THE EFFECT OF NDF CONTENT IN FINISHING DIETS ON PERFORMANCE AND MEAT QUALITY OF LAMBS

By

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ACRONYMS AND ABBREVIATIONS

a*	Redness factor of meat colour
AD	Acid detergent
ADF	Acid detergent fibre
ADG	Average daily gain
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine Tri-phosphate
b*	Yellowness factor of meat colour
BC	Buttock circumference
BE	Bruto energy
BH	Biohydrogenation
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
C	Carbon
c	*Cis*-configuration
C1	Blank bag correction
C4:0	Butyric acid
C6:0	Caproic acid
C10:0	Capric acid
C12:0	Lauric acid
C14:0	Myristic acid

C16:0	Palmitic acid
C16:1 *cis*-9	Palmitoleic acid
C18:0	Stearic acid
C18:1 *n*-9	Oleic acid
C18:1 *trans*-11	Vaccenic acid
C18:2 *cis*-9,*trans*-11	Conjugated linoleic acid
C18:2 *trans* 10, *cis* 12	Conjugated linoleic acid
C18:2*n*-6	Linoleic acid
C18:2*trans*11,*cis*15	Octadecadienoic acid
C18:3*n*-3	α-Linolenic acid
C20:4*n*-6	Arachidonic acid
C20:5*n*-3	Eicosapentaenoic acid
C22:6*n*-3	Docosahexaenoic acid
$C_{40}H_{80}NO_8P$	Dipalmitoyl lecithin
CH_4	Methane gas
CF	Crude fibre
CLA	Conjugated linoleic acid
cm	Centimeter
CO_2	Carbon dioxide gas
COOH	Carboxyl group
CP	Crude protein
CV	Coefficient of Variation
DE	Digestible energy

DHA	Docosahexaenoic acid
DM	Dry matter intake
DMI/lamb/week	Dry matter intake per lamb per week
EE	Ether extract
EL	External carcass length
EPA	Eicosapentaenoic acid
FA	Fatty acid
FAs	Fatty acids
FAME	Fatty acid methyl esters
FCR	Feed conversion ratio
FFA	Free fatty acid
FFAs	Free fatty acids
g	Gram
g/animal	Gram per animal
GC	Gas chromatograph
g DM	Gram dry matter
g/sheep/day	Gram per sheep per day
g/kg	Gram per kilogram
g/lamb/day	Gram per lamb per day
GE	Gross energy
GLM	General linear model
h	Hour
H^-	Hydrogen

H_2O	Water
HDL	High-density lipoprotein
HI	Heat increment
HSD	Tukey's honest significant difference
KOH	Potassium hydroxide
kg	Kilogram
Kg DM/day	Kilogram dry matter per day
Kg/lamb/day	Kilogram per lamb per day
km	Kilometer
KPa	Kilopascal
L*	Lightness factor of meat colour
LDL	Low-density lipoproteins
m	Meter
m_2	Meter squared (area)
ME	Metabolizable energy
MEI	Metabolizable energy intake
$Mg(ClO_4)_2$	Magnesium perclorate hexahydrate
mg	Milligram
mg/kg	Milligram per kilogram
min	Minute
MJ/kg DM	Mega joules per kilogram dry matter
MJ/kg	Mega joules per kilogram
MJ/sheep/day	Mega joule per sheep per day

MJ GE/kg DM	Mega joules gross energy per kilogram dry matter
mL	Milliliter
mm	Millimeter
mm/min	Millimetre per minute
mmol/l	Millimoles per liter
MMG	Metmyoglobin
MO	Micro-organism
MOs	Micro-organisms
MP	Microbial protein
MRT	Mean retention time
MUFA	Monounsaturated fatty acid
MUFAs	Monounsaturated fatty acids
n	Number
No.	Number
N_2	Nitrogen gas
n-3	Omega-3
n-6	Omega-6
n-6:*n-3*	Linoleic acid:α-Linolenic acid ratio
ND	Neutral-detergent
NDF	Neutral-detergent fibre
NDF%	Neutral-detergent fibre percentage
NDF/kg DM	Neutral-detergent fibre per kilogram dry matter
NE	Nett energy

NFC	Non-fibrous carbohydrates
NFE	Nitrogen-free extractives
NPN	Non-protein nitrogen
NRC	National Research Council
NSC	Non-structural carbohydrates
OM	Organic matter
OMG	Oxymyoglobin
P	Significance
peNDF	Physical effective neutral detergent fibre
pH	Hydrogen ion concentration
P_2O_5	Phosphorus pentoxide
psi	Pounds per square inch
PUFA	Polyunsaturated fatty acid
PUFAs	Polyunsaturated fatty acids
PUFA:SFA	Polyunsaturated fatty acids:Saturated fatty acid ratio
PVC	Polyvinyl chloride
RDP	Rumen degradable protein
SAMM	South African Mutton Merino
SARA	Sub-acute ruminal acidosis
SAS	Statistical analysis system
SC	Structural carbohydrates
SD	Standard deviation
SFA	Saturated fatty acid

SFAs	Saturated fatty acids
SI	Saturation index or chroma
T15	15% roughage inclusion
T30	30% roughage inclusion
T45	45% roughage inclusion
T60	60% roughage inclusion
T75	75% roughage inclusion
t	*Trans*-configuration
TBARS	Thiobarbituric acid reactive substances
TDN	Total digestible nutrients
TMR	Total mixed ration
UFA	Unsaturated fatty acid
UFAs	Unsaturated fatty acids
UFA:SFA	Unsaturated fatty acid:saturated fatty acid
VFA	Volatile fatty acid
VFAs	Volatile fatty acids
VLDL	Very low density lipoprotein
w/w	weight
W1	Bag tare weight
W2	Sample weight
W3	Dried weight of bag with fibre after extraction process
α	Alpha
Δ	Delta

µl	Micro liter
µm	Micro meter
%	Percentage
°C	Degrees Celsius
°C/min	Degrees Celsius per minute

CHAPTER 1

GENERAL INTRODUCTION

Recently there has been a lot of interest in finding different and more efficient techniques to manipulate the fatty acid (FA) composition of red meat. Currently, consumers are demanding safer, healthier and more convenient feed sources of reliable quality (McDonald *et al*., 2011). Ruminant products have traditionally been criticized for the perceived undesirable effects of saturated fatty acids (SFA) on human health and have therefore contributed to a decline in the consumption of red meat (Dewhurst *et al*., 2003). Most SFAs are associated with numerous diseases such as cardiovascular diseases and cancer (Wood *et al*., 2003). On the other hand, SFAs comprise more than 50% of cell membranes (providing cells with the needed rigidness) and contribute to the strength of bones by assimilating calcium effectively into the skeletal structure (Enig and Fallon, 1999). Most SFAs also lower lipoprotein levels (a blood-substance that shows proneness to heart diseases), provide liver protection due to alcohol abuse and toxins, improve the immune system and provide proper utilization of essential FAs [long chain omega-3 (*n-3*) FAs being better retained in tissue with a rich saturated fat diet] and asuitable energy substrate for the heart. Also some short- and medium chain SFAs have important antimicrobial properties (Enig and Fallon, 1999). Hence, both the amount and structure of a FA plays an important role in affecting and maintaining health (Jenkins *et al*., 2008).

There is a growing appreciation for the well-being benefits due in humans to the regular and particular consumption of polyunsaturated fatty acids (PUFA), such as *n*-3 and different conjugated linoleic acid (CLA) isomers (French *et al*., 2000). Conjugated linoleic acid has been discovered to decrease the possibility of cancer, cardiovascular disease, diabetes, improvement of the immune system and bone strength (Schmid *et al.,* 2006). Apart from the health benefits, PUFAs are also preferentially deposited in membrane phospholipids in ruminants, whereas the FA composition of phospholipids has been shown to be largely responsible for the susceptibility of meat to lipid oxidation and colour stability (Moloney *et al*., 2006; Whitney and Lupton, 2010). Strategies which alter the FA composition of meat could however also influence several aspects of meat quality, including its firmness, as well as meat colour and flavour (Wood *et al*., 2003).

The effect of nutrition on the FA composition of muscle and adipose tissue of red meat has been widely published and is mostly accredited to the FA composition of the diet fed to the animals (Whitney and Lupton, 2010). In addition, Cooper *et al*. (2004) stated that the basal diet also adversely affects ruminant FA composition. For example, when lambs were fed concentrate diets, the FA composition of the meat improved to more closely resemble what is recommended for the human diet as a whole. However, a lot of research has proven the contrary - the FA contents are more favourable in lambs finished on grassland or pasture (Aurousseau *et al.,* 2004; Poulson *et al.,* 2004). One of the main reasons for this positive effect is due to the general high *n*-3 and PUFA content of grass or pasture.

Lipids are notable energy supplement and provide about 2.25 times the digestible energy of carbohydrates (McDonald *et al*., 2011). An increase in the energy density of finishing diets is necessary in order to maintain higher levels of production. Thus, the inclusion of lipids in ruminant diets are in general practiced because of their high energy value (Chilliard, 1993; Bauman *et al*., 2003) and possible improvement of ruminant carcass quality (Bauchart *et al*., 1996) which could benefit animal and human health (McDonald *et al*., 2011). Standard ruminant diets contains on average 2.5 to 3% lipid. Higher levels may however disturb the rumen environment and adversely affect ruminal functions, consequently affecting animal performance parameters such as live weight gain and feed conversion (McDonald *et al*., 2011). Despite these concerns, most recent studies indicate that animal performance parameters are neither negatively affected by saturated nor unsaturated lipid source inclusion (Schollan *et al.,* 2006) if included at acceptable levels. One of the key concerns regarding unsaturated lipid supplementation is the negative effect it has on fibre digestion. The coating effect of free fatty acids (FFA) to cellulose may disrupt the attachment of bacterial cellulolytic enzymes (Jenkins, 1993), with a direct inhibition of rumen microbial activity (Maia *et al*., 2007). A common practise therefore is for total dietary fat to not exceed 6 to 7% of dietary dry matter (DM) (Jenkins, 1993; Doreau *et al*., 1997; NRC, 2001).

Unsaturated fatty acids have a negative effect on ruminal micro-organisms (MO) (Maia *et al*., 2007) and are therefore degraded to monounsaturated fatty acids (MUFA) and SFAs via microbial biohydrogenation (BH) (Wood *et al*., 2008). In other words, BH occurs when the unsaturated fatty acids (UFA) are hydrogenated to their saturated counterparts in the

rumen. The BH of UFAs in the rumen therefore leads to a high proportion of "health-threatening" SFAs in feed derived from ruminants (Li *et al*., 2010). Vaccenic acid (C18:1 *trans*-11) is one example and intermediate in the BH process of ruminal bacteria. From a dietary point of view, a high roughage diet fed to ruminants normally results in greater BH activities, affecting the carcass FA composition (McDonald *et al*., 2011). One of the main factors affecting the rate of BH depends on the composition of ruminal bacteria, which is adversely affected by the type of diet (chemical composition) fed to the animal (Lind and Molmann, 2011). Neutral-detergent fibre (NDF) is one of several important factors affecting the bacterial composition in the rumen. Ruminal pH and BH (Whitney and Lupton, 2010; McDonald *et al*., 2011) is positively related to the NDF content of the diet, as well as the NDF content of the roughage source fed to a ruminant (Galyean and Defoor, 2003). Ruminal pH is the most important ruminal factor affecting fibre digestion in the rumen (Nagaraja, 2012) and should be maintained at optimum levels to ensure rumen health and effective fermentation (Mertens, 1997).

In addition, altering the roughage:concentrate ratio in the diet of ruminants is a mechanism commonly suggested and used for synchronising available nutrients in the rumen in order to maximise the amount of microbial protein (MP), dietary protein and amino acids that passes to the small intestine of ruminants (Clark *et al*., 1992). As stated above, this ratio has the potential of not only affecting animal performance, but ruminant carcass FA composition as well. Therefore, it is important to acknowledge that the possibility exists that the dietary fibre, more specifically the NDF content of ruminant diets, could have an influence on ruminal bacteria composition (McDonald *et al*., 2011), hence BH and FA composition. Some research have been published in this regard focusing on total roughage inclusion and its effect on ruminal FA composition and outflow (Kucuk *et al*., 2001), but no data exists with special reference to the NDF content of lamb (and all ruminant) diets and its effect on carcass FA composition. The influence of roughage and concentrate inclusion in the diet on the FA composition of muscle and adipose tissue remains in abundant attentiveness (Aurousseau *et al.,* 2004) and there seems to be a lack of published studies (Helander, 2014). The aim of this study was therefore to determine the effect that increasing increments of dietary NDF could have on the diet digestibility, growth performance, meat quality and FA composition of muscle and adipose tissue in South African Mutton Merino lambs.

This dissertation is presented in the form of seven chapters that forms a single unit. Firstly the aim of the study is acquainted by a general introduction (Chapter 1), followed by a literature review (Chapter 2). The materials and methods used in this study are reported in detail in Chapter 3. In Chapter 4 the effect of altering NDF increments on the digestibility of finishing diets for lambs are evaluated. The influence of these factors on feed intake and production performance of lambs are discussed in Chapter 5, and on meat quality in Chapter 6. The general conclusions and recommendations are then summarized in Chapter 7. Although care has been taken to avoid repetition, some recurrence was inevitable to ensure transparency.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Both the amount and structure of fatty acids (FAs) play an important role in maintaining human health (Jenkins *et al*., 2008). There is this growing appreciation for the well-being benefits of FAs, due in part to the regular consumption of poly-unsaturated fatty acids (PUFA) (McDonald *et al*., 2011). Diets high in saturated fatty acids (SFA) are perceived to be unhealthy. Serious diseases has been detected from the regular consumption of hydrogenated fats, such as atherosclerosis, cancer, diabetes, immune system implications, obesity, low-birth weight babies, birth- defects, lactation difficulty, sterility, decreasing visual acuity and problems with bones and tendons (Enig and Fallon, 1999; McDonald *et al*., 2011). The FA composition of red meat also affects the quality properties of this product and includes taste, colour and shelf life (Booyens, 2012).

The effect of nutrition on the FA composition of muscle and adipose tissue of livestock is mostly the result of the FA composition of the diet fed (Whitney and Lupton, 2010). However, the type of diet fed to lambs also plays a major role in the composition of their meat. Research has shown that the FA content is more favourable in lambs finished on grassland or pasture, compared to those lambs finished on concentrate diets (Aurousseau *et al.,* 2004; Poulson *et al.,* 2004). The FA composition of ruminant meat, fed high levels in concentrate feeds contains more mono-unsaturated fatty acids (MUFAs) and SFAs due to microbial biohydrogenation (BH) of PUFAs in the rumen (Wood *et al*., 2008). This BH of unsaturated fatty acids (UFA) are more pronounced at higher ruminal pH levels (Wood *et al*., 2008). However, when lambs are fed concentrated diets, the FA content of the meat can be improved to more closely resemble what is recommended for the human diet as a whole (Cooper *et al*., 2004).

Ruminal pH is positively related to the neutral-detergent fibre (NDF) content of a roughage source, as well as the total NDF content of the diet (hence increased BH of UFAs) (Galyean and Defoor, 2003). Ruminal pH is very receptive to rumination which increases the pH due to the bicarbonate content of saliva which buffer's the rumen pH (Allen, 1997).

Ruminal pH is therefore one of the most important factors affecting fibre digestion in the rumen. The possibility exists that the dietary NDF level could have an influence on the ruminal bacteria composition (McDonald *et al*., 2011) and therefore ruminal fermentation dynamics, including BH of dietary lipids and the FAs leaving the rumen.

In addition, dietary roughage content (i.e. roughage:concentrate ratio) also affects feed utilization and animal performance (Whitney and Lupton, 2010). When a high-concentrate finishing diet is fed to lambs, dry matter intake (DMI) could increase. Care also has to be taken to include the minimum NDF for rumen health. However, greater levels of roughage in the same diet could reduce the average daily gain (ADG) (Whitney and Lupton, 2010). Fibre, or more specifically NDF, restricts DMI in ruminants because of the "bulk" or "fill" it gives to a diet (Mertens, 1992). When this happens ruminants consume less dry matter (DM) and the effectiveness of digestion normally increases (Tyrrell and Moe, 1981) due to a slower passage rate and longer retention time. On the other hand, a reduction in digestibility of DM, NDF, hemicelluloses, cellulose and energy per unit intake are noted on low roughage:concentrate diets, because of a shorter retention time (Colucci *et al*., 1982).

By measuring the influence of dietary composition (primarily roughage:concentrate ratios) and how it affects the ruminal pH, bacterial population and ultimately ruminal fermentation (biohydrogenation), may offer a better understanding as to how to possibly affect and alter the FA composition of muscle and adipose tissue (Du Toit, 2013). This dogma will remain in abundant attentiveness and no research exists where the NDF content of the diet was used as an indicator of the possible FA variation in the ruminant carcass. Only total fibre, or a ratio thereof, is presented in the literature (Helander, 2014). Therefore, in this review the possible effect that different levels of roughage (more specifically NDF content) and/or concentrates in diets have on rumen fermentation patterns, animal production, meat FA profiles and meat quality of ruminants will be discussed.

2.2 Fibre (NDF) in ruminant diets

Roughage evaluation is vital to assess its nutrient composition, as this allows farmers or nutritionists to develop feeding programs. Livestock utilisation of roughages can be improved if the nutrient composition, especially the crude protein (CP), fibre/NDF and

available energy, of it is known. A typical quality standard test for a roughage source consists in determining its acid-detergent fibre (ADF), NDF, CP and DM content (Orloff and Putnam, 2007). Roughage analysis can help decrease feed cost per animal, while production is maintained or even increased (Lemus, 2009).

