (AI) was higher in PRF of ewes (P<0.05). The increased AI was due to the higher C14:0 and C16:0 fatty acids, and lower C18:2n6c in ewes. Although DFAs did not differ as a concentration, there was evidence for an improvement in the DFA percent in rams (P<0.017). This

was due to UFA increasing by 6.14%. It may be possible that the high standard deviation (3.1 to 3.5) was the reason that UFA was not different between sexes (P>0.05).

f) Interactions between tannin and sex, and NPN source and sex in PRF

There were no interactions between tannin and sex or NPN source and sex (P>0.05; Appendix tables Table 7.1.5, 7.1.6, 7.1.11 and 7.1.12)

Table 4.3.22: Means (± SD) depicting the medium and long chain fatty acid profile of perirenal fat for the main effect of sex expressed as a percent of detected fatty acid (w/w%) and gravimetrically (mg of fatty acid per g of perirenal fat)

	w/w%		mg/g		P-Value	
	Female	Male	Female	Male	w/w%	mg/g
Replicates (n)	16	16	16	16		
Fatty acids						
C10:0	0.118±0.016	0.110±0.019	0.444±0.070	0.412±0.077	0.258	0.232
C12:0	0.131±0.047	0.110±0.039	0.487±0.172	0.409±0.144	0.256	0.233
C14:0	3.043±0.381	2.558±0.479	11.429±1.369	9.521±1.749	**0.016**	**0.008**
C14:1	0.036±0.012	0.028±0.012	0.135±0.042	0.105±0.044	0.134	0.109
C16:0	22.005±1.957	20.396±1.516	82.907±9.674	76.089±6.906	**0.022**	**0.032**
C16:1	0.650±0.098	0.607±0.105	2.434±0.314	2.261±0.384	0.255	0.175
C17:0	1.516±0.116	1.542±0.099	5.703±0.513	5.755±0.515	0.601	0.815
C18:0	136.110±2.632	135.946±3.621	136.040±13.906	134.105±15.547	0.914	0.787
C18:1t11	4.023±0.691	4.654±1.497	15.107±2.576	17.458±6.192	0.157	0.179
C18:1n9c	24.454±2.654	25.265±2.720	91.818±9.163	94.202±11.094	0.507	0.564
C18:1n11c	0.594±0.088	0.681±0.140	2.223±0.266	2.548±0.602	**0.063**	**0.07**
C18:2n6t	0.026±0.007	0.022±0.008	0.099±0.026	0.081±0.031	**0.051**	**0.055**
C18:2n6c	2.118±0.379	2.641±0.835	7.966±1.490	9.838±3.289	**0.024**	**0.041**
C20:0	0.204±0.027	0.228±0.030	0.766±0.100	0.849±0.106	**0.035**	**0.055**
C18:3n6	0.025±0.005	0.024±0.006	0.095±0.018	0.089±0.026	0.352	0.373
C20:1	0.051±0.009	0.055±0.013	0.192±0.029	0.204±0.051	0.36	0.391
C18:3n3	0.308±0.050	0.335±0.072	1.156±0.193	1.250±0.264	**0.092**	0.191

Table 4.3.22 continued…

CLA cis-9, trans-11/ trans-9, cis-12	0.448±0.088	0.438±0.086	1.679±0.309	1.629±0.305	0.625	0.55
CLA Trans-10, cis-12	0.007±0.003	0.009±0.003	0.027±0.009	0.033±0.011	0.105	**0.079**
CLA isomer 1	0.006±0.003	0.007±0.005	0.024±0.012	0.025±0.020	0.986	0.923
CLA isomer 3	0.075±0.020	0.065±0.013	0.245±0.047	0.287±0.104	0.14	0.144
C20:2	0.021±0.003	0.023±0.006	0.078±0.012	0.084±0.023	0.262	0.329
C22:0	0.020±0.005	0.025±0.006	0.073±0.017	0.092±0.021	**0.018**	**0.018**
C20:4n6	0.057±0.010	0.033±0.008	0.215±0.036	0.121±0.030	**<0.001**	**0**
UIP	3.965±0.583	4.187±0.336	15.189±1.492	15.584±1.011	0.296	0.303
Sums and ratios						
SFA	63.569±3.409	61.354±3.675	237.857±21.361	227.231±18.415	0.117	0.206
MUFA	29.807±2.927	31.291±3.127	111.909±9.749	116.777±13.995	0.251	0.278
PUFA	2.660±0.401	3.169±0.897	26.923±2.848	29.036±4.000	**0.036**	**0.04**
USF	32.466±3.142	34.460±3.570	138.702±11.577	145.913±16.481	0.143	0.157
SFA / UFA	1.986±0.315	1.808±0.286	1.734±0.276	1.580±0.244	0.136	0.132
MUFA/SFA	0.472±0.070	0.515±0.084	0.476±0.072	0.518±0.085	0.192	0.194
PUFA/SFA	0.042±0.008	0.052±0.018	0.114±0.018	0.129±0.024	**0.038**	**0.029**
CLA	0.527±0.102	0.530±0.098	1.975±0.356	1.975±0.371	0.873	0.821
n-3 PUFA	0.308±0.050	0.335±0.072	1.156±0.193	1.250±0.264	**0.092**	0.191
n-6 PUFA	2.227±0.386	2.719±0.848	8.375±1.515	10.131±3.347	**0.036**	**0.058**
n-6/n-3 ratio	7.262±0.649	8.142±1.716	7.262±0.649	8.122±1.718	0.102	0.111
Cis FA	28.314±3.035	29.691±2.953	106.381±10.448	110.803±12.328	0.276	0.312
Trans FA	4.576±0.771	5.206±1.523	17.180±2.846	19.514±6.320	0.177	0.198

Table 4.3.22 continued…

Trans/cis ratio	0.163±0.031	0.176±0.048	0.163±0.031	0.176±0.048	0.430	0.431
Total FA	-	-	376.559±19.745	737.044±19.728	-	0.684

Table 4.3.23: Means (± SD) for the enzyme and health indices of perirenal fat for the main effect of sex from the percent of detected fatty acid (%) and from the gravimetric concentration (mg of fatty acid per g of perirenal fat sample)

	w/w%		mg/g		P-value	
	Female	Male	Female	Male	w/w%	mg/g
Replicates (n)	16	16	16	16		
Enzyme indices						
Elongase	0.728±0.020	0.744±0.018	0.728±0.020	0.744±0.018	**0.030**	**0.030**
Delta 9 desaturase (C14+C16+C18)	29.156±3.354	30.566±3.396	29.156±3.354	30.566±3.396	0.338	0.338
Delta 9 desaturase C14	1.157±0.305	1.056±0.333	1.157±0.305	1.056±0.333	0.408	0.408
Delta 9 desaturase C16	2.894±0.531	2.900±0.495	2.894±0.531	2.900±0.495	0.901	0.901
Delta 9 desaturase C18	40.370±4.210	41.333±4.653	40.370±4.210	41.333±4.653	0.623	0.623
Delta 9 desaturase RA	10.039±1.378	8.997±2.007	10.039±1.376	9.337±1.962	0.146	0.146
Health indices						
AI	1.075±0.172	0.905±0.141	1.075±0.172	0.906±0.141	**0.010**	**0.010**
TI	3.635±0.599	3.294±0.516	3.657±0.605	3.313±0.522	0.119	0.119
DFA	68.576±1.655	70.406±1.881	274.742±14.181	279.918±16.725	**0.017**	0.356
h	31.729±3.049	33.769±3.493	135.940±11.327	143.243±16.206	0.125	0.139
H	25.048±2.063	22.954±1.899	94.336±10.191	85.610±8.168	**0.010**	**0.012**
h/H	1.280±0.196	1.484±0.227	1.461±0.222	1.687±0.244	**0.021**	**0.018**

4.3.5 The effect of weight on the fatty acid profile of Merino lamb

There were no changes to the total SFA, MUFA or PUFA percent or concentration regardless of the fatty acid depot (P>0.05; Tables 4.3.24, 4.3.26 and 4.3.28). This is consistent with the finding that these fatty acids are predominantly located in neutral lipids (triglyceride), which are affected to a greater extent by the level of fatness (Raes *et al*., 2004). In the current study, the percent of fat and ether extract did not differ between the heavy and light liveweights. Polyunsaturated fatty acids are mostly located in phospholipids, which are more consistent between fat levels, rather differing between the oxidative capacity of the muscle (Raes *et al*., 2004). Figure 4.3.15 is a chromatogram from subcutaneous fat showing the similarity between a heavy ram (black line) and light ram (pink line).

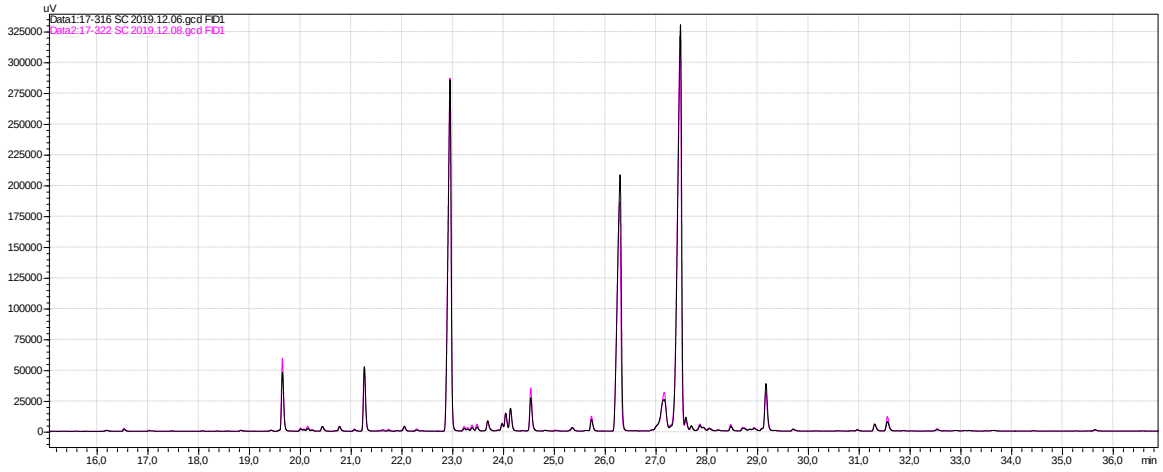

Figure 4.3.15: A chromatogram representing the effect of weight on fatty acid composition

4.3.5.1 The effect of weight on the fatty acid profile of IMF

The effect of weight on the fatty acid profile of freeze-dried *longissimus dorsi* (LD) was shown as a percent of identified fatty acid (w/w%) and as gravimetric concentrations (mg/g), with their sums and ratios of groups of fatty acids in Table 4.3.24. The Enzyme and health indices are presented in Table 3.25. The enzyme indices are used to describe the changes in fatty acids due to sex within the SFA, MUFA and PUFA sections.

a) The effect of weight on the SFA profile of IMF

The effect of weight on the SFA composition of SFA as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g PRF) are presented in Table 4.3.24. The amount of fat in the LD did not differ between weight categories (P>0.05). Therefore, it is unsurprising that there were few differences in the fatty acid profile between heavy and light lambs. As a percent of detected fatty acids, weight impacted C10:0 and C18:0 fatty acids (P>0.05). As a concentration there was a tendency for C10:0 and C16:0 to be higher in ewes (P=0.059 and P=0.071, respectively). Arsenos *et al*. (2006) found greater

differences in the fatty acid profile of sheep, including a higher unsaturated fatty acid profile with increasing maturity. Therefore, the limited differences may be as a result of similarity in the sheep's maturity at slaughter.

Although the increase in C16:0 was not significant as a percent of total fatty acid in the LD (P=0.101), the concentration tended to increase by 16.39% in the heavier lamb category (P=0.071). However, it was not enough to alter the AI or TI indices (P>0.05). Stearic acid (C18:0), the second largest contributor of SFA in IMF after C16:0, increased by 7.43% as a percent of detected fatty acid when lambs were slaughtered at a lighter weight (P<0.05). The difference was not significant as a concentration (P>0.05). The concentration tended to increase by 16.39% in heavier lambs (P=0.071). Capric acid (C10:0), present in small amounts, had a greater percent and tended to be higher as a concentration (P<0.05 and P=0.059, respectively). It increased by 12.75% as a percent of detected fatty acids and 24.19% as a concentration.

An increase in C16:0 with increased slaughter weight was also reported by Tejeda *et al*. (2008), when Merino lamb slaughter weights were increased from 24kg to 29kg. In accordance with our results, the concentration of IMF did not differ between weight categories. Therefore, there was another reason for its increase in concentration. This contrast reports where lighter lambs were slaughtered before weaning (Bas & Morand-Fehr, 2000). This is because a lamb's fatty acid profile is representative of the fatty acid composition presented in ewe milk (Beriain *et al*., 2000; Juarez *et al*., 2009). Therefore, if the ewe's milk was high in C12 and C14 saturated fatty acids, these fatty acids would drop in fat tissue following the introduction of solid feed (Beriain *et al*., 2000). Bas & Morand-Fehr (2000) reported typically higher C14:0 and C16:0 fatty acids in studies looking at the fatty acid composition of unweaned lambs. The increase in these fatty acids were related to the higher composition in ewe milk. It was suggested that these fatty acids increased C18:1n9c due to their elongation and desaturation. It was reported that C18:0 was higher in studies relating to weaned lambs due to the complete biohydrogenation potential in a fully functioning rumen, and C18:2 and C18: 3 increased as a result of the diet.

b) The effect of weight on the MUFA profile of IMF

The effect of weight on the MUFA composition of SFA as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g PRF) are presented in Table 4.3.24. Of the UFAs only C24:1 was influenced by the weight of the lambs. As a percent of total fatty acid, C24:1 tended to be higher in the LD of lighter lambs (P=0.079). Due to an interaction between weight and NPN (P<0.05), it only increased in light lambs on the calcium nitrate-based diet.

Landim *et al*. (2011) studied the effect of breed and weight on weaned lambs ranging in weight from 30 to 45kg. They reported a decrease in C14:0 and C18:0 but an increase in C18:1n9c when lambs were slaughtered at a heavier weight. It was suggested the decrease in C18:0 was a result of its conversion to C18:1n9c. Oleic acid (C18:1n9c) did not differ between weight categories in the current study (P>0.05). However, indices indicate that the weight category of lambs tended to have a potential effect on C18 delta 9 desaturase index and elongase (Table 4.3.25). The C18 delta 9 desaturase index indicated an increased desaturase activity in the lambs slaughtered at a heavier weight (P=0.064). This may explain the lower C18:0 in heavier lambs. There was also a tendency for increased elongase activity in lighter lambs (P=0.068; Table 4.3.25). This may explain the lower C16:0 in the lighter weight group. Although there were no changes in the elongation or desaturation of n-3 or n-6 fatty acids based on no changes to their products between heavy and light lambs (P>0.05).

c) The effect of weight on the PUFA profile of IMF

The effect of weight on the PUFA composition of SFA as a percent of detected fatty acids (w/w%) and gravimetrically (mg/g PRF) are presented in Table 4.3.24. There was no effect of weight on any of the PUFAs (P>0.05). From a health perspective neither the PUFA/SFA ratio nor the cis/trans ratio were affected by the weight category (P>0.05).

d) The effect of weight on the ratio of fatty acids in IMF

The effect of weight on ratios of fatty acids in the IMF from the percent of detected fatty acids (w/w%) and gravimetrically (mg/g PRF) are presented in Table 4.3.24. None of the ratios were affected by the weight at which the lambs were slaughtered (P>0.05).

e) The effect of weight on the health indices of IMF

The effect of weight on the health indices of IMF are presented on Table 4.3.25. Indices show that the overall health of the fatty acid profile was also unaffected, apart from the hypercholesterolemic index (H). It presented the risk of a tendency for the heavier lambs to increase the dominant cholesterol producing fatty acids (over the ones that do not) by 16.38% (P=0.084). This was not enough to affect the h/H ratio (P>0.05). Santos-Silva *et al*. (2002) reported a better h/H ratio in the *longissimus thoracis* of 24 as opposed to 30kg Merino Branco and Merino Branco x Ile de France ram lambs. The effect on the indices is possibly greater where a lighter group is slaughtered. The PUFA/SFA ratio was higher, and atherigenic (AI) and thrombogenic (TI) indices lower in the *longissimus* of the heavier weight category of lambs slaughtered between 10 and 25kg (Santos-Silva *et al*., 2002).

Table 4.3.24: Means (± SD) depicting the medium and long chain fatty acid profile of the longissimus dorsi between the 8th and 10th ribs for the main effect of weight expressed as a percent of detected fatty acid (w/w%) and gravimetrically (mg of fatty acid per g of *longissimus dorsi* on a DM basis)

	w/w%		Mg/g		P-values	
	Heavy	Light	Heavy	Light	w/w%	mg/g
Replicates (n)	16	16	17	15		
Fatty acids						
C10:0	0.115±0.020	0.102±0.016	0.077±0.022	0.062±0.022	**0.042**	**0.059**
C12:0	0.127±0.046	0.120±0.040	0.088±0.046	0.074±0.036	0.568	0.304
C13:0	0.011±0.004	0.011±0.003	0.007±0.004	0.007±0.003	0.752	0.463
C14:0	2.714±0.575	2.581±0.571	1.864±0.723	1.604±0.693	0.492	0.263
C14:1	0.080±0.026	0.072±0.019	0.055±0.026	0.045±0.022	0.420	0.283
C16:0	26.568±1.895	25.333±2.121	17.786±4.439	15.281±4.472	0.101	**0.071**
C16:1	1.444±1.225	1.384±0.136	0.972±0.288	0.837±0.259	0.568	0.177
C17:0	0.827±0.141	0.900±0.089	0.548±0.148	0.543±0.163	0.207	0.895
C18:0	16.484±1.244	17.708±1.211	10.899±2.207	10.561±2.615	**0.02**	0.69
C18:1t11	2.768±0.749	2.673±0.507	1.827±0.552	1.631±0.620	0.601	0.294
C18:1n9c	38.501±1.487	38.433±2.197	25.567±5.527	22.967±5.691	0.924	0.164
C18:1n11c	0.994±0.144	0.978±0.232	0.645±0.083	0.567±0.151	0.851	0.101
C18:2n6t	0.030±0.006	0.030±0.010	0.020±0.005	0.019±0.009	0.857	0.76
C18:2n6c	3.166±1.099	3.213±0.944	1.994±0.423	1.832±0.331	0.905	0.177
C20:0	0.082±0.010	0.086±0.012	0.054±0.010	0.051±0.012	0.372	0.36
C18:3n6	0.036±0.007	0.036±0.007	0.024±0.004	0.021±0.004	0.902	0.223
C20:1	0.056±0.009	0.056±0.009	0.036±0.007	0.033±0.007	0.738	0.119

Table 4.3.24 continued…

C18:3n3	0.284±0.102	0.287±0.071	0.183±0.064	0.169±0.047	0.976	0.532
CLA cis-9, trans-11/trans-9, cis-12	0.467±0.125	0.510±0.203	0.317±0.133	0.312±0.168	0.355	0.822
CLA cis-10, cis-12	0.006±0.002	0.006±0.002	0.004±0.002	0.004±0.001	0.970	0.357
CLA Trans-10, cis-12	0.011±0.005	0.014±0.013	0.008±0.005	0.009±0.009	0.295	0.483
CLA isomer 1	0.007±0.003	0.008±0.003	0.005±0.002	0.005±0.002	0.382	0.953
CLA isomer 3	0.158±0.034	0.184±0.103	0.108±0.058	0.112±0.073	0.320	0.666
C20:2	0.048±0.008	0.048±0.011	0.031±0.006	0.028±0.007	0.907	0.202
C22:0	0.029±0.011	0.034±0.010	0.018±0.005	0.019±0.003	0.121	0.507
C20:3n6	0.080±0.035	0.100±0.060	0.050±0.013	0.053±0.020	0.268	0.523
C22:1n9	0.015±0.006	0.068±0.166	0.009±0.002	0.041±0.108	0.255	0.287
C23:0	0.023±0.007	0.027±0.009	0.015±0.003	0.015±0.003	0.121	0.44
C20:4n6	0.845±0.397	0.951±0.476	0.528±0.163	0.512±0.133	0.474	0.984
C24:0	0.036±0.012	0.043±0.015	0.023±0.003	0.024±0.003	0.126	0.311
C20:5n3	0.060±0.042	0.078±0.055	0.037±0.019	0.042±0.020	0.203	0.322
C24:1	0.026±0.011	0.031±0.012	0.016±0.004	0.017±0.003	**0.079**	0.195
C22:6n3	0.049±0.031	0.060±0.040	0.031±0.017	0.032±0.017	0.143	0.362
UIP	3.842±1.156	3.837±0.558	2.565±0.982	2.285±0.612	0.865	0.243
Sums and ratios						
SFA	47.016±2.316	46.945±2.290	31.379±7.372	28.240±7.810	0.897	0.182
MUFA	43.883±1.176	43.695±1.922	29.127±6.169	26.138±6.529	0.886	0.148
PUFA	5.258±1.543	5.524±1.461	3.340±0.582	3.154±0.473	0.676	0.437
USF	49.142±2.029	49.219±2.177	32.467±6.420	29.292±6.725	0.815	0.138
SFA / UFA	0.960±0.083	0.957±0.089	0.960±0.083	0.957±0.089	0.858	0.854

Table 4.3.24 continued…

MUFA/SFA	0.936±0.064	0.934±0.078	0.936±0.064	0.934±0.078	0.985	0.985
PUFA/SFA	0.113±0.038	0.119±0.036	0.113±0.038	0.119±0.036	0.736	0.728
CLA	0.393±0.162	0.721±0.304	0.442±0.176	0.441±0.243	0.755	0.76
n-3 PUFA	0.393±0.162	0.425±0.146	0.250±0.088	0.242±0.064	0.300	0.914
n-6 PUFA	0.972±0.437	1.087±0.536	0.601±0.175	0.587±0.150	0.535	0.983
n6/n3 ratio	2.580±0.888	2.513±-.722	2.580±0.889	2.513±0.722	0.450	0.889
Cis FA	45.695±2.059	45.794±2.561	30.207±5.766	27.229±6.069	0.820	0.129
Trans FA	3.447±0.743	3.425±0.709	2.289±0.661	2.090±0.835	0.947	0.481
Trans/cis ratio	0.076±0.020	0.076±0.020	0.076±0.017	0.075±0.020	0.977	0.973
Total FA	-	-	63.846±13.426	57.532±14.355	-	0.155

Table 4.3.25: Means (± SD) for the enzyme and health indices of the *longissimus dorsi* between the 8th and 10th ribs for the main effect of weight from the percent of detected fatty acid (w/w%) and from the gravimetric concentration (mg of fatty acid per g of *longissimus dorsi* on a DM basis)

	%		mg/g		P-values	
	Heavy	Light	Heavy	Light	%	mg/g
Replicates (n)	16	16	17	15		
Enzyme indices						
Elongase	0.663±0.018	0.678±0.024	0.663±0.018	0.678±0.024	**0.068**	**0.068**
Delta 9 desaturase (C14+C16+C18)	46.670±1.757	46.650±2.328	46.670±1.757	46.650±2.328	0.911	0.911
Delta 9 desaturase C14	2.806±0.458	2.698±0.246	2.806±0.458	2.698±0.246	0.588	0.561
Delta 9 desaturase C16	5.144±0.612	5.192±0.461	5.144±0.612	5.192±0.461	0.676	0.676
Delta 9 desaturase C18	70.023±2.091	68.433±2.208	70.023±2.091	68.433±2.208	**0.064**	**0.064**
Delta 9 desaturase RA	14.858±3.791	15.765±4.743	14.858±3.790	15.765±4.743	0.362	0.362
Health indices						
AI	0.832±0.097	0.795±0.109	0.832±0.097	0.795±0.109	0.253	0.253
TI	0.910±0.164	1.901±0.182	1.893±0.167	1.879±0.185	0.782	0.721
DFA	65.626±2.136	66.926±2.228	43.366±8.274	39.852±9.191	0.100	0.224
h	47.521±2.065	47.609±2.204	31.379±5.958	28.319±6.447	0.822	0.137
H	29.282±2.250	27.913±2.473	19.650±5.104	16.885±5.126	0.111	**0.084**
h/H	1.637±0.196	1.725±0.239	1.637±0.196	1.725±0.238	0.234	0.233

f) Interaction between tannin and weight in IMF

The interaction between tannin and weight on IMF is presented in Table 7.1.1 (percent) and 7.1.7 (concentration). The percent of C20:0 and C20:1 fatty acids were affected by the interaction between tannin and weight ($P<0.05$). The C24:1 fatty acid was affected by the interaction between tannin and weight as a concentration ($P<0.05$). However, these fatty acids were present in very small amounts in meat. Elongase also differed from the concentration but numerically the difference was very small ($P<0.05$; Table 7.1.8).

g) *Interaction between NPN source and weight in IMF*

The interaction between NPN source and weight on IMF is presented in Table 7.1.1 (percent) and 7.1.7 (concentration). Non-protein nitrogen (NPN) interacted with weight as a percent of total lipid and concentration for C23:0 and C24:1 ($P<0.05$). The percent and concentration of C23:0 interacted between weight and NPN source ($P<0.05$). However, these fatty acids are present in very low quantities in intramuscular fat.

4.3.5.2 The effect of weight on the subcutaneous fatty acid profile of lamb

The effect of weight on the fatty acid profile of subcutaneous fat was shown as a percent of detected fatty acid (w/w%) and gravimetrically (mg/g SCF), as well as sums and ratios of groups of fatty acids in Table 4.3.26. Their enzyme and health indices are presented in Table 4.3.27.

Weight did not make any substantial changes to the SC fatty acid composition. Although there were a couple of tendencies, there was only one significant affect as a percent of detected fat and as a concentration (CLA isomer 1; $P<0.05$). Webb & Casey (1995) did not identify changes as a molar percent, but they did report increases in the concentration of C14:0, C16:0, C16:1, C18:0 and C18:1n9c in SCF of SA Mutton Merino wethers when the slaughter weight was increased from 37 to 43kg. They attributed these changes to an increase in total fatness due to the displacement of water molecules in adipocytes by saturated and unsaturated fatty acids. Although the concentration of all these fatty acids were higher in the current study, none of them were significant ($P>0.05$). This is perhaps due to the current study not having a higher total concentration of fat in SCF ($P>0.05$). Therefore, a larger weight gap may have been necessary to see meaningful results.

a) The effect of weight on the SFA composition of SCF

The effect of weight on the fatty acid profile of subcutaneous fat was shown as a percent of detected fatty acid (w/w%) and gravimetrically (mg/g SCF) in Table 4.3.26. The percent of C16:0 tended to be 5.75% higher in heavier lambs ($P=0.06$). Although the concentration also increased with weight (82.898mg/g±13.647 to 90.209mg/g±8.843), the variation was high, possibly also relating to its

insignificant difference (P>0.05). The only other saturated fatty acid to be affected was C10:0, and it tended to be higher as a percent and concentration in heavier lambs (P=0.065). The elongase indices decreased as a percent of total fatty acids by 1.91% in heavier lambs (P<0.05) and by 1.73% as a concentration (P=0.069). However, this did not affect the fatty acid profile (P>0.05).

b) The effect of weight on the MUFA composition of SCF

Weight did not affect the MUFA composition or its individual fatty acids in SCF (P>0.05; Table 4.3.26).

c) The effect of weight on the PUFA composition of SCF

The effect of weight on the fatty acid profile of MUFA was shown as a percent of detected fatty acid (w/w%) and gravimetrically (mg/g SCF) in Table 4.3.26. Of the PUFAs, the percent of CLA cis-9, trans-11 + trans-9, cis-12 tended to be higher in lighter lambs (P=0.095). Although rumenic acid was not affected (P>0.05), the concentration of CLA isomer 1 increased by 35.14% in lighter lambs (P<0.05). In contrast, Santos-silva *et al*. (2002) reported an increase in CLA in the *longissimus thoracis* of lambs between 24 and 30kg. This result may have been related to the feeding system and accumulation of CLA from pasture. The increase in calcium nitrate consumption due to a possibly higher feed intake in the heavier lambs may have reduced the absorption of this isomer. From a health perspective this isomer is only present in trace amounts. The concentration of C18:2n6t in the SCF also tended to be higher in the lighter lambs (P=0.07).