2.2.1 Roughage quality

Roughage quality can be defined as the potential of a fibre source to produce a desired animal response (Lemus, 2009). When feeding any roughage (hay) or fibre source to ruminants, one cannot expect that the quality of each can only be compared by its fibre content. Roughage quality does not only comprise of the fibre content of a hay source, but mainly its chemical content and physical form. This in turn affects how effectively it will be utilized by ruminants (Van Soest, 1965). Roughage sources consist of a large percentage of crude fibre (CF) which is the part of the roughage that is slowly digestible and has a great filling effect as previously mentioned. Crude fibre was primarily used as an indicator of the fibre content of feeds. Today, other parameters, like NDF and ADF, are identified to better indicate the fibre component of feeds (McDonald *et al*., 2011).

Roughage is made up of a large portion of cell-wall material. First, young herbage develops an outer primary layer and, as it matures, a thicker inner layer consisting of cellulose and hemicellulose both of which can be utilized by the ruminant animal. As the plant reaches maturity it develops a lignin layer between the primary and secondary layer which is relatively indigestible to the ruminant (lignification). Therefore, the fibre quality and digestibility of young herbage is superior to that of a mature plant (Holland and Kezar, 1999). The CP content, rate of digestion and voluntary intake of herbage also decreases as it matures and lignification sets in. Therefore, lignification reduces the nutritive availability of herbage and other feed taken in by ruminants (Van Soest, 1965).

The low net energy (NE) content of mature roughage is not just because of a low organic matter (OM) digestibility, but it is also associated with a high concentration of cellulose. The digestion of this polysaccharide in the rumen and the metabolism of its end products give rise to a high heat increment (HI) of digestion. Also, during rumen fermentation of OM, hydrogen is produced and methanogenic bacteria use this with carbon dioxide (CO_2) to produce methane (CH_4) and water (McDonald *et al*., 2011), which results in

a loss of feed energy. However, when the roughage content of feed increase, solid turnover in the rumen also increase without increasing DMI, which may be because of the increased rumination and salivation (Evans, 1981).

The quality of roughage can also be influenced by its palatability (voluntary intake) and anti-nutritional factors (Lemus, 2009). However, the chemical composition of any roughage is a better indication of its quality (Van Soest, 1965). It is thus important to note that roughage quality is a function of both animal and plant factors (McDonald *et al*., 2011).

2.2.2 Effective fibre

One problem with NDF as the only measurement of fibre quality is that NDF is a chemical feature of fibre and does not take the physical characteristics, such as particle size and density, into account (Mertens, 1997). Physical effective fibre is the part of a roughage responsible for stimulating mastication (Allen, 1997), where particle size determines the amount and time spent on rumination that will take place before the required diameter is reached for the animal species in question (Yang and Beauchemin, 2006).

Fibre, and therefore NDF plays an essential part in long term health and productivity of the animal (Mertens, 1997). Metabolic disturbances are then often the result of fibre requirements that are not sustained. Field investigations conducted at Penn State University have found that at least 10% of the feed material in a total mixed ration (TMR) should be greater than 19 mm in length (Heinrichs, 1996). Heinrichs (1996) then recommended that using the Penn State Particle Separator (PSPS) can give truthful outcomes concerning roughage particle size of a diet. The ideal proposed particle sizes are at least 10% of a TMR in the top sieve, 30 – 50% in the centre sieve and 40 – 60% in the base sieve. Effective fibre should be measured against a standard that produces a maximum amount of chewing (mastication) to maintain optimum rumen health and fermentation (Mertens, 1997). Fadlalla *et al*. (1987) reported a decline in diet fibre digestibility with finer ground roughage (2 to 5 mm) by sheep - which was associated with a relatively short mean retention time (MRT). The mean effective fibre content of fresh pasture should amount to 43% of the total NDF content (Kolver and de Veth, 2002).

2.2.3 Roughage:concentrate ratio in the diet

Altering the roughage:concentrate ratio in the diet of ruminants is a mechanism commonly suggested and used for synchronising the availabilities of energy and CP in the rumen in order to maximise the amount of microbial protein (MP), dietary protein and amino acids that passes to the small intestine of ruminants (Clark *et al*., 1992).

Digestibility of OM is one of the key components influencing the nutritive value of a ruminant's diet (McDonald *et al*., 2011). Organic matter digestibility in cows is affected by the level of fibre and concentrates (Rode *et al*., 1985). An increase in NDF degradation was observed when the roughage content increased in the diet. This suggests higher cellulolytic bacteria levels (Vlaeminck *et al.*, 2006). On the other hand, as the concentrate level of ruminants is increased, the total tract OM digestibility increases (Rode *et al*., 1985), until a point where it declines due to increased passage rate (McDonald *et al*., 2011). Increased flow of MP to the duodenum was recognised in one study with a roughage increase of 49% to 60% (Yang and Beauchemin, 2006). Duodenal flow of total or non-ammonia nitrogen did not necessarily change, even if the rumen nitrogen degradability increased from 35% to 55% of roughage content. Maximum bacterial flow is achieved at a minimum of 70% roughage content in sheep diets (even more when intake is increased) (Yang and Beauchemin, 2006). These effects observed may be related more to the amount and rate of OM fermentation in the rumen than to the specific roughage:concentrate ratio of the diet. Normally, available energy is the limiting factor affecting bacterial growth in the rumen. Any additional OM fermented in the rumen due to a change in the roughage:concentrate ratio of the diet, therefore probably increases MP synthesis by providing more energy - only when nitrogen is not limiting to the microbes (Clark *et al*., 1992).

2.2.4 Fibre and dry matter intake (DMI)

Acceptable voluntary feed intake is necessary for increased productivity. As stated earlier, NDF concentration is a major factor determining ruminal fill, which may explain decreased DMI, with increasing NDF concentration (Van Soest, 1965; McDonald *et al*., 2011). A lower feed intake due to the fibre filling effect and longer rumen retention time, is better defined by the NDF content of diets, especially when it increases from 22.5% to 45.8% in dairy cow diets. Thus, DMI could be influenced by the type and quality of roughage

provided to the animal. Nonetheless, the filling effect of a particular diet also would depend on factors affecting rate of digestion and flow from the reticulo-rumen, such as size and density of digesta particles, motility, reticulo-omasal orifice size, and rate of emptying of the abomasum (Allen, 2000). As mentioned, if the roughage particle size is reduced, the DMI will increase (Allen, 2000) due to less time spent on mastication by the ruminant (McDonald *et al*., 2011).

2.2.5 Lucerne hay as a roughage source in ruminant diets

Lucerne hay is becoming an ever more popular roughage choice because it has high rumen-degradable proteins (RDP), good palatability, high digestibility and has a high intake potential. The DMI of lucerne hay is higher than that of grass, despite the fact that the NDF digestibility of grass is often better than that of lucerne. The higher DMI of lucerne may be due to its higher particle brittleness and palatability (Allen, 2000). However, the palatability of the lucerne hay declines as it approaches later stages of bloom. Although lucerne hay is higher in lignin than most grass types, its fibre degradability in the rumen is superior to other roughage sources (DePeters, 2012). Protein content declines with maturity and fibre increases, and it seems that the best DM and CP content are captured when lucerne hay is harvested in the one-tenth to the first-half of bloom stage. Lucerne hay is good quality roughage and also provides energy, vitamins and minerals to livestock (Allen, 2000).

2.3. Ruminal pH

Rumen pH is a variable factor and has a great influence on the microbial population and fermentation patterns in the rumen. Ruminal pH is therefore a result of the concentrations of acids, bases and buffers present in the rumen at any given time (Mirzaei-Aghsaghali and Maheri-Sis, 2011).

As mentioned, the ability of a roughage to stimulate mastication and therefore the secretion of saliva which contains bicarbonate and phosphate buffers is of utmost importance for rumen health (McDonald *et al*., 2011). These buffers neutralize the volatile fatty acids (VFA) that are produced by OM fermentation in the rumen. The main factor affecting the pH of the rumen is therefore the ratio of VFAs to buffers in the rumen (Allen, 1997). When diets high in fermentable concentrates, or low in physically effective fibre are

fed, it causes a build-up of VFAs (especially lactic and propionic acid - see 2.4) which results in a decreased ruminal pH (Yang and Beauchemin, 2006). Therefore, rumen pH is positively related to the NDF level of the roughage, as well as the total NDF content of the diet in a ruminant. In contrast, a negative correlation between rumen pH and non-structural carbohydrates (NSC) is therefore observed (Kolver and de Veth, 2002). Whenever the pH of the rumen is too low, the feed intake of the animal will decrease due to sub-acute ruminal acidosis (SARA) (Garrett *et al*., 1999). Fibre digestibility and microbial yield will also decrease in the rumen. The above mentioned factors will all contribute to decreased animal production and increased feed costs (Allen, 1997).

Fibre digesting bacteria (cellulolytic) thrive at a ruminal pH of 6.2 to 6.8. Cellulytic- and methanogenic bacteria therefor prefer a higher pH and start to decrease when the pH falls below 6.0. Starch digesting bacteria (amilolytic), on the other hand, prefer a lower pH of between 5.2 and 6 (Mirzaei-Aghsaghali and Maheri-Sis, 2011).

2.4. Rumen Volatile Fatty Acids (VFA)

The VFAs important in ruminant production are propionic, butyric and acetic acid. As mentioned, the composition, as well as the physical form of the diet or fibre source does affect these VFA concentrations. However, when the ruminal pH falls to below 5.5, the total VFA production is depressed dramatically (Slyter, 1976) due to SARA (Garrett *et al*., 1999).

Butyric acid increases when there is a higher number of protozoa present in the rumen and reaches a maximum at a ruminal pH of 6.2 (Slyter, 1976). Propionic acid production is positively correlated to a more concentrated diet, whereas acetic acid production is positively correlated to a higher roughage based diet (McDonald *et al*., 2011). Acetic acid production is closely related to rumen pH and is maximal at a rumen pH of 7.4. Propionic acid production is less sensitive to ruminal pH than butyric acid and acetic acid (Slyter, 1976). Satter and Esdale (1968) stated the reason for this to be that the main end result for the metabolism of lactate is acetate, but the oxidation of lactate to pyruvate causes the synthesis of butyrate through acetate to sustain oxidation-reduction stability. The proportion of butyric acid also increases when the protein quantity in feed increases, and thus high protein roughages, such as lucerne hay, should cause an increase in the quantity of ruminal butyric acid (Slyter, 1976).

Absorption of VFAs at their site of production within the rumen is rapid, and large quantities are metabolized by the ruminal or large intestinal epithelium, before reaching the portal blood system (Bergman, 1990). Volatile fatty acids are readily absorbed into the blood stream and transported to body tissues where they are used for hepatic gluconeogenesis and lipogenesis in the peripheral tissues, as well as for milk synthesis (Tagang *et al*., 2010).

2.4.1 Formation of volatile fatty acids

Carbohydrates, structural- and non-structural (NSC) are the primary source of energy for ruminants. If the structural and NSC sources are limited, energy is mobilized from stored carcass fat and lastly from carcass protein. Non-fibrous carbohydrates (NFC), for example starches and simple sugars, ferment at a higher rate in the rumen and as a result the energy density of a diet increases. Non-fibrous carbohydrates provide a higher energy level for microbes and also determine the amount of bacterial protein produced in the rumen. Non-fibrous carbohydrates do not stimulate rumination or saliva production. They may impair fibre fermentation and could cause ruminal acidosis (McDonald *et al*., 2011). Glucose, fructose, maltose and cellobiose are firstly broken down by microbial fermentation to produce lactic acid. Secondly, another group of organisms ferments this lactic acid into the VFAs acetic and propionic acid, at a much slower speed with its possible negative consequences (Slyter, 1976).

On the other hand, structural carbohydrates are composed of cellulose, hemicellulose and lignin. Cellulose and hemicelluloses are broken down to simple sugars by micro-organisms (MO), whereas lignin is digested poorly, if at all (McDonald *et al*., 2011). Ruminants cannot digest cellulose or complex carbohydrates on their own (Bergman, 1990). Therefore they mainly rely on the function of microbes for the fermentation of feed in the rumen. Volatile fatty acids are produced at inconsistent rates during the day and are influenced by the feeding pattern and the nature of the diet, and its production increases with an increased feed intake (McDonald *et al*., 2011).

2.5. The basics of lipids and its metabolism

2.5.1 Lipids

Lipids (also known as fats) are a class of non-polar (not soluble in water) organic substances (Enig and Fallon, 1999; Vander *et al*., 2001; Zamora, 2005). These organic substances are primarily a combination of one glycerol molecule bound to three FAs via ester linkages (McDonald *et al*., 2011). Lipids can be divided into four subclasses: FAs, triacylglycerols, phospholipids and steroids. The most common functions of dietary fats include the supply of energy to body cells, to carry fat-soluble vitamins (vitamins A, D, E and K), and are a source of antioxidants and bioactive compounds. Fats are also integrated as structural components of the brain and cell membranes (Zamora, 2005).

2.5.2 Fatty acids (FA)

When a FA is not attached to another molecule, they are classified as free fatty acids (FFAs) (Zamora, 2005). Fatty acids are subdivided into different classes according to the presence and number of double bonds between carbon atoms (saturated, mono- or poly-unsaturated), and carbon chain length (short-, medium-, long- and very long chain) (McDonald *et al*., 2011). Table 2.1 sets out some basic information of the most commonly found FAs in nature.

The length of the carbon chain, together with the number of double bonds, determines the consistency of the lipid in which it is present. Therefore, as the number of carbon atoms and double bonds increase, the consistency of the lipid will tend to be liquid at room temperature, as is the case with vegetable and fish oils, which contains a high number of PUFAs. Animal fat (like tallow) contain more SFAs, compared to seed oils, and tend to be solid at room temperature (McDonald *et al*., 2011).

Roughage lipid composition basically consists of glycolipids and phospholipids with the main FA composition of roughages consisting of UFAs such as linoleic acid (C18:2*n*-6) and α-linolenic-acid (C18:3*n*-3). Concentrated feedstuffs' basic lipid composition, such as grains and oilseeds, are largely triglycerides consisting of linoleic and oleic (C18:1*n*-9) acid as the major FA constituents (Bauman *et al*., 1999).

Table 2.1 Basic information of commonly found fatty acids in nature (adapted from Zamora, 2005; Enig and Fallon, 2011)

Common name	Symbol*	Carbon atoms	Double bonds	Scientific name
Saturated fatty acids				
Butyric	C4:0	4	0	butanoic acid
Caprylic	C8:0	8	0	octanoic acid
Capric	C10:0	10	0	decanoic acid
Lauric	C12:0	12	0	dodecanoic acid
Myristic	C14:0	14	0	tetradecanoic acid
Palmitic	C16:0	16	0	hexadecanoic acid
Stearic	C18:0	18	0	octadecanoic acid
Arachidic	C20:0	20	0	eicosanoic acid
Monounsaturated fatty acids				
Palmitoleic	C16:1*n*-7	16	1	9-hexadecenoic acic
Oleic	C18:1*n*-9 (*cis*)	18	1	9-octadecenoic acid
Polyunsaturated fatty acids				
Linoleic	C18:2*n*-6 (all *cis*)	18	2	9,12-octadecadienoic acid
α-linolenic	C18:3*n*-3 (all *cis*)	18	3	9,12,15-octadecatrienoic acid
Arachidonic	C20:4*n*-6 (all *cis*)	20	4	5,8,11,14-eicosatetraenoic acid
Eicosapentaenoic	C20:5*n*-3 (all *cis*)	20	5	5,8,11,14,17-eicosapentaenoic acid
Docosahexaenoic	C22:6*n*-3 (all *cis*)	22	6	4,7,10,13,16,19-docosahexaenoic acid

* The figure before the colon indicates the number of carbon atoms which the fatty acid molecule contains, and the figure after the colon indicates the total number of double bonds.

The *n*-(omega) designation gives the position of the first double bond counting from the methyl end of the molecule.

One of the most common used oil to supplement animal diets is soybean oil. Soybean oil is composed out of 15% SFA and 85% UFA of which the SFAs are divided into 11% palmitic (C16:0) and 4% stearic acid (C18:0) - whereas the UFAs consist of 24% oleic, 54% linoleic and 7% α-linolenic acid. Soybean oil has an UFA:SFA ratio of 5.7 (Zamora, 2005; Enig and Fallon, 2011). Maize oil is comprised of 13% SFA and 87% UFA, of which the SFAs are divided into 11% palmitic and 2% stearic - whereas it's UFAs consist of 28% oleic, 58% linoleic and 1% α-linolenic acid. Maize oil has an UFA:SFA of 6.7 (Zamora, 2005; Enig and Fallon, 2011). Table 2.2 illustrates the FA composition of some common edible fats and oils.

Table 2.2 Fatty acid composition (percentage of total fatty acid content) of some common edible fats and oils (adapted from Zamora, 2005; Enig and Fallon, 2011)

		Saturated				Monounsaturated		Polyunsaturated	
Oil or Fat (% of total fatty acid content)	**Unsaturated /Saturated (ratio)**	**Capric acid (C10:0)**	**Lauric acid (C12:0)**	**Myristic acid (C14:0)**	**Palmitic acid (C16:0)**	**Stearic acid (C18:0)**	**Oleic acid (C18:1)**	**Linoleic acid (C18:2*n*-6)**	**α-Linolenic acid (C18:3*n*-3)**
Beef Tallow	0.9	-	-	3	24	19	43	3	1
Butterfat (cow)	0.5	3	3	11	27	12	2	2	1
Butterfat (goat)	0.5	7	3	9	25	12	27	3	1
Canola Oil	15.7	-	-	-	4	2	62	22	10
Maize Oil	**6.7**	**-**	**-**	**-**	**11**	**2**	**28**	**58**	**1**
Cottonseed Oil	2.8	-	-	1	22	3	19	54	1
Flaxseed Oil	9.0	-	-	-	3	7	21	16	53
Grape seed Oil	7.3	-	-	-	8	4	15	73	-
Olive Oil	4.6	-	-	-	13	3	71	10	1
Palm Oil	1.0	-	-	1	45	4	40	10	-
Palm Kernel Oil	0.2	4	48	16	8	3	15	2	-
Peanut Oil	4.0	-	-	-	11	2	48	32	-
Soybean Oil	**5.7**	**-**	**-**	**-**	**11**	**4**	**24**	**54**	**7**
Sunflower Oil*	7.3	-	-	-	7	5	19	68	1

* Not high-oleic acid variety.