Although CLA cis-9, trans-11 + trans-9, cis-12 tended to be higher in the lighter lambs, there were no indications of changes in the activity of the desaturase enzyme (P>0.05; Table 4.3.27). Therefore, its absorption may have been more efficient in the lighter lambs.

d) The effect of weight on the ratio of fatty acids in SCF

None of the ratios of fatty acids were affected by the weight of the lambs at slaughter (P>0.05; Table 4.3.26).

e) The effect of weight on the health indices of SCF

The effect of weight on the health indices of SCF are presented on Table 4.3.27. In terms of the overall health value of the SCF there were no changes in the AI, TI, DFA, h, H or h/H indices. Like IMF, the only exception was the hypercholesterolemic index (H), this time as a percent of total fatty acid, which tended to increase in the heavier lambs (P=0.090).

Table 4.3.26: Means (± SD) depicting the medium and long chain fatty acid profile of subcutaneous fat for the main effect of weight expressed as a percent of detected lipid (w/w%) and gravimetrically (mg of fatty acid per g of subcutaneous fat sample)

	%		mg/g		P-values	
	Heavy	Light	Heavy	Light	%	mg/g
Replicates (n)	17	15	17	15		
Fatty acids						
C10:0	0.152±0.023	0.137±0.021	0.548±0.081	0.484±0.094	**0.065**	**0.065**
C12:0	0.154±0.065	0.139±0.050	0.561±0.258	0.495±0.203	0.543	0.521
C13:0	0.021±0.005	0.028±-.020	0.077±0.018	0.090±0.045	0.231	0.307
C14:0	3.476±0.703	3.246±0.639	12.666±3.030	11.519±3.036	0.415	0.369
C14:1	0.081±0.023	0.089±0.027	0.293±0.091	0.302±0.075	0.428	0.700
C16:0	24.866±1.514	23.513±1.850	90.209±8.843	82.898±13.647	**0.06**	0.106
C16:1	1.017±0.204	1.031±0185	3.686±0.786	3.634±0.812	0.737	0.932
C17:0	1.677±0.301	1.885±0.695	6.053±1.016	6.405±1.224	0.326	0.429
C18:0	22.477±2.729	22.109±3.980	81.564±11.796	78.198±19.595	0.656	0.53
C18:1t11	3.982±1.606	3.829±0.729	14.355±5.521	13.670±3.316	0.753	0.772
C18:1n9c	32.927±2.784	33.666±2.882	119.324±12.415	117.601±14.252	0.37	0.899
C18:1n11c	0.618±0.125	0.645±0.102	2.228±0.398	2.262±0.418	0.453	0.644
C18:2n6t	0.043±0.011	0.049±0.018	0.157±0.039	0.180±0.077	**0.089**	**0.070**
C18:2n6c	2.066±0.999	1.988±0.423	7.391±3.402	7.023±1.359	0.644	0.632
C20:0	0.131±0.027	0.130±0.027	0.475±0.103	0.453±0.117	0.686	0.503
C18:3n6	0.025±0.005	0.026±0.005	0.092±0.018	0.092±0.026	0.899	0.868
C20:1	0.058±0.010	0.064±0.021	0.209±0.036	0.244±0.151	0.386	0.412
C18:3n3	0.329±0.081	0.334±0.057	1.185±0.254	1.231±0.351	0.825	0.527
CLA cis-9, trans-11/ trans-9, cis-12	0.558±0.125	0.631±0.136	2.020±0.474	2.187±0.576	**0.095**	0.206
CLA Trans-10, cis-12	0.014±0.009	0.014±0.009	0.049±0.029	0.049±0.030	0.893	0.868
CLA isomer 1	0.010±0.005	0.013±0.006	0.037±0.018	0.050±0.029	**0.023**	**0.022**
CLA isomer 3	0.076±0.026	0.089±0.047	0.273±0.086	0.301±0.163	0.482	0.686
C20:2	0.029±0.006	0.029±0.007	0.105±0.021	0.116±0.041	0.913	0.217
C22:0	0.011±0.003	0.012±0.005	0.042±0.014	0.039±0.015	0.793	0.527
C20:4n6	0.080±0.016	0.082±0.025	0.292±0.063	0.277±0.119	0.685	0.849
UIP	5.122±1.011	6.224±3.057	18.224±3.101	20.720±6.392	0.178	0.151
Sums and ratios						
SFA	52.964±3.626	51.917±5.065	192.195±20.178	180.582±33.380	0.219	0.217
MUFA	38.683±2.512	39.324±2.854	140.096±11.379	137.713±17.005	0.384	0.978

Table 4.3.26 continued…

PUFA	3.231±1.084	3.255±0.534	11.599±3.596	11.504±1.884	0.995	0.978
USF	41.914±2.862	42.579±3.053	151.695±11.270	149.217±18.065	0.411	0.984
SFA / UFA	1.275±0.172	1.214±0.191	1.275±0.172	1.210±0.190	0.26	0.228
MUFA/SFA	0.736±0.097	0.780±0.138	0.736±0.097	0.782±0.137	0.266	0.248
PUFA/SFA	0.062±0.024	0.065±0.015	0.062±0.024	0.066±0.014	0.729	0.628
CLA	0.658±0.126	0.747±0.163	135.907±10.765	133.533±16.290	**0.092**	0.99
n-3 PUFA	0.329±0.081	0.334±0.057	14.355±5.521	13.670±3.316	0.825	0.772
n-6 PUFA	2.172±1.005	2.095±0.436	121.552±12.279	119.863±14.549	0.656	0.889
n-6/n-3 ratio	6.496±1.935	6.310±1.008	9.254±2.598	9.247±2.264	0.606	0.976
Cis FA	37.231±2.738	37.053±3.147	134.804±11.506	132.781±15.835	0.395	0.95
Trans FA	4.684±1.626	4.625±0.794	16.890±5.580	16.436±3.684	0.94	0.929
Trans/cis ratio	0.127±0.048	0.123±0.025	0.127±0.048	0.124±0.025	0.779	0.81
Total FA	-	-	343.889±22.313	329.799±46.127	-	0.316

Table 4.3.27: Means (±SD) for the enzyme and health indices of subcutaneous fat for the main effect of weight from the percent of detected lipid (w/w%) and from the gravimetric concentration (mg of fatty acid per g of subcutaneous fat sample)

	%		mg/g		P-values	
	Heavy	Light	Heavy	Light	%	mg/g
Replicates (n)	17	15	17	15		
Enzyme indices						
Elongase	0.682±0.013	0.695±0.018	0.682±0.013	0.694±0.018	**0.052**	**0.069**
Delta 9 desaturase (C14+C16+C18)	40.120±3.396	41.722±4.742	40.120±3.396	41.764±4.729	0.235	0.224
Delta 9 desaturase C14	2.279±0.562	2.821±1.376	2.321±0.583	2.865±1.431	0.19	0.203
Delta 9 desaturase C16	3.925±0.749	4.211±0.730	3.925±0.749	4.215±0.728	0.22	0.214
Delta 9 desaturase C18	59.436±4.639	60.496±6.211	59.436±4.639	60.598±6.198	0.482	0.453
Delta 9 desaturase RA	12.969±3.030	14.361±3.225	12.969±3.030	14.165±3.615	0.145	0.215
Health indices						
AI	0.953±0.142	0.885±0.145	0.953±0.142	0.883±0.144	0.195	0.176
TI	0.624±0.030	0.617±0.042	2.389±0.330	2.255±0.376	0.709	0.216
DFA	64.391±1.428	64.687±2.492	233.259±13.553	222.415±32.094	0.584	0.654
h	40.759±2.754	41.395±2.920	147.506±10.735	145.037±17.365	0.415	0.996
H	28.342±1.959	26.759±2.386	102.875±11.170	94.417±16.438	**0.09**	0.126
h/H	1.449±0.190	1.564±0.228	1.449±0.190	1.567±0.226	0.149	0.138

f) Interaction between tannin and weight in SCF

The interaction between tannin and weight on SCF is presented in Table 7.1.3 (percent) and 7.1.9 (concentration). There was an interaction for C18:0 and C18:3n3 as a percent and concentration ($P<0.05$). There was also an interaction in the total amount of fatty acid present in the SCF ($P<0.05$). Tannin did not affect C18:0 and neither did weight ($P>0.05$). However, the desirable fatty acid index (DFA) from the w/w% was affected ($P=0.01$), as was the hypercholesterolemic fatty acid index (h; $P<0.05$).

As indicated in Figure 4.3.16, it can be speculated that when tannin was not included in the diet the lighter lambs had more C18:0. When tannin was included in the diet, the heavier lambs had more C18:0. Therefore, tannin had the greatest effect on lighter lambs.

When no tannin was added to the diet, C18:3n3 was higher in the lighter lambs (Figure 4.3.17). When tannin was supplied to the diet, the heavier lambs had a higher percent and concentration. Linolenic acid (C18:3n3) is used as a substrate for the synthesis of longer chain n-3 fatty acids through delta 5 and delta 6 desaturases and elongases (Garcia, 2011). These fatty acids are essential fatty acids and play a regulatory role in inflammatory response, blood pressure, brain development and cognitive function to name a few (Garcia, 2011). However, no elongation and desaturation products for C18:3n3 were detected in the SCF (Table 4.3.26). Therefore, the lighter lambs may have required some energy through beta oxidation of C18:3n3 when tannin was fed. The lighter lambs may have needed to replace the energy trapped by tannin due to reduced digestibility.

As suggested in Figure 4.3.18, the total amount of fatty acid (and therefore the ratio of fat to water) was higher in the heavier lambs when supplemented with tannin. This is because the lighter lambs dropped in fat when tannin was included in the diet. The heavy lambs increased in fat and were therefore more resistant to the negative effect of tannin.

Stearic acid (C18:0), a dominant fatty acid in SCF, showed a similar trend to the amount of fat present in SCF. This also meant that there was an interaction for the DFA ($P=0.01$). The numerical percent difference between w/w% was small. As indicated in Figure 4.3.19, the largest difference in variation in the hypocholesterolemic fatty acid (h) was a decrease in h in the lighter weight group when tannin was included in the diet.

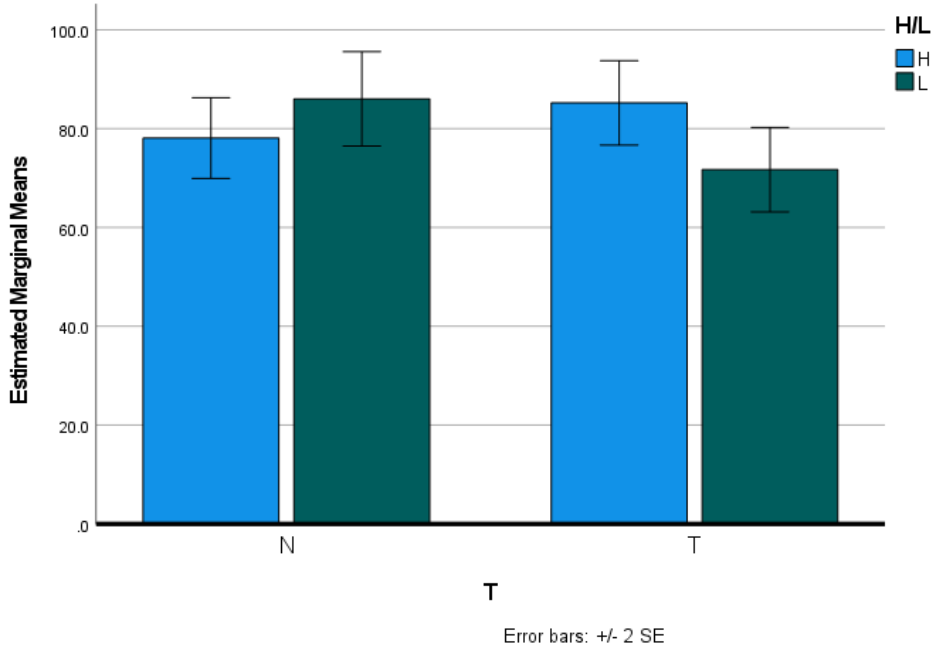

Figure 4.3.16: Estimated marginal means and standard error of C18:0 in the SCF of heavy and light lambs fed a TMR with (T) and without Acacia tannin (N; mg/g SCF)

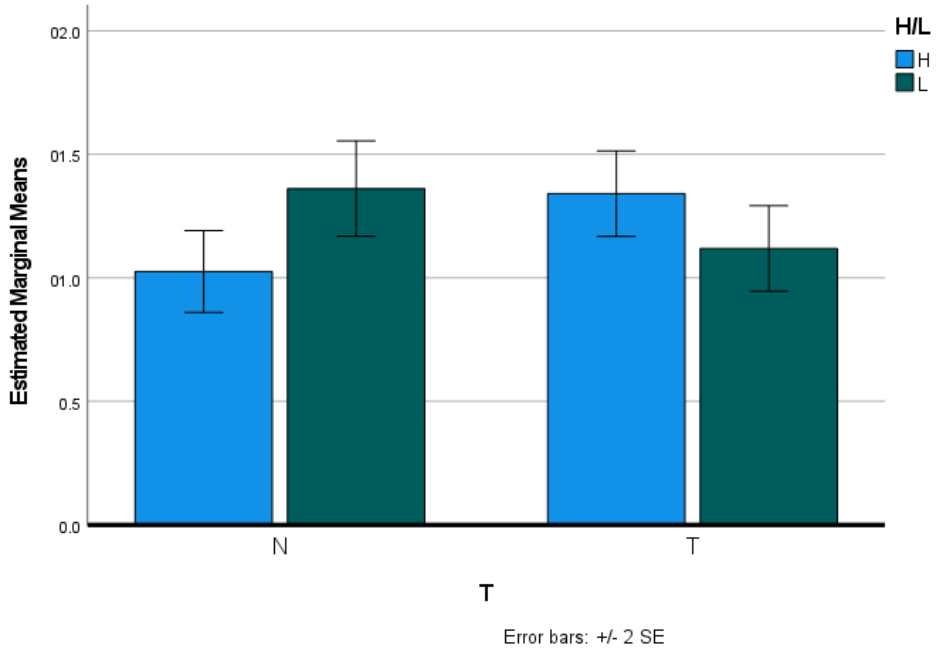

Figure 4.3.17: Estimated marginal means and standard error of C18:3n3 in the SCF of heavy and light lambs fed a TMR with (T) and without Acacia tannin (N; w/w%)

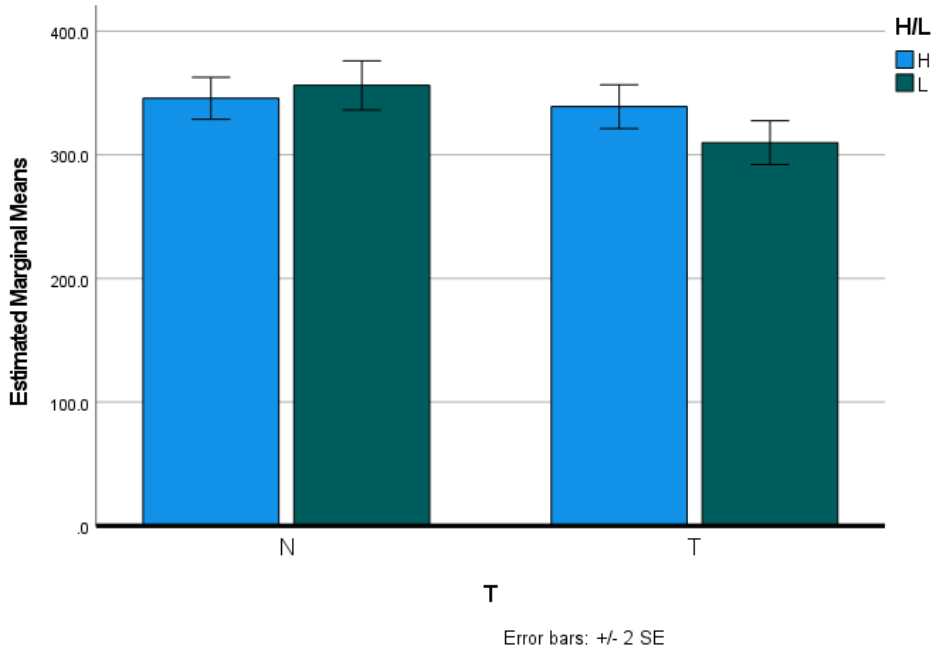

Figure 4.3.18: Estimated marginal means and standard error of total fat in SCF of heavy and light lambs fed a TMR with (T) and without Acacia tannin (N; mg/g SCF)

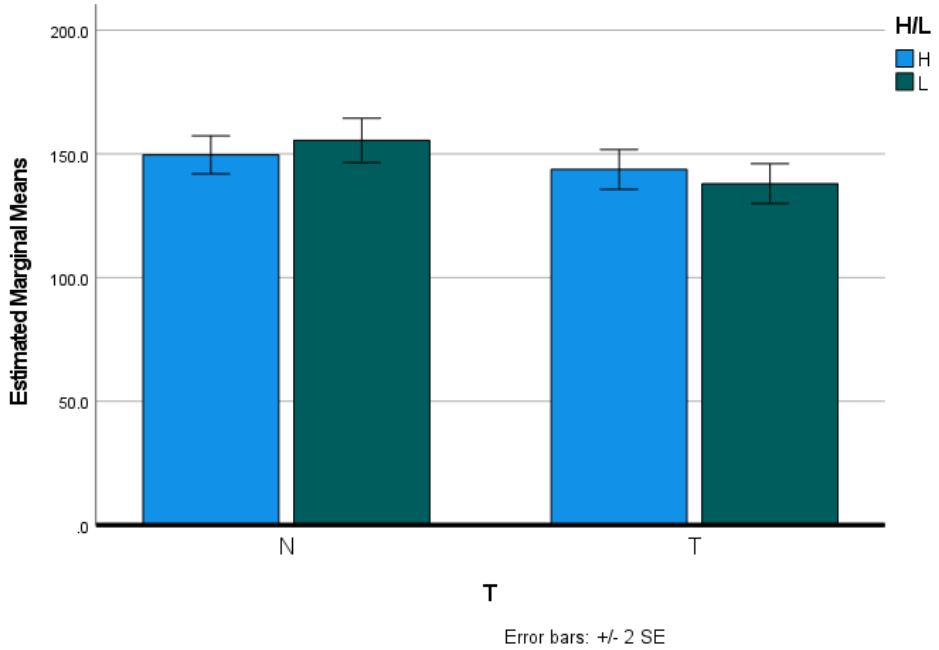

Figure 4.3.19: Estimated marginal means and standard error of hypercholesterolaemic fatty acid (h) in the SCF of heavy and light lambs fed a TMR with (T) and withou Acaciat tannin (N; from mg/g SCF)

g) *Interaction between NPN source and weight in SCF*

The interaction between NPN source and weight on SCF is presented in Table 7.1.3 (percent) and 7.1.9 (concentration). Linoleleidic acid (C18:2n6t) is the trans isomer of C18:2n6c. There was an interaction

between NPN source and weight for the percent and concentration of C18:2n6t (P<0.05). Numerically the difference was small.

4.3.5.3 The effect of weight on the fatty acid pofile of PRF

The effect of weight on the fatty acid profile of PRF was shown as a percent of detected fatty acid (w/w%) and gravimetrically (mg/g PRF) as well as sums and ratios of groups of fatty acids in Table 4.3.28. Enzyme and health indices are presented in Table 4.3.29 (from w/w%). Enzymes are discussed within the SFA, MUFA and/or PUFA section where it is applicable to explaining the fatty acid composition as affected by treatment.

Like the other fatty acid depots, the weight group did not change the total SFA, MUFA or PUFA percent or concentration. The effects on fatty acids were similar to the other fatty acid depot sites.

a) The effect of weight on the SFA composition of PRF

The effect of weight on the SFA composition of PRF was shown as a percent of detected fatty acid (w/w%) and gravimetrically (mg/g SCF) in Table 4.3.28. The C10:0 and C16:0 fatty acids were still consistently higher in the heavier lambs as a percent and concentration (P<0.05). Capric acid (C10:0) increased by 17.09% as a concentration (P<0.01) and 13.08% as a percent of fatty acids in the heavier category. The percent and concentration of C16:0 was better as a percent and concentration in the lighter lambs (P<0.05). It decreased by 6.38% and 8.96% respectively. Slaughtering at a heavier weight also decreased C22:0 by 20% as a percent and 18.68% as a concentration (P<0.05). Palmitic acid (C16:0) is atherogenic, thrombogenic and hypercholesterolemic (Chen & Liu, 2020). However, it only altered H (P<0.05; Table 4.3.29). Capric acid (C10:0) is a neutral fatty acid, does not alter the LDL cholesterol level, and is rapidly oxidised to acetyl CoA in the liver (Dietschy, 1998). Recently it has also been found to be part of a group of medium chain fatty acids with a positive effect on cognitive functioning, the ability to maintain optimal insulin sensitivity, and has shown the potential to regulate metabolic diseases through modifications of gut microbiota (Roopashree *et al*., 2021). The decrease in C22:0 is advantageous in heavier lambs as it raises LDL cholesterol concentrations (Cater & Denke, 2001). Despite its poor absorption, it has 2 to 3 times the potency for raising LDL cholesterol as C16:0 (Cater & Denke, 2001).

Palmitic acid (C16:0) did not increase in response to the concentration of lipid and water in PRF, as the concentration of lipid did not differ between weight groups (P>0.05). In agreement with lower C16:0 in lighter lambs, there was a 2.47% rise in the elongase index as a percent and as a concentration (P<0.05).

b) The effect of weight on the MUFA composition of PRF

Weight had no effect on the MUFA composition of PRF in Dohne Merino lambs (P>0.05; Table 4.3.28). Of the MUFAs, only C20:1 tended to increase as a concentration in heavier lambs (P=0.083; Table 4.3.28).

c) The effect of weight on the PUFA composition of PRF

Weight had no effect on the PUFA composition of PRF in Dohne Merino lambs (P>0.05; Table 4.3.28).

d) The effect of weight on the fatty acid ratios of PRF

Weight had no effect on the ratios of fatty acids (P>0.05; Table 4.3.28).

e) The effect of weight on the health indices of PRF

The effect of weight on the health indices of PRF are presented in Table 4.3.29. Of the equations determining the overall detrimental health effect of PRF, only the cholesterol index, H, increased by 6.87% in the heavier category (from the percent of detected lipid) and by 9.93% (from the concentration; P<0.05). The percent of DFA also showed a tendency to improve in the lambs slaughtered at a lighter weight (P=0.095). Therefore, the internal fat depot indicated that the lighter weight group was a better option from a health perspective. There is little research available on the effect of weight on peri renal fat. Kemp *et al*. (1981) identified no differences in the PR fatty acid profile of a combination of ewes and rams slaughtered at 41kg and 50kg.

Table 4.3.28: Means (± SD) depicting the medium and long chain fatty acid profile of perirenal fat for the main effect of eight expressed as a percent of detected lipid (w/w%) and gravimetrically (mg of fatty acid per g of perirenal fat sample)

	%		mg/g		P-value	
	Heavy	Light	Heavy	Light	%	mg/g
Replicates (n)	17	15	17	15		
Fatty acids						
C10:0	0.121±0.017	0.107±0.017	0.459±0.073	0.392±0.059	**0.02**	**0.007**
C12:0	0.124±0.044	0.117±0.044	0.468±0.166	0.426±0.146	0.731	0.535
C14:0	2.893±0.469	2.696±0.513	10.967±1.778	9.917±1.768	0.362	0.138
C14:1	0.033±0.012	0.032±0.013	0.123±0.046	±0.1150.045	0.932	0.74
C16:0	21.854±1.854	20.460±1.735	82.983±8.417	75.548±8.094	**0.05**	**0.018**
C16:1	0.621±1.103	0.637±0.104	2.353±0.392	2.342±0.325	0.532	0.893
C17:0	1.504±0.115	1.557±0.094	5.708±0.506	5.853±0.523	0.3	0.996
C18:0	35.286±3.290	36.869±2.773	133.961±14.472	136.332±15.024	0.221	0.772
C18:1t11	4.432±1.448	4.233±0.850	16.865±5.899	15.621±3.272	0.654	0.461
C18:1n9c	24.786±3.089	24.942±2.221	93.995±11.926	91.893±7.746	0.876	0.585
C18:1n11c	0.630±0.153	0.648±0.083	2.383±0.616	2.388±0.301	0.676	0.945
C18:2n6t	0.024±0.007	0.024±0.009	0.091±0.027	0.090±0.033	0.524	0.71
C18:2n6c	2.50±0.848	2.243±0.445	9.494±3.336	8.231±1.532	0.167	0.108
C20:0	0.208±0.031	0.225±0.030	0.789±0.111	0.828±0.108	0.284	0.598
C18:3n6	0.025±0.006	0.024±0.005	0.094±0.026	0.091±0.018	0.978	0.712
C20:1	0.055±0.011	0.051±0.011	0.208±0.046	0.187±0.033	0.211	**0.083**
C18:3n3	0.326±0.064	0.317±0.063	1.235±0.240	1.167±0.226	0.514	0.301
CLA cis-9, trans-11/ trans-9, cis-12	0.438±0.090	0.449±0.083	1.655±0.322	1.653±0.291	0.556	0.785
CLA Trans-10, cis-12	0.008±0.003	0.008±0.002	0.029±0.012	0.031±0.009	0.351	0.477
CLA isomer 1	0.007±0.005	0.006±0.003	0.026±0.019	0.023±0.012	0.752	0.667
CLA isomer 3	0.071±0.025	0.071±0.016	0.269±0.100	0.262±0.060	0.864	0.828
C20:2	0.022±0.005	0.021±0.004	0.083±0.022	0.079±0.013	0.782	0.479
C22:0	0.020±0.004	0.025±0.006	0.074±0.015	0.091±0.024	**0.026**	**0.046**
C20:4n6	0.047±0.015	0.043±0.016	0.178±0.057	0.157±0.059	0.381	0.182
UIP	3.971±0.564	4.194±0.349	15.341±1.528	15.437±0.946	0.207	0.628
Sums and ratios						
SFA	62.447±4.455	62.478±2.653	235.414±21.133	229.292±19.621	0.975	0.394
MUFA	30.554±3.689	30.543±2.315	115.928±14.867	112.547±8.130	0.985	0.425
PUFA	3.028±0.912	2.785±0.446	28.512±4.363	27.237±2.456	0.208	0.197
USF	33.582±4.203	33.328±2.504	144.440±17.921	139.784±9.173	0.827	0.322

Table 4.3.28 continued…

SFA / UFA	1.903±0.372	1.891±0.231	1.664±0.322	1.649±0.201	0.894	0.837
MUFA/SFA	0.496±0.096	0.491±0.057	0.499±0.098	0.495±0.058	0.874	0.874
PUFA/SFA	0.049±0.018	0.045±0.008	0.123±0.026	0.120±0.017	0.242	0.599
CLA	0.523±0.105	0.535±0.094	7.906±1.524	7.449±1.123	0.57	0.284
n-3 PUFA	0.326±0.064	0.317±0.063	16.865±5.899	15.621±3.272	0.514	0.461
n-6 PUFA	2.595±0.854	2.334±0.444	96.378±12.184	94.281±7.807	0.172	0.588
n-6/n-3 ratio	7.906±1.524	7.471±1.133	6.068±1.342	6.301±1.500	0.3	0.729
Cis FA	29.041±3.526	28.959±2.466	110.213±13.752	106.755±8.248	0.923	0.385
Trans FA	4.978±1.477	4.792±0.914	18.936±6.019	17.679±3.505	0.706	0.482
Trans/cis ratio	0.172±0.045	0.167±0.035	0.172±0.045	0.167±0.035	0.785	0.782
Total FA	-	-	379.854±17.908	369.076±20.228	-	0.653

Table 4.3.29: Means (± SD) for the enzyme and health indices of perirenal fat for the main effect of weight from the percent of detected lipid (w/w%) and from the gravimetric concentration (mg of fatty acid per g of perirenal fat sample)

	%		mg/g		P-value	
	Heavy	Light	Heavy	Light	%	mg/g
Replicates (n)	17	15	17	15		
Enzyme indices						
Elongase	0.728±0.017	0.746±0.021	0.728±0.017	0.746±0.021	**0.027**	**0.027**
Delta 9 desaturase (C14+C16+C18)	29.813±3.976	29.915±2.735=4	29.813±3.976	29.915±2.734	0.931	0.931
Delta 9 desaturase C14	1.096±0.330	1.119±0.315	1.096±0.330	1.119±0.315	0.78	0.781
Delta 9 desaturase C16	2.779±0.514	3.031±0.476	2.779±0.514	3.031±0.476	0.155	0.155
Delta 9 desaturase C18	41.271±5.022	40.376±3.665	41.271±5.022	40.376±3.665	0.654	0.654
Delta 9 desaturase RA	9.337±1.962	9.723±1.577	9.337±1.962	9.723±1.577	0.504	0.504
Health indices						
AI	1.024±0.201	0.952±0.143	1.024±0.201	0.952±0.143	0.256	0.257
TI	3.473±0.691	3.455±0.436	3.493±0.201	3.476±0.442	0.908	0.915
DFA	68.868±1.816	70.197±1.966	278.4±14.643	276.116±16.807	**0.095**	0.592
h	32.874±4.127	32.608±2.430	141.755±17.624	137.139±9.066	0.81	0.316
H	24.747±2.116	23.156±2.092	93.950±9.409	85.465±9.172	**0.051**	**0.014**
h/H	1.347±0.264	1.422±0.194	1.530±0.286	1.624±0.219	0.408	0.325

f) Interaction between tannin and weight in PRF

There were no interactions between tannin and weight in PRF (Table 7.1.5 and Table 7.1.11).

g) Interaction between NPN source and weight in PRF

The interaction between NPN source and weight on PRF is presented in Table 7.1.5 (percent) and 7.1.11 (concentration). The NPN source and weight interacted for the percent and concentration of C20:1 ($P<0.05$). However, this fatty acid is present in small concentrations in PRF.

4.3.6 Interaction between weight and sex

Despite lambs slaughtered at different weights based on their sex, the weight at which they were slaughtered meant that there were not very many interactions between these two factors (see appendix). However, there was an interaction between weight and sex for the concentration of C24:1 in IMF ($P<0.01$; Table 7.1.7), C18:1n11c in SCF (Table 7.1.9) and the PUFA/SFA ratio in PRF (Table 7.1.11; $P<0.05$).

4.3.7 Conclusion

- *Calcium nitrate's effect on the percent and concentation of fatty acids:*
  - *The effect of NPN source on the percent of IMF*

As a percent of detected lipid, calcium nitrate improved the fatty acid profile and its indices. Atherogentic (AI), thrombogenic (TI) and hypercholesterolaemic (H) fatty acids decreased upon supplementation, and the hypocholestrolaemic/ hypocholesterolaemic ratio (h/H) and PUFA/SFA (P/S) ratios also improved. It also improved the fatty acid profile by reducing SFA, particularly in the diets supplemented with tannin. In addition, it showed the potential to reduce unhealthy C16:0 fatty acids. Monounsaturated fatty acids (MUFAs) were also higher with the increase in C18:1n9c due to higher delta 9 desaturase activity on C18:0. C18:1n9c is not a harmful fatty acid, hence its place in the desirable fatty acid (DFA) index. Total PUFA did not significantly increase and neither did its individual PUFAs.

  - *The effect of NPN source on the concentration of fatty acids in IMF*

As a concentration the calcium nitrate-based diet improved the fatty acid profile by reducing the AI and TI indices and increasing the h/H ratio. It also showed a tendency to reduce SFA. This is likely largely a result of a tendency for lower C18:0 in the calcium nitrate-based diet. This may have been because of an increase in delta 9 desaturase activity on C18:0. This in turn improved the MUFA/SFA ratio, particularly when it was used in the diets containing tannin. PUFA and its major individual fatty

acids were not significantly affected by calcium nitrate. However, it did significantly improve C20:4n6 and CLA cis10, cis12, particularly between the urea-based diet with tannin and the calcium nitrate-based diet without tannin.

- *The effect of NPN source on the percent and concentration of detected fatty acid in SCF*

Calcium nitrate had no significant effect on the percent of SCF besides decreasing the delta 9 desaturase RA indices. Calcium nitrate had no significant effect on SCF apart from an increase in C20:2.

- *The effect of NPN source on the percent of detected fatty acid in PRF*

There were no major effects on the nutrirional value of the fatty acid profile of PRF when lambs were fed a calcium nitrate-based diet. The SFA, MUFA, PUFA, P/S ratio and health indices did not significantly improve or worsen. Of the individual fatty acids, it increased vaccenic acid, which increased total trans fatty acids in peri-renal fat. This was particularly evident between the urea-based diet without tannin and the calcium nitrate-based diet with tannin. However, vaccenic acid is not a health concern as it has many beneficial properties. Although PUFA was not affected, it did increase C18:2n6t and CLA isomer 1 and 3.

- *The effect of NPN source on the concentration of fatty acid in PRF*

Calcium nitrate had a larger effect on PRF as a concentration. It improved the DFA and h indices, increased the MUFA profile, and showed a tendency for an improved PUFA concentration. This resulted in a higher UFA profile with a significant difference between the urea-based diet without tannin and the calcium nitrate-base diet with tannin. Like the percent, vaccenic acid also increased, thus raising the trans fatty acid concentration. The largest difference was between the urea-based diet without tannin and the calcium nitrate-based diet with tannin. Of the PUFAs, C18:2n6t, CLA and all its isomers increased in the calcium nitrate-based diet. This is advantageous, as CLA has many health benefits. Total cis fatty acid was higher in the calcium nitrate-based diet. Calcium nitrate significantly increased the fatty acid concentration of PRF.

- *The effect of NPN source on rumen fluid*

Non-protein nitrogen source did not significantly affect the SFA, MUFA or PUFA composition. However, it showed a tendency to increase MUFA through an increase in C18:1 fatty acids. The fatty acid profile of rumen fluid of lambs on the calcium nitrate-based diet may have been the reason for an increase in vaccenic acid in PRF, total trans fatty acids in PRF, a decrease in C13:0 in IMF and a tendency for reduced trans/cis ratio in PRF. A tendency for increased MUFA in the calcium supplemented lambs rumen fluid may have contributed to higher MUFA in IMF and PRF. An increase

in C18:1n9t, C20:3n3 and C12:0, and a decrease in CLA trans-10, cis-12 and UFA was not reciprocated in the fatty acid depot sites. Besides an increase in the VA/LNA ratio, the fatty acid profile of rumen fluid suggests the microbial population was mostly unaffected by the use of calcium nitrate as an NPN source. The ratios show that the biohydrogenation patterns of C18:2n6c and the saturation of oleic acid to stearic acid were unaffected by calcium nitrate.

- *Tannins effect on the percent and concentation of fatty acids:*
  - *The effect of tannin on the percent of detected fatty acids in IMF*

Tannin did not significantly improve or worsen the health indices, SFA, MUFA or PUFA but it improved the PUFA/SFA ratio. The total amount of trans fatty acid and the trans/cis ratio increased significantly. This was because of an increase in vaccenic acid, which is a health promoting trans fatty acid. Although the total amount of PUFA did not change significantly, individual fatty acids such as C18:2n6t increased and C20:5n2, C22:6n3 and C20:2 decreased.

  - *The effect of tannin on the concentration of fatty acids in IMF*

Tannin did not have a significant effect on the health indices, SFA, MUFA or PUFA/SFA ratio. It tended to increase PUFA with higher C18:2n6c, C18:2n6t and CLA cis-9, trans-11 + CLA trans-9, cis-12. Although the total trans fatty acids and trans/cis ratio was higher, it was because of an increased vaccenic acid concentration. Therefore, it would not pose a threat to human health upon consumption.

  - **T***he effect of tannin on the percent of detected fatty acids in SCF*

Tannin did not change the health indices of subcutaneous fat, SFA or MUFA but it did increase the concentration of PUFA due to an increase in C18:2n6c and C18:3n3 fatty acids. This also improved the P/S ratio. This was not a result of changes in biohydrogenation patterns. The increase in C18:2n6c increased the total n-6 fatty acid percent. Tannin did not affect the n-6/n-3 ratio. The fatty acid profile was also improved by an increase in CLA trans-10, cis-12. Tannin also showed possible potential for reducing C16:0 through a tendency to reduce it in SCF. This is advantageous due to its association with coronary heart disease.

  - *The effect of tannin on the concentration of fatty acids in SCF*

Tannin had no effect on the SFA and PUFA profile, but it did decrease the concentration of MUFA and improved the fatty acid profile through a higher PUFA/SFA ratio. Tannin improved the health of SCF by reducing the cholesterol producing fatty acids in H. Tannin reduced C16:0, particularly between the urea-based diet with tannin and the calcium nitrate-based diet without tannin. However, it also

decreased DFA and h (the fatty acids that do not produce cholesterol). The AI and TI indices as well as h/H ratio were not improved or made worse through the supplementation of tannin. Total cis fatty acid decreased when tannin was supplemented to the calcium nitrate-based diet as a result of a decrease in C18:1n9c. C16:1 also decreased. Although it did not affect the total PUFA concentration, C18:2n6t and CLA isomer 1 decreased. A decrease in these fatty acids and indices may have been a result of a decrease in the amount of fat relative to water in the SCF sample.

- *The effect of tannin on the percent of detected fatty acids in PRF*

Tannin improved the fatty acid profile of SCF by increasing PUFA, PUFA/SFA ratio, and improving the DFA and h indices. The PUFA percent increased in the urea-based diet. This was because the total amount of n-6 fatty acids, C18:2n6c and C20:4n6 also increased in the urea-based diet when tannin was supplemented. C18:3n6 and C18:3n3 were also improved in the diets containing tannin. Tannin tended to increase the vaccenic acid percent so that the urea-based diet without tannin had a lower percent than the calcium nitrate-based diet with tannin. C18:1n11c was also significantly higher when tannin was supplemented.

- *The effect of tannin on the concentration of fatty acids in PRF*

Tannin improved the fatty acid profile of peri-renal fat by increasing the PUFA concentration, particularly in the urea-based diet. It also increased the DFA and h indices. Hypocholestrolaemic fatty acids (h) were only significant between the urea-based diet without tannin and the calcium nitrate-based diet with tannin. n-6 fatty acids were higher because of an increased C18:2n6c, C20:4n6 and C18:3n6 fatty acids. C18:3n3 also increased with tannin supplementation, particularly in the urea-based diet. However, the rumen fluid results suggest that the C18:2n6c biohydrogenation pathway was not affected. Therefore, there is another reason for its increase. The concentration of n-3 fatty acids were less in rumen fluid, possibly as a result of an increase in the VA/LNA ratio. Although total SFA and MUFAs were unaffected by tannin supplementation, C18:1n11c increased.

- *The effect of tannin on rumen fluid*

Tannin tended to decrease the SFA and MUFA percent, but had no effect on PUFA. This may have been partially responsible for the decrease in SFA and MUFA in SCF. A decrease in C18:1n9c in SCF, C24:1 in IMF, and CLA trans-10, cis-12 in SCF may have been as a result of a decrease in these fatty acids in the rumen fluid in the tannin supplemented diets. A decrease in total cis fatty acids, C20:3n3, total n-3 fatty acids and C12, C13, C17, C14, C20, C23 and C24 saturated fatty acids in rumen fluid were not reciprocated in the fatty acid depot sites. The biohydrogenation ratios suggest that rumen microbes in the C18:2n6c pathway and the saturated of C18:1n9c to C18:0 were unaffected by tannin.

❖ *The effect of sex on the percent and concentration of fatty acids:*

- Effect of sex on fatty acid composition

The fatty acid composition of IMF, SCF and PRF of Dohne Merino lambs were affected by sex. It affected the SFA, MUFA and PUFA profiles, individual fatty acids, ratios, health indices and enzymes. However, the internal fatty acid depot, PRF, had the least differences between ewe and ram lambs. All fatty acid depots sites consistently showed that ram lamb fat was healthier due to less C14:0, C16:0, decreased fatty acids associated with AI and H indices as well as increased PUFA/SFA ratio regardless of whether the w/w% or mg/g was analysed. There were few instances where calcium nitrate or tannin affected the outcome of the effect of sex.

- *Effect of sex on the percent of detected lipid IMF*

Ram lambs had a fatty acid profile that was healthier to the consumer than that of ewes. It tended to lower SFA and was significantly higher in PUFA which improved the PUFA/SFA ratio. The n-3 and n-6 fatty acids as well as C20:2 were also higher in rams but the n-3/n-6 ratio was unaffected. Eicosapentaenoic acid (C20:5n3), a product of the n-3 series, was highest in rams not fed tannin. The AI, h, H and h/H indices were also better. However, rams had a lower DFA index as a result of decreased C18:0. There was great improvement in the saturated fatty acid profile with lower C14:0 and C16:0 fatty acids in rams. Elongase activity was highest in rams particularly when there was no tannin in the diet. Tannin reduced the difference between the sexes. Although total MUFA was not affected, there was a decrease in C14:1, C16:1, C20:1 and C24:1 fatty acids in rams. Rams decreased delta 9 desaturase activity on C18:0. Calcium nitrate did not cause any differences between the sexes, besides on CLA trans-10, cis-12.

- *Effect of sex on the concentration of fatty acid in IMF*

Ram lambs had less intramuscular fat than ewe lambs which may be the reason for many of the lower significant fatty acids and parameters. For example, the major contributing fatty acids such as C16:0, C18:0, C18:1n9c and C18:1n11t were all lower in rams. Saturated fatty acid and MUFA were also lower. The total polyunsaturated fatty acid was not affected, but the PUFA/SFA ratio was better in rams. Due to a decrease in C18:1n11t, the total amount of trans fat was also lower. The concentration of cis fatty acids were also lower due to decreased C18:1n9c. Therefore, the trans/cis ratio was not affected. AI and h were better in rams, but DFA, h and h/H were better in ewe lambs. The concentration of CLA and its isomers were also better in ewe lambs. However, CLA isomer 1 was better in rams when lambs were fed tannin. The C20:2 fatty acid decreased more in rams when the lambs were on the calcium nitrate-based diet. Delta-9 desaturase C18 was higher in ewes and elongase was higher in rams.

Elongase was higher in rams than ewes particularly in the diets not containing tannin. Therefore, tannin reduced the difference.

- *Effect of sex on the percent of detected fatty acid in SCF*

The subcutaneous fat of rams was better than that of ewes. The AI, TI, h, H and h/H indices were all better in rams. SFA was also lower, MUFA higher and there was a tendency for PUFA to be higher as well. A tendency for an increase in PUFA was as a result of a tendency for an increased C18:2n6c. This meant the PUFA/SFA ratio was also better in rams. The atherogenic, thrombogenic and hypercholesterolaemic fatty acid, C14:0, decreased significantly in rams thereby improving the fatty acid profile further. The significant decrease in SFA was largely because of a decrease in C18:0. An increase in C18:0 in ewes is not a concern as C18:0 is a neutral fatty acid. Of the CLA isomers, CLA trans-10, cis-12 and CLA isomer 3 were also higher in rams. The amount of cis fatty acid was also higher in rams. Sex effected the enzymes involved in *de novo* fatty acid synthesis. The activity of delta 9 desaturase on C14, C16 and C18 SFAs was greater in rams. This would have contributed to the overall decrease in SFAs. Sex was influenced by the NPN source for several of the sums, ratios and desaturase indices due its influence on C18:1n9c. In the urea-based diet ram lambs had a higher percent, and in the calcium nitrate-based diet ewes had a higher percent. This influenced MUFA, UFA, SFA/UFA, MUFA/SFA, cis FA, delta 9 desaturase C18 and h index. Palmitoleic acid (C16:1) was also higher in ram lambs when lambs were on the urea-based diet, but lower in rams when lambs were fed the calcium nitrate-based diet.

- *Effect of sex on the concentration of fatty acid in SCF*

Ram lamb subcutaneous fat had less fat and more water. This led to less total SFA and MUFA. C12:0, C14:0, C16:0 and C18:0 decreased in ram lambs. The C14:0 and C16:0 fatty acids are atherogenic, thrombogenic and hypercholesterolaemic. Therefore, there was also an improved AI, TI, H and h/H indices. DFA and h were better in ewe lambs. The PUFA/SFA ratio was also significantly better in rams. On the calcium nitrate-based diet, C18:1n9c was higher in ewes. On the urea-based diet it was higher in rams. This led to a pronounced difference in MUFA between ewes and rams on the calcium nitrate-based diet. This also caused interactions for total cis fatty acid. Arachidonic acid (C20:4n6), a product of the n-6 series, was higher in ewe lambs but CLA trans-10, cis-12 was higher in ram lambs. Enzyme indices suggested fatty acids could be altered by the sex of the lamb. Delta 9 desaturase and activity at C14, C16 and C18 SFAs were significantly higher in rams. It was much higher in rams when lambs were fed a urea-based diet. The ewes and lambs differed much less within the calcium nitrate-based diet and were within the urea-based diet values.

- *Effect of sex on the percent of detected fatty acid in PRF*

Ram lambs had a better internal fatty acid composition with an improved PUFA percent, PUFA/SFA ratio, decreased AI and H, and increased DFA and h/H indices. Linoleic acid (C18:2n6c) increased the total n-6 fatty acids in rams. However, C18:2n6t and C20:4n6 (present as a smaller percent) were lower. Although total SFA was not affected, C14:0 and C16:0 fatty acids were lower in the SFA profile of ram lambs. The longer chain C20 and C22:0 fatty acids were higher in rams. The decrease in C14:0 and C16:0 may have been because of an increase in elongase activity. There were no significant interactions between tannin and sex.

- *Effect of sex on the concentration of fatty acid in PRF*

The concentration of fatty acids in PRF were affected by sex in a very similar way to its effect on the percent of detected lipid. Rams had a more desirable PUFA concentration, PUFA/SFA ratio, AI, DFA, H and h/H indices. Although total SFA was not affected, C14:0 and C16:0 decreased in ram lambs and C20:0 and C22:0 increased in ram lambs. This may have been because of a significant increase of elongase activity in rams. Of the PUFAs, C18:2n6c increased and C18:2n6t and C20:4n6 decreased in ram lambs.

❖ *The effect of weight on the percent and concentration of fatty acids*

- The effect of weight on the fatty acid composition

Weight did not affect the healthfulness of the fatty acid profile. There were no changes in the SFA, MUFA, PUFA or UFA fractions besides an increase in the concentration of UFA in heavier lambs. All fatty acid depots indicated a potential (significant or tendency) for reduced C10:0 and C16:0 fatty acids in lighter carcasses as a percent and/or concentration, with the latter a result of increased elongase activity.

- *Effect of weight as a percent of detected fatty acid in IMF*

The weight at which lambs were slaughtered did not affect he fatty acid profile, ratios or indices. However, the percent of C10:0 and C18:0 fatty acids were significantly higher in heavier lambs. Interactions between sex and tannin were identified for C20:0 and C20:1 present as a small percent of IMF. Therefore, it did not have a large effect on the fatty acid profile. An interaction between NPN source and weight affected the C23:0 and C24:1 fatty acids.

- *Effect of weight on the concentration of fatty acid in IMF*

Weight did not significantly affect the health of the fatty acid profile as a concentration. However, C24:1 and elongase were affected by the interaction between tannin and weight. In the diet containing

tannin, C18:3n3 and DFA were higher in heavier lambs but when no tannin was fed it was higher in lighter lambs.

- *Effect of weight as a percent of detected fatty acid in SCF*

Weight did not significantly affect the fatty acid composition and health values from the percent of detected fatty acids in SCF. The only exception was an increase in CLA isomer 1 and elongase activity in lighter sheep. On the diet not containing tannin C18:0, C18:3n3 and DFA were higher in rams than ewes. However, it was higher in rams when lambs were not supplemented with tannin. In the calcium nitrate-based diet C18:2n6t increased to a greater extent in lighter lambs.

- Effect of weight on the concentration of fatty acid in SCF

Weight did little affect the fatty acid concentrations and health values in SCF. It only increased CLA isomer 1. In the diet containing tannin the percent of C18:0, C18:3n3, h index and total fatty acids were higher in heavier lambs. The opposite was true when tannin was not supplied to the diet. The difference in CLA isomer 1 was also greater in the calcium nitrate-based diet where lighter lambs had a higher concentration. Linoleleidic acid (C18:2n6t) was highest in the heavy lambs on the urea-based diet.

- *Effect of weight as a percent of detected fatty acid in PRF*

Weight did not have many effects on the fatty acid health parameters. Lighter lambs had less C10:0 and C16:0 fatty acids. The latter because of increased elongase activity. The longer chain C22:0 increased. This also resulted in a lower H index, thereby improving the cholesterol concentration. There was no interaction between tannin and weight. However, within the calcium nitrate-based diet, lighter lambs had less C20:1 and C14:1.

- *Effect of weight on the concentration of fatty acid in PRF*

The C10:0, C16:0, C22:0 fatty acids and H fatty acid index were affected in the same way as the percent of detected lipid. However, elongase was not significantly affected. The C14:1 and C20:1 fatty acids were also affected by the interaction between calcium nitrate and weight in the same way as the percent of detected fatty acid.

4.4 The effect treatment, sex and weight of fat and muscle colour

4.4.1 Longissimus dorsi muscle colour

4.4.1.1 Colour parameters on day one and six

As indicated in Table 4.4.1, the number of days after slaughter affected redness (a*) and chroma (C*) (P<0.001) but not lightness (L*), yellowness (b*) or hue angle (h*) (P>0.05). Redness (a*) and Chroma increased redness by 7.4% and chroma by 7.6%, respectively. On both days the meat was within the optimal L* and a* range specified by Khliji *et al*. (2010) of equal to or above 34 and 9.5 respectively.

Regardless of the treatment groups, the current study experienced an unexpected increase in a* value on day six. Reports of meat redness increasing after day zero colour readings were also reported by McKenna *et al.* (2005) and Rant *et al*. (2019). Intrinsic factors such as the percent of oxygen, partial pressure, temperature and length of bloom can cause differences in colour stability (Jacob, 2020). Jakobson & Bertelsen (2000) recorded the best preservation of a* and colour stability when storing meat at 3°C with an oxygen level of between 55% and 80%; a* values increased until day four and then decreased to day zero values by day eight. It is possible for aged meat to have a more desirable red colour directly after blooming due to light scatter and a deeper penetration of oxygen (MacDougall, 1981). However, colour is not maintained long due to inherently poor colour stability in meat (MacDougall, 1981). A blooming time of between six and 24 hours will result in a 100% bloom but changes in colour stability may become a concern (Jacob, 2020). Therefore, a new cut through the *longissimus dorsi* of the rib cut on day 6, a shorter blooming time relative to most studies, zip lock packaging, storage temperature of 3°C to 5°C and optimal partial pressure may have resulted in less stress on the meat. Therefore, there was good colour stability up until day six, regardless of whether tannin or calcium nitrate was used in the TMR.

Hue angle was surprisingly low on day one and six (4.52 ±2.64 and 5.36±2.45, respectively) and was not different between days (P>0.05). This indicates that colour on both days was closest to the red end of the spectrum. Other studies have indicated a more orange or yellow hue. South African Mutton Merinos from Cloete *et al*. (2008), Chulayo *et al*. (2013) and Muller *et al*. (2019) had much higher hue values. The hue of SA Mutton Merinos slaughtered by Cloete *et al*. (2008) were in the range of 37.6. Luciano *et al*. (2009b) was in the region of 16.7 on day zero and increased to 59.6 (a more yellow hue) on day 7. Therefore, their b* values significantly increased from 3.9 to 9.8 from day zero to day seven. In the current study, yellowness (b*) increased, although not significantly, from 1.44±1.08 to 1.62±0.75 (P>0.05). A higher hue angle is correlated to an increase in metmyoglobin on the surface of meat, and therefore increased meat browning (Luciano *et al*., 2011). Therefore, the meat oxidised more slowly in the current study.

The Chroma (C), also called the saturation index, increased from 16.1 to 17.3 (P<0.05). According to MacDougall (1982) it usually decreases with age.

Table 4.4.1: The effect of day on the colour parameters of the longissimus dorsi

	Day 1	Day 6	P-values
L*	34.93±3.65	34.56±1.42	0.574
a*	16.09±1.15	17.28±0.94	**<0.001**
b*	1.44±1.08	1.62±0.75	0.536
Chroma	16.14±1.15	17.36±0.94	**<0.001**
Hue	4.52±2.64	5.36±2.45	0.237

4.4.1.2 The effect of tannin and calcium nitrate on meat colour

Meat colour is important for consumer purchasing habits and determines the freshness and wholesomeness of meat (Mancini *et al*., 2005). Luciano *et al*. (2009a) reported the benefit of tannin on improving colour and oxidative stability of meat. As indicate in Table 4.4.2, 4.4.3 and 4.4.4 the current study did not show that tannin supplementation affected meat colour regardless of whether it was sampled on day one or six (P>0.05). Table 4.4.2 and 4.4.4 indicated that the supplementation of the diet with calcium nitrate as a NPN source instead of urea significantly lowered redness (a*) (P<0.05) as an average of day 1 and 6 and on day 6. The average of both days on the chroma also differed (P<0.05; Table 4.4.2). The effect of treatment on colour parameters of the *longissimus dorsi* did not differ with sex of the lamb or its weight category (Table 4.4.6 and 4.4.8: P>0.05).

Table 4.4.2: The effect of NPN source and condensed tannin on the colour parameters of the longissimus dorsi as the mean average and STD of day 1 and 6

	Urea diet		Nitrate diet		P-value			
	None	Tannin	None	Tannin	Treat	T	N	T*N
L*	33.88±4.50	34.64±1.64	35.57±1.57	34.90±2.21	0.331	0.983	0.134	0.262
a*	17.02±1.13ab	17.10±1.13a	16.12±1.17b	16.50±1.21ab	0.038	0.439	**0.005**	0.641
b*	1.34±0.81	1.87±1.24	1.28±-0.72	1.62±0.80	0.300	**0.082**	0.565	0.625
Chroma	17.02±1.14	17.14±1.16	16.23±1.20	16.,60±1.23	0.103	0.432	**0.018**	0.742
Hue	4.69±2.75	4.99±2.50	4.48±2.43	5.58±2.67	0.648	0.291	0.680	0.569

Table 4.4.3: Means (± SD) of the effect of NPN source and condensed tannin on colour parameters of the longissimus dorsi on day 1

	Urea		Calcium nitrate		P-value			
	None	Tannin	None	Tannin	Treat	N	T	N*T
L*	33.458± 6.347	35.012± 1.856	36.443± 1.273	34.813± 2.90	0.411	0.258	0.958	0.199
a*	16.419± 0.855	16.454± 1.142	15.296± 0.906	16.186± 1.394	0.180	0.084	0.257	0.299
b*	1.351± 0.910	1.958± 1.629	1.095± 0.815	1.336± 0.729	0.526	0.334	0.353	0.542
Chroma	16.501± 0.866	16.429± 1.106	15.368± 0.981	16.257± 1.398	0.262	0.129	0.362	0.275
Hue	5.111± 3.234	4.262± 2.377	3.993± 2.733	4.696± 2.553	0.863	0.843	0.745	0.460

Table 4.4.4: Means (± SD) of the effect of NPN source and condensed tannin on colour parameters of the longissimus dorsi on day 6

	Urea diet		Nitrate diet		P-values			
	None	Tannin	None	Tannin	Treat	N	T	N*T
L*	34.3± 1.633	34.263± 1.405	34.706± 1.384	34.979± 1.416	0.666	0.247	0.851	0.793
a*	17.629± 1.085	17.743± 0.678	16.953± 0.754	16.808± 0.987	0.150	**0.029**	0.915	0.653
b*	1.322± 0.763	1.788± 0.795	1.457± 0.610	1.906± 0.806	0.374	0.689	**0.097**	0.978
Chroma	17.545± 1.181	17.850± 0.696	17.094± 0.646	16.938± 1.012	0.263	**0.072**	0.873	0.479
Hue	4.276± 2.308	5.716± 2.562	4.972± 2.163	6.458± 2.637	0.343	0.476	0.105	0.920

Table 4.4.5: Interactions between the factors of treatments (NPN source and condensed tannin), day, sex and weight as indicated by significance (P-values)

	P-values						
	N*T	N*Day	T*Day	N*MF	T*MF	N*HL	T*HL
L*	0.262	0.527	0.905	0.540	0.801	0.434	0.166
a*	0.641	0.770	0.358	0.896	0.562	0.182	0.146
b*	0.625	0.302	0.822	0.543	0.821	0.095	0.800
Chroma	0.742	0.884	0.569	0.765	0.717	0.330	0.169
Hue	0.569	0.500	0.141	0.888	0.430	0.124	0.601

a) The effect of tannin on meat colour

The effect of tannin on colour of the *longissimus dorsi* is shown in table 4.4.2 (average), 4.4.3 (day one) and 4.4.4 (day six). As depicted in Table 4.4.3, tannin did not affect any of the colour parameters on day one (P>0.05). However, Table 4.4.4 indicated that by day six there was a tendency towards an increase in yellowness (b*; P=0.097) and hue angle (h*; P=0.105). After 24 hours post-mortem (PM) and a 24-hour bloom, Luciano *et al*. (2009b) identified a higher b* and hue angle on day 7 with a more gradual increase in yellowness and hue in the tannin supplemented sheep. Therefore, the concentration of tannin may have needed to be higher or may have needed exposure to oxygen for a longer period to exhibit obvious antioxidant properties in the current study.