Note: Percentages may not add to 100% due to rounding and other constituents not listed. Where percentage varies, average values were used.

Zamora (2005) acknowledged that FAs are an important source of fuel for body cells, because their metabolism yields large quantities of energy in the form of Adenosine Tri-phosphate (ATP). Many cell types can use either glucose or a FA for this purpose - in particular, heart and skeletal muscle prefer FAs.

2.5.2.1 Saturated fatty acids (SFAs)

A FA is termed saturated when all accessible carbon bonds are bound to a hydrogen (H^+) atom (McDonald *et al*., 2011). They are extremely stable as all the carbon-atom linkages are filled (or saturated) with hydrogen atoms. This way they do not generally go rancid (or oxidize easily), even when heated for cooking purposes. Capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic and stearic acid are examples of SFAs (see Table 2.1) (Enig and Fallon, 1999, Zamora, 2005; Enig and Fallon, 2011).

2.5.2.2 Monounsaturated fatty acids (MUFAs)

Monounsaturated fatty acids have one double bond linking two carbon atoms and, as a result, are short of two hydrogen atoms, e.g. oleic acid (see Table 2.1) (McDonald *et al*., 2011). Monounsaturated fats have a kink or bend at the position of the double bond so that they do not clump together as easily as saturated fats and, therefore, tend to be a liquid at room temperature. Like saturated fats, they are quite stable. They do not go rancid easily and can be used for cooking purposes (e.g. olive oil) (Enig and Fallon, 1999, Zamora, 2005; Enig and Fallon, 2011).

2.5.2.3 Polyunsaturated fatty acids (PUFA)

Polyunsaturated fatty acids have two or more pairs of double bonds between each pair of carbon atoms. The two most commonly found PUFAs in food products are linoleic acid [also called omega-6 (*n*-6)] and α-linolenic acid [also called omega-3 (*n*-3)] (see Table 2.1).

Mammals cannot produce PUFAs and hence they are termed essential FAs. They are liquid at room temperature, even when refrigerated. The unpaired electrons at the double bonds make these oils highly reactive. They go rancid easily, particularly linolenic acid. In nature, PUFAs are usually found in the *cis*- form (Enig and Fallon, 1999). When the hydrogen atoms lie on the same side of the double bond, the acid is said to be in the *cis*-

configuration, while it is said to be in the *trans*- form when the atoms lie on opposite sides, as shown in Figure 2.1 (McDonald *et al*., 2011).

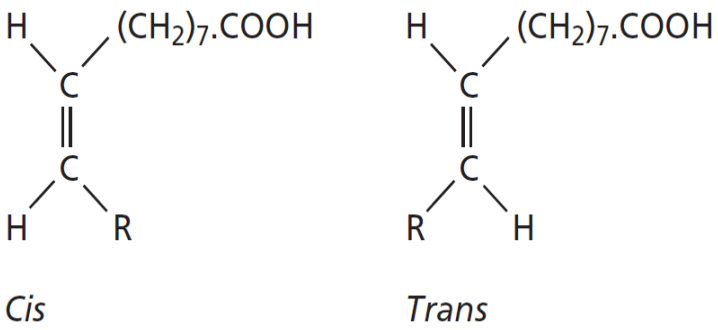


Figure 2.1 Chemical structure of a *cis*- and *trans*- form of a fatty acid [adapted from McDonald *et al*. (2011)].

The chemical structure of some of the mentioned FAs is illustrated in Figure 2.2. Enig and Fallon (1999) acknowledged that all fats and oils, whether of vegetable or animal origin, are composed of a combination of SFAs, MUFAs and PUFAs. In general, animal fats such as lard and tallow contains about 40 to 60% saturated fats.

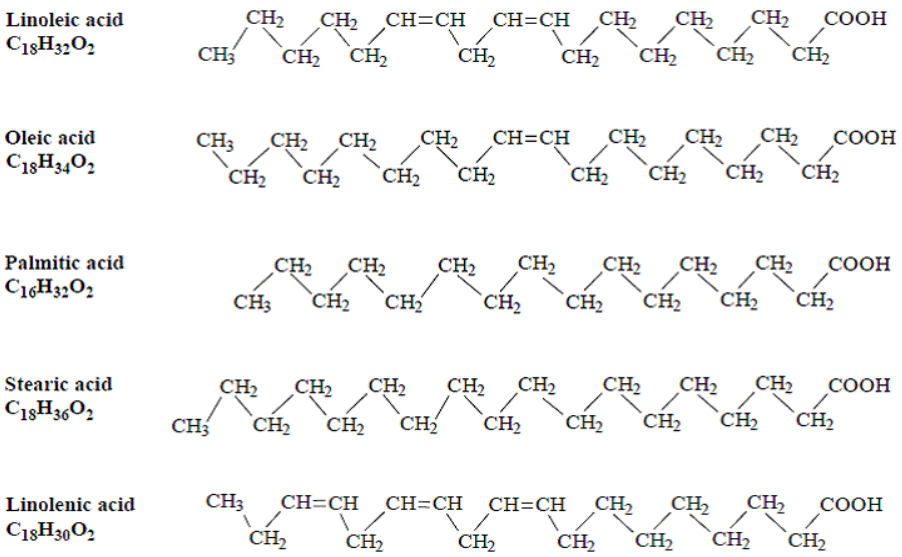


Figure 2.2 Chemical structures of certain fatty acids [adapted from McDonald *et al*. (2011)].

2.5.2.4 Phospholipids

Phospholipids are vital constituents of the molecular association of tissue, particularly membranes (Vander *et al*., 2001). Phospholipids appear in all animals and plantcells and are composed out of one glycerol molecule while one FA is replaced by a phosphorus molecule (Schollan *et al.,* 2006). They contain substances such as lecithin, cephalin and sphingomyelin. Lecithin, also called phosphatidylcholine, is essential for nerve and brain tissue that consist of a combination of stearic, palmitic and oleic acid, connected to the choline ester of phosphoric acid. The chemical structure of dipalmitoyl lecithin ($C_{40}H_{80}NO_8P$) is typical of the phosphatides found in the brain, lung, and spleen tissue (Zamora, 2005).

2.5.3 Lipid inclusion in ruminant diets

Lipids are primarily an energy supplement in ruminant diets and provide about 2.25 times the digestible energy of carbohydrates. The metabolism of lipids result in a lower HI, which is an advantage in hot weather and animal production – i.e. lower methane emissions and heat lost (via metabolism) (McDonald *et al*., 2011). Although the inclusion of lipids in ruminant diets is not only for their high energy value (Chilliard, 1993; Bauman *et al*., 2003), but also for the possible improvement of ruminant carcass quality (Bauchart *et al*., 1996) which could benefit animal and human health (McDonald *et al*., 2011).

Ruminant diets usually contain 2% lipid content on average, mostly derived from cereal grains, crops, oilseeds or their extracts. The form of lipids contained in roughages and grains are generally in the form of triacylglycerols and phospholipids, with linoleic and α-linolenic acid being the principle FAs (Kennelly, 1996). The common practise is for the total dietary fat to not exceed 6 to 7% of feed DM (Jenkins, 1993; Doreau *et al*., 1997; NRC, 2001). The standard inclusion of lipids, above that already present in a basal diet is 3%, where protected lipids in the form of calcium-soaps could provide an additional 3-4% energy (McDonald *et al*., 2011).

One of the key concerns about lipid supplementation is the negative effect it has on fibre digestion (Gulati *et al*., 1997). The coating effect of FFAs to cellulose may get in the way of the attachment of bacterial cellulolytic enzymes (Jenkins, 1993). Also, PUFAs are poisonous to numerous bacterial species present in the rumen, and as end result could weaken their growth and function (Maia *et al*., 2007).

2.5.4 Ruminal metabolism of lipids

The capability of ruminal MOs to digest lipids is limited and only a reasonable amount of dietary lipid can be metabolised (McDonald *et al.,* 2011). The rumen is a site of powerful microbial metabolism and its effect on dietary lipids will be discussed in the following sections.

2.5.4.1 Hydrolysis of ruminal dietary lipids

Hydrolysis of lipids (lipolysis) by microbial lipase is the first step in the transformation of dietary lipids entering the rumen and entails the breakage of the ester linkages between glycerol and FAs to yield FFAs (Harfoot and Hazlewood, 1988; Bauman *et al.,* 1999; Jenkins *et al.,* 2008). These FFAs are used as an energy source by certain ruminal microbes, and some are used as carbon donors for amino acid synthesis. The lipases are mostly derived from bacteria and moulds, which are primarily responsible for spoilage (rancidity) of fat (McDonald *et al.,* 2011). This process is essential for the first step of BH to occur (Harfoot and Hazlewood, 1988).

In an industrial process, lipids may also be hydrolysed by boiling with an alkaline source to result in glycerol and soap (sodium- and potassium salts of the FAs). Under natural conditions the products of lipolysis are usually a mixture of mono- and di-acylglycerol with FFAs. Most of these fats are odourless and tasteless, but some of them [e.g. butyric (C4:0) and caproic (C6:0) acid] have extreme taste and smells.

2.5.4.2 Ruminal fatty acid hydrogenation

Unsaturated fatty acids are toxic to MOs (if present in high concentration) by manifesting bactericidal and bacteriostatic effects (mentioned earlier) (Maia *et al*., 2007). Hence these UFAs are further hydrogenated to SFAs to a large extent as a defence mechanism to detoxify those. The industrial BH process is comprised of an isomerisation reaction, where an H^+ atom is added to the double bond of an unsaturated acid of a fat, thereby converting them to their saturated (single bond) analogues (McDonald *et al*., 2011).

Consumed dietary lipids, first undergo lipolysis and BH in the rumen, followed by advanced BH of the UFAs (mainly linoleic and α-linolenic acid) to stearic acid (Bauman *et al*.,

1999; McDonald *et al*., 2011). Ruminal BH of dietary lipids is responsible for the production of *trans*-FAs, as well as high levels of SFAs in the body fat of ruminants (Bauman *et al*., 1999; Enig and Fallon, 1999; McDonald *et al*., 2011), a feature considered undesirable for human health (Bauman *et al*., 1999).

2.5.5 Factors affecting ruminal biohydrogenation

Several factors are known to affect the BH pattern and FA composition in the rumen, including roughage:concentrate ratio, level and type of lipid supplementation (the concentration of UFAs entering the rumen), ruminal pH and ionophores (Whitney and Lupton, 2010; Du Toit, 2013). Changes in the rumen environment lead to changes in the microbial population and activity, which affects rumen lipid digestion and as a result the end products of BH (Martin and Jenkins, 2002).

In terms of dietary composition, the amount of readily and slow fermentable carbohydrates has the greatest impact on the pH of the rumen (Harfoot and Hazlewood, 1988). A low ruminal pH inhibits the growth of cellulolytic bacteria, the main ruminal biohydrogenating bacteria (Martin and Jenkins, 2002). For example, one of the main products formed as a result of BH include conjugated linoleic acid (CLA) (C18:2*cis*-9, *trans*-11) isomers. CLA is produced by different bacterial species in the rumen through the isomerisation (hydrogenation) of linoleic acid, but also through endogenous synthesis from vaccenic acid (C18:1*trans*11) via Δ 9-desaturase enzymes (Mulvihill, 2001; Evans *et al.,* 1981; Schmid *et al.,* 2006; Webb and O'Neill, 2008; Woods and Fearon, 2009). If the rumen pH is altered, this process is normally affected. Ionophore antibiotics and other antimicrobials on the other hand manipulate BH and lipolysis of UFAs in the rumen environment by selectively inhibiting bacterial populations (Bauman *et al.,* 1999; Demeyer and Doreau, 1999).

2.5.6 Microbes of interest in ruminal lipid metabolism

As mentioned, bacteria are mainly accountable for lipolysis and the hydrogenation of lipids in the rumen (Harfoot and Hazlewood, 1988). There are two groups of bacteria of particular interest: (i) lipolytic bacteria that hydrolyse triacylglycerides to FFAs, and (ii) hydrogenating bacteria which take part in the BH of UFAs released during hydrolysis (Harfoot and Hazlewood, 1988; Jenkins *et al*., 2008).

Lipolytic bacteria include gram positive *Anaerobica lypolitica* and gram negative *Butyrivibrio fibrisolvens* bacteria (Harfoot and Hazlewood, 1988). Hydrogenating bacteria on the other hand are divided into two different populations due to the distinct end products and reactions of BH (Bauman *et al*., 1999): Cellulolytic bacteria hydrogenate for example α-linoleic and linoleic acid and produce vaccenic acid as an end-product (Du Toit, 2013). Some of the most common bacteria are *Micrococcus sp*., *Butyrivibrio fibrisolvens spp.*, a few *Eubacterium spp*. and *Ruminococcus albus* amongst others (Harfoot and Hazlewood, 1988). These bacteria prefer a pH of 6.2 to 6.8. Amylolytic bacteria again utilize vaccenic, linoleic and other octadenoic acids to produce stearic acid (Du Toit, 2013) and include *Fusocillus* T344 and *Fusocillus babrahamensis* P2/2 (Harfoot and Hazlewood, 1988). For complete BH to occur, both the fibre and starch fermenting bacteria should be present in the rumen in adequate numbers. The preferable pH for the latter bacteria is between 5.2 and 6 (Harfoot and Hazlewood, 1988).

Apart from bacteria there are also protozoa (a second contributor) in the rumen. Protozoa are most commonly found in the rumen and can make out up to 50% of the rumen's biomass (Jenkins *et al.,* 2008), but their existence is not a requirement for BH and their contribution are believed to be limited (Harfoot and Hazlewood, 1988). They may even restrain BH by assimilating UFAs prior to transformation by bacteria (Devilliard *et al.,* 2006). However, protozoa, mainly *Epidinium spp.*, are estimated to be held responsible for 30 and 40% of the lipolytic action in the rumen (Harfoot and Hazlewood, 1988).

One-third of the rumen biomass is made out of anaerobic fungi. It has a limited input to BH, even though a few species, for example *Neocallimastix frontalis*, metabolizes linoleic acid to a large extent (Jenkins *et al.,* 2008).

2.6 Evaluation of the nutritional value of lipids in animal tissue

Cholesterol levels and FA composition plays a major role in meat quality and human health. As mentioned, the type of FA present is one of the indicators used to evaluate ruminant tissue quality. The ratios of polyunsaturated fatty acids: saturated fatty acids (PUFA:SFA) and linoleic acid:α-linolenic acid (*n*-6:*n*-3) are also commonly used for the evaluation of the nutritional value of a fat and considered important (McDonald *et al*., 2011). From a consumer's health point of view the suggested ratio of *n*-6:*n*-3 is below 4.0 in

muscle tissue, while the suggested value for the PUFA:SFA ratio is 0.4 or higher (Wood *et al*., 2003).

2.6.1 Fatty acids important for human and animal health

Apart from the mentioned ratios, most SFAs are perceived to be bad for human health for they are associated with numerous diseases such as cardiovascular disease and cancer (Wood *et al*., 2003). A SFA has a high-risk pattern of blood lipoproteins; stearic-, and myristic acid. Also all *trans*-acids are considered to be the most harmful with increased consumption of SFA, whereas blood levels of cholesterol and low-density lipoproteins (LDL) are raised (McDonald *et al*., 2011). In contrast to and against popular belief, SFAs comprise more than 50% of cell membranes (providing cells with the needed rigidness and durability), and are important to contribute to the strength of bones by assimilating calcium effectively into the skeletal structure (at least 50% of the dietary fats should be saturated). Most SFAs lower lipoprotein levels (a blood-substance that indicate proneness to heart disease), provide liver protection due to alcohol and toxins, improve the immune system, provide proper utilization of essential FAs (long chain *n*-3 FAs are better retained in tissue with a rich saturated fat diet), are the preferred energy substrate for heart muscle, and some short- and medium chain SFAs have important antimicrobial properties (Enig and Fallon, 1999).

On the other hand, UFAs (especially PUFAs) are apparent to be beneficial for human health, because of its association with a lower risk of hypertension, coronary heart disease, renal disease, Type 2 diabetes, chronic obstructive pulmonary disease, ulcerative colitis and Crohn's disease (Wood *et al*., 2003). When increasing this approved FA content of animal diets (MUFAs and PUFAs), it could retain the same advantageous properties as in the case in human health (McDonald *et al*., 2011). Omega-6 PUFA (which mainly occurs in plant lipids) decrease blood concentration of LDL ("bad" cholesterol) and *n*-3 PUFA (from fish lipids) decrease very low-density lipo-proteins (VLDL). A MUFA, such as the oleic acid in olive oil, tends to be a long-chain FA that is short enough to be transformed into high-density lipo-protein (HDL) ("good") cholesterol and not long enough to be transformed into LDL. Polyunsaturated fatty acids, such as linoleic acid and α-linolenic acid, are short chain FAs and therefore easily transformed into HDL (McDonald *et al*., 2011).