Hopkins *et al*. (1996) and Khliji *et al*. (2010) identified redness (a*) and lightness (L*) as the most important colour determinants for consumer satisfaction. On average, consumers identify meat as fresh when a* and L* values are equal to or above 9.5 and 34, respectively. Ninety five percent of customers are satisfied when a* is equal to or above 14.4 and L is equal to or above 44% (Khliji *et al*., 2010). In general, regardless of the treatment, the day one and six L* and a* values were within the acceptable range (Table 4.4.3 and 4.4.4). Redness on day one ranged from 15.296±0.906 to 16.454±1.142 and on day six it ranged from 16.808±0.987 to 17.629±1.085 (Table 4.4.3 and 4.4.4, respectively). Lightness ranged from 33.458±6.347 to 36.443±1.273 on day one and between 34.3±1.633 to 34.979±1.416 on day six (Table 4.4.3 and Table 4.4.4, respectively).

Table 4.4.3 and 4.4.4 indicated that when tannin was supplemented to the diet, there were no signs of change in colour stability (P>0.05). As indicated in Table 4.4.2, due to calcium nitrate decreasing a* the urea-based diet with tannin was the reddest out of all the treatments and was higher than the calcium nitrate-based diet without tannin (P<0.05). Priolo *et al*. (2000; 2002a; 2002b; 2005) and Guerreiro *et al*. (2020) also indicated no changes in redness (a*) in meat from tannin. In contrast to these researchers, Luciano *et al*. (2009a; 2009b) reported redder meat (a*) in tannin supplemented

sheep with a slower rate of decrease in its value upon ageing. The rapid change in colour in that study may be as a result of over exposure to oxygen due to the larger surface area of minced meat and a highly oxidised storage environment (packaged in 80% oxygen).

Lightness (L*) was not affected by tannin supplementation on either day one or day six post-mortem (P>0.05; Table 4.4.3 and Table 4.4.4 respectively). This contrasted with Priolo *et al*. (2002a; 2002b) and Costa *et al*. (2021) who determined that tannin increased the lightness of sheep meat. Priolo *et al*. (2000) suggested this was due to lower haemoglobin and myoglobin concentrations. Priolo & Vasta (2007) hypothesised that it was because of a decrease in microbial biosynthesis of vitamin B12 needed for the synthesis of haemoglobin. This would indicate that a higher level of tannin may be necessary to disrupt microbial biosynthesis. Differences amongst studies may also be due to the part of the carcass analysed. In comparison to other muscle groups, the *longissimus dorsi* is one of the lightest due to its good colour stability (Tschirhart-Hoelocher *et al*., 2006).

In agreement with studies that analysed meat colour between 24 and 72 hours post slaughter with 1 to 2 hours of bloom, no differences in yellowness (b*) were identified through tannin supplementation in Table 4.4.3 and 4.4.4 (P>0.05; Priolo *et al*., 2000, Priolo *et al*., 2002a; Guerreiro *et al*., 2020; Costa *et al*., 2021). However, Luciano *et al*. (2009b) reported that by day 14 of storage sheep fed the Quebracho tannin had a significantly lower b* value than concentrate fed sheep. In the current study there was a tendency for b* to be higher in tannin supplemented sheep (P=0.098; Table 4.4.4). However, the rate of increase in b* between day one and six was less in the tannin supplemented sheep (12% increase) than the unsupplemented sheep (13.65% increase). Regardless of the treatment or the day, the value of b* was low with the highest value on day one reaching 1.958±1.629 and on day six reaching 1.906±0.806 in comparison to SA Mutton Merino recorded by Chulayo *et al*. (2013) (12.2) and Muller *et al*. (2019) (9.87). Holman *et al*. (2016) recorded a relationship between lightness (L*) and b* and minimum and maximum acceptability based on the value of b*. For L* values between 35 and 50, there was an increase in acceptability for b* values as b* increased from 13 to 22, at which point it plutoed or slowly declined. Yellowness typically increases during storage due to rising levels of methaemoglobin (MacDougall, 1982). Agbeniga (2018) reported a negative correlation between proteolytic enzymes and b*. A study conducted by Kadri *et al*. (2019) reported the inhibitory activity of epicatechin against u-calpain on good binding affinity and free binding energy. Although epicatechin absorbs well through the duodenum, it would have to avoid metabolization by microbes in the rumen (Wein *et al*., 2016).

Table 4.4.3 and 4.4.4 showed that the analysis of the chroma (C*) determined no effect of tannin on colour intensity or vividness of the meat on either day one or day six of storage, respectively (P>0.05).

Of the studies mentioned above, only Costa *et al*. (2021) identified a significant decrease in C* when tannin was supplemented to the diet. The actual colour of the meat (hue) did not vary with tannin supplementation except on day six where there was a tendency for tannin supplemented diets to have a higher (and therefore redder) hue value. In agreement with the current study, Priolo *et al*. (2000; 2002a ;2002b) and Guerrier *et al*. (2020) identified no differences in hue angle (p>0.05). However, when Priolo *et al*. (2005) extended the bloom time to 24h, lightness, yellowness and hue were affected due to increased oxidation.

b) The effect of NPN source on meat colour

The structure of muscle and its myofilament lattice spacing affects light scattering and therefore paleness of meat (Hughes *et al*., 2014). Calcium dependant proteolytic enzymes are natural destructors of muscle tissue and loosen myofibrils (van Rensburg & Ogutta, 2013). The effect of calcium nitrate on colour of the *longissimus dorsi* is shown in table 4.4.2 (average), 4.4.3 (day one) and 4.4.4 (day 6). The additional calcium nitrate did not affect the lightness of meat. The lightness and redness values of the urea based TMR are in the range of Wang *et al*. (2016). However, the yellowness was a lot lower in the current study (between one and two compared to 11.85). When urea is supplied at 2.5%, in comparison to the 1% in the current study, the meat is typically darker, and redness is reduced (Wang *et al*., 2016). Hegarty *et al*. (2016) reported that calcium nitrate makes meat (but not fat) lighter with increased supplementation. Although little research has been done comparing the effects of NPN sources on the colour of subcutaneous fat and muscle, research from Hegarty *et al*. (2016) suggests that the level of supplementation effects colour. When low urea and calcium nitrate concentrations were supplied to cattle feed, the former was shown to have a significantly lighter colour in both fat and meat of cattle, but at higher concentrations it made no difference (Hegarty *et al*., 2016). Although there was no interaction between the NPN sources and day of the colour readings (Table 4.5.5), significance was only detected on day six (Table 4.4.4). Lightness (L*), b* and h* were unaffected, but redness was lower (P<0.05) and Chroma tended to be lower (P=0.072) in the calcium nitrate-based diets. Therefore, the calcium nitrate-based diet on day six may be potentially less appealing to the consumer due to the redder more vivid colour in the urea-based diet. However, the differences between the two treatments were very small in scale. Our results may be an indication of the level of supplementation as well as other extrinsic and intrinsic factors.

4.4.1.3 The effect of sex on meat colour

As depicted in Table 4.4.6 (average) and 4.4.7 (day one and six), there was an effect of sex on lamb lightness (L*), redness (a*) and Chroma (C*) on average and on day one (P<0.05). This is contradictory to research conducted by Diaz *et al*. (2003) and Tejeda *et al*. (2008). In the current study day one

colour readings identified ewe meat as darker than that of rams (P<0.05). It was also redder (a*) and had a higher C* (P<0.05). On day six the differences in lightness, redness and C* were less (P=0.108, P=0.062 and P=0.100, respectively). Ewes tended to have yellower carcasses on day six (P=0.081).

In agreement with research conducted by Sañudo *et al*. (1998) and Facciolongo *et al*. (2018) ram meat was lighter on average (P<0.006; Table 4.4.6) and on day one than ewes (P<0.05; Table 4.4.7). According to Lazzaroni (2007) ewe meat is in generally darker than rams at equal slaughter weight as a result of earlier deposition of fat (Fiems *et al*., 2000; Lazzaroni, 2007). Sabbioni *et al*. (2019) identified lightness as an earlier developing parameter in rams.

Table 4.4.6 indicated that ewe *Longissimus dorsi* was redder a* (P<0.001) with a higher saturation index (P=0.004). Although redness is typically higher in ewes, most studies have not found it to be significant (Sañudo *et al*., 1998; Craigie *et al*., 2012; Facciolongo *et al*., 2018) except for Johnson *et al*. (2005). Ledward and Shorthose (1971) suggested that it is possible due to a 10% higher haem pigment in ewes than wethers. The day of the colour reading may also have made a difference (Table 4.4.7). L*, a*, and C* were all significant on day one (P<0.05). By day 6 none of the colour parameters were significant anymore (P>0.05).

Table 4.4.6: The effect of sex on the colour parameters of the longissimus dorsi and their interactions

	Sex		P-Value	Interactions		
	F	M	MF	Treat*MF	HL*MF	Day*MF
L*	33.770±3.050	35.723±2.029	**0.006**	0.245	0.876	0.102
a*	17.087±1.150	16.285±1.132	**0.001**	0.750	0.606	0.452
b*	1.544±0.728	1.509±1.1	0.813	0.899	0.566	0.066
Chroma	17.120±1.165	16.375±1.151	**0.004**	0.849	0.907	0.467
Hue	5.158±2.389	4.713±2.745	0.402	0.514	0.769	0.111

Table 4.4.7: The effect of sex on the colour parameters of the *longissimus dorsi* on day 1 and 6

	Day 1		Day 6		P-value		
	F	M	F	M	Day 1	Day 6	Day*MF
L*	33.41±4.25	36.46±2.10	34.13±0.92	34.99±1.72	**0.024**	0.108	0.102
a*	16.57±1.18	15.60±0.91	17.60±0.89	16.97±0.91	**0.010**	**0.062**	0.452
b*	1.21±0.55	1.66±1.41	1.88±0.75	1.36±0.68	0.325	**0.081**	0.066
Chroma	16.59±1.16	15.68±0.97	17.65±0.93	17.07±0.97	**0.018**	0.100	0.467
Hue	4.19±1.99	4.84±3.20	6.13±2.41	4.58±2.30	0.558	0.118	0.111

4.4.1.4 The effect of weight on meat colour

As indicated in Table 4.4.8 (average between day one and six), Lightness (L*), yellowness (b*) and hue (h*) were affected by the weight at which sheep were slaughtered (P<0.05). As presented in Table 4.4.9 (the effect of weight on day one and day six), on day one yellowness (b*) and hue (h*) were higher in the lighter weight category (P<0.05). On day six there were no longer any differences between the heavy and light weight groups (P>0.05).

Heavier carcasses with darker meat confirm previous reports by Sañudo *et al*. (1996), Vergara & Gallego (1999), Zgur *et al*. (2003), Abdulla & Quudsieh (2009) and Mathlouthi *et al*. (2013). There are inconsistencies between studies with the other colour parameters possibly due to additional factors such as weight and diet. However, a decrease in hue on day one (P<0.01) with increased liveweight was also reported by Abdulla & Quudsieh (2009). Unlike the current study the decrease was not significant. The increase in b* in lighter carcasses was unexpected on average (P<0.05; Table 4.4.8) and on day one (P<0.05; Table 4.4.9). Santos-Silva *et al*. (2002) and Teixeira *et al*. (2005) also reported lighter and yellower carcasses when lambs were slaughtered at a lighter weight.

Table 4.4.8: The effect of weight category on the colour parameters of the longissimus dori and their interactions

	Weight		P-Value	Interactions		
	H	L	HL	Treat*HL	HL*MF	Day*HL
L*	34.13±3.18	35.44±1.99	**0.040**	0.274	0.876	0.410
a*	16.68±1.23	16.69±1.19	0.969	0.098	0.606	0.258
b*	1.29±0.69	1.79±1.09	0.045	0.198	0.566	0.054
Chroma	16.76±1.25	16.73±1.18	0.904	0.120	0.907	0.195
Hue	4.32±2.27	5.63±2.73	0.034	0.349	0.769	0.056

Table 4.4.9: The effect of weight category on the colour parameters of the longissimus dorsi on day 1 and 6

	Day 1		Day 6		P-value		
	H	L	H	L	Day 1	Day 2	Day*HL
L*	34.09±4.37	35.88±2.40	34.17±1.35	35.00±1.42	0.129	0.101	0.410
a*	15.96±1.11	16.24±1.20	17.41±0.86	17.13±1.04	0.457	0.396	0.258
b*	0.97±0.49	1.96±1.32	1.61±0.72	1.63±0.81	**0.024**	0.943	**0.054**
Chroma	15.99±1.12	16.30±1.19	17.53±0.85	17.16±1.02	0.423	0.305	0.195
Hue	3.34±1.69	5.85±2.93	5.30±2.40	5.42±2.59	**0.006**	0.882	0.056

4.4.1.5 The effect of Interactions on meat colour

As indicated in Table 4.4.5 there were no interactions between any of the factors (P>0.05). The NPN source nor the inclusion of tannin influenced the colour parameters between the different days. This contradicts Luciano *et al*. (2009b), where colour deterioration occurred over time and tannin supplementation delayed it. There was no interaction between the age of the meat and the sex of the lamb (P>0.05). Table 4.4.7 shows that by day six post-mortem ewe meat had not started decreasing in lightness values yet, however, rams had decreased by 4.2%. By day six the yellowness and hue had already started to increase by 55.37% and 46.3% respectively in ewes. Redness and chroma were increasing on day six regardless of sex. There was no interaction between the weight category and the age of the meat. There was a high tendency for yellowness (P=0.054) and hue (P=0.056) to increase at a faster rate in the heavier weight category. On day one the heavier carcasses were less yellow than the lighter carcasses. By day six the differences were insignificant (P>0.05). There was no interaction between weight and sex (P>0.05). Differences between studies may be due to slaughter weight. Sabbioni *et al*. (2019) determined that differences in a*, b* and C* between sexes were slaughter weight specific. Although a* and C* were also higher in this study when they were slaughtered below 50.7kg, none of the colour values were significant (P>0.05; Table 4.4.8 and Table 4.4.9). However, at heavier weights redness, yellowness and Chroma were greater in rams (P>0.05). Therefore, the weight at slaughter might have been the reason for differences in the sex interaction between studies.

4.4.2 Subcutaneous Fat Colour

The effect of treatment, sex and weight affected SCF differently to muscle. The average subcutaneous fat is depicted in Table 4.4.10. The lightness, redness, yellowness and hue of subcutaneous fat were 67.5, 3.7, 6.7, 7.9 and 61.2, respectively. This is consistent with results of suckling lambs reported by Diaz *et al*. (2003). The low yellow values were consistent with the feeding system. When grazed on pasture, fat is typically yellower with a higher hue angle and Chroma (Priolo *et al*., 2002b) due to carotenoid pigmentation (Kirton *et al*.,1975).

4.4.2.1 The effect of treatment on subcutaneous fat colour

There are minimal studies researching the effect of tannin and calcium nitrate on subcutaneous fat colour since it can be removed. Table 4.4.10 shows that subcutaneous fat colour was not affected by tannin supplied to the TMR (P<0.05). This is consistent with a report from Rivaroli *et al*. (2019) who indicated no differences when tannin-rich sainfoin pellets were supplemented to lambs grazing alfalfa pastures. The calcium nitrate-based diet did, however, increase the hue (P<0.05). Although not

significant, the supplementation of the urea-based diet with tannin resulted in an 11% reduction in hue.

Table 4.4.10: The effect of NPN source and condensed tannin on the colour parameters of subcutaneous fat

	Urea		CaN		P-value			
	None	Tannin	None	Tannin	Treat	T	N	T*N
L*	66.57±2.59	67.36±1.91	67.92±2.20	68.27±2.97	0.530	0.601	0.193	0.733
a*	4.65±2.02	3.74±2.42	3.49±1.61	3.22±1.40	0.455	0.433	0.207	0.588
b*	8.09±1.57	5.96±1.70	6.34±2.42	6.41±1.53	0.221	0.197	0.394	0.161
Chroma	9.42±2.27	7.20±2.57	7.61±2.50	7.27±1.77	0.250	0.194	0.292	0.262
Hue	61.18±6.97	55.11±13.25	63.79±7.88	64.60±8.15	0.086	0.345	**0.039**	0.215

4.4.2.2 The effect of sex on subcutaneous fat colour

As shown in Table 4.4.11, the subcutaneous fat of rams was 58% redder (a*) than ewes (P<0.023) and the hue was higher in ram than ewe lambs (P<0.002). This contrasts Scerra *et al*. (2001) who reported no significant change in redness between sexes. In contrast to the current study, they did report lighter fat in ewes.

Table 4.4.11: The effect of sex and weight on the colour parameters of subcutaneous fat

	Sex		Weight		P-Values	
	F	M	H	L	MF	HL
L*	67.383±2.20	67.682±2.685	67.59±2.67	67.46±2.19	0.821	0.844
a*	4.618±2.204	2.930±0.997	4.11±2.27	3.39±1.32	**0.027**	0.375
b*	6.796±2.280	6.611±1.592	6.58±2.06	6.83±1.85	0.823	0.706
Chroma	8.459±2.850	7.295±1.667	8.02±2.70	7.72±2.01	0.239	0.846
Hue	56.488±9.901	65.851±7.027	59.33±10.91	63.26±7.95	**0.003**	0.173

4.4.2.3 The effect of weight on subcutaneous fat colour

As show in Table 4.4.11, the subcutaneous fat colour parameters were not affected by the slaughter weight of lambs (P>0.05).

4.4.3 The effect of interactions on subcutaneous fat colour

As indicated in Table 4.4.12 there were no significant interactions between the different factors (P>0.05).

Table 4.4.12: Interactions between treatment, weight and sex expressed as P-values

	Interactions		
	Treat*MF	Treat*HL	HL*MF
L*	0.358	0.526	0.959
a*	0.843	0.570	0.562
b*	0.520	0.884	0.451
Chroma	0.499	0.795	0.325
Hue	0.423	0.097	0.886

4.4.4 Conclusion

In general, there was good colour stability between day one and six regardless of the treatment, sex or weight. This was possibly a result of the longissimus dorsi inherently having good colour stability as well as cutting and storage conditions. The a* and L* values were within a desirable consumer acceptability range regardless of the treatment, sex and weight at which the lambs were slaughtered. At the current dose, *Acacia mearnsii* tannin did not affect the colour stability of meat over the six days post slaughter. It therefore did not show antioxidant properties through its colour. However, by day 6 calcium nitrate had a higher redness value a* within the longissimus dorsi than the urea-based diet. Other factors that played a role in the colour of the longissimus dorsi were sex, weight and day at which the readings were taken. Lightness was higher in rams but redness and chroma were higher in ewe lambs. Differences between sex decreased by day six. Yellowness (b*) and h* were higher in the heavier lambs on day one, but by day six they were no longer significantly different. Redness (a*) and C* were higher on day six than day one. This was likely a result of storage conditions, the way the *longissimus dorsi* was cut, as well as the shorter length of bloom in comparison to other studies.

4.5 Principle Component Analysis for the *longissimus dorsi* muscle and subcutaneous fat

The results of the principal component analysis (PCA) of carcass characteristics and fatty acids are presented on Figure 4.5.1 and Figure 4.5.2 (intramuscular fat) and Figure 4.5.3 (subcutaneous fat). The principle component scores are presented in 4.5.3 (intramuscular fat) and 4.5.5 (subcutaneous fat) and principle component loadings in 4.5.4 (intramuscular fat) and 4.5.6 (subcutaneous fat). The PCA was only done for intramuscular fat (IMF) and subcutaneous fat (SCF), the most important fatty acid depots when it comes to changes in sex and nutrition. The loadings with the highest scores are discussed. The objective of this was to get an overall impression of the results from a different angle.

Table 4.5.1 indicates that 81% of the total variation in IMF could be explained by PCA 1, 2 and 3. Figure 4.5.2 and table 4.5.3 showed that heavy ewes on the urea-based diet without tannin (UOHF) and light ewes fed urea with no tannin (UOLF) were directly associated with PC1 values, and light rams on the calcium nitrate-based diet without tannin (NOLM) and heavy rams fed a urea-based diet with tannin (U1HM) were associated with inverse PC1 values. Therefore, table 4.5.4 indicated that there was a direct association with muscle percent and liveweight and an inverse relationship with fat percent. Therefore, higher PC1 values were associated with rams and lower values associated with ewes. This suggests that rams had a greater liveweight, percent of muscle and lower fat content than ewes. This confirms preveous results in table 4.1.3.

Table 4.5.4 showed that principle component 2 was directly correlated with fat%, eye muscle area (EMA) and liveweight but inversely correlated with ether extract (EE%), temperature and muscle percent. It was also inveresely correlated with light ewes fed tannin on the urea-based diet (U1LF) and heavy ewes fed tannin on the urea-based diet (U1HF) and directly correlated with light rams on the calcium nitrate-based diet without tannin (NOLM) and and heavy rams on the calcium nitrate-based diet without tannin (NOHM; Table 4.5.3). Therefore sex, nitrate supplementation and tannin in these four treatments affected fat percent, EE, temperature and muscle %.

Figure 4.5.2 and Table 4.5.4 indicated that PC3 was directly associated with delta 9 desaturase and temperature, but inversely correlated associated with EMA, SFA, and liveweight. The replacement of urea with calcium nitrate is directly associated with PC1 (Table 4.5.3; Figure 4.5.2) and therefore replacing urea with calcium nitrate will result in lower SFA, EMA and an increase delta 9 desaturase in muscle. This was particularly evident in heavy ewes on the calcium nitrate-based diet with tannin (N1HF) and light ewes on a calcium nitrate-based diet with tannins (N1LF) and negatively correlated with heavy ewes on a urea-based diet without tannins (UOHF) and light rams on a urea-based diet with tannins (U1LM). Therefore, calcium nitrate increased delta 9 desaturase and temperature, and deceased liveweight, EMA, and SFAs. The oppositie was true for the U1HF and U1LF.

PCA 1 and 2 made up 92% of the variance in subcutaneous fat (Figure 4.5.3 and Table 4.5.2). PCA 1 was positively correlated to the desirable fatty acid index (DFA1), SFA, MUFA, cis fatty acid, hypocholesterolaemic fatty acids and hypercholesterolaemic fatty acids (Figure 4.5.3 and Table 4.5.6). NOLF and NOHF were positively associated with PCA 1 and N1LM and U1LM were inversely associated (Figure 4.5.3 amd Table 4.5.6). Therefore, tannin and sex played a role in these parameters. PCA2 was inversely associated with SFA, and hypercholesterolaemic fatty acids but positively associated with MUFA, cis fatty acids and hypercholesterolaemic fatty acids (Figure 4.5.4 and Table 4.5.6). Therefore, light ewes on the urea-based diet without tannin (UOLF) and heavy ewes on the urea-based diet with tannin (U1HF) were positively associated with these parameters and light rams on the urea-based diet without tannin (UOLM) and heavy rams on the urea-based diet without tannin (UOHM) were inversely associated. This suggests that rams have an advantage because they have less SFA but on closer examination they have less hypocholesterolaemic fatty acids but more MUFA and Cis fatty acids. This effect was also confirmed in the MANOVA analysis.

Conclusion:

Due to the combination of factors, not all the results were confirmation of the MANOVA used prior to this analysis. However, the importance of the effect of sex was reiterated in this analysis. This analysis did give a larger insite into the combination of factors driving change in carcass characteristics.

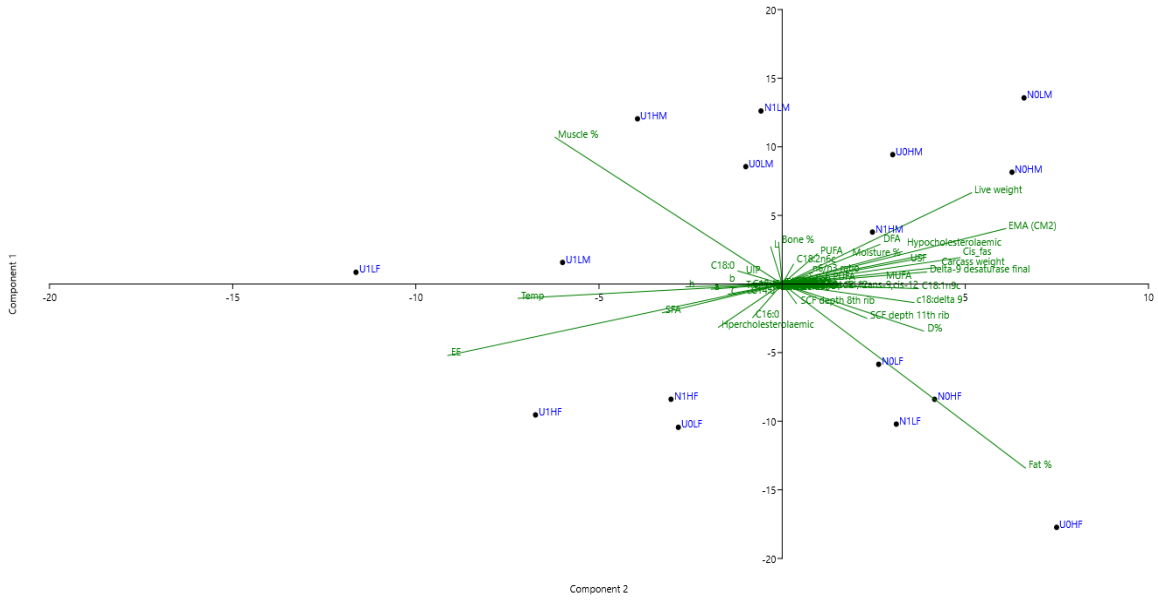

Figure 4.5.1: Projections of carcass quality and fatty acid parameters of intramuscular fat on a plane defined by PC1 and PC2

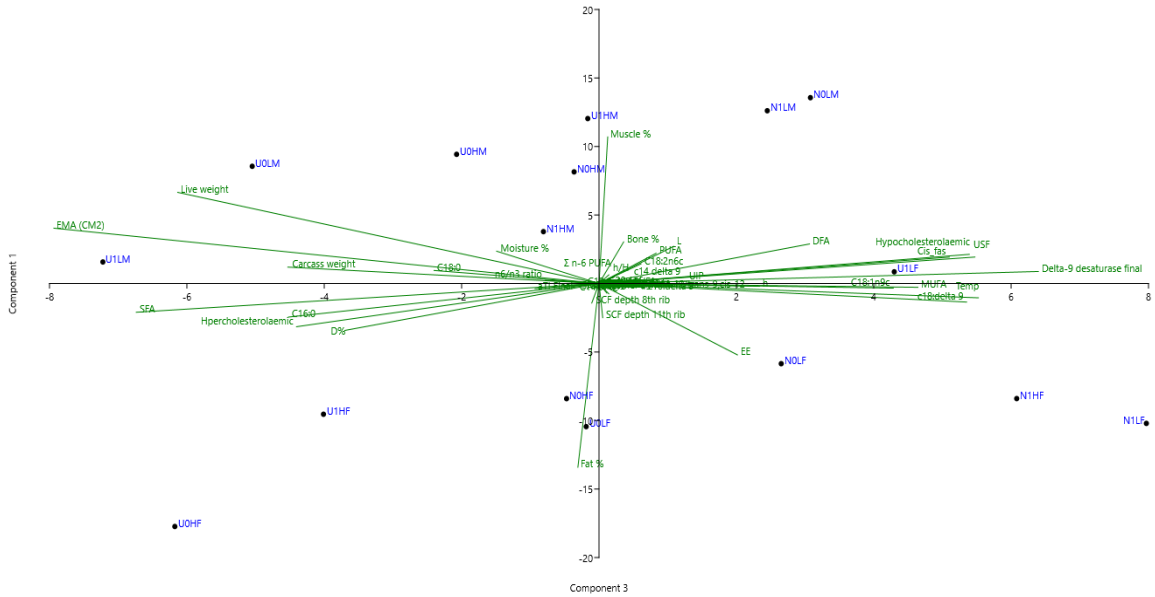

Figure 4.5.2: Projections of carcass quality and fatty acid parameters of intramuscular fat on a plane defined by PC1 and PC3

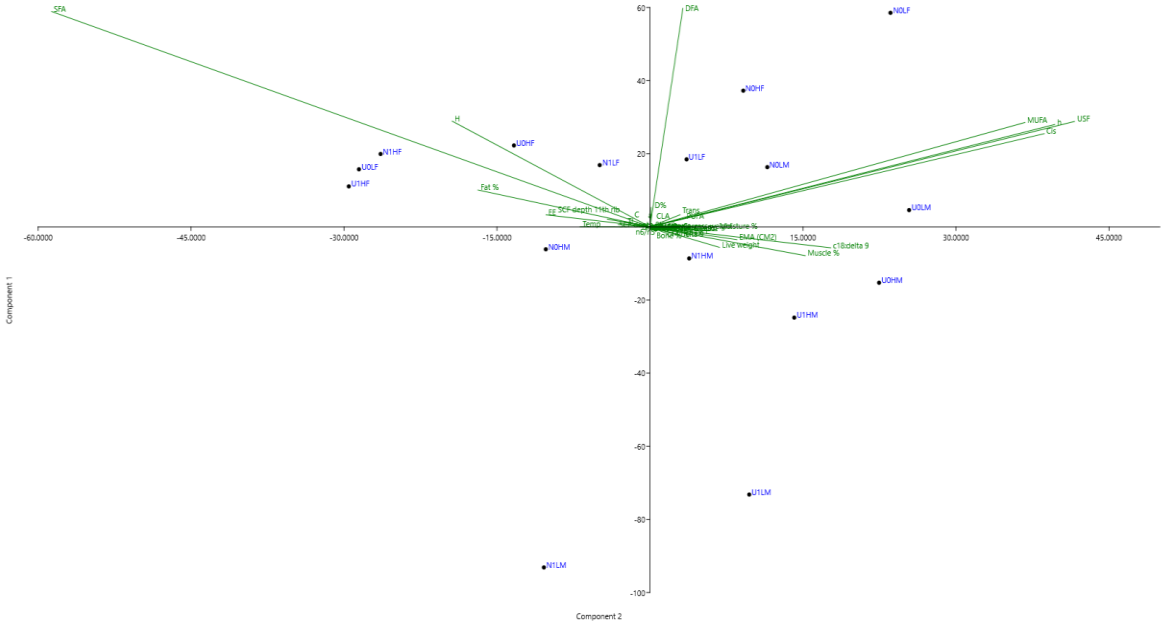

Figure 4.5.3: Projections of carcass quality and fatty acid parameters of subcutaneous fat on a plane defined by PC1 and PC2

Table 4.5.1: Eigenvalue and variance percent for intramuscular fat

PCA	Eigenvalue	% Variance
1	101.886	55.174
2	29.265	15.848
3	18.5813	10.063
Total		81.084

Table 4.5.2: Eigenvalue and variance percent for subcutaneous fat

PCA	Eigenvalue	% Variance
1	1465.96	75.014
2	336.19	17.203
Total		92.217

Table 4.5.3: Principal component scores for intramuscular fat

Treatment	Abbreviation	PC 1	PC 2	PC 3
Light ewes in the calcium nitrate-based diet without tannin	N0LF	-585	263	265
Light ewes on the calcium nitrate-based diet with tannin	N1LF	-1021	312	797
Heavy ewes on the calcium nitrate-based duet without tannin	N0HF	-840	416	-47
Heavy ewes on the calcium nitrate-based diet with tannin	N1HF	-840	-304	608
Light rams in the calcium nitrate-based diet without tannin	N0LM	1357	660	308
Light rams on the calcium nitrate-based diet with tannin	N1LM	1261	-58	245

Continuation Table 4.5.3				
Heavy rams on the calcium nitrate-based diet without tannin	N0HM	815	627	-36
Heavy rams on the calcium nitrate-based diet with tannin	N1HM	379	246	-81
Light ewes on the urea-based diet without tannin	U0LF	-1044	-284	-19
Light ewes on the urea-based diet with tannin	U1LF	86	-1163	430
Heavy ewes on the urea-based diet without tannin	U0HF	-1774	749	-618
Heavy ewes on the urea-based diet with tannin	U1HF	-954	-673	-401
Light rams on the urea-based diet without tannin	U0LM	856	-99	-505
Light rams on the urea-based diet with tannin	U1LM	157	-600	-722
Heavy rams on the urea-based diet without tannin	U0HM	943	302	-207
Heavy rams on the urea-based diet with tannin	U1HM	1204	-395	-16

Table 4.5.4: Principal component loading for intramuscular fat

	PC 1	PC 2	PC 3
Live weight	30.4	23.6	-28.0
Carcass weight	5.5	19.3	-20.7
D%	-15.6	17.6	-16.9
SCF depth 8th rib	-6.5	1.8	-0.5
SCF depth 11th rib	-11.4	10.5	0.2
EE	-23.8	-41.7	9.2
Bone %	13.9	-0.4	1.6
Muscle %	48.8	-28.3	0.6
Fat %	-61.2	30.3	-1.4
EMA (CM2)	18.4	27.9	-36.2
Temp	-4.8	-32.9	25.2
pH	0.0	-0.1	-0.4
Moisture %	10.8	15.0	-6.8
Ash	-0.1	-0.2	0.2
L*	12.4	-1.4	5.0
a*	-1.7	-8.8	-4.3
b*	-0.1	-5.4	0.3
C*	-3.4	-6.8	0.7
h*	-0.9	-12.0	10.7
C10:0	0.0	-0.1	-0.1
C12:0	-0.1	-0.5	0.0
C13:0	0.0	0.0	0.0
C14:0	-3.2	-4.2	0.5
C14:1	-0.1	-0.1	0.1
C16:0	-11.2	-3.7	-20.6
C16:1	-0.6	-0.2	0.1
C17:0	0.3	-1.0	0.5
C18:0	4.3	-5.5	-11.0
C18:1t11	0.3	-3.7	2.0
C18:1n9c	-1.5	17.0	19.6
C18:1n11c	0.9	0.2	-0.8
C18:2n6t	0.0	0.0	0.0
C18:2n6c	6.6	1.4	2.8
C20:0	0.1	0.0	-0.1
C18:3n6	0.0	0.0	0.0
C20:1	0.0	0.0	0.0
C18:3n3	0.5	0.0	0.5
CLA cis-9, trans-11/trans-9,cis-12	-0.1	-0.6	0.4
CLA cis 10, cis 12	0.0	0.0	0.0
CLA Trans-10, cis-12	0.0	0.0	0.0
CLA isomer 1	0.0	0.0	0.0

Table 4.5.4 continued			
CLA isomer 3	-0.1	-0.2	-0.6
C20:2	0.0	0.1	-0.1
C22:0	0.1	0.0	0.0
C20:3n6	0.3	0.2	0.0
C22:1n9	-0.1	-0.2	0.0
C23:0	0.0	0.0	0.0
C20:4n6	1.2	1.5	0.7
C24:0	0.1	0.1	0.0
C20:5n3	0.3	0.3	0.1
C24:1	0.1	0.1	0.0
C22:6n3	0.1	0.3	0.0
UIP	0.7	-2.8	5.8
SFA	-9.6	-14.9	-30.8
MUFA	-1.3	13.4	21.2
PUFA	10.1	4.4	3.8
USF	8.8	17.7	25.0
SFA / UFA	-0.4	-0.6	-1.1
CLA	-0.3	-0.8	-0.3
Σ n-3 PUFA	0.9	0.5	0.7
Σ n-6 PUFA	2.9	3.2	0.7
n-6/n-3 ratio	1.1	6.7	-3.6
Cis fas	8.8	22.2	23.3
Trans fas	0.0	-4.5	1.7
Trans/ cis ratio	0.0	-0.1	0.0
MUFA/SFA	0.2	0.6	1.0
Elongase final	0.1	0.1	0.2
AI	-0.6	-0.8	-0.8
TI	-0.6	-1.2	-2.4
DFA	13.2	12.2	14.0
Hypocholesterolaemic (h)	9.7	17.9	24.6
Hpercholesterolaemic (H)	-14.4	-7.9	-20.1
h/H	1.2	1.2	2.0
Delta-9 desaturase	4.0	18.0	29.2
c14 delta 9 desaturase	0.1	-0.5	2.4
c16:delta 9 desaturase	-0.7	1.0	4.8
c18:delta 9 desaturase	-6.2	16.4	24.4
RA delta 9 desaturase	0.0	0.1	0.0
PUFA/SFA	0.2	0.1	0.2

D%: Dressing percent, SCF depth of the 8th rib: subcutaneous fat depth of the 8th rib, EMA: Eye muscle area; Temp: Temperature, L*: lightness, a*: redness, b*: yellowness, C*: Chroma, h*: Hue angle, UIP: Unidentified peak, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, UFA: Unsaturated fatty acids, Σ n-3 PUFA: Sum of the n-3 polyunsaturated fatty acids, Σ n-6 PUFA; Sum of the n-6 polyunaturated fatty acids, AI: Atherogenic index, TI: Thrombogenic index, DFA: Desirable fatty acid index, h/H: ratio of hypocholesterolaemic fatty acid to hypocholesterolaemic fatty acid, RA delta 9 desaturase: Rumenic acid delta 9 desaturase, PC 1: Principle component 1, PC 2: Principle component 2, PC 3: Principle component 3.

Table 4.5.5: Principal component scores for subcutaneous fat

Light ewes in the calcium nitrate- based diet without tannin	N0LF	5854.8	2357.6
Light ewes on the calcium nitrate- based diet with tannin	N1LF	1690.4	-493.07
Heavy ewes on the calcium nitrate-based duet without tannin	N0HF	3726.2	914.23
Heavy ewes on the calcium nitrate-based diet with tannin	N1HF	1994.5	-2641.4
Light rams in the calcium nitrate-based diet without tannin	N0LM	1634.2	1149.9
Light rams on the calcium nitrate- based diet with tannin	N1LM	-9313.4	-1040.4
Heavy rams on the calcium nitrate-based diet without tannin	N0HM	-612.02	-1021.5
Heavy rams on the calcium nitrate-based diet with tannin	N1HM	-862.38	383.77
Light ewes on the urea-based diet without tannin	U0LF	1575.5	-2853.8
Light ewes on the urea-based diet with tannin	U1LF	1847.1	358.48
Heavy ewes on the urea-based diet without tannin	U0HF	2227.2	-1334
Heavy ewes on the urea-based diet with tannin	U1HF	1107.5	-2953.3
Light rams on the urea-based diet without tannin	U0LM	459.18	2540.3
Light rams on the urea-based diet with tannin	U1LM	-7320.2	973.09
Heavy rams on the urea-based diet without tannin	U0HM	-1527.2	2246
Heavy rams on the urea-based diet with tannin	U1HM	-2481.2	1414.1

Table 4.5.6: Principal component loadings for subcutaneous fat

	PC 1	PC 2
Live weight	-5.2685	6.4098
Carcass weight	0.28111	2.9536
D%	5.0301	0.10539
SCF depth 8th rib	0.60302	-2.9308
SCF depth 11th rib	1.9999	-3.9432
EE	3.1114	-9.6043
Bone %	-2.0708	2.8034
Muscle %	-7.4066	14.343
Fat %	9.5045	-15.885
EMA (CM2)	-3.2856	8.0252
Temp	0.044732	-6.4546
pH	-0.02904	0.11373
Moisture %	-0.9895	6.1503
Ash	0.015339	-0.04197
L*	-1.0079	5.3663
a*	0.55641	0.16629
b*	-0.21471	0.35246
C*	0.6527	-1.012
h*	-0.17279	0.079803
SFA	55.478	-55.26
MUFA	26.9	34.629
PUFA	0.2597	4.5694
USF	27.159	39.198
PUFA/SFA ratio	-0.0206	0.041563
MUFA/SFA ratio	-0.11561	0.40229
SFA / UFA ratio	0.17518	-0.68406
CLA	0.31154	1.1144
Σ n-3 PUFA	0.17216	0.41926
Σ n-6 PUFA	-0.32154	2.8233
n6/n3	-1.203	0.53736
Cis FA	24.02	36.411
Trans FA	3.1391	2.7861
Trans/Cis	0.001983	-0.01658
Elongase	-0.00122	0.021016
Delta-9 desaturase	-0.04132	0.1336
AI	0.13981	-0.47543
TI	0.37693	-1.2974
DFA	56.3	3.0358
Hypocholestrolaemic FA (h)	26.378	37.376
Hypercholesterolaemic FA (H)	27.236	-18.265
h/H final	-0.21296	0.63892
c14 delta 9 desaturase	-1.7348	1.602

Table 4.5.6 continued…		
c16:delta 9 desaturase	-0.21864	2.3642
c18:delta 9 desaturase	-5.3862	16.687
RA desaturase	-1.5726	3.3599

D%: Dressing percent, SCF depth of the 8th rib: subcutaneous fat depth of the 8th rib, EMA: Eye muscle area, Temp: Temperature, L*: lightness, a*: redness, b*: yellowness, C*: Chroma, h*: Hue angle, UIP: Unidentified peak, SFA: Saturated fatty acids, MUFA; Monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, UFA: Unsaturated fatty acids, Σ n-3 PUFA: Sum of the n-3 polyunsaturated fatty acids, Σ n-6 PUFA: Sum of the n-6 polyunaturated fatty acids, AI: Atherogenic index, TI: Thrombogenic index, DFA: Desirable fatty acid index, h/H: ratio of hypocholesterolaemic fatty acid to hypocholesterolaemic fatty acid, RA delta 9 desaturase: Rumenic acid delta 9 desaturase, PC 1: Principle component 1, PC 2: Principle component 2.

4.6 References

Abdullah, A. Y. & Qudsieh, R. I., 2009. Effect of slaughter weight and aging time on the quality of meat from Awassi ram lambs. Meat Science, 82(3), 309-316.

Adejoro, F. A., Hassen, A., Akanmu, A. M. & Morgavi, D. P., 2020. Replacing urea with nitrate as a non-protein nitrogen source increases lambs' growth and reduces methane production, whereas acacia tannin has no effect. Animal Feed Science and Technology, 259, 114360.

Agbeniga, B., 2018. Effects of timing and duration of low voltage electrical stimulation of light and heavy carcasses on meat quality of South African feedlot cattle. PhD (Agric) dissertation, University of Pretoria, South Africa. pp. 198.

Ahmed, S. T., Islam, M. M., Bostami, A. R., Mun, H. S., Kim, Y. J. & Yang, C. J., 2015. Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (Punica granatum L.) by-products. Food Chemistry, 188, 481-488.

Akanmu, A. M., Hassen, A. & Adejoro, F. A., 2020. Haematology and serum biochemical indices of lambs supplemented with Moringa oleifera, Jatropha curcas and Aloe vera leaf extract as anti-methanogenic additives. Antibiotics, 9(9), 601.

Alves, S. P., Francisco, A., Costa, M., Santos-Silva, J. & Bessa, R. J., 2017. Biohydrogenation patterns in digestive contents and plasma of lambs fed increasing levels of a tanniferous bush (Cistus ladanifer L.) and vegetable oils. Animal Feed Science and Technology, 225, 157-172.

Araujo, R. C., Pereira, M. L., Couto, V. R., Lemos, B. J., da Cunha, P. H. J., Arnhold, E., Silva, J. A. & Fernandes, J. J., 2021. Dose-response effect of encapsulated nitrate replacing soybean meal on growth performance, ingestive behavior, and blood metabolites of feedlot finishing bulls. Livestock Science, 247, 104475.

Archibeque, S. L., Lunt, D. K., Gilbert, C. D., Tume, R. K. & Smith, S. B., 2005. Fatty acid indices of stearoyl-CoA desaturase do not reflect actual stearoyl-CoA desaturase enzyme activities in adipose tissues of beef steers finished with corn-, flaxseed-, or sorghum-based diets. Journal of Animal Science, 83(5), 1153-1166.

Arnold, A. M. & Meyer, H. H., 1988. Influences of gender, time of castration, genotype, birthtype and feeding regimen on lamb longissimus fiber type proportions. Journal of Animal Science, 66(10), 2476-2483.

Arsenos, G., Kufidis, D., Zygoyiannis, D., Katsaounis, N. & Stamataris, C., 2006. Fatty acid composition of lambs of indigenous dairy Greek breeds of sheep as affected by post-weaning nutritional management and weight at slaughter. Meat Science, 73(1), 55-65.

Banskalieva, V., Sahlu, T. A. & Goetsch, A. L., 2000. Fatty acid composition of goat muscles and fat depots: A review. Small Ruminant Research, 37(3), 255-268.

Ban-Tokuda, T., Orden, E. A., Barrio, A. N., Lapitan, R. M., Delavaud, C., Chilliard, Y., Fujihara, T., Cruz, L. C., Homma, H. & Kanai, Y., 2007. Effects of species and sex on plasma hormone and metabolite concentrations in crossbred Brahman cattle and crossbred water buffalo. Livestock Science, 107(2-3), 244-252.

Bas, P. & Morand-Fehr, P., 2000. Effect of nutritional factors on fatty acid composition of lamb fat deposits. Livestock Production Science, 64(1), 61-79.

Bas, P., Berthelot, V., Pottier, E. & Normand, J., 2007. Effect of level of linseed on fatty acid composition of muscles and adipose tissues of lambs with emphasis on trans fatty acids. Meat Science, 77(4), 678-688.

Bauman, D. E., Baumgard, L. H., Corl, B. A. & Griinari, D. J., 1999. Biosynthesis of conjugated linoleic acid in ruminants. In: Proceedings of the American Society of Animal Science, 77, 1-14.

Beriain, M. J., Horcada, A., Purroy, A., Lizaso, G., Chasco, J. & Mendizabal, J. A., 2000. Characteristics of Lacha and Rasa Aragonesa lambs slaughtered at three live weights. Journal of Animal Science, 78(12), 3070-3077.

Bessa, R. J., Alves, S. P. & Santos-Silva, J., 2015. Constraints and potentials for the nutritional modulation of the fatty acid composition of ruminant meat. European Journal of Lipid Science and Technology, 117(9), 1325-1344.

Bhatt, R. S., Sahoo, A., Sarkar, S., Soni, L., Sharma, P. & Gadekar, Y. P., 2020. Dietary supplementation of plant bioactive-enriched aniseed straw and eucalyptus leaves modulates tissue fatty acid profile and nuggets quality of lambs. Animal, 14(12), 2642-2651.

Bond, J. J., Can, L. A. & Warner, R. D., 2004. The effect of exercise stress, adrenaline injection and electrical stimulation on changes in quality attributes and proteins in Semimembranosus muscle of lamb. Meat Science, 68(3), 469-477.

Booyens, K. E., Einkamerer, O. B., Hugo, A., Van der Merwe, H. J., Slippers, S. C. & Fair, M. D., 2012. Fatty acid composition and oxidative stability of lambs' meat as affected by a bioflavonoid antioxidant and fat sources. South African Journal of Animal Science, 42(5), 483-487.

Brand, T. S., Van Der Westhuizen, E. J., van Der Merwe, D. A. & Hoffman, L. C., 2018. Analysis of carcass characteristics and fat deposition of Merino, South African Mutton Merino and Dorper lambs housed in a feedlot. South African Journal of Animal Science, 48(3), 477-488.

Buccioni, A., Antongiovanni, M., Petacchi, F., Mele, M., Serra, A., Secchiari, P. & Benvenuti, D., 2006. Effect of dietary fat quality on C18: 1 fatty acids and conjugated linoleic acid production: An in vitro rumen fermentation study. Animal Feed Science and Technology, 127(3-4), 268-282.

Buccioni, A., Minieri, S., Rapaccini, S., Antongiovanni, M. & Mele, M., 2011. Effect of chestnut and quebracho tannins on fatty acid profile in rumen liquid-and solid-associated bacteria: An in vitro study. Animal, 5(10), 1521-1530.

Buccioni, A., Pauselli, M., Minieri, S., Roscini, V., Mannelli, F., Rapaccini, S., Lupi, P., Conte, G., Cappucci, A., Brufani, L., Ciucci, F. & Mele, M., 2017. Chestnut or quebracho tannins in the diet of grazing ewes supplemented with soybean oil: Effects on animal performances, blood parameters and fatty acid composition of plasma and milk lipids. Small Ruminant Research, 153, 23-30.

Busboom, J. R., Miller, G. J., Field, R. A., Crouse, J. D., Riley, M. L., Nelms, G. E. & Ferrell, C. L., 1981. Characteristics of fat from heavy ram and wether lambs. Journal of Animal Science, 52(1), 83-92.

Carreño, D., Hervás, G., Toral, P. G., Belenguer, A. & Frutos, P., 2015. Ability of different types and doses of tannin extracts to modulate in vitro ruminal biohydrogenation in sheep. Animal Feed Science and Technology, 202, 42-51.

Casey, N. H., Van Niekerk, W. A. & Spreeth, E. B., 1988. Fatty acid composition of subcutaneous fat of sheep grazed on eight different pastures. Meat Science, 23(1), 55-63.

Castro, T., Manso, T., Mantecón, A. R., Guirao, J. & Jimeno, V., 2005. Fatty acid composition and carcass characteristics of growing lambs fed diets containing palm oil supplements. Meat Science, 69(4), 757-764.

Cater, N. B. & Denke, M. A., 2001. Behenic acid is a cholesterol-raising saturated fatty acid in humans. The American Journal of Clinical Nutrition, 73(1), 41-44.

Chen, J. & Liu, H., 2020. Nutritional indices for assessing fatty acids: A mini-review. International Journal of Molecular Sciences, 21(16), 5695.

Chikwanha, O. C., Vahmani, P., Muchenje, V., Dugan, M. E. & Mapiye, C., 2018. Nutritional enhancement of sheep meat fatty acid profile for human health and wellbeing. Food Research International, 104, 25-38.

Chulayo, A. Y. & Muchenje, V., 2013. Effect of pre-slaughter conditions on physico-chemical characteristics of mutton from three sheep breeds slaughtered at a smallholder rural abattoir. South African Journal of Animal Science, 43(5), S64-S68.

Cloete, J. J. E., Hoffman, L. C. & Cloete, S. W. P., 2008. Carcass characteristics and meat quality of progeny of five Merino dam lines, crossed with Dormer and Suffolk sires. South African Journal of Animal Science, 38(4), 355-366.

Cloete, J. J. E., Hoffman, L. C. & Cloete, S. W. P., 2012. A comparison between slaughter traits and meat quality of various sheep breeds: Wool, dual-purpose and mutton, Meat Science, 91(3), 318-324.

Cloete, J. J. E., Hoffman, L. C., Cloete, S. W. P. & Fourie, J. E., 2004. A comparison between the body composition, carcass characteristics and retail cuts of South African Mutton Merino and Dormer sheep. South African Journal of Animal Science, 34(1), 44-51.

Costa, E. D. S., Ribeiro, C. D. M., Silva, T. M., Batista, A. S. M., Vieira, J. F., Barbosa, A. M., da Silva Júnior., Bezerra, L. R., Pereira, E. S. & Oliveira, R. L., 2021. Effect of dietary condensed tannins inclusion from Acacia mearnsii extract on the growth performance, carcass traits and meat quality of lambs. Livestock Science, 104717.

Craigie, C. R., Lambe, N. R., Richardson, R. I., Haresign, W., Maltin, C. A., Rehfeldt, C., Roehe, R., Morris, S. T. & Bunger, L., 2012. The effect of sex on some carcass and meat quality traits in Texel ewe and ram lambs. Animal Production Science, 52(7), 601-607.

De Klerk, I., 2016. The effect of NDF content in finishing diets on performance and meat quality of lambs. PhD (Agric) dissertation, University of the Free State, South Africa. pp104

De Smet, S., Raes, K. & Demeyer, D., 2004. Meat fatty acid composition as affected by fatness and genetic factors: A review. Animal Research, 53(2), 81-98.

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

Demirel, G., Ozpinar, H., Nazli, B. & Keser, O., 2006. Fatty acids of lamb meat from two breeds fed different forage: Concentrate ratio. Meat Science, 72(2), 229-235.

Devillard, E., McIntosh, F. M., Newbold, C. J. & Wallace, R. J., 2006. Rumen ciliate protozoa contain high concentrations of conjugated linoleic acids and vaccenic acid, yet do not hydrogenate linoleic acid or desaturate stearic acid. British Journal of Nutrition, 96(4), 697-704.

Devine, C. E., Graafhuis, A. E., Muir, P. D. & Chrystall, B. B., 1993. The effect of growth rate and ultimate pH on meat quality of lambs. Meat Science, 35(1), 63-77.

Dıaz, M. T., Velasco, S., Pérez, C., Lauzurica, S., Huidobro, F. & Cañeque, V., 2003. Physico-chemical characteristics of carcass and meat Manchego-breed suckling lambs slaughtered at different weights. Meat Science, 65(3), 1085-1093.

Dietschy, J. M., 1998. Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. The Journal of Nutrition, 128(2), 444S-448S.

Don, B. R. & Kaysen, G., 2004. Serum albumin: Relationship to inflammation and nutrition. Seminars in Dialysis, 17(6), 432-437. https://doi.org/10.1111/j.0894-0959.2004.17603.x

Durmic, Z., McSweeney, C. S., Kemp, G. W., Hutton, P., Wallace, R. J. & Vercoe, P. E., 2008. Australian plants with potential to inhibit bacteria and processes involved in ruminal biohydrogenation of fatty acids. Animal Feed Science and Technology, 145(1-4), 271-284.

Elliot, J. I. & Bowland, J. P., 1969. Correlation of melting point with the sum of the unsaturated fatty acids in samples of porcine depot fat. Canadian Journal of Animal Science, 49(3), 397-398.

Enoch, H. G., Catala, A. & Strittmatter, P., 1976. Mechanism of rat liver microsomal stearyl-CoA desaturase. Studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid. Journal of Biological Chemistry, 251(16), 5095-5103.

Enser, M., Hallett, K. G., Hewett, B., Fursey, G. A. J., Wood, J. D. & Harrington, G., 1998. Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. Meat Science, 49(3), 329-341.

Etim, N. N., Williams, M. E., Akpabio, U. & Offiong, E. E., 2014. Haematological parameters and factors affecting their values. Agricultural Science, 2(1), 37-47.