Short-chain FAs have up to six carbon atoms. They float through the bloodstream easily and they are quickly burned as fuel. Medium-chain FAs have more than six, but no more

than 12 carbon atoms. They readily form the triglycerides stored inside fat cells. Long-chain FAs have more than 18 carbon atoms. They are the saturated fats that aren't easy for the body to burn and aren't easy to store, either. However, because SFAs are hard to burn and hard to store, the body usually uses them to make cholesterol (McDonald *et al*., 2011).

2.6.2 Fatty acid composition of ruminant meat and adipose tissue

The lipid tissue composition of ruminants is a direct reflection of the rumen metabolism of the dietary FA (Demeyer and Doreau, 1999). Due to this, the meat composition is largely composed out of SFAs and MUFAs (Wood *et al.,* 2008). Polyunsaturated fatty acids are mainly restrictedto the phospholipid part of the muscle and represent cell membrane formation (Demeyer and Doreau, 1999; Wood *et al.,* 2003). Linoleic and α-linolenic acid are largely the main PUFAs present in muscle and adipose tissue. The PUFA:SFA ratio is low (0.1) for lamb and beef (Schollan *et al.,* 2006). As mentioned, from a human health perspective, the suggested ratio should be approximately 0.4 and above (Wood *et al.,* 2003; Webb and O'Neill, 2008). On the other hand, looking at the *n*-6:*n*-3 ratio of 1.32 for lamb would be acceptable. The suggested ratio is less than 4 (Wood *et al.,* 2003; Webb and O'Neill, 2008). Beef with the most desirable flavour has lower percentages of SFAs and PUFAs, and higher percentages of MUFAs present in the carcass fat (McDonald *et al*., 2011).

As stated previously, BH decreases the UFA and increases the SFA content of rumen digesta (McDonald *et al*., 2011). Polyunsaturated fatty acids are extensively hydrogenated in the rumen (including CLA), resulting in the formation of stearic acid as the major end product (McDonald *et al*., 2011) which can lead to elevated stearic acid in the muscle content. The natural level of CLA in the tissue of ruminants is between 4 and 19 g/kg of the total FA for sheep, and between 1.2 and 10 g/kg for beef. Most CLA isomers come in the form of *cis*-9,*trans*-11 octadecadienoic acid – a result of ruminal BH (McDonald *et al.,* 2011). Oleic acid, formed from stearic acid by the enzyme stearoyl Co-A desaturase, is a major component of neutral lipid and in ruminants the same enzyme forms CLA, an important nutrient in human nutrition. The human diet has been given a new dimension by the discovery that in particular CLA has a beneficial role in the human body. Conjugated linolenic acid has been shown to be antiatherogenic and anticarcinogenic, and also to limit obesity and stimulate immune function. As mentioned earlier, CLA is produced in the rumen as an intermediate in the bacterial hydrogenation of UFAs present in the diet, but it

may also be synthesised in animal tissues. It is therefore present in both milk and meat from ruminants. Ruminants given feed that contain relatively high concentrations of UFAs, such as young pasture herbage, produce fats with particularly high contents of CLA (McDonald *et al*., 2011).

Linoleic acid is a major FA of feeds for all species. Its incorporation into adipose tissue and muscle, in relation to the amount contained in the diet, is greater than for any other FA. It is deposited in muscle phospholipid at a high level where it, and its long chain products [e.g. aracidonic acid (C20:4*n*-6)], compete well for incorporation into phospholipid molecules (Wood *et al*., 2008).

In all species the proportion of linoleic acid declines in muscle as fat deposition increases. The main reason being that phospholipid linoleic acid declines as the proportion of muscle and neutral lipid, with its higher content of SFA and MUFA, increases. Like linoleic acid, α-linolenic acid is an essential FA and is important to ruminants since it is the major FA in grass (Wood *et al*., 2008). However, it does not compete well for insertion into phospholipid compared with linoleic acid and its incorporation into adipose tissue and muscle is less efficient. Greater BH of α-linolenic acid and a long rumen transit time for roughage diets also limits the amount available for tissue uptake compared with linoleic acid from concentrate diets. However, the amount of these FAs consumed over time has an effect on meat FA content (Wood *et al*., 2008). A positive feature of feeding grass is that levels of the nutritionally important long chain *n*-3 PUFA are increased, e.g. eicosopentaenoic acid (EPA) (C20:5*n*-3) and docosohexaenoic acid (DHA) (C22:6*n*-3) over time (Wood *et al*., 2008). Ruminant meat is a relatively good source of the *n*-3 PUFA. Wood *et al*. (2008) compared the FA composition and total FA content of subcutaneous adipose tissue of the *M*. *longissimus* muscle from loin chops or steaks of pigs, sheep and cattle. Sheep and beef containshigher unfavourable SFA - especially myristic acid-, less of the favourable UFAs (linoleic- and α-linolenic acid), and nearby the same MUFA content of pork muscle- and adipose tissue. Sheep muscle contains significantly higher proportions of α-linolenic acid compared to that of cattle (Wood *et al*., 2008). Some of the main FAs and its presence in pig, sheep and beef carcasses are presented in Table 2.3.

Table 2.3 Main fatty acid composition (g/100 g fatty acids) of subcutaneous adipose tissue and muscle of loin steaks/chops in pigs, sheep and cattle [adapted from Wood *et al*. (2008)]

		Adipose tissue (g/100g)			Muscle (g/100g)		
Fatty acid	**Symbol**	**Pork**	**Mutton**	**Beef**	**Pork**	**Mutton**	**Beef**
Myristic acid	C14:0	1.6[a]	4.1[b]	3.7[b]	1.3[a]	3.3[c]	2.7[b]
Palmitic acid	C16:0	23.9[b]	21.9[a]	26.1[c]	23.2[b]	22.2[a]	25.0[c]
Stearic acid	C18:0	12.8[a]	22.6[b]	12.2[a]	12.2[a]	18.1[c]	13.4[b]
Palmitoleic acid	C16:l *cis*	2.4[a]	2.4[a]	6.2[b]	2.7[b]	2.2[a]	4.5[c]
Oleic acid	C18:1 *cis*-9	35.8[b]	28.7[a]	35.3[b]	32.8[a]	32.5[a]	36.1[b]
Linoleic acid	C18:2*n*-6	14.3[b]	1.3[a]	1.1[a]	14.2[b]	2.7[a]	2.4[a]
α-linolenicacid	C18:3*n*-3	1.4[c]	1.0[b]	0.5[a]	0.95[b]	1.37[c]	0.70[a]
Arachidonic acid	C20:4*n*-6	0.2	-	-	2.21[b]	0.64[a]	0.63[a]
Eicosapentaenoic acid	C20:5*n*-3	-	-	-	0.31[b]	0.45[c]	0.28[a]
Linoleic acid:α-linolenic acid	*n-6:n-3*	7.6	1.4	2.3	7.2	1.3	2.1
PUFA:SFA*	P/S	0.61	0.09	0.05	0.58	0.15	0.11
Total		**65.0**	**70.6**	**70.0**	**2.2**	**4.9**	**3.8**

* Polyunsaturated:saturated fatty acid ratio.

2.6.3 Factors affecting fatty acid composition of ruminant meat

There are several factors that have an influence on the FA composition of ruminant meat, such as gender, age, breed, carcass cuts, feed supplements, hydrogenation (see 2.5.4.2) as well as feed composition (roughage:concentrate ratio) (see 2.2.5) (McDonald *et al*., 2011).

Despite BH that occurs in the rumen, the effect of a diet on FA composition of muscle and adipose tissue is mainly due to the FA composition of the diet fed (Aurousseau *et al*., 2004; Poulson *et al*., 2004; Webb and O'Neill, 2008; Woods and Fearon, 2009; McDonald *et al*., 2011). Researchers determined that there are differences between the carcass FA composition of ruminants raised on natural veld and those finished on concentrate diets.

Those raised on pasture have a more approving FA composition than intensively finished ruminants (Wood *et al*., 2008) because of the fact that grass is usually more rich in *n*-3 PUFAs (mentioned previously), even though it has a low lipid content (Webb and O'Neill, 2008). As a result, the effect of the dietary lipid composition on the FA composition of muscle and adipose tissue of this latter feeding system remains of great interest (Du Toit, 2013).

Carcass percentages of oleic- and linoleic acid increase with animal age and weight, whereas α-linolenic acid decreases. Although more of an indirect effect, as animals grow older the MUFA content of carcass fat may increase markedly and the PUFA content slightly (due to the increase in linoleic acid), whereas the SFA content declines in the carcass. The age, as well as breed type of an animal specifically affects the concentration of MUFAs by affecting stearoyl-CoA desaturase gene expression and activity, whereas diet is the sole source of the essential FAs (Huerta-Leidenz *et al*., 1996).

2.6.4 Lipid oxidation

Oxidation of fat is the process where FAs are broken down by oxygen to give shorter chain products, including free radicals which attack other FAs much more readily than does the original oxygen. When this happens more free radicals are produced with the result that the speed of oxidation increases exponentially. The products of oxidation include shorter chain FAs, FA polymers, aldehydes (alkanals), ketones (alkanones), epoxides and hydro-carbons. These acids and alkanals are major contributors to the smell and flavours associated with oxidized fat and reduce its palatability (McDonald *et al*., 2011).

Fat can be spoiled (oxidized) due to a number of different degradation processes, the reaction rate of which is influenced by the impact of oxygen, high temperatures and long storage periods (Guillén and Cabo, 2002; López-Duarte and Vidal-Quintanar, 2009). Buckley and Morrissey (1992) reported that the rate and extent of lipid oxidation are dependent on a number of factors, the most important being the level of PUFA present in the particular muscle system. MUFA is more resistant to oxidative modification than PUFA (McDonald *et al*., 2011).

2.6.4.1 The influence of fatty acid composition on meat lipid oxidation

Strategies which alter the FA composition of the lipid and muscle fractions of meat could also affect a number of aspects of meat quality - firmness of fat, meat and fat colour, lipid stability and taste of the meat. This in turn is influenced by FA proportions (Wood *et al.,* 2003; Schollan *et al.,* 2006).

Lipid oxidation is the main deteriorating reaction in loss of meat quality. The most important number of factors depends on the vulnerability of muscle tissue to lipid oxidation is being the level of PUFA present in the particular muscle system (. Unsaturation of FAs make lipids vulnerable to oxygen attack with harmful implications on meat quality and customer health due to lipid peroxidation. Phospholipids are more vulnerable to oxidation than triglycerides and cholesterol esters, because of their high presence of PUFA (Buckley *et al*., 1995). Oxidative weakening leads to the manufacturing of hydro peroxides, which are vulnerable to further oxidation or breakdown to secondary products such as short-chain aldehydes, ketones and other oxygenated compounds. These may harmfully affect lipids, pigments, proteins, carbohydrates, vitamins and the general quality of meat by causing loss of colour and nutritive value, hence limiting shelf-life. Therefore, lipids containing increased levels of PUFAs are highly sensitive to oxidation reactions during storage and are likely to turn rancid at high environmental temperatures, exposure to oxygen and light and also microbial growth. It is important to mention again that MUFAs are more resistant to oxidative breakdown than PUFAs (McDonald *et al*., 2011). Dietary manipulation of ruminant meat to try and increase the PUFA content and the PUFA:SFA ratio thereof may inadvertently lead to a higher susceptibility to oxidative breakdown, decreased colour stability and reduced shelf-life (Webb and O'Neill, 2008; Wood *et al.,* 2008).

As mentioned, the colour of meat is one of the most important quality attributes influencing the initial selection by the consumer because of the bright red colour suggesting freshness and quality. The generation of free radicals in lipid oxidation may trigger colour deterioration due to the oxidation of red myoglobin to unattractive brown metmyoglobin (McDonald *et al*., 2011).

The stability of meat to oxidation is also the result of the balance between pro- and antioxidants (Wood *et al.*, 2003). Although plant oils used in the nutritional manipulation of ruminant FA composition contain antioxidants, it is advisable to incorporate chemical or

natural antioxidants in animal diets as well (McDonald *et al*., 2011). This is not only to decrease the oxidation of nutrients (especially the beneficial PUFAs) in feed sources, but also to add to the oxidative durability of meat products (Wood *et al.,* 2008). It is an unmistakable fact that the extent of lipid oxidation is restricted by the antioxidant content of tissue. However, when animals are fed a similar basal diet and the tissue FA composition are altered by the addition of different oils to the diet, lipid and colour stability can be accepted to be more related to the FA composition than the antioxidant concentration (Schollan *et al.,* 2006). Vitamin E is an essential nutrient, which stabilises PUFAs and has a central role in meat quality, particularly in ruminants (Wood *et al*., 2008).

McDonald *et al*. (2011) recommended that CLA in animal tissues could condense the formation of FA free radicals and subsequent oxidation reactions as it is supposed to have not only superior oxidative steadiness than other PUFAs, but also antioxidant-like properties. The CLA content in pork was found to be stable and CLA did not participate in the oxidative processes, while other PUFAs decreased as a result of oxidation. There is however little information on the effects of CLA supplementation on colour stability (Wood *et al.*, 2003).

2.7. Meat and carcass quality

2.7.1 Meat quality

Meat quality depends not only on the degree of marbling, but also on its FA composition, age, breed type, gender of the animal and its diet. Variation in FA composition, in particular variation in saturation, affects firmness of fat, which in turn affects the economics of meat processing and consumer acceptance of meat. In pork, beef and lamb the melting point of lipid and the firmness/hardness of carcass fat is closely related to the concentration of stearic acid (Wood *et al*., 2008).

Maree and Casey (1993) stated that the value of meat is determined by its composition of muscle, connective tissue and fat, the chemical and physical attributes of these components, and finally by contamination and spoilage. Meat quality can be improved by incorporating natural antioxidants into animal diets (as mentioned earlier) or adding these compounds onto the meat surface as well (Velasco and Williams, 2011). Meat colour has been reported as the main factor when consumers assess meat quality, since they relate

colour to freshness. However, colour does not match differences in eating satisfaction (McDonald *et al*., 2011).

2.7.2 Nutritional qualities of lamb meat

Lamb is an outstanding source of high quality protein and important vitamins and minerals (Maree and Casey, 1993; ANON, 2011). Red meat provides an ideal source of copper, manganese, selenium, iron, 45% of the recommended daily allowance of zinc. It is also a great source of B-vitamins (especially thiamine) and vital FAs where half is unsaturated (ANON, 2011).

Many people are concerned with the drenched fat and cholesterol substance in meat, which may result in lower consumption (Maree and Casey, 1993). However, the enclosure of specific lipid sources and antioxidants in ruminant diets may end in healthier lamb carcasses for human utilization. Lamb contains very little marbling (fat within the meat) (ANON, 2011), compared to other meats (McDonald *et al*., 2011).

2.7.3 Consumer preferences in terms of meat quality

Texture and flavour are two of the sensory properties by which customers most eagerly judge meat superiority. As mentioned, consumers discriminate against meat cuts that have lost their fresh look as well (oxidized nutrients, especially UFAs). Discoloured meat is often ground and marketed in a reduced value form (Gray *et al*., 1996). The consumer's purchase decision is strongly influenced by the product's visual appearance (colour). In most parts of the world, leanness is a vital decisive factor when customers purchase meat (Strydom *et al*., 2009). While a small amount of fat is desired to maintain tastiness, it adds tenderness and reduces the risk of the meat drying out. Thus, animals that are selected for leanness, are fed to give maximal growth of muscle and are slaughtered when immature (and hence have less fat) (McDonald *et al*., 2011).

A comparison between vegetarians and meat eaters in Britain showed that vegetarians had lower levels of blood cholesterol (4.88 mmol/l vs. 5.31 mmol/l) and were 24% less likely to die from coronary heart disease. Over the last quarter-century that has passed since dietary fat was first linked to coronary heart disease, many developed countries have shown

a decrease in these incidences. On the other hand, the richer inhabitants of developing countries, who can afford the fat-rich diets of developed countries, are now experiencing coronary heart disease (McDonald *et al*., 2011).

2.7.4 Stability of meat

2.7.4.1 Thiobarbituric acid reactive substances (TBARS)

The thiobarbituric acid reactive substances (TBARS) can be used to determine the lipid peroxidation and oxidative tension within meat (Jensen *et al*., 1997). As stated, the stability in meat can be improved by chemical and natural antioxidants, eventually leading to decreased TBARS values (Buckley *et al*., 1995). One of the major advantages of measuring the TBARS content of meat is that the results are highly negatively correlated with sensory evaluation scores. A malonaldehyde level of 0.5 mg/kg and above is considered to be the turning point from where lipid oxidation causes a rancid odour and taste of meat detected by consumers (Wood *et al*., 2008). A lower TBARS value indicates lower susceptibility of meat to oxidation (Buckley *et al*., 1995). Jensen *et al*. (1997) revealed that only FAs with three or more double bonds are believed to cause more rapid malonaldehyde formation. As mentioned before, increasing the PUFA content of meat may lead to an increased oxidation of these FAs, leading to an unwanted product by the consumer.

2.7.4.2 Meat colour

As mentioned, the main cause affecting meat product acceptability at the time of consumer purchase is the colour of the meat (Gray *et al*., 1996; Jensen *et al*., 1997; Karami *et al*., 2010; Karamucki *et al*., 2011). The measurement of colour is usually presented as the result of three different components, depending on the scale used – for example L*, a*, b* on the CIELAB scale or L*, C*, h° on the CIELCh scale (Karamucki *et al*., 2011). The quality traits of each colour parameter have a certain meaning, such as the contents of basic chemical components in the meat, pH, and water holding capacity. The colour of meat depends on different factors such as the number of haeminic pigments (particularly myoglobin), the chemical state of these pigments, the physical characteristics of the meat and pH (Karamucki *et al*., 2011).