Facciolongo, A. M., Lestingi, A., Colonna, M. A., Nicastro, F., De Marzo, D. & Toteda, F., 2018. Effect of diet lipid source (linseed vs. soybean) and gender on performance, meat quality and intramuscular fatty acid composition in fattening lambs. Small Ruminant Research, 159, 11-17.

Fernandes, J., Pereira Filho, J., Menezes, D., Caldas, A. C., Cavalcante, I., Oliveira, J., Júnior, J.S., Cézar, M. & Bezerra, L., 2021. Carcass and meat quality in lambs receiving natural tannins from Mimosa tenuiflora hay. Small Ruminant Research, 198, 106362.

Fievez, V., Colman, E., Castro-Montoya, J. M., Stefanov, I. & Vlaeminck, B., 2012. Milk odd-and branched-chain fatty acids as biomarkers of rumen function—An update. Animal Feed Science and Technology, 172(1-2), 51-65.

Francisco, A., Dentinho, M. T., Alves, S. P., Portugal, P. V., Fernandes, F., Sengo, S., Jerónimo, E., Oliveira, M. A, Costa, P., Sequeira, A., Bessa, R. J. B. & Santos-Silva, J., 2015. Growth performance, carcass and meat quality of lambs supplemented with increasing levels of a tanniferous bush (Cistus ladanifer L.) and vegetable oils. Meat Science, 100, 275-282.-

García, E. M., López, A., Zimerman, M., Hernández, O., Arroquy, J. I. & Nazareno, M. A., 2019. Enhanced oxidative stability of meat by including tannin-rich leaves of woody plants in goat diet. Asian-Australasian Journal of Animal Sciences, 32(9), 1439.

Garcia, P. T., 2011. Metabolism of α-linolenic acid (ALA) in meat animals. Soybean and Nutrition, 349-368.

Geay, Y., Bauchart, D., Hocquette, J. F. & Culioli, J., 2001. Effect of nutritional factors on biochemical, structural and metabolic characteristics of muscles in ruminants, consequences on dietetic value and sensorial qualities of meat. Reproduction Nutrition Development, 41(1), 1-26.

Gesteira, S. M., Oliveira, R. L., Silva, T. M., Ribeiro, R. D., Ribeiro, C. V., Pereira, E. S., Lanna, D.P., Pinto, L. F., Rocha, T. C., Vieira, J. F. & Bezerra, L. R., 2018. Physicochemical quality, fatty acid composition, and sensory analysis of Nellore steers meat fed with inclusion of condensed tannin in the diet. Journal of Food Science, 83(5), 1366-1372.

Grundy, S. M., 1994. Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. The American Journal of Clinical Nutrition, 60(6), 986S-990S.

Guerreiro, O., Alves, S. P., Soldado, D., Cachucho, L., Almeida, J. M., Francisco, A., Santos-Silva, J., Bessa, R. J. B. & Jerónimo, E., 2020. Inclusion of the aerial part and condensed tannin extract from Cistus ladanifer L. in lamb diets–Effects on growth performance, carcass and meat quality and fatty acid composition of intramuscular and subcutaneous fat. Meat Science, 160, 107945.

Guler, G. O. & Aktumsek, A., 2011. Effect of feeding regime on fatty acid composition and conjugated linoleic acid content of perirenal, omental and tail fat in Akkaraman lambs. African Journal of Biotechnology, 10(36), 7099-7108.

Guo, Y., Li, M., Tang, Z., Peng, L., Peng, K., Xie, F., Xie, H. & Yang, C., 2021. Effects of different doses of sodium nitrate on fatty acid composition and microbial population in in vitro simulation of buffalo rumen fermentation with added linoleic acid. Acta Prataculturae Sinica, 30(9), 159-167.

Hammond, A. C., 1983. The use of blood urea nitrogen concentration as an indicator of protein status in cattle. The Bovine Practitioner, 114-118.

Harfoot, C. G., 1981. Lipid metabolism in the rumen. In: Lipid metabolism in ruminant animals Ed: Christie, W. C., Pergamon. pp. 21-55.

Hegarty, R. S., Miller, J., Oelbrandt, N., Li, L., Luijben, J. P. M., Robinson, D. L., Nolan, J. V. & Perdok, H. B., 2016. Feed intake, growth, and body and carcass attributes of feedlot steers supplemented with two levels of calcium nitrate or urea. Journal of Animal Science, 94(12), 5372-5381.

Hess, H. D., Tiemann, T. T., Noto, F., Carulla, J. E. & Kreuzer, M., 2006. Strategic use of tannins as means to limit methane emission from ruminant livestock. International Congress Series. 1293, 164-167.

Hoffman, L. C., Schmidt, D., Muller, M. M., Cloete, J. J. E. & Cloete, S. W. P., 2003. Sensory and objective mutton quality characteristics of SA Merino sheep selected for and against reproductive fitness. South African Journal of Animal Science, 33(1), 52-64.

Honkanen, A. M., Griinari, J. M., Vanhatalo, A., Ahvenjärvi, S., Toivonen, V. & Shingfield, K. J., 2012. Characterization of the disappearance and formation of biohydrogenation intermediates during incubations of linoleic acid with rumen fluid in vitro. Journal of dairy science, 95(3), 1376-1394.

Hopkins, D. L., 1996. Assessment of lamb meat colour. Meat Focus International, 5(11), 400-401.

Horcada, A., Beriain, M. J., Purroy, A., Lizaso, G. & Chasco, J., 1998. Effect of sex on meat quality of Spanish lamb breeds (Lacha and Rasa Aragonesa). Animal Science, 67(3), 541-547.

Horrocks, L. A. & Yeo, Y. K., 1999. Health benefits of docosahexaenoic acid (DHA). Pharmacological Research, 40(3), 211-225.

Hulshof, R.B.A., Berndt, A., Gerrits, W.J.J., Dijkstra, J., van Zijderveld, S.M., Newbold, J.R. & Perdok, H.B., 2012. Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based diets. Journal of Animal Science, 90(7), 2317-2323.

Ishlak, A., Günal, M. & AbuGhazaleh, A. A., 2015. The effects of cinnamaldehyde, monensin and quebracho condensed tannin on rumen fermentation, biohydrogenation and bacteria in continuous culture system. Animal Feed Science and Technology, 207, 31-40.

Jacob, R. (2020). Implications of the variation in bloom properties of red meat: A review. Meat Science, 162, 108040.

Jacome-Sosa, M., Vacca, C., Mangat, R., Diane, A., Nelson, R. C., Reaney, M. J., Shen, J., Curtis, J. M., Vine, D. F., Field, C. J., Igarashi, M., Piomelli, D., Banni, S. & Proctor, S. D., 2016. Vaccenic acid suppresses intestinal inflammation by increasing anandamide and related N-acylethanolamines in the JCR: LA-cp rat. Journal of Lipid Research, 57(4), 638-649.

Jayanegara, A., Ridla, M., Laconi, E. B. & Nahrowi, N., 2018. Tannin as a feed additive for mitigating enteric methane emission from livestock: Meta-analysis from RUSITEC experiments. In: IOP Conference Series: Materials Science and Engineering, 434, 012108.

Jenkins, T. C., Wallace, R. J., Moate, P. J. & Mosley, E. E., 2008. Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. Journal of Animal Science, 86(2), 397-412.

Jerónimo, E., Alfaia, C. M., Alves, S. P., Dentinho, M. T., Prates, J. A., Vasta, V., Santos-Silva, J. & Bessa, R. J., 2012. Effect of dietary grape seed extract and Cistus ladanifer L. in combination with vegetable oil supplementation on lamb meat quality. Meat Science, 92(4), 841-847.

Johnson, P. L., Purchas, R. W., McEwan, J. C. & Blair, H. T., 2005. Carcass composition and meat quality differences between pasture-reared ewe and ram lambs. Meat Science, 71(2), 383-391.

Juárez, M., Horcada, A., Alcalde, M. J., Valera, M., Polvillo, O. & Molina, A., 2009. Meat and fat quality of unweaned lambs as affected by slaughter weight and breed. Meat Science, 83(2), 308-313.

Junior, F. M. V., Martins, C. F., Feijó, G. L. D., Teixeira, A., Leonardo, A. P., de Almeida Ricardo, H., Fernandes, A. R. M. & Reis, F. A., 2019. Evaluation of genotype on fatty acid profile and sensory of meat of indigenous Pantaneiro sheep and Texel or Santa Inês crossbred finished on feedlot. Small Ruminant Research, 173, 17-22.

Kadri, S., El Ayed, M., Mabrouk, M., Limam, F., Elkahoui, S., Aouani, E. & Mokni, M. 2019. Characterization, anti-oxidative effect of grape seed powder and in silico affinity profiling of polyphenolic and extra-phenolic compounds for calpain inhibition. Journal of Pharmaceutical and Biomedical Analysis, 164, 365-372.

Kafle, D., Lee, J. H., Min, B. R. & Kouakou, B., 2021. Carcass and meat quality of goats supplemented with tannin-rich peanut skin. Journal of Agriculture and Food Research, 5, 100159

Kamel, H. E. M., Al-Dobaib, S. N., Salem, A. Z. M., López, S. & Alaba, P. A., 2018. Influence of dietary supplementation with sunflower oil and quebracho tannins on growth performance and meat fatty acid profile of Awassi lambs. Animal Feed Science and Technology, 235, 97-104.

Kasap, A., Kaić, A., Širić, I., Antunović, Z. & Boro Mioč, B., 2018. Proximate and mineral composition of M. longissimus thoracis et lumborum of suckling lambs from three Croatian indigenous breeds reared in outdoor conditions. Italian Journal of Animal Science, 17(2), 274-278. DOI: 10.1080/1828051X.2017.1377643

Kaysen, G. A., Jones Jr, H. & Hutchinson, F. N., 1989. High protein diets stimulate albumin synthesis at the site of albumin mRNA transcription. Kidney International Supplement. 26, 168-172.

Kemp, J. D., Johnson, A. E., Stewart, D. F., Ely, D. G. & Fox, J. D., 1976. Effect of dietary protein, slaughter weight and sex on carcass composition, organoleptic properties and cooking losses of lamb. Journal of Animal Science, 42(3), 575-583.

Kemp, J. D., Mahyuddin, M., Ely, D. G., Fox, J. D. & Moody, W. G., 1981. Effect of feeding systems, slaughter weight and sex on organoleptic properties, and fatty acid composition of lamb. Journal of Animal Science, 51(2), 321-330.

Kemp, P., White, R. W. & Lander, D. J., 1975. The hydrogenation of unsaturated fatty acids by five bacterial isolates from the sheep rumen, including a new species. Microbiology, 90(1), 100-114.

Khiaosa-Ard, R., Bryner, S. F., Scheeder, M. R. L., Wettstein, H. R., Leiber, F., Kreuzer, M. & Soliva, C. R., 2009. Evidence for the inhibition of the terminal step of ruminal α-linolenic acid biohydrogenation by condensed tannins. Journal of Dairy Science, 92(1), 177-188.

Khliji, S., Van de Ven, R., Lamb, T. A., Lanza, M. & Hopkins, D. L., 2010. Relationship between consumer ranking of lamb colour and objective measures of colour. Meat Science, 85(2), 224-229.

Kim, J. H., Kim, Y., Kim, Y. J. & Park, Y., 2016. Conjugated linoleic acid: Potential health benefits as a functional food ingredient. Annual Review of Food Science and Technology, 7(1), 221-244.

Kirkpatrick, R. L., Buckland, D. E., Abler, W. A., Scanlon, P. F., Whelan, J. B. & Burkhart, H. E., 1975. Energy and protein influences on blood urea nitrogen of white-tailed deer fawns. The journal of wildlife management, 692-698.

Kirton, A. H., Crane, B., Paterson, D. J. & Clare, N. T., 1975. Yellow fat in lambs caused by carotenoid pigmentation. New Zealand Journal of Agricultural Research, 18(3), 267-272.

Kronberg, S. L., Scholljegerdes, E. J., Barceló-Coblijn, G. & Murphy, E. J., 2007. Flaxseed treatments to reduce biohydrogenation of α-linolenic acid by rumen microbes in cattle. Lipids, 42(12), 1105-1111.

Krueger, W. K., Gutierrez-Bañuelos, H., Carstens, G. E., Min, B. R., Pinchak, W. E., Gomez, R. R., Anderson, R. C., Krueger, N. A. & Forbes, T. D. A., 2010. Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steers fed a high-grain diet. Animal Feed Science and Technology, 159(1-2), 1-9.

Landim, A. V., Cardoso, M. T. M., Castanheira, M., Fioravanti, M. C. S., Louvandini, H. & McManus, C., 2011. Fatty acid profile of hair lambs and their crossbreds slaughtered at different weights. Tropical Animal Health and Production, 43(8), 1561-1566.

Latham, E. A., Anderson, R. C., Pinchak, W. E. & Nisbet, D. J., 2016. Insights on alterations to the rumen ecosystem by nitrate and nitrocompounds. Frontiers in Microbiology, 7, 228.

Ledward, D. A. & Shorthose, W. R., 1971. A note on the haem pigment concentration of lamb as influenced by age and sex. Animal Science, 13(1), 193-195.

Le, H. D., Meisel, J. A., de Meijer, V. E., Gura, K. M. & Puder, M., 2009. The essentiality of arachidonic acid and docosahexaenoic acid. Prostaglandins, Leukotrienes and Essential Fatty Acids, 81(2-3), 165-170.

Lee, C. & Beauchemin, K. A., 2014. A review of feeding supplementary nitrate to ruminant animals: Nitrate toxicity, methane emissions, and production performance. Canadian Journal of Animal Science, 94(4), 557-570.

Lee, C. E., Kang, J. S. & Kim, K. I., 2008. Effects of gender, gonadectomy and sex hormones on growth and plasma cholesterol level in rats. Annals of Nutrition and Metabolism, 53(1), 1-5.

Lee, J. H., Min, B. R. & Lemma, B. B., 2017. Quality characteristics of goat meat as influenced by condensed tannins-containing pine bark. Small Ruminant Research, 146, 28-32.

Lee, J. H., Yamamoto, I., JEONG, J. S., Nade, T., Arai, T., & Kimura, N., 2011. Relationship between adipose maturity and fatty acid composition in various adipose tissues of Japanese Black, Holstein and Crossbred (F1) steers. Animal Science Journal, 82(5), 689-697.

Lemaitre, R. N., McKnight, B., Sotoodehnia, N., Fretts, A. M., Qureshi, W. T., Song, X., King, I. B., Sitlani, C. M., Siscovick, D. S., Patsy, B. M. & Mozaffarian, D., 2018. Circulating very long-chain saturated fatty acids and heart failure: The cardiovascular health study. Journal of the American Heart Association, 7(21), e010019.

Lewis, D., Hill, K. J. & Annison, E. F., 1957. Studies on the portal blood of sheep. 1. Absorption of ammonia from the rumen of the sheep. Biochemical Journal, 66(4), 587.

Li, L., Davis, J., Nolan, J. & Hegarty, R., 2012. An initial investigation on rumen fermentation pattern and methane emission of sheep offered diets containing urea or nitrate as the nitrogen source. Animal Production Science, 52(7), 653-658.

Link, B. A., Bray, R. W., Cassens, R. G. & Kauffman, R. G., 1970. Fatty acid composition of bovine skeletal muscle lipids during growth. Journal of Animal Science, 30(5), 726-731.

Liu, M., Zuo, L. S. Y., Sun, T. Y., Wu, Y. Y., Liu, Y. P., Zeng, F. F. & Chen, Y. M., 2020. Circulating very-long-chain saturated fatty acids were inversely associated with cardiovascular health: A prospective cohort study and meta-analysis. Nutrients, 12(9), 2709.

Lourenço, M., Ramos-Morales, E. & Wallace, R. J., 2010. The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. Animal, 4(7), 1008-1023.

Lourenço, M., Van Ranst, G., De Smet, S., Raes, K., & Fievez, V., 2007. Effect of grazing pastures with different botanical composition by lambs on rumen fatty acid metabolism and fatty acid pattern of longissimus muscle and subcutaneous fat. Animal, 1(4), 537-545.

Louvandini, H., Cenci, F. B., Issakowicz, J., Sampaio, A. C. K., do Prado Paim, T., de Araújo, S. C. & McManus, C., 2014. Carcass traits in sheep receiving Acacia mearnsii condensed tannin extract to control endoparasites. Journal of Agricultural Science, 6, (10), 128-134.

Luciano, G., Monahan, F. J., Vasta, V., Biondi, L., Lanza, M. & Priolo, A., 2009a. Dietary tannins improve lamb meat colour stability. Meat Science, 81(1), 120-125.

Luciano, G., Monahan, F., Vasta, V., Biondi, L., Lanza, M., Pennisi, P. & Priolo, A., 2009b. Lamb meat colour stability as affected by dietary tannins. Italian Journal of Animal Science, 8(sup2), 507-509.

Luciano, G., Vasta, V., Monahan, F. J., López-Andrés, P., Biondi, L., Lanza, M. & Priolo, A., 2011. Antioxidant status, colour stability and myoglobin resistance to oxidation of longissimus dorsi muscle from lambs fed a tannin-containing diet. Food Chemistry, 124(3), 1036-1042.

Ma, X., Jiang, Z. & Lai, C., 2016. Significance of increasing n-3 PUFA content in pork on human health. Critical Reviews in Food Science and Nutrition, 56(5), 858-870.

MacDougall, D. B., 2010. Colour measurement of food: Principles and practice. In: Colour Measurement. Ed: Gulrajani, M. L., Woodhead Publishing. pp. 312-342.

Majewska, M. P. & Kowalik, B., 2020. Growth performance, carcass characteristics, fatty acid composition, and blood biochemical parameters of lamb fed diet with the addition of lingonberry leaves and oak bark. European Journal of Lipid Science and Technology, 122(2), 1900273.

Malau-Aduli, A. E. O., Holman, B. W. B., Kashani, A. & Nichols, P. D., 2015. Sire breed and sex effects on the fatty acid composition and content of heart, kidney, liver, adipose and muscle tissues of purebred and first-cross prime lambs. Animal Production Science, 56(12), 2122-2132.

Mancini, R. A. & Hunt, M., 2005. Current research in meat color. Meat Science, 71(1), 100-121.

Mapiye, C., Chimonyo, M., Dzama, K. & Marufu, M. C., 2010. Protein status of indigenous Nguni and crossbred cattle in the semi-arid communal rangelands in South Africa. Asian-Australasian Journal of Animal Sciences, 23(2), 213-225.

Mapiye, C., Vahmani, P., Aalhus, J. L., Rolland, D. C., Baron, V. S., Block, H. C., Uttaro, B., Dugan, M. E. R. & McAllister, T. A., 2015. Fatty acid composition of beef steers as affected by diet and fat depot. South African Journal of Animal Science, 45(4), 386-394.

Marmer, W. N., Maxwell, R. J. & Wagner, D. G., 1985. Effects of dietary monensin on bovine fatty acid profiles. Journal of Agricultural and Food Chemistry, 33(1), 67-70.

Marra, C. A. & de Alaniz, M. J., 2000. Calcium deficiency modifies polyunsaturated fatty acid metabolism in growing rats. Lipids, 35(9), 983-990.

Marra, C. A., Rimoldi, O. & de Alaniz, M. J., 2002. Correlation between fatty acyl composition in neutral and polar lipids and enzyme activities from various tissues of calcium-deficient rats. Lipids, 37(7), 701-714.

Mattos, R., Staples, C. R. & Thatcher, W. W., 2000. Effects of dietary fatty acids on reproduction in ruminants. Reviews of Reproduction, 5(1), 38-45.

McClelland, T. H., Bonaiti, B. & Taylor, C. S., 1976. Breed differences in body composition of equally mature sheep. Animal Science, 23(3), 281-293.

McKenna, D. R., Mies, P. D., Baird, B. E., Pfeiffer, K. D., Ellebracht, J. W., & Savell, J. W. (2005). Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. Meat science, 70(4), 665-682.

Meat Safety Act No. 40 of 2000. Department of Agriculture, Forestory and Fisheries, Republic of South Africa.

Meynadier, A., Zened, A., Farizon, Y., Chemit, M. L. & Enjalbert, F., 2018. Enzymatic study of linoleic and alpha-linolenic acids biohydrogenation by chloramphenicol-treated mixed rumen bacterial species. Frontiers in Microbiology, 9, 1452.

Min, B. R., Solaiman, S., Taha, E. & Lee, J., 2015. Effect of plant tannin-containing diet on fatty acid profile in meat goats. Journal of Animal Research and Nutrition, 1, 1-5.

Minieri, S., Buccioni, A., Rapaccini, S., Pezzati, A., Benvenuti, D., Serra, A. & Mele, M., 2014. Effect of Quebracho tannin extract on soybean and linseed oil biohydrogenation by solid associated bacteria: An in vitro study. Italian Journal of Animal Science, 13(3), 3267.

Morales, R. & Ungerfeld, E. M., 2015. Use of tannins to improve fatty acids profile of meat and milk quality in ruminants: A review. Chilean Journal of Agricultural Research, 75(2), 239-248.

Mortensen, M. B. & Nordestgaard, B. G., 2020. Elevated LDL cholesterol and increased risk of myocardial infarction and atherosclerotic cardiovascular disease in individuals aged 70–100 years: A contemporary primary prevention cohort. The Lancet, 396(10263), 1644-1652.

Mosley, E. E., Powell, G. L., Riley, M. B. & Jenkins, T. C., 2002. Microbial biohydrogenation of oleic acid to trans isomers in vitro. Journal of Lipid Research, 43(2), 290-296.

Mozaffarian, D., Micha, R. & Wallace, S., 2010. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: A systematic review and meta-analysis of randomized controlled trials. PLoS medicine, 7(3), e1000252.

Mukhoty, H., Combs, W. & Groves, T. D. D., 1969. Growth-dependent changes in blood urea nitrogen levels in Lincoln and Southdown lambs. Canadian Journal of Animal Science, 49(2), 197-204.

Muller, A., Brand, T. S., Cloete, J. J. E. & Cloete, S. W. P., 2019. Growth, carcass and meat quality traits of dormer and South African Mutton Merino lambs. Proceedings of the Association in the Advancement of Animal Breeding and Genetics, 23, 119-122.

Mulvihill, B., 2001. Ruminant meat as a source of conjugated linoleic acid (CLA). Nutrition Bulletin, 26(4), 295-299.

Nagao, K. & Yanagita, T., 2005. Conjugated fatty acids in food and their health benefits. Journal of bioscience and bioengineering, 100(2), 152-157.

Nagaraja, T.G., Newbold, C.J., van Nevel, C.J. & Demeyer, D.I., 1997. Manipulation of ruminal fermentation. In: The Rumen Microbial Ecosystem. Eds: Hobson, P.N. & Stewart, C.S., Springer, Dordrecht. pp. 523-632.

Natali, F., Siculella, L., Salvati, S. & Gnoni, G. V., 2007. Oleic acid is a potent inhibitor of fatty acid and cholesterol synthesis in C6 glioma cells. Journal of Lipid Research, 48(9), 1966-1975.

Nestel, P., Clifton, P. & Noakes, M., 1994. Effects of increasing dietary palmitoleic acid compared with palmitic and oleic acids on plasma lipids of hypercholesterolemic men. Journal of Lipid Research, 35(4), 656-662.

Obsen, T., Faergeman, N. J., Chung, S., Martinez, K., Gobern, S., Loreau, O., Wabitsch, M., Mandrup, S. & McIntosh, M., 2012. Trans-10, cis-12 conjugated linoleic acid decreases de novo lipid synthesis in human adipocytes. The Journal of Nutritional Biochemistry, 23(6), 580-590.

Okeudo, N. J. & Moss, B. W., 2007. Intramuscular lipid and fatty acid profile of sheep comprising four sex-types and seven slaughter weights produced following commercial procedure. Meat Science, 76(2), 195-200.

Oteng, A. B. & Kersten, S., 2020. Mechanisms of action of trans fatty acids. Advances in Nutrition, 11(3), 697-708.

Priolo, A., Bella, M., Lanza, M., Galofaro, V., Biondi, L., Barbagallo, D., Salem, H. B., & Pennisi, P., 2005. Carcass and meat quality of lambs fed fresh sulla (Hedysarum coronarium L.) with or without polyethylene glycol or concentrate. Small Ruminant Research, 59(2-3). 281-288.

Priolo, A., Lanza, M., Bella, M., Pennisi, P., Fasone, V. & Biondi, L., 2002. Reducing the impact of condensed tannins in a diet based on carob pulp using two levels of polyethylene glycol: Lamb growth, digestion and meat quality. Animal Research, 51(4), 305-313.

Priolo, A., Salem, H. B., Atti, N. & Nefzaoui, A., 2002. Polyethylene glycol in concentrate or feedblocks to deactivate condensed tannins in Acacia cyanophylla Lindl. foliage 2. Effects on meat quality of Barbarine lambs. Animal Science, 75(1), 137-140.

Priolo, A. & Vasta, V., 2007. Effects of tannin-containing diets on small ruminant meat quality. Italian Journal of Animal Science, 6(sup1), 527-530.

Priolo, A., Waghorn, G. C., Lanza, M., Biondi, L. & Pennisi, P., 2000. Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. Journal of Animal Science, 78(4), 810-816.

Queiroz, L. D. O., Barbosa, A. M., Mourão, G. B., Fonseca, M. A., Pinto, L. F. B., da Silva Júnior, J. M., Silva, T. M., Lima, A. G. V. O., Bezerra, L. R. & Oliveira, R. L., 2021. Performance, carcass traits and meat quality of lambs fed with different roughage: Concentrate ratios associated with

variable physically effective neutral detergent fibre content. The Journal of Agricultural Science, 159(3-4), 293-303.

Raes, K., De Smet, S. & Demeyer, D., 2004. Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review. Animal Feed Science and Technology, 113(1-4), 199-221.

Rana, M. S., Tyagi, A., Hossain, S. A. & Tyagi, A. K., 2012. Effect of tanniniferous Terminalia chebula extract on rumen biohydrogenation, Δ 9-desaturase activity, CLA content and fatty acid composition in longissimus dorsi muscle of kids. Meat Science, 90(3), 558-563.

Rant, W., Radzik-Rant, A., Świątek, M., Niżnikowski, R., Szymańska, Ż., Bednarczyk, M., Orlowski, E., Morales-Villavicencio, A. & Ślęzak, M., 2019. The effect of aging and muscle type on the quality characteristics and lipid oxidation of lamb meat. Archives Animal Breeding. 62(2), 383-391.

Ratnayake, W. N. & Galli, C., 2009. Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism. Annals of Nutrition & Metabolism, 55(1/3), 8-43.

Rhee, K. S., 2000. Fatty acids in meats and meat products. In: Fatty Acids in Foods and their Health Implications (2nd ed.). Ed: Chow, C. K., Marcel Dekker Inc., New York. pp. 83-108.

Rivaroli, D., Prunier, A., Meteau, K., do Prado, I. N. & Prache, S., 2019. Tannin-rich sainfoin pellet supplementation reduces fat volatile indoles content and delays digestive parasitism in lambs grazing alfalfa. Animal, 13(9), 1883-1890.

Roberts, W. K., 1966. Effects of diet, degree of fatness, and sex upon fatty acid composition of cattle tissues. Canadian Journal of Animal Science, 46(3), 181-190.

Rojas-Román, L. A., Castro-Pérez, B. I., Estrada-Angulo, A., Angulo-Montoya, C., Yocupicio-Rocha, J. A., López-Soto, M. A., Barreras, A., Zinn, R. A. & Plascencia, A., 2017. Influence of long-term supplementation of tannins on growth performance, dietary net energy and carcass characteristics: Finishing lambs. Small Ruminant Research, 153, 137-141.

Roopashree, P. G., Shetty, S. S. & Kumari, N. S., 2021. Effect of medium chain fatty acid in human health and disease. Journal of Functional Foods, 87, 104724.

Rothschild, M. A., Oratz, M. & Schreiber, S. S., 1977. Albumin synthesis. In: Albumin: Structure, Function and Uses. Eds: Rosenoer, V. M., Oratz, M., Rothschild, M. A., Pergamon, 227-253.

Sabbioni, A., Beretti, V., Zambini, E. M., Superchi, P. & Ablondi, M., 2019. Allometric coefficients for physical-chemical parameters of meat in a local sheep breed. Small Ruminant Research, 174, 141-147.

Safari, E., Sefidbakht, N. & Farid, A., 1988. Effect of castration and cryptorchidism on fatty acid content of ovine adipose tissue. Meat Science, 23(1), 65-69.

Sakurama, H., Kishino, S., Mihara, K., Ando, A., Kita, K., Takahashi, S., Shimizu, S., & Ogawa, J., 2014. Biohydrogenation of C20 polyunsaturated fatty acids by anaerobic bacteria. Journal of Lipid Research, 55(9), 1855-1863.

Sales-Campos, H., Reis de Souza, P., Crema Peghini, B., Santana da Silva, J. & Ribeiro Cardoso, C., 2013. An overview of the modulatory effects of oleic acid in health and disease. Mini Reviews in Medicinal Chemistry, 13(2), 201-210.

Sampath, H. & Ntambi, J. M., 2005. The fate and intermediary metabolism of stearic acid. Lipids, 40(12), 1187-1191.

Santos-Silva, J., Bessa, R. J. B. & Santos-Silva, F. J. L. P. S., 2002. Effect of genotype, feeding system and slaughter weight on the quality of light lambs: II. Fatty acid composition of meat. Livestock Production Science, 77(2-3), 187-194.

Sañudo, C., Santolaria, M. P., Maria, G., Osorio, M. & Sierra, I., 1996. Influence of carcass weight on instrumental and sensory lamb meat quality in intensive production systems. Meat Science, 42(2), 195-202.

Sañudo, C., Sierra, I., Olleta, J. L., Martin, L., Campo, M. M., Santolaria, P., Wood, J.D. & Nute, G. R. (1998). Influence of weaning on carcass quality, fatty acid composition and meat quality in intensive lamb production systems. Animal Science, 66(1), 175-187.

Scerra, V., Caparra, P., Foti, F., Lanza, M. & Priolo, A., 2001. Citrus pulp and wheat straw silage as an ingredient in lamb diets: effects on growth and carcass and meat quality. Small Ruminant Research, 40(1), 51-56.

Schönfeldt, H.C., van Heerden, S.M., Sainsbury, J. & Gibson, N., 2011. Nutrient content of uncooked and cooked meat from South African classes of A2 lamb and C2 mutton. South African Journal of Animal Science, 41, 142-145.

Seoni, E., Battacone, G., Silacci, P., Kragten, S. A., Chelali, J. M., Dohme-Meier, F. & Bee, G., 2018. Effect of condensed tannins from Birdsfoot trefoil and dietary protein level on growth performance, carcass composition and meat quality of ram lambs. Small Ruminant Research, 169, 118-126.

Shingfield, K. J., Bernard, L., Leroux, C. & Chilliard, Y., 2010. Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal, 4(7), 1140-1166.

Shingfield, K. J., Bonnet, M. & Scollan, N. D., 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal, 7(s1), 132-162.

Silanikove, N., Landau, S., Or, D., Kababya, D., Bruckental, I. & Nitsan, Z., 2006. Analytical approach and effects of condensed tannins in carob pods (Ceratonia siliqua) on feed intake, digestive and metabolic responses of kids. Livestock Science, 99(1), 29-38.

Sinclair, A. J., Slattery, W. J. & O'Dea, K.,1982. The analysis of polyunsaturated fatty acids in meat by capillary gas-liquid chromatography. Journal of the Science of Food and Agriculture, 33(8), 771-776.

Smith, S. B., 2016. Marbling and its nutritional impact on risk factors for cardiovascular disease. Korean Journal for Food Science of Animal Resources, 36(4), 435.

Smith, S. B., Lunt, D. K., Chung, K. Y., Choi, C. B., Tume, R. K. & Zembayashi, M., 2006. Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. Animal Science Journal, 77(5), 478-486.

Solomon, M. B., Lynch, G. P., Ono, K. & Paroczay, E., 1990. Lipid composition of muscle and adipose tissue from crossbred ram, wether and cryptorchid lambs. Journal of Animal Science, 68(1), 137-142.

Song, J., Wang, Y., Fan, X., Wu, H., Han, J., Yang, M., Lu, L. & Nie, G., 2019. Trans-vaccenic acid inhibits proliferation and induces apoptosis of human nasopharyngeal carcinoma cells via a mitochondrial-mediated apoptosis pathway. Lipids in Health and Disease, 18(1), 1-9.

Staerfl, S. M., Soliva, C. R., Leiber, F. & Kreuzer, M., 2011. Fatty acid profile and oxidative stability of the perirenal fat of bulls fattened on grass silage and maize silage supplemented with tannins, garlic, maca and lupines. Meat Science, 89(1), 98-104.

Sykes, A. R. & Thompson, R., 1978. The relationship between serum albumin concentration and body protein loss in pregnant sheep. The Journal of Agricultural Science, 91(1), 173-179.

Tanaka, K., 2005. Occurrence of conjugated linoleic acid in ruminant products and its physiological functions. Animal Science Journal, 76(4), 291-303.

Teixeira, A., Batista, S., Delfa, R. & Cadavez, V., 2005. Lamb meat quality of two breeds with protected origin designation. Influence of breed, sex and live weight. Meat Science, 71(3), 530-536.

Tejeda, J. F., Peña, R. E. & Andrés, A. I., 2008. Effect of live weight and sex on physico-chemical and sensorial characteristics of Merino lamb meat. Meat Science, 80(4), 1061-1067.

Thompson, J. M., Butterfield, R. M. & Perry, D., 1985. Food intake, growth and body composition in Australian Merino sheep selected for high and low weaning weight. 2. Chemical and dissectible body composition. Animal Science, 40(1), 71-84.

Torres, R. N. S., Ghedini, C. P., Paschoaloto, J. R., da Silva, D. A. V., Coelho, L. M., Junior, G. A., Ezequiel, J. M. B., Neto, O. R. M. & Almeida, M. T. C., 2022. Effects of tannins supplementation to sheep diets on their performance, carcass parameters and meat fatty acid profile: A meta-analysis study. Small Ruminant Research, 206, 106585.

Turpeinen, A. M., Mutanen, M., Aro, A., Salminen, I., Basu, S., Palmquist, D. L. & Griinari, J. M., 2002. Bioconversion of vaccenic acid to conjugated linoleic acid in humans. The American Journal of Clinical Nutrition, 76(3), 504-510.

Vahmani, P., Mapiye, C., Prieto, N., Rolland, D. C., McAllister, T. A., Aalhus, J. L. & Dugan, M. E., 2015. The scope for manipulating the polyunsaturated fatty acid content of beef: A review. Journal of Animal Science and Biotechnology, 6(1), 1-13.

Vahmani, P., Ponnampalam, E. N., Kraft, J., Mapiye, C., Bermingham, E. N., Watkins, P. J., Proctor, S. D. & Dugan, M. E., 2020. Bioactivity and health effects of ruminant meat lipids. Invited Review. Meat Science, 165, 108114.

Van der Merwe, D. A., Brand, T. S. & Hoffman, L. C., 2019. Application of growth models to different sheep breed types in South Africa. Small Ruminant Research, 178, 70-78.

Van der Merwe, D. A., Brand, T. S. & Hoffman, L. C., 2020. Slaughter characteristics of feedlot-finished premium South African lamb: effects of sex and breed type. Foods, 9(5), 648.

Van Rensburg, E. & Oguttu, J. W., 2013. Calpain and Calpastatin activity postmortem and meat tenderness: Are the two related. Journal of Animal and Veterinary Advances, 12(6), 683-688.

Van Zyl, L. C., 1967. Contributions to the study of blood constituents in domestic animals in South Africa. 6. Normal values for serum protein fractions in sheep as obtained by electrophoresis

on cellulose acetate strips., 1967. Onderstepoort Journal of Veterinary Research, 34 (2), 633-646.

Vasta, V., Makkar, H., Mele, M. & Priolo, A., 2008. Ruminal biohydrogenation as affected by tannins in vitro. British Journal of Nutrition, 102(1), 82-92.

Vasta, V., Mele, M., Serra, A., Scerra, M., Luciano, G., Lanza, M. & Priolo, A., 2009a. Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. Journal of Animal Science, 87(8), 2674-2684.

Vasta, V., Pennisi, P., Lanza, M., Barbagallo, D., Bella, M. & Priolo, A., 2007. Intramuscular fatty acid composition of lambs given a tanniniferous diet with or without polyethylene glycol supplementation. Meat Science, 76(4), 739-745.

Vasta, V., Yáñez-Ruiz, D. R., Mele, M., Serra, A., Luciano, G., Lanza, M., Biondi, L. & Priolo, A., 2010. Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. Applied and Environmental Microbiology, 76(8), 2549-2555.

Villar, M. L., Godwin, I. R., Hegarty, R. S., Erler, D. V., Farid, H. T. & Nolan, J. V., 2021. Nitrate and nitrite absorption, recycling and retention in tissues of sheep. Small Ruminant Research, 200, 106392.

Vlaeminck, B., Fievez, V., Cabrita, A. R. J., Fonseca, A. J. M. & Dewhurst, R. J., 2006a. Factors affecting odd-and branched-chain fatty acids in milk: A review. Animal Feed Science and Technology, 131(3-4), 389-417.

Vlaeminck, B., Fievez, V., Demeyer, D. & Dewhurst, R. J., 2006b. Effect of forage: concentrate ratio on fatty acid composition of rumen bacteria isolated from ruminal and duodenal digesta. Journal of Dairy Science, 89(7), 2668-2678.

Wang, B., Ma, T., Deng, K. D., Jiang, C. G. & Diao, Q. Y., 2016. Effect of urea supplementation on performance and safety in diets of Dorper crossbred sheep. Journal of Animal Physiology and Animal Nutrition, 100(5), 902-910.

Wang, X., Gupta, J., Kerslake, M., Rayat, G., Proctor, S. D. & Chan, C. B., 2016. Trans-11 vaccenic acid improves insulin secretion in models of type 2 diabetes in vivo and in vitro. Molecular Nutrition & Food Research, 60(4), 846-857.

Wang, Y., Lu, J., Ruth, M. R., Goruk, S. D., Reaney, M. J., Glimm, D. R., Vine, D. F., Field, C. J. & Proctor, S. D., 2008. Trans-11 vaccenic acid dietary supplementation induces hypolipidemic effects in JCR: LA-cp rats. The Journal of Nutrition, 138(11), 2117-2122.

Webb, E. C. & Casey, N. H., 1995. Genetic differences in fatty acid composition of subcutaneous adipose tissue in Dorper and SA Mutton Merino wethers at different live weights. Small Ruminant Research, 18(1), 81-88.

Webb, E. C. & O'neill, H. A., 2008. The animal fat paradox and meat quality. Meat Science, 80(1), 28-36.

Webb, E. C., 2021. Cis/Trans-Fatty Acid Content of Red Meats and the Related Effects on Meat Quality and Human Health. In: Meat and Nutrition. Ed: Ranabhat, C. L., InechOpen, London, United Kingdom. pp. 121-138.

Webb, E. C., Casey, N. H. & Simela, L., 2005. Goat meat quality. Small Ruminant Research, 60(1-2), 153-166.

Webb, E. C., Casey, N. H. & Van Niekerk, W. A., 1994. Fatty acids in the subcutaneous adipose tissue of intensively fed SA Mutton Merino and Dorper wethers. Meat Science, 38(1), 123-131.

Webb, E. C., Hassen, A., Olaniyi, M. O. & Pophiwa, P., 2022. Effect of dietary inclusion of azadirachta indica and moringa oleifera leaf extracts on the carcass quality and fatty acid composition of lambs fed high forage total mixed rations. Animals, 12(16), 2039.

Wein, S., Beyer, B., Gohlke, A., Blank, R., Metges, C. C. & Wolffram, S., 2016. Systemic absorption of catechins after intraruminal or intraduodenal application of a green tea extract in cows. PLoS One, 11(7), e0159428.

World Health Organization (WHO)., 2003. Diet, Nutrition and the Prevention of Chronic Diseases. Report of a joint WHO/FAO expert consultation. WHO technical report series 916, WHO, Geneva, Switzerland.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S.I. & Whittington, F. M., 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Science, 78(4), 343-358.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Whittington, F. M. & Richardson, R. I., 2003. Effects of diets on fatty acids and meat quality. Options Méditerranéennes, Series A, 67, 133-141.

Yang, C., McKain, N., McCartney, C. A. & Wallace, R. J., 2019. Consequences of inhibiting methanogenesis on the biohydrogenation of fatty acids in bovine ruminal digesta. Animal Feed Science and Technology, 254, 114189.

Yu, L. P., & Lee, Y. B., 1986. Effects of postmortem pH and temperature muscle structure and meat tenderness. Journal of Food Science, 51(3), 774-780.

Yusiati, L. M., Kurniawati, A., Hanim, C. & Anas, M. A., 2018. Protein binding capacity of different forages tannin. In: IOP Conference Series: Earth and Environmental Science, 119, 012007

Zeller, W. E., 2019. Activity, purification, and analysis of condensed tannins: Current state of affairs and future endeavors. Crop Science, 59(3), 886-904.

Zhao, L., Meng, Q., Ren, L., Liu, W., Zhang, X., Huo, Y. & Zhou, Z., 2015. Effects of nitrate addition on rumen fermentation, bacterial biodiversity and abundance. Asian-Australasian Journal of Animal Sciences, 28(10), 1433.

Zimmer, N., Cordesse, R., Inesta, M. & Fabre, D., 1995. Influence des tanins hydrolysables de châtaignier sur le métabolisme azoté des ovins et des caprins. In: Annales de zootechnie, 44(1), 227-227.

Zock, P. L., De Vries, J. H. & Katan, M. B., 1994. Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. Arteriosclerosis and thrombosis: A journal of vascular biology, 14(4), 567-575.

CHAPTER 5

5 GENERAL CONCLUSION AND IMPLICATIONS

Research has shown that tannin can be used to reduce methane emissions in ruminants. This is advantageous as it is a contributing factor in global warming. Calcium nitrate, apart from being used as a non-protein-nitrogen (NPN) source, has also been used to reduce methane emissions. Although there are a few disadvantages to these supplements, they mostly either have no effect or even positive effects on carcass characteristics, proximate analysis, blood and colour. Tannin and calcium nitrate can safely be used for the reduction of enteric methane emissions while simultaneously having minimal effect or overall improvement in the fatty acid profile. This is advantageous due to the stigma surrounding the fatty acid profile of ruminant meat. The fatty acid depot sites were not all as sensitive to dietary change and did not all react in the same manner. Not all changes in the depot sites were caused by changes in biohydrogenation patterns. In some cases, this could be explained by changes in enzyme activity. Tannin had more of an overall impact on the fatty acid profile than calcium nitrate. Blood parameters indicated that the sheep were healthy. The higher BUN concentration did not appear to affect the sheep on the calcium nitrate-based diet negatively in terms of the carcass, quality of the meat or health of the lambs.

The largest disadvantage of tannin at the dose rate currently utilised is the extra month required to reach slaughter weight, its lower cold carcass weight and dressing percent and higher EE. Tannin did not affect other carcass characteristics or proximate analysis (body composition, fat depth, eye muscle area, carcass pH, moisture percent or ash percent). Tannin did not change the antioxidant properties and shelf life, evident by no significant differences in colour stability. An advantage to supplementation is its faster elevation in *longissimus dorsi* muscle temperature that may improve meat tenderness. Tannin improved the fatty acid profile from a health perspective, showing the potential to increase consumer acceptability. For two or more fat depots, as a percent of detected fatty acid and/or gravimetric concentration, tannin increased polyunsaturated fatty acid (PUFA), vaccenic acid, trans fatty acids, C18:1n11c, C18:2n6c, n-3 and n-6 fatty acids. The fat of lambs fed tannin was better due to the higher PUFA and PUFA/SFA ratio. In terms of the health indices, desirable fatty acids (DFA) and hypocholesterolaemic fatty acids (h) increased in perirenal fat (mg/g). However, due to less total fat in subcutaneous fat of sheep fed tannin, desirable fatty acids (DFA), hypocholesterolaemic fatty acids (h) and hypercholesterolaemic fatty acids (H) decreased. The higher trans fatty acid was not a concern due to vaccenic acid being a major constituent, as it has many health benefits. The higher n-6 fatty acids did not change the n-6/n-3 ratio which was within the recommended range of below 4 in IMF or reduce it where it was above 4 in subcutaneous fat and perirenal fat.

Calcium nitrate supplementation could be used for the reduction of methane emissions and as a NPN source without affecting proximate analysis or carcass characteristics (with the acceptation of a decrease in dressing percent). It did not show any antioxidant properties between days, but redness (a*) was lower on day six. Fatty acids were not affected consistently across all fatty acid depots sites. Neither the health indices nor fatty acid profile of SCF was affected by calcium nitrate supplementation. IMF decreased SFA and increased MUFA and PUFA/SFA ratio. A decrease in C18:0 could be associated with an increase in delta-9 desaturase activity. The atherogenic (AI), thrombogenic (TI), h/H ratio, h and H were significantly improved as a percent, and all but h and H also significantly improved as a concentration. The concentration of PRF had improved h and DFA indices, and MUFA was significantly higher due to an increase in vaccenic acid (mg/g).

The sex of lambs had a major effect on the carcass characteristics, colour and fatty acid profile. Ewe lambs took much longer to reach their slaughter weights than rams and were already depositing fat at time of slaughter (between 45 and 50kg). They were therefore closer to their mature size. This was evident by the body condition ratio (fat:muscle:bone) which was higher in fat and lower in muscle and bone, increased SCF depth, EE and D% in ewes and lowering moisture percent. Liveweight, CCW and EMA were higher in rams. Therefore, if ewe lambs were slaughtered earlier, they would have had a similar body composition as rams. Lightness (L*) was higher in rams, and lower in a* and Chroma. However, these differences were only significant on day one. By day six there was only a tendency for ewes to start going yellower (b*), possibly because of them being fatter. All fatty acid depot sites were affected by lamb sex. However, PRF was the least affected. SFA, MUFA and PUFA profiles, individual fatty acids, ratios, enzymes and health indices were all influenced by sex. All fatty acid depot sites consistently indicated that ram lambs had a healthier fatty acid profile due to lower C14:0 and C16:0 fatty acids, lower AI and H indices and a higher PUFA/SFA ratio. The fatty acids consistently affected by sex in two or more of the fatty acid depots (as a percent of detected fatty acid and/or concentration) were saturated fatty acid (SFA), C14:0, C16:0, C22:0, C18:2n6t and total fatty acid which were lower in rams; and PUFA, C18:2n6c, n-6, PUFA/SFA (P/S) ratio, total cis FA and elongase activity which were lower in ewes. There were a few instances where ewes and rams were affected differently when fed calcium nitrate and tannin.

The weight of lambs at slaughter was predetermined and lambs slaughtered at a heavier weight took longer to reach their target weight. Heavier lambs had increased CCW and EMA but had already started depositing more fat into the subcutaneous region. However, it had not yet increased its deposition of fat into the intramuscular fat region, evident by no significant increase in EE%. Yellowness (b*) and hue angle (h) were higher in the lighter sheep on day one but by day six there were no differences between weight categories. Weight did not have a substantial effect on the

healthfulness of the fatty acid profile. Therefore, a greater difference in weight would have been required for an effect. Interactions between weight and treatment were found. There were a few instances where tannin and calcium nitrate affected heavy and light lambs differently.

Tannin and calcium nitrate can be used for the reduction of methane emissions without having a negative effect on meat quality. However, lambs on the tannin diet will take longer to reach slaughter weight and have a lower CCW and D% at the weight slaughtered and this will have an effect on the overall profitability of the feedlotting activities. Ewes and rams differed significantly, emphasizing the difference in physiological growth rates and the importance of determining a lighter slaughter weight for ewes than rams for the best quality carcass. The weight at which lambs were slaughtered were close enough together to pose no major differences in the carcass or its quality.

CHAPTER 6

6 CRITICAL EVALUATION AND RECOMMENDATIONS

- Following this study, a DNA sample should be conducted on the rumen fluid and digesta to confirm the biohydrogenating microbes affected by tannin and calcium nitrate.
- The feaces of tannin supplemented lambs had a coating around their droppings suggesting attachment to the proteins of the digestive tract. Further research should examine the effect of Acacia mearnsii at the current dose on the effect on the rumen and intestinal tract lining. The purpose of this would be to ensure absorption of nutrients is not compromised.
- The rumen fluid fatty acid was very diluted, and the dilution differed depending on how much the lambs drank before slaughter. A possible solution to standardize it could be to freeze dry a rumen fluid sample to create a more concentrated sample. The risk would be a change in the fatty acid profile. Multiple samples from fistulated sheep may also be a consideration.
- This study had a very low polyunsaturated fatty acid concentration. A possible reason for this could have been the method used. To validate the results a follow up study using the chloroform: methanol method by Folch et al. (1957) with modifications by Webb & Casey (1995) should be considered.
- Acacia mearsii may have different binding afficacies to proteins amongst other phytochemical properties which may make these results vary from other studies. Therefore, studies with the same concentration of tannin are not always comparable. Other factors like production system, diet etc. will also have an effect.
- This is a broad study researching the effect of many factors that need to be considered in conjunction with using calcium nitrate and tannin to reduce methane emissions– further research should aim to have a larger sample size with a more specific focus on its affect within each factor (i.e effect of tannin on animals in different stages of maturity and a separate study on the effect of tannin on rams and effect of tannins on ewes.
- As weight and carcass weight increase, the percent of muscle and bone would ordinarily decrease, and fat would increase. The differences between this study and others may have to do with the stage of maturity being very similar within the heavy and light weight catagories. To compare with previous studies starting with weights at an earlier maturity would be needed to model the data – that was not the purpose of this study. Further research should examine the effect of calcium nitrate and tannin supplements on lambs at an earlier state of maturity.

Folch, J., Lees, M. & Sloane Stanley, G. H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemiatry. *226*(1), 497-509.

Webb, E. C. & Casey, N. H., 1995. Genetic differences in fatty acid composition of subcutaneous adipose tissue in Dorper and SA Mutton Merino wethers at different live weights. *Small Ruminant Research*. *18*(1), 81-88.

Beitz, D. C., 2004. Lipid Metabolism. In: Dukes' Physiology of Domestic Animals. Ed: Reece, W.O., Cornell University Press, Ithaca, New York. pp. 453-471.

CHAPTER 7

7 APPENDIX

7.1 Fatty acid interacions

Tables indicating the fatty acid interactions (p-values) for treatment and sex (Treat*MF), treatment and weight (Treat*wight), tannin and sex (T*MF), none protein nitrogen source and sex (N*MF), tannin and weight (T*HL), none protein nitrogen source and weight (N*HL), and sex and weight (MF*HL) in the different fatty acid depot sites. Significant differences are highlighted in bold where P<0.05.

Gravimetric interaction tables:

Table 7.1.1: P-values indicating the gravimetric interactions between the factors of treatment, sex and weight on the concentration of medium and long chain fatty acids of the *longissimus dorsi* between the 8th and 10th ribs.

	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL	MF*HL
Fatty acids							
C10:0	0.84	0.591	0.798	0.4	0.911	0.186	0.633
C12:0	0.964	0.329	0.63	0.856	0.416	0.108	0.518
C13:0	0.818	0.355	0.409	0.729	0.507	0.111	0.832
C14:0	0.921	0.696	0.801	0.941	0.912	0.25	0.732
C14:1	0.971	0.563	0.852	0.889	0.686	0.188	0.944
C16:0	0.391	0.743	0.113	0.98	0.678	0.309	0.518
C16:1	0.462	0.834	0.14	0.797	0.841	0.381	0.83
C17:0	0.901	0.404	0.929	0.796	0.612	0.127	0.978
C18:0	0.71	0.504	0.426	0.845	0.855	0.17	0.567
C18:1t11	0.863	0.162	0.428	0.952	0.426	0.086	0.621
C18:1n9c	0.607	0.801	0.424	0.633	0.682	0.384	0.889
C18:1n11c	0.175	0.179	0.062	0.367	0.266	0.126	0.763
C18:2n6t	0.859	0.538	0.54	0.896	0.625	0.926	0.098
C18:2n6c	0.961	0.748	0.848	0.692	0.852	0.295	0.308
C20:0	0.94	0.326	0.81	0.986	0.336	0.131	0.9
C18:3n6	0.531	0.497	0.915	0.393	0.253	0.49	0.153
C20:1	0.485	0.352	0.676	0.598	0.289	0.259	0.918
C18:3n3	0.62	0.488	0.222	0.836	0.577	0.17	0.358
CLA cis-9, trans-11/ trans-9, cis-12	0.492	0.52	0.518	0.165	0.677	0.265	0.917
CLA cis-10, cis-12	0.23	0.591	0.126	0.321	0.924	0.947	0.615
CLA trans-10, cis-12	0.095	0.448	0.057	0.075	0.594	0.546	0.995
CLA isomer 1	0.972	0.818	0.755	0.744	0.789	0.47	0.371
CLA isomer 3	0.072	0.65	**0.041**	0.12	0.808	0.777	0.531
C20:2	0.17	0.233	0.267	**0.049**	0.732	0.114	0.615
C22:0	0.423	0.603	0.181	0.593	0.72	0.367	0.482
C20:3n6	0.662	0.972	0.872	0.877	0.797	0.79	0.801
C22:1n9	0.39	0.439	0.286	0.296	0.392	0.408	0.383
C23:0	0.375	0.058	0.122	0.481	0.373	**0.011**	0.476
C20:4n6	0.61	0.951	0.895	0.7	0.753	0.644	0.487

Continuation Table 7.1…							
C24:0	0.675	0.326	0.414	0.551	0.833	0.089	0.971
C20:5n3	0.236	0.255	0.075	0.459	0.255	0.334	0.577
C24:1	0.236	**0.029**	0.176	0.111	**0.023**	0.036	**0.008**
C22:6n3	0.176	0.779	0.529	0.088	0.351	0.728	0.385
UIP	0.197	0.169	0.352	0.333	0.376	0.084	0.377
Sums and ratios							
SFA	0.555	0.635	0.221	0.962	0.788	0.219	0.542
MUFA	0.59	0.714	0.351	0.681	0.805	0.289	0.969
PUFA	0.885	0.533	0.905	0.6	0.631	0.189	0.377
USF	0.625	0.651	0.360	0.645	0.856	0.239	0.945
SFA / UFA	0.675	0.727	0.243	0.882	0.680	0.297	0.093
MUFA/SFA	0.495	0.638	0.234	0.923	0.839	0.213	0.098
PUFA/SFA	0.837	0.911	0.483	0.739	0.519	0.801	0.517
CLA	0.314	0.541	0.25	0.131	0.697	0.355	0.788
n-3 PUFA	0.449	0.463	0.179	0.556	0.432	0.212	0.357
n-6 PUFA	0.598	0.959	0.921	0.702	0.736	0.687	0.527
n6/n3 ratio	0.582	0.652	0.219	0.804	0.459	0.473	0.406
Cis FA	0.641	0.73	0.393	0.668	0.76	0.293	0.974
Trans FA	0.722	0.192	0.33	0.583	0.465	0.111	0.761
Trans/cis ratio	0.786	0.193	0.368	0.708	0.299	0.177	0.651
Total FA	0.591	0.634	0.273	0.808	0.817	0.222	0.765

Table 7.1.2: P-values indicating the interaction between the factors of treatment, sex and weight on the enzyme and health indices from the concentration of medium and long chain fatty acids of the *longissimus dorsi* between the 8th and 10th ribs.