The changes in meat colour are primarily due to the oxidation of red oxymyoglobin (OMG) to metmyoglobin (MMG), which gives meat an unattractive brown colour (Karami *et al*., 2010; Velasco and Williams, 2011). As muscle has a closed structure, it appears dark and the meat tends to be tough at a high pH. It is necessary to delay pigment oxidation and/or enhance reduction of oxidized myoglobin to sustain an acceptable fresh meat colour over prolonged periods of time (Buckley *et al*., 1995; Gray *et al*., 1996). The amount of chemical (Karami *et al*., 2010) or natural (Gray *et al*., 1996; Velasco and Williams, 2011) antioxidants within meat can delay meat colour loss by dropping the oxidation process, extending the red colour (a*-values) and lightness (L* and b*) of meat, as well as delaying MMG formation to finally improve retail shelf-life.

Meat from cattle raised on pasture was reported to be darker than meat from animals raised on concentrates, when measured by objective (P <0.001) as well as subjective (P <0.05) methods. Several factors, not a specific one, are responsible for these different variations. Therefore, diet also affects meat colour and flavour. In sheep a pastoral flavour is mostly determined by the branched-chain FA and 3-methylindole (Velasco and Williams, 2011).

2.7.4.3 Meat shelf life

Shelf life of food is defined as the period from when a product is produced, until to a cut-off point for utilization, where the food product remains safe and nutritious under recommended conditions. The oxidative stability of oils is a vital sign of the recital and shelf life of meat (Jensen *et al*., 1997) - where the oxidation of UFAs unfavourably affects the colour, texture, nutritive value and safety of meat (Buckley and Morrissey, 1992).

2.7.4.4 Meat pH

The biochemical changes that accompany post-slaughter metabolism and post-mortem ageing in the conversion of muscle to meat give rise to conditions whereby the process of lipid oxidation is no longer firmly controlled and the balance of pro-oxidative factors to antioxidant capacity, favours oxidation (Gray *et al*., 1996). Normal metabolism and cellular integrity cease when an animal is slaughtered and the metabolism is maintained only by residual metabolites. These are gradually used up by the energy metabolism in the muscle. Glycogen is anaerobically converted to lactic acid which accumulates in the muscle due to

the absence of a circulatory system. This accumulated lactic acid causes the pH to decline. Animals that are not stressed or suffer pre-slaughtered exhaustion prior to slaughter have an ultimate meat pH value around 5.4 (Maree and Casey, 1993).

The susceptibility of meat and meat products to undergo oxidation also depends on several factors, including pre-slaughter events such as stress, and post-slaughter events such as early post-mortem pH, carcass temperature, cold shortening, and techniques such as electrical stimulation (Buckley *et al*., 1995; Gray *et al*., 1996). In the post-slaughter phase, it is highly unlikely that the armoury of antioxidant defensive systems (superoxide dismutase, glutathione peroxidases, ceruloplasmin and transferrin) available to the cell in the live animal still function because of quantitative changes in several metabolites and physical properties (Buckley *et al*., 1995).

Tenderness of meat is related both directly and indirectly to the ultimate pH. The ultimate pH in its turn is related to the time and onset of *rigor mortis* and the related cooling of the carcass and individual muscles, thereby to the extent of myofibrillar contraction. Muscles with high glycogen content at slaughter proceed slowly into *rigor* and have a low ultimate pH (5.4). The latter thus have a lower chance of cold shortening (Maree and Casey, 1993), resulting in tender meat (Booyens, 2012). A higher pH is less beneficial due to a darker colour meat and a higher susceptibility to bacterial spoilage with a reduced flavour. The post-mortem softening process of muscle is more efficient at a lower meat pH level (5.4) (McDonald *et al*., 2011).

2.8. Conclusion

There is credit of human health benefits from the standard utilization of PUFAs, particularly those exerted by *n*-3 FAs and diverse CLA isomers. These benefits include reduced risk of cardiovascular disease and diabetes, lower risk of cancer, improved visual and nervous system development and maintenance, as well as having anti-inflammatory effects and improving the immune system and bone health. Due to the positive effects of MUFAs and PUFAs on human health there has been an increased interest in recent years to positively manipulate the FA composition of meat by increasing the MUFA, PUFA and in particular *n*-3 FA content of ruminant meat and lowering the less beneficial SFA content. However, there are still a lot of questions unanswered, referring to the favourable FA content of the carcass on the health of the consumer.

Roughage evaluation is vital to assess its nutrient composition. Livestock utilisation of roughages can be improved if the nutrient composition, especially the CP, fibre/NDF and available energy of it is known. A typical quality standard test for a roughage source consists in determining its ADF, NDF; CP and DM content. Roughage analysis results can help decrease feed cost per animal, while production is maintained or even increased. The NDF content and amount of physical effective fibre in the diet does have an influence on the voluntary DMI of ruminants. Feeding sheep mixed diets of high nutritional quality is not a common practice world-wide-which is reflected in the lack of published studies focusing on the effects of mixing roughage, especially focused on NDF, with concentrate diets on feed intake, performance of sheep and meat quality.

Roughage also plays a major role in the FA composition of ruminant meat. Unfortunately there is lack of proof on the effect of different levels of NDF on animal performance and FA composition. One study (Kucuk *et al*., 2001) evaluated the forage content that would provide the greatest duodenal flow of UFAs in ewes to determine the optimal escape of these important FAs from ruminal BH. However, its effect on carcass FA composition was not measured. The influence of roughage and concentrate inclusion in the diet on the FA composition of muscle and adipose tissue of ruminants therefore remains of abundant attentiveness.

Strategies which alter the FA composition, to hopefully increase the PUFA content of ruminant lipid and muscle fractions, can affect the meat quality such as colour, firmness, flavour and lipid stability. However, the oxidative stability of the muscle tissue reduces as the degree of unsaturation increases in muscle membranes. Thus, escalating the PUFA content and the PUFA:SFA ratio of ruminant meat may unintentionally lead to a higher vulnerability to oxidative breakdown, decreased colour stability and condensed shelf-life.

Literature regarding the effect of lipid source and its inclusion on ruminant FA composition is abundant. However, there seems to be a lack of knowledge how basal diet constituents, especially fibre, affects ruminant FA tissue composition. No literature evaluating the effect of specifically the NDF content of ruminant diets on meat FA composition was found and will remain the focus of this research.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Introduction

Both a production study (August to October 2014) and digestibility study (October 2014) was conducted on the experimental farm (Paradys) of the University of the Free State. The farm is situated approximately 20 km south of Bloemfontein in the Free State province of South Africa at 29°13'17.45" latitude, 26°12'26.28" longitude with an altitude of 1424 m above sea level. The climate during the production and digestibility study was the normal seasonal occurrence for the end of spring, and the onset of the summer season. The average minimum and maximum temperatures recorded during the duration of the study were between 13.2°C and 27.1°C, respectively.

All procedures conducted during this study were approved by the Interfaculty Animal Ethics Committee for Animal Experimentation at the University of the Free State (Animal Experiment No. 15/2014).

3.2 Experimental animals

The production study consisted of sixty (60) South African Mutton Merino (SAMM) whether lambs. The lambs, approximately three months of age and with an initial fasted live weight of 29.3±1.8 kg, were randomly and equally allocated (12 lambs per treatment) to five dietary treatments (see paragraph 3.5.1). Accordingly the lambs were randomly placed individually within each of the 60 pens (see paragraph 3.6.1) in a closed but well ventilated building. The method of random allocation of the lambs used in this study reduced the probability of animals of the same treatment being penned next to each other, and thus decreased the probability that a specific treatment may have been affected either positively or negatively by environmental factors due to pen location and/or stall conditions. At termination of the production study, all 60 lambs were slaughtered and carcass characteristics and meat quality were evaluated as discussed later. Thirty-five lambs were randomly allocated (7 lambs per treatment) and used in the digestibility study (see paragraph 3.7).

3.2.1 Preparation of experimental animals

All animals were subjected to a standard health and vaccination program four weeks prior to the onset of the production study, as commonly practiced in the commercial feedlot sector of South Africa. The animals were injected with an antiparasitic remedy for cattle and sheep (Reg. No. G1463; Act 36 of 1947; Batch no 17399) with residual activity against certain important round worms. All lambs were injected with a multivitamin (Reg. No. G2731; Act 36 of 1947) and dosed with a tapeworm remedy (Reg. No. G447; Act 36 of 1947). Animals were also treated for the active immunization against pulpy kidney, malignant oedema, blackquarter, tetanus and pasteurellosis. In particular the vaccine was recommended as an aid in the prevention of pneumonic and septicaemic pasteurellosis in lambs (Reg. No. G1517; Act 36 of 1947; Batch no D087XA02). All the animals were weaned about four weeks prior to entering the trial.

3.2.2 Weighing of lambs

At the onset and end of the production study, all the animals were fasted overnight (minimum of 12 hours) and the individual empty stomach body weight recorded the next morning, in order to calculate the production performance of the lambs. During the production study the full stomach body weight of all lambs were recorded on a weekly basis at the same time. The scale with its crate to weigh the lambs is shown in Figure 3.1.

Figure 3.1 Scale used to weigh the lambs.

3.3 Housing

Animals were housed in adjustable pens (n=1 lamb/pen; 1.404 m^2) on wooden slatted floors (Figure 3.2), which ensured a clean and hygienic environment within a naturally ventilated building. Recommendations for space allowance in confinement sheep for production purposes do vary, but is mostly accepted to fall within the margins of 0.6 to 1.1 m^2/sheep (Færevik *et al.*, 2005). The elevated slatted floor allowed urine and faeces to accumulate on a concrete floor below. The adjacent constructed pens allowed animals to have visual and limited contact with each other. However, the construction of the pens prevented access to the feed troughs by adjacent animals. The pens were constructed and separated from each other by partitions constructed with steel pipes. All pens were clearly marked with a number (Figure 3.3). The house was properly washed and disinfected with a quaternary ammonium compound (GNR 592/30268) before the onset of the study. Each pen was also cleaned once a week to ensure and maintain a good hygienic environment.

Figure 3.2 Pens constructed for housing of the experimental animals.

Figure 3.3 Distinct markings of each pen to clearly identify treatment allocation.

3.4 Feeding troughs and water buckets

Each pen was equipped with its own feed trough and water bucket (Figure 3.4).

Figure 3.4 Feed troughs and water buckets used.

The feed troughs were designed to limit feed wastage to a minimum for more accurate feed intake measurement results. The feed troughs were placed on the gate of each pen. The partitioning between each pen was reinforced to prevent animals from reaching feed from the adjacent pen. Each water bucket was located opposite to the feeding trough in the corner with a pressure filling system regulated with a ball valve. These water buckets were brushed and flushed every second day.

3.5 Experimental diets

Five experimental diets were formulated to facilitate an incremental increase of neutral-detergent fibre (NDF) content between treatments (Table 3.1). All experimental diets were mixed on the experimental farm with a commercial feed mixer.

3.5.1 Physical and chemical composition of the experimental diets

The calculated physical and chemical compositions of the experimental diets are presented in Table 3.1. The five dietary treatments were formulated to contain a similar nutrient composition differing only in respect to the incremental increasing NDF content as the primary parameter, as well as decreasing non-structural carbohydrate (NSC) content as a result thereof, representing a dose-response experimental design. Due to these differences, the decreasing calculated metabolizeable energy (ME) content of the experimental diets from low (15%) to high (75%) roughage inclusion was therefore unavoidable.

Tabel 3.1 Mean calculated physical and chemical composition of experimental diets

	Treatment*				
Parameters	**T15**	**T30**	**T45**	**T60**	**T75**
Physical composition (% as fed):					
Yellow maize-coarse	64.35	53.75	43.40	32.40	21.72
Lucerne	15.00	30.00	45.00	59.30	67.40
Molasses	8.00	4.20	0.59	0.00	0.51
Soybean meal	6.00	6.00	5.50	1.87	0.00
Soybean oil	3.00	3.00	3.00	3.00	3.00
Maize oil	0.00	0.20	0.40	0.70	1.00
Maize cobs	0.00	0.00	0.00	0.80	4.20
Ammonium chloride	1.00	1.00	1.00	1.00	1.00
Limestone powder/fine	1.00	0.60	0.30	0.00	0.00
Urea	0.90	0.45	0.00	0.08	0.15
Salt-fine	0.50	0.50	0.50	0.50	0.50
Premix B	0.25	0.25	0.25	0.25	0.25
Mono Calcium phosphate (MCP)	0.00	0.05	0.06	0.10	0.27
Chemical composition (% DM basis):					
Dry matter (DM)	88.73	89.43	90.11	90.54	90.89
Organic matter (OM)	93.93	93.22	92.48	91.63	90.68
Non-structural carbohydrates (NSC)	57.88	52.09	46.56	40.72	34.95
Crude protein (CP)	16.94	17.07	16.98	16.95	16.82
Neutral detergent fibre (NDF)	12.76	17.69	22.53	27.48	32.40
Acid detergent fibre (ADF)	6.41	10.82	15.16	19.46	23.04
Metabolizable energy (ME MJ/kg DM	11.93	11.57	11.19	10.70	10.22
Ash	6.09	6.78	7.52	8.37	9.32
Ether extract (EE)	6.32	6.36	6.40	6.47	6.49

* Treatment diets: Increments of percentage neutral-detergent fibre (NDF) depicted as % roughage included.Note: The feeding value of feeds used to calculate chemical composition of diets was according to a commercial feed manufacturer's data.

As mentioned, the NDF content increased incrementally from a low roughage (15% lucerne hay) inclusion to a high inclusion rate (primarily lucerne hay with added maize cobs) in the following order: 12.76% (T15), 17.69% (T30), 22.53% (T45), 27.48% (T60) and 32.40% (T75) NDF/kg dry matter (DM), respectively.

Even though the NDF content was the primary parameter used as basis for formulating the diets, lucerne hay was the roughage source used and included incrementally. Therefore, all references to treatments and treatment effects will be focused on T15, T30, T45, T60 and T75, respectively. In addition, due to the fact that the lucerne hay used to formulate each treatment diet contained high amounts of crude protein (CP: 21.51% on a DM basis), the lucerne hay content of T75 were limited to 67.40% and maize cobs (low in CP content: 3.13% on a DM basis) included to formulate for the correct NDF content to result in similar NDF increments (average of 4.91±0.05% between diets on a DM basis) (Table 3.1).

Soya oil was included at 3% in all treatment diets (as is basis). However, due to the fact that maize decreased with increasing lucerne hay inclusion (from T15 to T75), maize germ oil was added using the difference (maize lipid contribution to experimental diets subtracted by the standard 3% soya oil inclusion; Table 3.1) to keep the total lipid content between all treatments similar, as well as similar fatty acid (FA) compositions (see Table 6.1, Chapter 6). The maize meal used to formulate the diets contained on average 4.49% lipid on a DM basis. However, lucerne hay, as most fibre sources, did not contribute much to the lipid content between the diets (1.61% on a DM basis) (McDonald *et al*., 2011).

Similar to the case where maize cob inclusion was used in treatment T75 to formulate for similar CP content and correct NDF increment (discussed previously), molasses was used to limit maize inclusion of certain treatments to ensure similar lipid content between the diets (Table 3.1) as molasses contains no lipid.

The formulated CP content between the diets was higher (16.96% on a DM basis) than that recommended by NRC (1985) for finishing lambs (14.70% on a DM basis) weighing 30 kg (4 to 7 months old). The reason for this was the high inclusion of lucerne hay in treatment T75 (67.40%) with its high CP content (mentioned earlier). Even though the NRC (1985) nutrient requirements for finishing lambs in a feedlot were used as a guideline to formulate the experimental diets, some deviation regarding these nutrient requirements

due to maximum and/or minimum maize vs. lucerne hay inclusion levels were unavoidable (CP and energy content).

In addition, urea inclusion to formulate for similar CP content between diets was limited due to its possible effect on ruminal pH (Briggs, 1967; Church, 1973). The recommendation for urea inclusion was no more than 15 to 25% of total CP in cattle and sheep fattening diets (Briggs, 1967). Hence, soya oilcake was used to limit urea inclusion to not have a possible effect on ruminal pH.

No additives or rumen modifiers that may affect ruminal pH, microbial composition or FA BH were included in the diets. Only dietary affects may be assumed as a causative effect whether any significant results were noted in the data sets presented later.

A synthetic antioxidant was included in the five basal diets to prevent the oxidation of unsaturated fatty acids (UFA) in the different diets. The antioxidant used contained a combination of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin and trisodium citrate.

Lucerne hay was milled by a hammer mill through a 20 mm sieve before included in each diet. Each respective diet was carefully mixed, using a standard commercial feed mixer (Fig. 3.5).

Figure 3.5 Commercial feed mixer used to mix the experimental diets.

Special care was taken to prevent any cross contamination between treatments. This was achieved by thoroughly cleaning the feed mixer after mixing a specific batch. Each diet was fed to the lambs in the same form (ground mash). The diets were not pelleted as the heat from the pelleting process might lead to unwanted oxidation of especially the UFAs contained within the diets.

Feed samples were taken at the start (day 0) and at the end of the production study (day 61) and stored at -15°C, until the FA profile for the five treatment diets could be determined. Figure 3.6 represents the physical appearance of the different treatments.

Figure 3.6 Visual appearances of the five dietary treatments.

3.6 Production study

As indicated, the production study was conducted over a period of 61 days (including a ten-day adaptation period) to investigate the influence of incrementally increasing NDF levels on the production performance of finishing lambs.

3.6.1 Experimental design

The production study was compiled out of five dietary treatments (n=12 lambs/treatment) (see paragraph 3.5.1), subdivided into twelve replicates per treatment (n=1 lamb/replicate). Each treatment was compiled according to increasing NDF increments to represent a dose-response trial design.