	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL	MF*HL
Enzyme indices							
Elongase	0.182	0.772	**0.049**	0.836	0.612	0.478	**0.050**
Delta 9 desaturase (C14+C16+C18)	0.400	0.479	0.175	0.748	0.876	0.131	0.752
Delta 9 desaturase C14	0.997	0.691	0.937	0.839	0.562	0.368	0.601
Delta 9 desaturase C16	0.976	0.963	0.864	0.845	0.644	0.992	0.218
Delta 9 desaturase C18	0.987	0.490	0.895	0.889	0.483	0.292	0.046
Delta 9 desaturase RA	0.307	0.948	0.965	0.105	0.641	0.792	0.425
Health indices							
AI	0.722	0.629	0.300	0.809	0.723	0.277	**0.010**
TI	0.370	0.731	0.089	0.884	0.489	0.382	0.107
DFA	0.636	0.594	0.368	0.693	0.853	0.208	**<0.001**
h	0.645	0.654	0.389	0.631	0.843	0.245	**<0.001**
H	0.479	0.736	0.159	0.973	0.734	0.292	**<0.001**
h/H	0.398	0.624	0.115	0.841	0.407	0.367	**0.011**

Table 7.1.3: P-values indicating the interactions between the factors of treatment, sex and weight on the concentration of subcutaneous medium and long chain fatty acids.

	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL	MF*HL
Fatty acids							
C10:0	0.334	0.746	0.264	0.136	0.656	0.388	0.543
C12:0	0.803	0.459	0.473	0.596	0.515	0.159	0.875
C13:0	0.534	0.345	0.172	0.886	0.085	0.717	0.129
C14:0	0.803	0.453	0.366	0.706	0.426	0.25	0.906
C14:1	0.463	0.329	0.871	0.153	0.434	0.155	0.694
C16:0	.0.791	0.763	0.343	0.742	0.387	0.928	0.491
C16:1	0.139	0.523	0.31	0.049	0.893	0.283	0.263
C17:0	0.37	0.109	0.098	0.792	0.087	0.282	0.134
C18:0	0.175	0.136	0.097	0.195	**0.026**	0.713	0.554
C18:1t11	0.621	0.149	0.664	0.597	0.088	0.686	0.438
C18:1n9c	0.082	0.87	0.31	0.022	0.796	0.691	0.413
C18:1n11c	**0.045**	0.088	0.296	0.626	0.117	0.822	**0.017**
C18:2n6t	**0.099**	**0.017**	0.046	0.331	0.163	**0.025**	0.178
C18:2n6c	0.273	0.616	0.445	0.267	0.232	0.902	0.229
C20:0	0.243	0.218	0.175	0.146	0.069	0.34	0.737
C18:3n6	0.401	0.137	0.271	0.254	0.059	0.58	0.653
C20:1	0.506	0.28	0.335	0.285	0.214	0.46	0.448
C18:3n3	0.991	**0.028**	0.942	0.771	**0.006**	0.79	0.826
CLA cis-9, trans-11/ trans-9, cis-12	0.135	0.839	0.303	0.064	0.683	0.718	0.663
CLA Trans-10, cis-12	0.724	0.519	0.767	0.819	0.149	0.969	0.323
CLA isomer 1	0.999	**0.046**	0.999	0.895	0.118	0.051	0.399
CLA isomer 3	0.646	0.688	0.499	0.355	0.458	0.796	0.252
C20:2	0.37	0.147	0.107	0.604	0.117	0.107	0.298
C22:0	0.543	0.744	0.598	0.203	0.397	0.559	0.367
C20:4n6	0.27	0.691	0.676	0.627	0.308	0.948	0.229
UIP	0.247	0.279	0.093	0.286	0.093	0.542	0.676
Sums and ratios							
SFA	0.327	0.31	0.138	0.311	0.074	0.696	0.564
MUFA	0.102	0.457	0.384	0.037	0.321	0.891	0.235
PUFA	0.29	0.381	0.39	0.57	0.119	0.946	0.236
USF	0.124	0.321	0.523	0.059	0.197	0.908	0.165
SFA / UFA	0.107	0.468	0.256	**0.049**	0.266	0.628	0.753
MUFA/SFA	0.160	0.686	0.419	0.056	0.346	0.782	0.875
PUFA/SFA	0.223	0.798	0.115	0.975	0.536	0.882	0.523
CLA	0.159	0.872	0.437	0.067	0.523	0.839	0.956
n-3 PUFA	0.991	0.028	0.942	0.771	**0.006**	0.79	0.826
n-6 PUFA	0.269	0.601	0.464	0.276	0.219	0.905	0.219
n6/n3 ratio	0.22	0.682	0.498	0.213	0.291	0.849	0.302
Cis FA	0.089	0.752	0.345	**0.03**	0.513	0.762	0.224
Trans FA	0.597	0.152	0.595	0.807	0.089	0.703	0.452
Trans/cis ratio	0.556	0.27	0.505	0.403	0.135	0.612	0.718
Total FA	0.484	0.195	0.134	0.909	**0.044**	0.792	0.260

Table 7.1.4: P-values indicating the interactions between the factors of treatment, sex and weight on the enzyme and health indices from the concentration of subcutaneous medium and long chain fatty acids.

	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL	MF*HL
Enzyme indices							
Elongase	0.706	0.379	0.662	0.339	0.479	0.881	0.84
Delta 9 desaturase (C14+C16+C18)	0.173	0.434	0.423	0.046	0.141	0.628	0.783
Delta 9 desaturase C14	0.398	0.524	0.364	0.273	0.814	0.778	0.412
Delta 9 desaturase C16	0.529	0.462	0.382	0.014	0.131	0.872	0.403
Delta 9 desaturase C18	0.071	0.528	0.589	0.057	0.479	0.269	0.753
Delta 9 desaturase RA	0.207	0.401	0.224	0.077	0.145	0.604	0.78
Health indices							
AI	0.166	0.311	0.333	0.158	0.076	0.650	0.735
TI	0.406	0.416	0.441	0.066	0.628	0.441	0.882
DFA	0.134	0.458	0.240	0.637	0.286	0.607	0.154
h	0.346	0.054	0.096	0.063	0.013	0.851	0.164
H	0.131	0.267	0.552	0.720	0.177	0.847	0.592
h/H	0.769	0.699	0.325	0.191	0.372	0.726	0.958

Table 7.1.5: P-values indicating the interactions between the factors of treatment, sex and weight on the concentration of perirenal medium and long chain fatty acids.

	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL	MF*HL
Fatty acids							
C10:0	0.24	0.279	0.711	0.21	0.769	0.494	0.087
C12:0	0.743	0.334	0.724	0.752	0.877	0.085	0.767
C14:0	0.845	0.244	0.784	0.857	0.281	0.155	0.806
C14:1	0.632	0.222	0.427	0.876	0.837	**0.055**	0.199
C16:0	0.956	0.153	0.708	0.84	0.074	0.544	0.402
C16:1	0.348	0.42	0.256	0.55	0.307	0.259	0.167
C17:0	0.452	0.447	0.754	0.143	0.474	0.184	0.969
C18:0	0.654	0.277	0.877	0.596	0.3	0.115	0.177
C18:1t11	0.286	0.612	0.192	0.357	0.67	0.649	0.338
C18:1n9c	0.295	0.611	0.083	0.428	0.378	0.58	0.354
C18:1n11c	0.168	0.574	0.537	0.131	0.307	0.595	0.359
C18:2n6t	0.23	0.8	0.119	0.643	0.937	0.533	0.131
C18:2n6c	0.557	0.688	0.514	0.41	0.261	0.872	0.3
C20:0	0.901	0.926	0.633	0.574	0.835	0.949	0.273
C18:3n6	0.551	0.836	0.87	0.251	0.391	0.831	0.467
C20:1	0.391	0.075	0.101	0.637	0.117	0.039	0.066
C18:3n3	0.791	0.933	0.351	0.704	0.843	0.559	0.391
CLA cis-9, trans-11/ trans-9, cis-12	0.896	0.975	0.83	0.795	0.668	0.875	0.299
CLA Trans-10, cis-12	0.258	0.398	0.453	0.172	0.16	0.352	0.514
CLA isomer 1	0.156	0.565	0.251	0.314	0.349	0.984	0.293
CLA isomer 3	0.236	0.42	0.71	0.069	0.305	0.178	0.104
C20:2	0.384	0.236	0.324	0.189	0.627	0.13	0.341
C22:0	0.689	0.991	0.679	0.304	0.849	0.994	0.754
C20:4n6	0.405	0.665	0.516	0.615	0.528	0.806	0.708
UIP	0.737	0.414	0.534	0.732	0.382	0.204	0.102
Sums and ratios							
SFA	0.845	0.171	0.969	0.581	0.12	0.176	0.168
MUFA	0.359	0.595	0.247	0.245	0.292	0.448	0.191
PUFA	0.355	0.546	0.381	0.382	0.179	0.684	0.082
USF	0.372	0.514	0.393	0.221	0.22	0.444	0.125
SFA / UFA	0.606	0.226	0.47	0.909	0.093	0.249	0.115
MUFA/SFA	0.686	0.303	0.454	0.593	0.174	0.221	0.11
PUFA/SFA	0.538	0.178	0.436	0.745	0.084	0.261	**0.034**
CLA	0.835	0.995	0.876	0.857	0.836	0.899	0.201
n-3 PUFA	0.791	0.933	0.351	0.704	0.843	0.559	0.391
n-6 PUFA	0.547	0.69	0.513	0.412	0.261	0.868	0.294
n6/n3 ratio	0.528	0.639	0.95	0.274	0.342	0.44	0.761
Total cis	0.324	0.542	0.133	0.327	0.258	0.573	0.24
Total trans	0.274	0.616	0.193	0.357	0.663	0.65	0.292
Trans/cis ratio	0.263	0.623	**0.077**	0.688	0.895	0.846	0.655
Total FA	0.481	0.377	0.573	0.148	0.371	0.324	0.653

Table 7.1.6: P-values indicating the interactions between the factors of treatment, sex and weight on the enzyme and health indices from the concentration of perirenal medium and long chain fatty acids.

	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL	MF*HL
Enzyme indices							
Elongase	0.373	0.496	0.207	0.614	0.179	0.699	0.851
Delta 9 desaturase (C14+C16+C18)	0.590	0.368	0.230	0.792	0.174	0.286	0.176
Delta 9 desaturase C14	0.532	0.335	0.249	0.784	0.783	0.102	0.078
Delta 9 desaturase C16	0.484	0.206	0.224	0.572	0.090	0.226	0.131
Delta 9 desaturase C18	0.595	0.394	0.322	0.900	0.272	0.207	0.172
Delta 9 desaturase RA	0.772	0.723	0.400	0.574	0.529	0.974	0.767
Health indices							
AI	0.711	0.293	0.279	0.804	0.074	0.938	0.209
TI	0.613	0.261	0.444	0.920	0.106	0.275	0.132
DFA	0.374	0.708	0.390	0.130	0.981	0.318	0.931
h	0.383	0.530	0.407	0.220	0.224	0.463	0.13
H	0.923	0.137	0.687	0.823	0.063	0.795	0.409
h/H	0.774	0.215	0.482	0.438	0.068	0.610	0.199

Molar interaction tables:

Table 7.1.7: P-values indicating the interactions between the factors of treatment, sex and weight on the medium and long chain fatty acid percent of total lipid in the longissimus dorsi between the 8th and 10th ribs.

	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL	MF*HL
Fatty acid							
C10:0	0.34	0.626	0.758	0.146	0.915	0.265	0.862
C12:0	0.721	0.232	0.343	0.79	0.331	0.086	0.579
C13:0	0.568	0.344	0.198	0.565	0.491	0.105	0.958
C14:0	0.997	0.666	0.899	0.87	0.987	0.288	0.954
C14:1	1.000	0.574	0.959	0.954	0.677	0.218	0.632
C16:0	0.179	0.67	0.043	0.876	0.31	0.869	0.478
C16:1	0.604	0.997	0.192	0.856	0.908	0.884	0.419
C17:0	0.886	0.552	0.452	0.956	0.32	0.343	0.8
C18:0	0.923	0.804	0.507	0.955	0.746	0.813	0.321
C18:1t11	0.863	0.237	0.535	0.681	0.282	0.237	0.29
C18:1n9c	0.703	0.225	0.47	0.671	0.319	0.081	0.437
C18:1n11c	0.414	0.547	0.542	0.32	0.225	0.692	0.69
C18:2n6t	0.591	0.339	0.574	0.975	0.61	0.321	**0.022**
C18:2n6c	0.776	0.974	0.64	0.426	0.797	0.801	0.488
C20:0	0.438	0.074	0.124	0.613	0.012	0.584	0.948
C18:3n6	0.379	0.489	0.418	0.802	0.17	0.486	0.123
C20:1	0.517	0.175	0.261	0.9	**0.045**	0.72	0.621
C18:3n3	0.361	0.744	0.099	0.781	0.514	0.609	0.24
CLA cis-9, trans-11/ trans-9, cis-12	0.366	0.646	0.757	0.113	0.458	0.438	0.999
CLA cis-10, cis-12	0.544	0.535	0.362	0.343	0.68	0.72	0.831
CLA trans-10, cis-12	0.089	0.465	0.09	**0.048**	0.372	0.63	0.936
CLA isomer 1	0.786	0.979	0.343	0.955	0.873	0.861	0.43
CLA isomer 3	0.136	0.719	0.112	0.081	0.542	0.936	0.534
C20:2	0.208	0.469	0.613	0.058	0.344	0.56	0.896
C22:0	0.958	0.212	0.833	0.86	0.491	0.099	0.761
C20:3n6	0.659	0.771	0.737	0.764	0.748	0.483	0.646
C22:1n9	0.393	0.462	0.287	0.272	0.426	0.468	0.433
C23:0	1	0.09	0.978	0.921	0.355	**0.021**	0.909
C20:4n6	0.627	0.895	0.419	0.827	0.541	0.803	0.854
C24:0	0.944	0.305	0.563	0.863	0.593	0.093	0.734
C20:5n3	0.221	0.38	**0.045**	0.882	0.231	0.797	0.981
C24:1	0.937	0.052	0.631	0.706	0.059	0.034	0.204
C22:6n3	0.171	0.584	0.116	0.252	0.201	0.964	0.657
UIP	0.115	0.355	0.07	0.554	0.243	0.282	0.452
Sums and ratios							
SFA	0.412	0.788	0.134	0.846	0.503	0.469	0.183
MUFA	0.894	0.43	0.801	0.897	0.622	0.127	0.094
PUFA	0.783	0.925	0.553	0.805	0.538	0.907	0.564
USF	0.911	0.582	0.484	0.920	0.908	0.177	0.063
SFA / UFA	0.671	0.726	0.241	0.880	0.680	0.296	0.093
MUFA/SFA	0.496	0.638	0.234	0.923	0.839	0.213	0.098
PUFA/SFA	0.834	0.911	0.475	0.743	0.518	0.798	0.518
CLA	0.272	0.633	0.405	0.077	0.444	0.552	0.825
n-3 PUFA	0.251	0.585	0.062	0.933	0.327	0.701	0.411
n-6 PUFA	0.625	0.886	0.443	0.824	0.548	0.759	0.888
n6/n3 ratio	0.582	0.652	0.219	0.804	0.46	0.472	0.407

Continuation Table 7.7…							
Cis FA	0.816	0.345	0.357	0.834	0.711	0.108	0.199
Trans FA	0.795	0.182	0.403	0.748	0.231	0.224	0.39
Trans/cis ratio	0.787	0.193	0.37	0.706	0.298	0.178	0.65

Table 7.1.8: P-values indicating the interactions between the factors of treatment, sex and weight on the enzyme and health indices of the longissimus dorsi between the 8th and 10th ribs from the percent of total lipid (%).

	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL	MF*HL
Enzyme indices							
Elongase	0.182	0.772	**0.049**	0.836	0.612	0.478	0.65
Delta 9 desaturase (C14+C16+C18)	0.400	0.479	0.175	0.748	0.876	0.131	0.175
Delta 9 desaturase C14	0.997	0.696	0.938	0.840	0.566	0.373	0.328
Delta 9 desaturase C16	0.976	0.963	0.864	0.845	0.644	0.992	0.204
Delta 9 desaturase C18	0.987	0.490	0.895	0.889	0.483	0.292	0.256
Delta 9 desaturase RA	0.307	0.948	0.965	0.105	0.641	0.792	0.694
Health indices							
AI	0.722	0.629	0.300	0.809	0.723	0.277	0.357
TI	0.402	0.752	0.102	0.876	0.503	0.395	0.082
DFA	0.801	0.732	0.332	0.960	0.772	0.321	0.332
H	0.822	0.547	0.358	0.963	0.962	0.158	0.096
H	0.300	0.681	0.080	0.860	0.381	0.672	0.53
h/H	0.396	0.624	0.115	0.843	0.406	0.367	0.442

Table 7.1.9: P-values indicating the interactions between the factors of treatment, sex and weight on the medium and long chain fatty acid profile of the subcutaneous fat from the percent of total lipid (%).

	Treat*MF	T*MF	N*MF	Treat*HL	T*HL	N*HL	MF*HL
Fatty acids							
C10:0	0.371	0.544	0.101	0.75	0.635	0.326	0.385
C12:0	0.783	0.464	0.527	0.476	0.569	0.161	0.837
C13:0	0.524	0.152	0.961	0.375	0.089	0.885	0.126
C14:0	0.799	0.411	0.572	0.443	0.612	0.246	0.752
C14:1	0.63	0.834	0.236	0.345	0.25	0.27	0.442
C16:0	0.779	0.603	0.398	0.474	0.791	0.982	0.773
C16:1	0.132	0.486	**0.033**	0.324	0.443	0.27	0.341
C17:0	0.345	0.082	0.809	0.194	0.056	0.473	0.139
C18:0	0.101	0.12	0.095	0.237	**0.049**	0.775	0.811
C18:1t11	0.534	0.465	0.566	0.285	0.178	0.633	0.499
C18:1n9c	0.143	0.991	0.024	0.507	0.176	0.573	0.903
C18:1n11c	0.083	0.858	0.695	0.352	0.563	0.852	0.059
C18:2n6t	0.037	0.016	0.254	0.05	0.481	**0.034**	0.197
C18:2n6c	0.26	0.231	0.357	0.853	0.462	0.768	0.291
C20:0	0.171	0.194	0.069	0.248	0.073	0.407	0.979
C18:3n6	0.51	0.522	0.249	0.351	0.165	0.725	0.674
C20:1	0.726	0.762	0.287	0.411	0.458	0.665	0.408
C18:3n3	0.623	0.219	0.672	0.136	0.031	0.633	0.518
CLA cis-9, trans-11/ trans-9, cis-12	0.129	0.181	0.073	0.924	0.88	0.785	0.942
CLA Trans-10, cis-12	0.573	0.496	0.847	0.679	0.275	0.993	0.329
CLA isomer 1	0.967	0.677	0.905	0.128	0.329	0.068	0.409
CLA isomer 3	0.793	0.635	0.487	0.617	0.545	0.579	0.148
C20:2	0.898	0.943	0.599	0.932	0.911	0.692	0.1
C22:0	0.583	0.27	0.394	0.886	0.457	0.895	0.97
C20:4n6	0.449	0.459	0.997	0.564	0.205	0.641	0.515
UIP	0.336	0.092	0.545	0.295	0.073	0.648	0.362
Sums and ratios							
SFA	0.131	0.18	0.08	0.504	0.204	0.72	0.957
MUFA	0.094	0.739	**0.021**	0.686	0.41	0.778	0.735
PUFA	0.228	0.16	0.682	0.773	0.371	0.8	0.338
USF	0.095	0.467	**0.036**	0.767	0.603	0.848	0.551
SFA / UFA	0.09	0.232	**0.044**	0.48	0.241	0.683	0.7
MUFA/SFA	0.145	0.686	0.396	0.053	0.326	0.816	0.91
PUFA/SFA	0.189	0.868	0.100	0.876	0.633	0.788	0.457
CLA	0.156	0.262	0.08	0.992	0.922	0.955	0.623
n-3 PUFA	0.623	0.219	0.672	0.136	0.031	0.633	0.518
n-6 PUFA	0.26	0.245	0.358	0.842	0.44	0.782	0.287
n6/n3 ratio	0.207	0.562	0.16	0.724	0.328	0.747	0.342
Cis FA	0.132	0.769	**0.027**	0.64	0.245	0.682	0.77
Trans FA	0.498	0.392	0.747	0.302	0.19	0.659	0.547
Trans/Cis ratio	0.55	0.482	0.43	0.293	0.149	0.586	0.689

Table 7.1.10: P-values indicating the interactions between the factors of treatment, sex and weight on the enzyme and health indices of subcutaneous fat from the percent of total lipid (%).

	Treat*MF	T*MF	N*MF	Treat*HL	T*HL	N*HL	MF*HL
Enzyme indices							
Elongase	0.684	0.306	0.596	0.362	0.419	0.815	0.773
Delta 9 desaturase (C14+C16+C18)	0.162	0.429	0.408	**0.044**	0.135	0.649	0.805
Delta 9 desaturase C14	0.560	0.481	0.392	0.288	0.137	0.897	0.392
Delta 9 desaturase C16	0.070	0.519	0.599	**0.014**	0.473	0.265	0.398
Delta 9 desaturase C18	0.143	0.292	0.306	**0.051**	0.068	0.687	0.792
Delta 9 desaturase RA	0.253	0.486	0.265	0.088	0.170	0.707	0.642
Health indices							
AI	0.379	0.447	0.419	0.150	0.599	0.474	0.703
TI	0.306	0.225	0.389	0.228	0.077	0.966	0.663
DFA	0.386	0.033	0.112	0.580	**0.011**	0.846	0.179
H	0.102	0.708	0.423	0.042	0.632	0.775	0.571
H	0.763	0.498	0.517	0.413	0.962	0.732	0.901
h/H	0.381	0.546	0.353	0.185	0.792	0.804	0.934

Table 7.1.11: P-values indicating the interactions between the factors of treatment, sex and weight on the medium and long chain fatty acid profile of perirenal fat from the percent of total lipid (%).

	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL	MF*HL
Fatty acids							
C10:0	0.264	0.307	0.815	0.426	0.935	0.284	0.094
C12:0	0.756	0.314	0.768	0.904	0.96	0.071	0.729
C14:0	0.804	0.306	0.686	0.835	0.458	0.101	0.918
C14:1	0.628	0.219	0.482	0.775	0.912	**0.046**	0.202
C16:0	0.711	0.335	0.411	0.523	0.114	0.928	0.452
C16:1	0.479	0.369	0.357	0.887	0.261	0.194	0.177
C17:0	0.614	0.42	0.438	0.396	0.687	0.326	0.798
C18:0	0.515	0.351	0.858	0.908	0.382	0.128	0.122
C18:1t11	0.285	0.449	0.148	0.514	0.598	0.551	0.279
C18:1n9c	0.488	0.475	0.14	0.905	0.237	0.353	0.3
C18:1n11c	0.243	0.361	0.488	0.271	0.231	0.43	0.294
C18:2n6t	0.221	0.763	0.113	0.79	0.844	0.6	0.108
C18:2n6c	0.629	0.553	0.423	0.613	0.198	0.961	0.251
C20:0	0.985	0.881	0.737	0.963	0.599	0.633	0.322
C18:3n6	0.616	0.707	0.797	0.349	0.291	0.687	0.366
C20:1	0.465	**0.039**	0.121	0.997	0.081	**0.023**	0.056
C18:3n3	0.391	0.645	0.164	0.29	0.668	0.309	0.225
CLA cis-9, trans-11/ trans-9, cis-12	0.823	0.932	0.723	0.57	0.583	0.934	0.284
CLA Trans-10, cis-12	0.336	0.475	0.52	0.294	0.235	0.293	0.482
CLA isomer 1	0.15	0.513	0.244	0.373	0.321	0.965	0.274
CLA isomer 3	0.313	0.37	0.777	0.116	0.373	0.131	0.09
C20:2	0.459	0.146	0.331	0.299	0.46	0.065	0.272
C22:0	0.821	0.94	0.657	0.477	0.726	0.781	0.857
C20:4n6	0.375	0.788	0.592	0.471	0.485	0.953	0.526
UIP	0.983	0.522	0.747	0.885	0.273	0.395	0.146
Sums and ratios							
SFA	0.691	0.22	0.536	0.659	0.103	0.224	0.083
MUFA	0.641	0.329	0.364	0.698	0.171	0.245	0.153
PUFA	0.562	0.517	0.329	0.559	0.173	0.841	0.167
USF	0.668	0.277	0.497	0.637	0.127	0.258	0.113
SFA / UFA	0.6	0.248	0.422	0.846	0.096	0.281	0.128
MUFA/SFA	0.684	0.297	0.451	0.584	0.171	0.217	0.11
PUFA/SFA	0.567	0.391	0.395	0.494	0.136	0.612	0.116
CLA	0.826	0.934	0.761	0.845	0.717	0.726	0.197
n-3 PUFA	0.391	0.645	0.164	0.29	0.668	0.309	0.225
n-6 PUFA	0.615	0.553	0.42	0.617	0.197	0.96	0.242
n6/n3 ratio	0.521	0.599	0.917	0.256	0.321	0.415	0.791
Cis FA	0.563	0.365	0.211	0.813	0.153	0.333	0.196
Trans FA	0.276	0.441	0.149	0.536	0.583	0.542	0.237
Trans/cis ratio	0.262	0.624	0.077	0.689	0.893	0.847	0.655

Table 7.1.12: P-values indicating the interactions between the factors of treatment, sex and weight on the enzyme and health indices of perirenal fat from the percent of total lipid (%).

	Treat*MF	Treat*HL	T*MF	N*MF	T*HL	N*HL	MF*HL
Enzyme indices							
Elongase	0.373	0.496	0.207	0.614	0.179	0.699	0.851
Delta 9 desaturase (C14+C16+C18)	0.590	0.368	0.230	0.792	0.174	0.286	0.176
Delta 9 desaturase C14	0.533	0.335	0.250	0.784	0.783	0.101	0.078
Delta 9 desaturase C16	0.484	0.206	0.224	0.572	0.090	0.226	0.131
Delta 9 desaturase C18	0.595	0.394	0.322	0.900	0.272	0.207	0.172
Delta 9 desaturase RA	0.772	0.723	0.400	0.574	0.529	0.974	0.767
Health indices							
AI	0.710	0.292	0.278	0.802	0.074	0.940	0.21
TI	0.618	0.258	0.446	0.922	0.106	0.269	0.132
DFA	0.560	0.425	0.342	0.499	0.168	0.568	0.763
h	0.682	0.285	0.510	0.631	0.128	0.271	0.116
H	0.673	0.320	0.410	0.539	0.116	0.720	0.492
h/H	0.732	0.234	0.448	0.403	0.073	0.628	0.216

7.2 Chemicals

Table 7.2: Chemicals used for medium and long chain fatty acid analysis

Chemical	Supplier	Ref #
Diethyl ether for analysis Emsure ACS.ISO.REAG	Sigma Aldrich	1.00921.25
Petroleum benzene boiling range 40 60 grade C for gas chromatography Suprasolv	Sigma Aldrich	1.01772.25
Ethanol 95% (AR) Absolute	Ethanol SA 95/ES	
Chloroform grade for analysis ACS.ISO.REAG	Sigma Aldrich	1.02445.25
Anyhydrous Na_2SO_4 (AR)	Sigma Aldrich	238597
32% HCl	Sigma Aldrich	Emplura 1.00313.25
2%Sulphuric acid in methanol	Made up	
Methanol	Sigma Aldrich	LiChrosolv Gradient grade for liquid chromatography 1.06007.25
Sulphuric acid	Sigma Aldrich	30743.25
n- hexane	Sigma Aldrich	1.04371.25 n- hexane for gas chromatography Suprasolv
Toluene anhydrous 99.8% ACS reagent	Sigma Aldrich	244511
Pyrogallol	Sigma Aldrich	16040

Supelco 37 component FAME mix	Sigma Aldrich	CRM47885
Pentadecanoic acid internal std	Sigma Aldrich	P6125
9(z), 11(E) octadecadienoate (rumenic acid)	Laradon	20-1823-7
Cis/trans FAME mix	Restek	35079
Octadecadienoic acid, conjugated, methyl ester (CLA)	Sigma Aldrich	05632