3.6.2 Adaptation of the lambs

Before the onset of the study all animals (60) had free access to ground lucerne hay (milled by means of a hammer mill through a 20 mm sieve) as discussed previously. At the onset of the production study (day 0) the lambs were subjected to a 10-day adaptation period procedure as described, by Du Toit (2013). Each treatment diet was increased incrementally with 200 g/animal every second day, fed on top of the lucerne hay to ensure the intake thereof, until an average total trial feed intake of 1 kg/lamb/day was accomplished. The lucerne hay was provided on an *ad libitum* basis to all animals. Total feed intake was recorded during the adaptation period (trial feed, including the lucerne hay). This adaptation procedure ensured that the animals were less prone to metabolic disorders caused by fast ingestion of high energy diets, containing feed ingredients such as maize meal or molasses that are easily fermentable in the rumen (McDonald *et al*., 2011).

3.6.3 Feeding the lambs

After dietary adaptation the lucerne hay was removed and the animals were fed the respective experimental diets on an *ad libitum* basis for the remainder of the experimental period (51 days), until the majority of lambs attained an average empty live weight of ±50 kg prior to slaughter.

The animals were fed twice daily, at 08h00 and at 16h00, not only to reduce feed wastage, but also to increase feeding frequency and hence the dry matter intake (DMI) of the animals. The feeding periods were followed precisely to ensure a healthy constant rumen environment. The feed refusals from each pen were collected on a weekly basis (before weighing of the animals), in order to calculate the average DMI/lamb/week. Feed intake was subsequently determined on a weekly basis by subtracting the feed refusal weight from the total amount of feed provided.

3.7 Digestibility study

The digestibility study was conducted after the completion of the production study over a period of 12 days (4-day adaptation to the faecal bags followed by a consecutive 8-day collection period) to investigate the effecte of the NDF content on feed digestibility and digestible nutrient content of each diet.

Thirty-five lambs (mean 48.11±2.94 kg live weight; seven lambs per treatment) were randomly allocated between the five treatments. The lambs were housed individually in the same pens (with the same feeding trough and water bucket) within the same building used during the production study (see paragraph 3.3, 3.4) (Figure 3.4). The pens remained in exactly the same manner as for the production study.

3.7.1 Experimental design

The digestibility study was compiled according to the same trial design and experimental treatments as discussed in paragraph 3.5.1, culminating into five dietary treatments (n=7 lambs/treatment), subdivided into seven replicates per treatment (n=1 lamb/replicate).

3.7.2 Adaptation of the lambs

The same feed adaptation procedures as discussed in paragraph3.6.2 were followed for the digestibility study. As already mentioned the lambs were allowed for the first four days within the 12 days to adapt to the faecal bags, before the faecal collection period commenced.

3.7.3 Feeding and feed refusals

The lambs were offered the same experimental diets (see paragraph 3.5.1) used in the production study. To try and avoid variation in assessing the voluntary feed intake of sheep, a sequential method of feed allocation was followed by providing each animal a 15% refusal level of intake. Calculations have been done on a daily basis by using a preceding 3-day moving average of feed intake. This method was expected to provide a 15% excess of feed at all times to each animal. This method differed slightly from that explained by Blaxter *et al*. (1961) - it consisted of feeding each animal 1.15 times its mean consumption of the preceding two days. Adjustments of feed offered were made only if the amount of feed refused was less than 15% of that offered to the same animal. If so, the feed offered was increased to 1.15 times of that consumed during the previous day.

The lambs were treated the same as during the production study (see paragraph 3.6.3) and fed twice daily (at 08h00 and 16h00). In order to calculate actual intake and feed digestibility, the feed refusals were collected every morning just before the 08h00 feeding period. Only half of the calculated feed required for every lamb was provided at 08h00 and the rest at the 16h00 feeding period. As discussed, for whatever reason any lamb ate more than its allocated amount of feed/day, more was then provided to the animal.

Daily feed refusals of each animal was dried in a force draught oven at 100°C for at least 16 hours and weighed. After thorough mixing, representative samples were taken from the pooled feed refusals (eight days) of each individual animal, ground to pass through a 1 mm sieve and stored in plastic jars with airtight screw tops for chemical analyses. Accordingly, a composite feed sample from each of the five treatment diets was collected on a daily basis. After thorough mixing, representative samples were taken from the pooled feed samples (eight days) of each treatment, ground and stored for chemical analysis as described earlier.

Representative samples of feed and feed refusals were obtained by means of the quartering method (McDonald *et al*., 2011).

3.7.4 Faeces collection

As mentioned, all lambs were fitted with a harness and a faecal collecting bag (Figure 3.7), four days prior to the collection period to become accustomed. The faeces voided were collected twice daily before feeding at 08h00 and 16h00, respectively, to avoid over accumulation of faeces inside the bag (Figure 3.8). Each day's faecal output was weighed fresh and a 10% representative sample collected (Wilke and Van der Merwe, 1975) and composited. Faecal collection commenced for a total of eight days. Collected faeces was placed in marked paper bags and dried for 16 hours at 100°C. The total amount of dried faeces was then weighed, whereas the total faecal DM excreted was calculated by multiplying the value by a factor of 10.

Figure 3.7 Lamb fitted with faecal bag and harness.

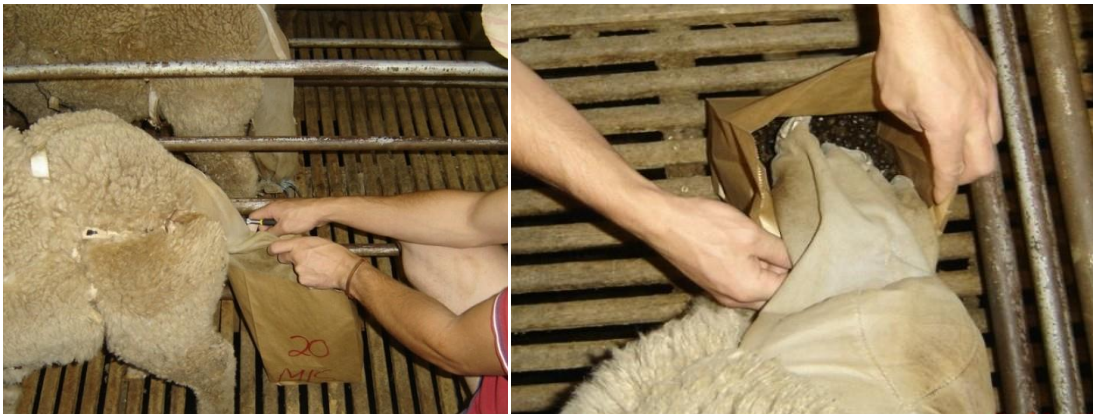


Figure 3.8 Collection of the faeces.

After weighing, smaller representative samples of the dried faeces from each lamb were obtained by the quartering method (McDonald *et al*., 2011), ground to pass through a 1 mm sieve and stored in sealed bottles, pending chemical analysis.

3.8 Water

Fresh, clean water was freely available to all the animals during the production and digestibility studies. The water troughs were cleaned and the system was flushed every second day.

3.9 Chemical analysis

Milled feed, refusal and faecal samples were analysed for DM, CP, NDF, gross energy (GE), ash, organic matter (OM) and ether extract (EE), whereas the NSC was determined by difference (Van Soest *et al*., 1991). Any analysis was repeated if the value between each duplicate differed more than 3%.

3.9.1 Dry matter (DM)

Dry matter content of the feed samples was analysed according to the Association of Official Analytical Chemists (AOAC) official method 934.01 for chemical procedures (AOAC, 2000). The DM content of the feed was determined in the physical form which the feed was presented to the lambs. Approximately 200 g of each sample was collected, weighed in a porcelain crucible and dried in a force draught oven at 100°C for a minimum period of 16 hours to a constant weight. After drying, the samples were placed in desiccators to cool and weighed immediately afterwards.

DM was calculated as follow:

% Moisture = [Weight loss after drying (g) / Weight of test sample (g)] × 100

%Dry matter = 100 - % Moisture content

The weight of individual crucibles was deducted to determine the weight loss after drying, as all crucibles did not have exactly the same weight.

3.9.2 Ash

Ash content of the feed, feed refusal and faecal samples were measured according to the AOAC official method 942.05 for chemical procedures (AOAC, 2000). Ash content was determined by complete incineration of each sample using a muffle furnace. Approximately 2 g of the ground sample (DM basis) was weighed in porcelain crucibles. A porcelain lid was then placed on each crucible containing the samples and placed in a cold muffle furnace and heated to a constant temperature of 600°C. Samples were kept at this temperature for 3 hours before switching the furnace off and allowing it to cool before the oven could be opened and samples handled. The samples were then transferred to desiccators to cool and weighed immediately afterwards. The ash content was calculated as follow:

% Ash = [Weight of ash (g DM) / Weight of test sample (g DM)] × 100

The weight of the individual crucibles were deducted to determine the weight of the ash and the weight of the test sample, as all crucibles did not have exactly the same weight.

3.9.3 Organic matter (OM)

The OM of the feed, feed refusal and faecal samples were determined by subtracting the ash content (%) of each sample from 100.

% Organic matter (OM) = 100 - % Ash

3.9.4 Crude Protein (CP)

The CP content of the feed, feed refusal and faecal samples were determined according to the AOAC official method 990.03 for chemical procedures (AOAC, 2000) with a Leco® FP-528 instrument for nitrogen analysis. Approximately 0.12 g of each sample (DM) was accurately weighed and placed into aluminium foil cups that were sealed and placed on the carousel of the instrument, which did sample analyses routinely. The principle of the Dumas method is that nitrogen (N_2) is freed by pyrolysis and subsequent combustion and is swept by carbon dioxide (CO_2) as carrier into the nitrometer. The carbon dioxide is absorbed by

potassium hydroxide (KOH) and the residual nitrogen volume measured. The nitrogen content is then converted to a protein equivalent by multiplying the percentage nitrogen with a factor of 6.25 (McDonald *et al*., 2011). Protein values were recorded on a computer, which was connected to the scale, as well as the analyzing instrument. The protein equivalent was calculated by the computer program from the numerical factor obtained, as described.

3.9.5 Neutral-detergent fibre (NDF)

The NDF content of the feed, feed refusal and faecal samples was determined according to the method of Van Soest *et al*. (1991), using the ANCOM $^{200/220}$ Fibre Analyser (ANCOM Technology Corp., Fairport, NY, USA).

The experimental procedures for the analyses were as follows: Firstly the filter bag (W1) was weighed and tarred to zero. Approximately 0.45 to 0.55 g of the prepared sample (W2) was weighed directly into the filter bag. By using a heat sealer, the upper edge of the filter bag was completely sealed, within 4 mm of the top. One blank bag (C1) was weighed and included in the run to determine the blank bag correction factor. The bag suspender with bags was inserted into the fibre analyser vessel and a weight placed on top in order to keep it submerged. A 100 ml/bag of neutral-detergent (ND) was then added (a minimum of 1500 mL was used to ensure the bag suspender remained submerged). 20 g (0.5 g/50 mL of ND solution) of sodium sulphite and 4.0 mL of α-amylase were added to the solution in the vessel. The vessel was rotated, agitated and heated. The timer was set for 75 minutes (min), where after the lid was closed. At the end of extraction, the heater and agitator were turned off. The drain valve was opened (slowly at first) and the hot solution emptied before opening the lid. After the solution had drained, the exhaust valve was closed and the lid opened. Then 1900 mL of de-ionized water (70 - 90°C) and 4.0 mL α-amylase was added to the first and second rinses. It was rotated, stirred and rinsed for 5 min. Each lid was sealed with the heat on, or left open with the heat off. The hot water rinses were repeated three times. After the rinsing process was completed, the samples were removed and gently pressed out to remove excess water from the bags. The bags were placed in a 250 mL beaker and enough acetone added to cover the bags and soaked for 3 - 5 min. The bags were then removed from the acetone and placed on a wire screen to dry. The bags were then placed in an oven at 102±2°C for 4 hours until a constant weight is reached. When

removed from the oven, they were placed directly into a collapsible desiccant pouch and flattened to remove the air. Finally, the bags were cooled to ambient temperature and weighed (W3).

% NDF (as-received basis) = {[W3 - (W1 x C1)] / W2} x 100

W1 = Bag tare weight (g)

W2 = Sample weight (g)

W3 = Dried weight of bag with fibre after extraction process (g)

C1 = Blank bag correction (running average of final oven-dried weight divided by the original blank bag weight).

3.9.6 Acid-detergent fibre (ADF)

The ADF content of the feed, feed refusal and faecal samples were determined according to the official AOAC method 973.18 for chemical procedures (AOAC, 2000).

The experimental procedure for the analyses was as follow: Dry empty crucibles were placed in an oven at 105°C for >4 hours (1 hour if transferred from the ashing furnace) and record tare weight (W1). Weigh (W2) ca 1 g test portion of dried or as-received material (materials with >15% moisture should have the weight adjusted to provide an equivalent amount of DM into Berzelius beakers. For conversion results to DM basis, weigh test portion for moisture determination of the test sample at the same time. Allocate 2 blanks (no test material) for the first set of 24 analyses and 1 additional blank for each additional 12 analyses within a run. Immediately before refluxing (do not add acid detergent >10 min before refluxing) add 100mL of acid-detergent (AD) solution at room temperature. Heat to boiling in 5–10 min and, if necessary, reduce heat slightly to avoid foaming, but provide moderate particle agitation. After 5–10 min of refluxing, rinse down sides of beaker using a fine stream of AD solution (add <5mL). Reflux for 60±5 min from the time of onset of boiling. Remove each beaker from the heating unit, swirl, and filter into the crucible. Without the inverting beaker, use a fine stream of boiling water to rinse all particles into the crucible. Remove AD and rinse water using minimum vacuum. Close vacuum and fill crucible with ca 40 mL of hot (90–100°C) water, stir to break up the residue filter mat, and let soak for 3–5 min. Repeat water soaking twice and vacuum dry. Rinse sides and bottom of crucible to be sure that all traces of acid are removed (any residual acid will be concentrated during drying and cause charring of residues and low fibre values). Add 30–40

mL of acetone, stir to break up all clumps and expose all particles to acetone, soak 3–5 min and repeat until no colour is removed (typically 2 acetone soakings are sufficient). Remove residual acetone with vacuum. Dry >3 hours, preferably overnight, at 105°C in a forced draught oven and weighed (W3). Cool for 15 min in a desiccator over P_2O_5 or $Mg(ClO_4)_2$ and always weigh crucibles in the same order. Check that the balance is zero after each weighing if crucibles are warm. The following formula is used to calculate ADF: % ADF (DM basis):

$ADF_{DM} = 100 * [(W3 – W1) – (B3 – B1)] / (W2 * \text{test sample DM})$

Where B1 and B3 are average weights of all blanks after oven drying before and after AD extraction, respectively, and test sample DM = test sample portion weight after oven drying/test sample portion weight before drying.

3.9.7 Ether extract (EE)

The EE content of the feed, feed refusal and faecal samples were determined by means of the Soxhet extraction method (No 920.39), using petroleum ether (40 - 65°C boiling point) as a solvent (AOAC, 2000). A 2 g sample (DM-basis) was closed inside a pre-weighed Schleicher and Schull No. 589/2 filter paper and was carefully placed within the extraction thimble. The thimble was then closed properly with a clean cotton wool plug and placed inside the glass extractor that was connected with the pre-weighed glass flask before pouring the 150 mL solvent (petroleum ether) gently into the thimble. The extractor unit was placed into the individual heating units and connected with the water cooling unit. The heating was set to ensure a droplet speed of approximately 5 to 6 drops of solvent per second and the samples were extracted for 6 hours. After the extraction process, glass flasks were removed from the heating units and all remaining solvent was damped off over a warm water bath before drying overnight in an oven at 60°C. After drying, each glass flask containing the lipid fraction was placed inside a desiccator until reaching room temperature and weighted accurately to the 4^{th} decimal. The EE fraction of samples was calculated as follows:

% EE = [Weight of EE (g DM) / Weight of test sample (g DM)] × 100

3.9.8 Gross energy (GE)

Gross energy content of feed, feed refusals and faecal samples were determined using a Leco® AC500 Isoperibol Calorimeter (Leco Corp., St. Joseph, MI) following ASTM standard D5865 (ASTM, 2009) (Cantrell *et al*., 2010). Approximately 0.2 g (DM-basis) of each sample (feed, feed refusal and faeces) was weighed accurately to the 4^{th} decimal and placed in a steel crucible. A platinum wire (5 cm) was connected to the electrodes of the bomb calorimeter and the steel crucible containing the sample was carefully placed inside the bomb vessel before filling it with oxygen to a pressure of 3000 kPa. Special attention was given to ensure that the platinum wire was in contact with the sample and to avoid contact with the steel crucible itself. The sample weight was entered into a computer. The vessel was placed into a water bath and electrodes connected to the vessel. An operating system recorded the temperature every six seconds accurately to 0.0001°C using an electronic thermometer. The GE was expressed as mega joules per kilogram DM (MJ/kg DM).

3.9.9 Non-structural carbohydrates (NSC)

The NSC content of the feed, feed refusal and faecal samples was determined according to the equation described by Van Soest *et al*. (1991). The net fraction can be reasonably calculated by the following formula:

NSC = 100% – (%NDF + %protein + %fat + %ash)

3.10 Apparent digestibility of feed nutrients

The apparent digestibility of feed constituents is best defined as the proportion of ingested feed or nutrients not excreted in the faeces and therefore assumed to be absorbed by the animal. The following formula was used to calculate apparent digestibility (McDonald *et al*., 2011):

$$\text{Apparent digestibility (\%)} = \frac{\text{Feed or nutrient intake (g DM)} - \text{Feed or nutrient excreted in faeces (g DM)}}{\text{Feed or nutrient intake (g DM)}} \times 100$$

Metabolisable energy was calculated from digestible energy (DE) values by multiplying the DE by a factor of 0.8 (McDonald *et al*., 2011) to compensate for energy losses in the urine and methane gas (CH_4). The apparent digestible nutrients of a feed is the specific nutrient content of a unit weight of the feed less the specific nutrient content of the faeces (digestion study) resulting from the consumption of any unit weight of that feed. The digestible nutrient content of the diet was calculated as follows (McDonald *et al*., 2011):

$$\text{Apparent digestible nutrient content (\%)} = \frac{\text{Nutrient intake (g DM)} - \text{Nutrient excreted in faeces (g DM)}}{\text{Feed intake (g DM)}} \times 100$$

3.11 Carcass evaluation

At the end of the production study all lambs (mean 48.11±2.94 kg live weight) were slaughtered at a commercial abattoir. Cold carcass weights were recorded 24 hours after refrigeration at 2-4^{o}C according to the methods described by Fisher and De Boer (1993). The cold carcass weight was then used to determine the dressing percentage:

Dressing percentage (%) = [Cold carcass weight (kg) / Live weight (kg)] x 100

The external length (EL), shoulder circumference and buttock circumference (BC) of each carcass wasalso measured [(a), (b) and (c), respectively; Figure 3.9].

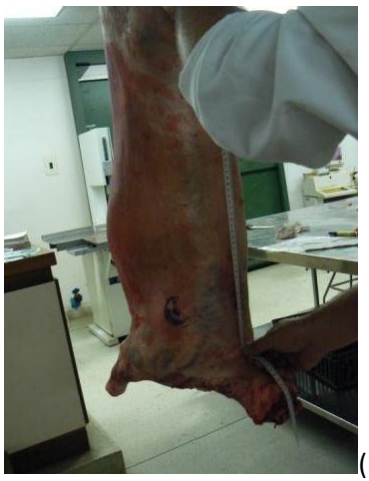
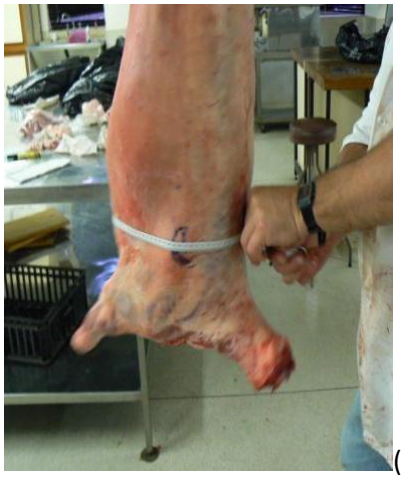
(a)  (b)  (c) 

Figure 3.9 Measuring the external length (a), shoulder circumference (b) and buttock circumference (c) of the lamb carcass.

Meat evaluation was performed on the left side of each carcass. All carcasses were split between the 12th and 13th rib (thoracic vertebra) and fat depth measured with a calliper (Electronic digital calliper; Omni-Tech), 45 mm (a) and 110 mm (b) from the mid dorsal line (Figure 3.10) (Carson *et al*., 1999).

(a)  (b) 

Figure 3.10 Fat thickness measured between the 12th and 13th rib at 45 mm (a) and 110 mm (b) from the mid dorsal line.

To measure the area of the Longissimus muscle (*Musculus longissimus dorsi*) between the 12th and 13th rib, the longissimus muscle was traced directly onto transparent film (Edwards *et al*., 1989; Figure 3.11). The traced outline was scanned with a scale bar and the eye muscle area measured using a video image analysis system (Soft Imaging System: analysis® 3.0). The video image analysing system was calibrated with the scale bar.

Figure 3.11 Tracing of the eye muscle between the 12th and 13th rib on to transparent paper.

3.12 Meat quality evaluation

Three loin chops (the left 9th to the 11th rib) from the same carcasses used for carcass measurements (see paragraph 3.11) were collected for meat quality evaluation.

3.12.1 Fatty acid profile determination

Total lipid from feed material was extracted according to the AOAC official method 920.39 for chemical procedures (AOAC, 2000). Total lipid from muscle and subcutaneous fat samples [using the first (fresh) loin chop; chop 1] were quantitatively extracted according to the method of Folch *et al*. (1957), using chloroform and methanol in a ratio of 2:1. Total extractable intramuscular fat was determined gravimetrically from the extracted fat and expressed as percentage fat (w/w) per 100 g tissue. An antioxidant, BHT, was added at a concentration of 0.001% to the chloroform:methanol mixture. A rotary evaporator was used to dry the fat extracts under vacuum. The extracts were dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as a moisture adsorbent.

The extracted fat from the feed, muscle and subcutaneous fat was stored in a polytope (glass vial, with push-in top) under a blanket of nitrogen and frozen at –20°C pending FA analyses. A lipid aliquot (20 mg) of feed, muscle and subcutaneous lipid were converted to methyl esters by base-catalysed transesterification with sodium methoxide (0.5 M solution in anhydrous methanol) for 2 hours at 30 °C - in order to avoid conjugated linoleic acid (CLA) isomerisation, as proposed by Park *et al*. (2001), Kramer *et al*. (2002) and Alfaia *et al*. (2007). Fatty acid methyl esters (FAME) from the feed, muscle and subcutaneous lipid were quantified using a Varian 430 flame ionization gas chromatograph (GC), with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thicknesses). Analysis was performed using an initial isothermic period (40°C for 2 min). Thereafter, temperature was increased at a rate of 4°C/min to 230°C. Finally an isothermic period of 230°C for 10 min followed. Fatty acid methyl esters n-hexane (1µl) was injected into the column using a Varian CP-8400 Autosampler. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software recorded the chromatograms.

Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from the Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Conjugated linoleic acid standards were obtained from Matreya Inc. (Pleasant Gap, Unites States of America). These standards included: *cis*-9, *trans*-11 and *trans*-10, *cis*-12-C18:2 isomers. All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, Johannesburg, South Africa). Fatty acids were expressed as the proportion of each individual FA to the total of all FAs present in the sample. The following FA combinations were calculated: omega-3 (*n*-3), omega-6 (*n*-6), total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), PUFA/SFA ratio and *n*-6/*n*-3 ratio.

3.12.2 Stability of fresh and frozen lamb chops

The second loin chop (chop 2) from each lamb carcass was individually placed in a polystyrene tray containing an absorbent pad, overwrapped with oxygen-permeable polyvinyl chloride (PVC) meat stretch wrap (Figure 3.12) and stored for seven days at 4^{o}C under fluorescent light for fresh meat stability studies.

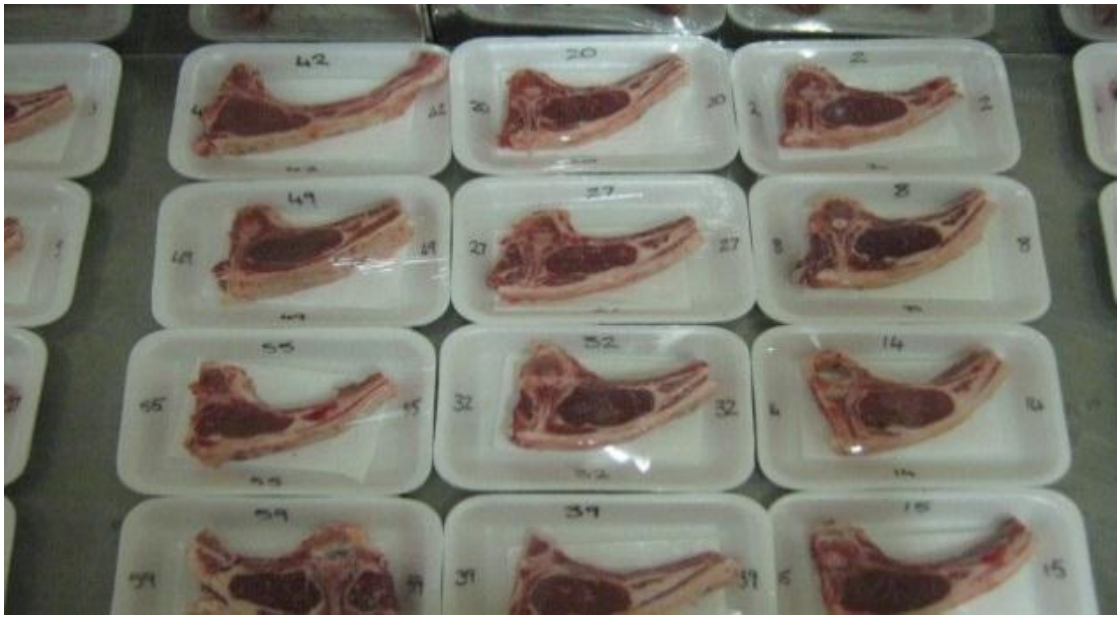


Figure 3.12 The second loin chop (Chop 2) placed in polystyrene trays containing absorbent pads, overwrapped with PVC meat stretch wrap and stored for seven days.

The third loin chop (chop 3) was vacuum sealed (Figure 3.13) and stored for 90 days at -18^{o}C in the dark for frozen storage stability evaluation.

Figure 3.13 Vacuum sealing third loin chop (chop 3) and stored for 90 days at -18^{o}C.

3.12.3 Meat colour

The colour (L*, a* and b* values) of muscle was subsequently determined in triplicate using the second loin chop (chop 2; see paragraph 3.12.2) on days 0, 1, 2, 3, 4, 5, 6 and 7 using a Minolta chromo meter. Chroma or saturation index (SI) which is related to colour intensity of meat, was calculated according to the formula: SI = (a*2 + b*2)*0.5 for muscle (Ripoll *et al*., 2011). Hue angle was also calculated according to the formula: tan-1(b*/a*) (Rippoll *et al*., 2011).

3.12.4 Thiobarbituric acid reactive substance (TBARS) determination

A 5 g sample of lean meat was removed from the middle of each loin chop on day 0 (chop 1; the fresh loin chop), day 7 (chop 2 stored at 4^{o}C) and day 90 (chop 3 stored at -18^{o}C) to determine the malonaldehyde content/kg meat using the aqueous acid extraction method of Raharjo *et al*. (1992). This method is used to determine the magnitude of lipid oxidation.

3.12.5 Meat tenderness evaluation

For meat tenderness measurement, the *M. longissimus lumborum* (LL) (11th to 13th rib) was removed from one side of the carcass, vacuum sealed and frozen at -20°C. Frozen samples were thawed at 4°C for 18 hours before preparation. Thawed cuts were prepared according to an oven-broiling method using direct heat (AMSA, 1978). An electric oven was set on "broil" 10 min prior to preparation (260°C). Steaks were placed on an oven pan on a rack to allow meat juices to drain during cooking and placed in the pre-heated oven 9 cm below the heat source. The cuts were cooked to an internal temperature of 35°C, then turned over and finished to 70°C. Cuts were cooled down at room temperature for at least two to three hours before measuring shear force. Four cylindrical samples (12.5 mm core diameter) of each sample were cored parallel to the grain of the meat, and sheared perpendicular to the fibre direction using a Warner Bratzler shear device mounted on an Universal Instron apparatus (cross head speed = 200 mm/min; one shear in the centre of each core). The reported value in kg represents the average of the peak force measurements of each sample.

3.13 Statistical analysis

The data was subjected to analysis of variance (PROC ANOVA) and analyzed according to a complete random design representing a dose-response trial formulated to comply with increasing incremental NDF levels and tested for significant differences using the General Linear Model (GLM) procedures of the Statistical Analysis System (SAS) program. Tukey's honest significant difference (HSD) test was used to identify significant differences (P <0.05) between treatments (SAS, 1999).

CHAPTER 4

THE EFFECT OF NEUTRAL-DETERGENT FIBRE CONTENT ON THE DIGESTIBILITY OF FINISHING DIETS FOR LAMBS

4.1 Introduction

A ruminant's fermentative nature mainly relies on the function of microbes for the digestion and fermentation of feed in the rumen (Slyter, 1976). The neutral-detergent fibre (NDF) content is one of several important dietary factors affecting voluntary feed intake, feed digestibility, ruminal pH and the bacterial composition in the rumen. As the feed intake of an animal increases, the metabolizability of the feed declines, owing to an increase in rate of passage and a reduction in rumen retention time. This is also true for a higher energy diet, as well as a diet containing lower amounts of roughage and of smaller particle size (McDonald *et al*., 2011). For example, when roughage with a high NDF digestibility is fed to ruminants, dry matter intake (DMI) is increased followed by a resultant lower dietary NDF digestibility due to a shorter rumen retention time (Oba and Allen, 1990). However, as the roughage level of ruminant diets is decreased, the total tract organic matter (OM) digestibility increases (Rode *et al*., 1985). The rumen cellulolytic bacterial population increases when a diet high in NDF content is fed to ruminants (Weimer *et al*., 1999). Therefore, NDF degradation is affected positively when the roughage content increases in the diet, which suggests a slower rate of passage and higher cellulolytic bacteria levels in the latter (Vlaeminck *et al.*, 2006). When the concentrate level increases, the total OM digestibility increases (Clark *et al*., 1992) due to a larger number of amilolytic bacteria and a lower rumen pH. The low nett energy (NE) of mature roughage is not only because of a low OM digestibility, but it is also associated with a high concentration of cellulose (McDonald *et al*., 2011). The digestion (fermentation) of this polysaccharide in the rumen and the metabolism of its end products give rise to a high heat increment (HI), hence loss of energy. Also, during rumen fermentation of OM, hydrogen is produced and methanogenic bacteria use it with carbon dioxide (CO_2) to produce methane (CH_4) and water (H_2O) and a further loss of energy occurs. Neutral-detergent fibre may also restrict DMI of ruminants due to its "bulkiness" or "fill" effect it provides to a diet (Dado and Allen, 1995; McDonald *et al.,* 2011).

Rumen pH is positively correlated to the NDF content of a roughage source, as well as the total NDF content of the ruminant's diet (Galyean and Defoor, 2003). Ruminal pH is the most important factor affecting fibre digestion in the rumen. The change in microbial fermentation in dairy cows fed a low-fibre diet is characterized by a decline in ruminal pH and reduction in the acetate:propionate ratio (Nagaraja, 2012). Therefore, a positive correlation between rumen pH and roughage digestibility is commonly observed in literature. Animals with a ruminal pH of below 5.5 stand a good chance of developing sub-acute ruminal acidosis (SARA) (McDonald *et al*., 2011).

Altering the roughage:concentrate ratio in the diet is a mechanism commonly suggested and used for synchronising the availabilities of energy and crude protein (CP) simultaneously in the rumen in order to maximise the amount of microbial protein, dietary protein and amino acids that passes through to the small intestine (Clark *et al*., 1992). Any additional OM fermented in the rumen due to a change in the roughage:concentrate ratio of the diet therefore probably increases microbial protein synthesis by providing more energy. Non-fibrous carbohydrates (NFC), for example starches and simple sugars, ferment at a faster rate in the rumen compared to NDF, and as a result the energy density of a diet increases when its inclusion is increased. Normally, available energy is one of the limiting factors which affect bacterial growth in the rumen (Clark *et al*., 1992). Non-fibrous carbohydrates provide a higher energy level, faster availability of energy to microbes and also determine the amount of bacterial protein produced in the rumen (McDonald *et al*., 2011). Non-fibrous carbohydrates do not stimulate rumination and therefore sufficient saliva production and it may impair fibre fermentation and could cause SARA (McDonald *et al*., 2011).

Lipids are a noteworthy energy supplement that provides about 2.25 times the digestible energy (DE) of carbohydrates (McDonald *et al*., 2011). Thus the inclusions of lipids in ruminant diets are in general practiced because of their high energy value (Chilliard, 1993; Bauman *et al*., 2003). In addition, the possible improvement of ruminant carcass quality (Bauchart *et al*., 1996) due to more healthy fatty acids (FA) could also benefit animal and human health (McDonald *et al*., 2011). A common practise is for total dietary fat to not exceed 6 to 7% of dietary DM (Jenkins, 1993; Doreau *et al*., 1997; NRC, 2001).

One of the key concerns regarding lipid supplementation is the negative effect it has on fibre digestion. The coating effect of free fatty acids (FFA) to cellulose may impair the attachment of bacterial cellulolytic enzymes (Jenkins, 1993). Also, polyunsaturated fatty acids (PUFA) are poisonous to numerous bacterial species present in the rumen, and as an end result could weaken their growth and function (Maia *et al*., 2007). The standard inclusion of a lipid source in a basal ruminant diet is 3%, whereas protected lipids could increase diet lipid content with an additional 3-4% (McDonald *et al*., 2011).

Even though the main purpose of increasing the NDF increments in lamb diets has been to determine the effect thereof on FA biohydrogenation (BH) and carcass FA content, its effect on diet digestibility and available nutrients cannot be ignored. The aim of this study was therefore to determine and explain the effect of increasing incremental NDF levels on the nutrient digestibility and digestible nutrient content of finishing diets fed to lambs.

4.2 Materials and Methods

The materials and methods used in the digestibility study have been described in Chapter 3 and were briefly as follows: The digestibility study was conducted after the completion of the production study over a period of 12 days (4-day adaptation to the faecal bags followed by an consecutive eight-day collection period) to investigate the effect of the NDF level on nutrient digestibility and digestible nutrient content of each diet. Seven lambs (mean 48.11±2.94 kg live weight; total of thirty-five) were randomly allocated to each treatment (n=7 lambs/treatment). The lambs were housed individually in pens. The five dietary treatments were formulated to contain a similar nutrient composition, differing only in respect to the NDF content as the primary treatment parameter. The NDF content increased from low roughage (primarily lucerne hay) inclusion to a high inclusion rate representing a dose-response trial in the following order: 12.76% (T15), 17.69% (T30), 22.53% (T45), 27.48% (T60) and 32.40% (T75) NDF/kg DM, respectively.

To try and avoid variation in assessing the voluntary feed intake of sheep, a sequential method of feed allocation was followed by providing each animal with a 15% refusal level of feed intake. Calculations were made on a daily basis by using a preceding 3-day moving average of feed intake. Daily feed refusals (orts) of each animal were also collected. The faeces voided were collected twice daily before feeding. Collected feed, feed refusals and faecal samples were analysed for DM, CP, NDF, acid-detergent fibre (ADF), gross energy

(GE), ash, OM and ether extract (EE), whereas the non-structural carbohydrate (NSC) content was calculated using the difference. Apparent digestibility calculations were done accordingly.

The data was subjected to analysis of variance (PROC ANOVA) of the SAS program, version 9.2 (SAS, 2008). Tukey's honest significant difference (HSD) test was used to identify significant differences (P <0.05) between treatments.

Null hypothesis: An increase in NDF percentage would result in a decrease in total tract DM digestibility and dietary ME content, as well as an increase in NDF digestibility. The null hypothesis will be accepted where P <0.05 was recorded and influenced by these mentioned parameters.

4.3 Results and Discussion

4.3.1 Chemical composition of experimental diets

The actual chemical composition of the experimental diets is presented in Table 4.1. No chemical analysis of the individual feed ingredients was conducted before formulating the experimental diets. Hence some nutrient variation between the formulated (Table 3.1; Chapter 3) and actual values (Table 4.1) could be expected. Actual values of the experimental diets in Table 4.1 indicated a slightly higher NDF and ADF content, compared to the formulated values (Table 3.1). Most important is that the NDF increments were constant and compared well with the formulated values (average of 5.89±0.71% DM, compared to 4.91±0.05% DM in Chapter 3), even though total values may differ slightly. It was important for the NDF increments to remain the same between treatments as lucerne hay inclusion increased from treatment T15 to T75, respectively. A small variation did however occur. In contrast, a slightly lower lipid (EE) content of the actual diets (Table 4.1) was recorded compared to those formulated in Table 3.1 (Chapter 3), but were still consistent. The tabulated lipid content of maize meal used to calculate feed lipid content and determine maize germ oil's inclusion for comparable total lipid and fatty acid (FA) composition (explained in Chapter 3) was probably higher, which resulted in this small difference. The lipid contribution of lucerne hay (1.61% on a DM basis) (McDonald *et al*., 2011) could also explain this slight difference.

Table 4.1 The mean chemical composition of the five experimental diets used during the digestibility study (DM basis)

	Treatment diets*				
Parameter (DM basis)	**T15**	**T30**	**T45**	**T60**	**T75**
Dry matter (%)	89.71	90.31	90.92	90.63	91.58
Organic matter (%)	94.01	92.44	91.27	89.46	89.63
Non-structural carbohydrates (%)	59.13	50.66	44.24	36.81	31.24
Crude protein (%)	15.16	16.75	16.35	16.22	15.53
Neutral detergent fibre (%)	14.33	19.21	25.12	31.41	37.89
Acid detergent fibre (%)	7.54	11.60	16.11	21.62	25.60
Ash (%)	5.99	7.56	8.73	10.54	10.37
Ether extract (%)	5.38	5.81	5.55	5.02	4.97

* Treatment diets: Increments of neutral-detergent fibre (NDF) depicted as percentage roughage included.

The calculated NSC content of the analysed T60 and T75 treatments (36.81 and 31.24%, respectively; Table 4.1) as well as CP content of T15 and T75 (15.16 and 15.53%, respectively; Table 4.1) were slightly lower than that of the same formulated (Table 3.1) treatments (NSC - 40.72 and 34.95%; CP - 16.49 and 16.82%, respectively). In contrast, the ash content of the actual T45, T60 and T75 treatments (8.73, 10.54 and 10.37%, respectively; Table 4.1) were somewhat higher than the same formulated treatments (7.52, 8.37 and 9.32%, respectively; Table 3.1). It has to be borne in mind that the NSC content of the formulated and analysed values was determined using the formula as proposed by Van Soest *et al*. (1991).

The ash content increased consistently from T15 to T75 (Table 4.1), probably due to the higher mineral content of lucerne hay (9.5% DM) compared to that of maize (1.3% DM) (McDonald *et al*., 2011). Roughage source may also contribute to nutrient variation in animal diets as there were large differences found in nutrient densities within the same roughage source due to various factors such as locality, climate, soil and production practices (Smith, 2008). Due to this accepted variation in roughage nutrient composition

(i.e. lucerne hay), as well as some by-products, its inclusion in the experimental diets could affect diet nutrient composition which may explain the limited and small variability in the NSC, CP and ash content (%), the constant and comparable higher NDF and ADF, as well as the lower EE content between formulated and analysed diets. Scholtz (2001) also indicated that the NDF content of lucerne hay cultivated in South Africa varies between 26.70 and 69.82%. Hence, when included within animal diets, these differences may result in some nutrient variability. In addition, the CP content of lucerne hay has also been reported to vary between 15 to 22% (Hanson *et al*., 1988). Therefore, the small variation in CP content between analysed and formulated values (discussed previously) could explain the lower chemical values depicted in the experimental diets.

It is however evident from Table 4.1 that the chemical composition of the various experimental diets compared well with that of the calculated values in Table 3.1 (Chapter 3). Thorough mixing and efficient sampling probably ensured that the chemical composition of the different diets compared well with one another.

4.3.2 Apparent digestibility and digestible nutrient content of experimental diets

Dry matter intake, apparent digestibility and digestible nutrient content of experimental diets are presented in Table 4.2. From Table 4.2 it is evident that incremental dietary NDF content did affect the voluntary DMI of lambs. The DMI of lambs receiving the T15 treatment was significantly lower (P =0.0015) compared to the other treatments. Nonetheless, even though the feed intake of lambs from only one treatment was affected, it is probable to accept that the level of feeding should therefore not influence overall apparent digestibility of the diets. The lower intake of lambs fed T15 could be attributed to the high maize inclusion (64.35%; Table 3.1) and possible resultant lower ruminal pH that may end up in SARA and an accompanied lower intake (McDonald *et al*., 2011). Acidosis reduces ruminant voluntary intake (Smith, 2008). Roughage physical effectiveness is also correlated to ruminal pH as it stimulates rumination when the particle size is large enough (Allen, 1997). As discussed in Chapter 3 (see paragraph 3.5.1), no ruminal buffers were added to the diets, therefore the NDF content of the other treatments probably contained enough effective fibre to stimulate mastication and buffer the rumen (Wilcox and Van Horn, 1992).

Table 4.2 The effect of dietary NDF content on DMI, apparent digestibility and apparent digestible nutrient content of finishing diets fed to lambs (mean±SD)

	Treatment diets*						
Parameter (DM Basis)	**T15**	**T30**	**T45**	**T60**	**T75**	**Significance (*P*-value)**	**CV[#]**
DM intake (g/sheep/day)	1159[b]±210	1464[a]±151	1601[a]±187	1587[a]±221	1472[a]±205	0.0015	13.49
Apparent digestibility coefficients:							
Dry matter	0.86[a]±0.04	0.81[a,b]±0.01	0.76[b,c]±0.03	0.74[c,d]±0.06	0.69[d]±0.06	<.0001	5.71
Organic matter	0.87[a]±0.04	0.83[a,b]±0.02	0.79[b,c]±0.03	0.77[b,c]±0.06	0.72[c]±0.06	<.0001	5.28
Non-structural carbohydrates	0.94[a]±0.04	0.93[a]±0.02	0.91[a,b]±0.03	0.92[a,b]±0.05	0.86[b]±0.07	0.0144	4.93
Crude protein	0.82[a]±0.04	0.79[a,b]±0.02	0.75[b,c]±0.03	0.76[a,b,c]±0.06	0.72[c]±0.06	0.0018	5.84
Neutral-detergent fibre	0.65±0.04	0.60±0.03	0.58±0.04	0.60±0.08	0.60±0.07	0.1930	9.07
Acid-detergent fibre	0.67[a]±0.10	0.57[a,b]±0.04	0.54[b]±0.04	0.57[a,b]±0.08	0.56[a,b]±0.08	0.0288	12.40
Ash	0.67[a]±0.04	0.59[a,b]±0.04	0.50[b,c]±0.10	0.48[b,c]±0.10	0.36[c]±0.15	<.0001	17.38
Ether extract	0.91[a]±0.03	0.90[a,b]±0.02	0.87[b]±0.03	0.86[b]±0.05	0.87[b]±0.03	0.0355	3.59
Gross energy	0.85[a]±0.04	0.81[a,b]±0.02	0.76[b,c]±0.03	0.74[b,c]±0.07	0.69[c]±0.06	<.0001	5.91

Table 4.2 (Cont.)

Parameter (DM basis)	Treatment diets*					Significance (*P*-Value)	CV
	T15	T30	T45	T60	T75		
Apparent digestible nutrient content of diet:							
Organic matter (%)	82.46^{a}±3.53	77.31^{a,b}±1.22	72.33^{b,c}±3.21	69.57^{c,d}±5.17	64.88^{d}±4.85	<.0001	5.27
Non-structural carbohydrates (%)	55.60^{a}±2.54	47.17^{b}±0.85	40.71^{c}±2.30	33.68^{d}±2.30	26.53^{e}±1.91	<.0001	5.08
Crude protein (%)	12.29^{a,b}±0.72	13.18^{a}±0.38	12.10^{a,b}±0.43	12.30^{a,b} ±1.00	11.13^{b}±1.05	0.0008	6.29
Neutral-detergent fibre (%)	9.81^{d}±0.52	11.89^{d}±0.62	14.73^{c}±0.97	19.32^{b}±2.45	22.87^{a}±2.52	<.0001	10.63
Acid-detergent fibre (%)	5.34^{c}±0.74	6.87^{b,c}±0.47	8.81^{b}±0.67	12.62^{a}±1.88	14.58^{a}±1.84	<.0001	13.23
Ash (%)	3.75±0.19	4.15±0.23	4.16±1.19	4.97±1.12	3.73±1.45	0.1488	23.70
Ether extract (%)	4.76^{b}±0.17	5.08^{a}±0.13	4.79^{b}±0.18	4.28^{c}±0.22	4.34^{c}±0.18	<.0001	3.80
ME* (MJ/kg DM	11.45^{a}±0.52	11.05^{a,b}±0.22	10.18^{b,c}±0.40	9.98^{c}±0.88	9.36^{c}±0.83	<.0001	5.98

^{a,b,c} Mean values with different superscripts differ significantly (P <0.05).

$^{\#}$ CV = Coefficient of variation (%).

* Treatment diets: Increments of neutral-detergent fibre (NDF) depicted as percentage roughage included; ME = Metabolizable energy = Digestible energy × 0.8 (McDonald *et al*., 2011).

The results of a study conducted by Smith (2008) indicated that the inclusion of 15 to 20% NDF and/or 13 to 15% physical effective NDF (peNDF) of *Medicago sativa* (lucerne) hay provided in the NDF requirements of lambs fed finishing diets when the hay was milled through a 12.5 mm screen. The NDF content of T15 (14.33% DM; Table 4.1) was however below this recommended NDF value for finishing lambs. If rumen buffers or modifiers were added to all the diets, DMI of T15 would probably have improved and compared well with all the other treatments.

It has long been recognised that in ruminants there is a negative relationship between digestibility of feed and their intake (McDonald *et al.,* 2011). Dry matter intake is also affected by dietary NDF content and therefore digestibility (Mertens, 1992). Expressed in a different way, feed that are digested rapidly and have a higher digestibility encourage higher intakes. Higher digestion means a faster flow rate leaving more rumen space for an increased intake. This increased intake again could however lead to a lower digestibility (Freer and Dove, 2002). This means that even a highly digestible feed is then exposed to the action of digestive enzymes for a shorter period of time and digestibility is reduced - even with a proposed higher digestibility to start with (McDonald *et al.,* 2011). The DMI of lambs from only one treatment (T15; low fibre diet) were negatively affected (P <0.05) (Table 4.2) by dietary treatment. It is however difficult to make conclusive recommendations when only one set of lambs were affected over a short period of time.

Neutral-detergent fibre limits DMI of ruminants due to its “bulky” or “fill” nature (Dado and Allen, 1995; McDonald *et al.,* 2011). Roughage sources, e.g. lucerne hay, have a greater fill effect and are bulkier than concentrated diets like maize in this instance. In contrast to the present study, Dado and Allen (1995) found a reduction in DMI with a higher NDF percentage. Beauchemin (1991) also reported that when the roughage content of a diet increased, DMI was negatively affected. In other words, roughage with a low NDF content result in a higher DMI compared to roughages with a high NDF content due to a shorter rumen retention time (Arelovich *et al*., 2008). Due to this explanation, the lack of a significant effect of treatments T30, T45, T60 and T75 on DMI is difficult to explain. It has to be mentioned again that it is difficult to make meaningful conclusions regarding the intake of sheep over a short period of time (Blaxter *et al*., 1961) especially when animal numbers

are restricted. There were also no signs of dietary selection by the sheep. Therefore feed ingredient selectivity could not have had an effect on diet intake and digestibility.

Mertens (1997) used NDF as a feed characteristic to predict the filling effect and energy content of diets. It was found that DMI was positively correlated with NDF concentration when energy limits intake, but negatively correlated with NDF concentration when "fill" limits intake, which was probably not the case in the present study. Van Soest (1965) also explained that roughage NDF content was more highly related to DMI of sheep compared to other chemical measures, and Waldo (1986) suggested that NDF content is the best single chemical predictor of DMI by ruminants. It should be mentioned that cellulose is only part of the plant's fibre fraction and does not necessarily represent a more indigestible fraction of the structural polysaccharides in the fibre source (Van Soest, 1965).

Even though not entirely consistent between treatments, from Table 4.2 it is well evident that the DM, OM, NSC, GE, CP, ADF and EE digestibility, as well as ash solubility were significantly affected (P <0.05) following incremental lucerne hay (NDF) inclusion in the diets. Both DM and OM digestibility decreased (P <0.05) following the increasing NDF increments. Dry matter and OM digestibility were associated and could be related to each other (Du Toit, 2013), which is evident from the data presented in the present study (Table 4.2). This data is consistent with Rode *et al*. (1985) who stated that as the roughage level of ruminant diets is increased, the total tract OM digestibility decreases. Organic matter digestibility is affected by the level of not only the roughage intake, but the concentrates as well (Clark *et al*., 1992).

Non-structural carbohydrate digestibility of T75 was significantly lower (P <0.05) compared to T15 and T30 (Table 4.2). Non-fibrous carbohydrates (NFC) (sugar, starch, organic acids, pectin, β-glucans, galactans, and fructans) are known to be the most common sources of energy for high producing ruminants like dairy cows, and are used to optimize production in an intensive system (Huntington, 1997). Non-fibrous carbohydrates are also more palatable and digestible when compared to NDF in dietary fibre and are practically fully fermented in the rumen (90-100%) (Van Soest *et al*., 1991). The NFC fraction of a diet relates to the NSC fraction used in the current study, and could explain the lower (P <0.05) NSC digestibility of T75, compared to T15 and T30. Due to the fact that rumen pH is positively correlated with the NDF content of a ruminant's diet, it may also explain this

significant lower digestibility (Galyean and Defoor, 2003). In addition, Kucuk *et al*. (2001) stated that ruminal pH (dietary fibre content) is positively related to fibrolytic (fibre digesting) bacterial activity.

The GE and CP digestibility significantly decreased (P <0.05) following increasing incremental NDF content (Table 4.2). As stated above, NFC are more digestible compared to dietary fibre and are practically fully fermented in the rumen (Van Soest *et al*., 1991), hence a possible reason for the decreasing (P <0.05) GE digestibility following incremental NDF content. The decreased (P <0.05) CP digestibility following lucerne hay inclusion on the other hand could probably be attributed to the higher degradability of plant protein sources and non-protein nitrogen (NPN) sources (like urea) (McDonald *et al*., 2011) included in higher quantities in T15 (soya bean meal and urea) and decreased following lucerne hay inclusion (T15 to T75) (Table 3.1). In contrast, the protein content of lucerne hay has a high degradability (rumen-degradable protein - RDP) (Allen, 2000), which was probably initiated by a decreased (P <0.05) total tract digestibility (Table 4.2). Yang and Beauchemin (2006) found that by changing the roughage content of a diet from a low 35% to as high as 55% roughage inclusion, increased ruminal nitrogen degradability significantly, which was not the case in the present study.

According to McDonald *et al*. (2011) the NE system was incorporated in what was at one time the standard reference work on the feeding of livestock in the USA, but was not much used in practice. The preferred system in America was for many years the total digestible nutrients (TDN) system. The TDN content of a feed was calculated as the combined weight in 100 kg of feed of digestible CP and digestible carbohydrate [crude fibre (CF) plus nitrogen-free extractives (NFE)], plus 2.25 times the weight of digestible EE. The EE is multiplied by 2.25 because the energy value of fat is approximately 2.25 times higher than that of carbohydrate. Therefore, any significant effect on CP, ADF, NSC or EE digestibility (P <0.05; Table 4.2) in the current study could be associated with GE digestibility, hence ME content of the diet.

In contrast, the lack of a significant effect on NDF digestibility (P >0.05; Table 4.2) was not expected as a higher NDF content results in a higher ruminal pH (McDonald *et al*., 2011) which positively effects ruminal fibre digestibility due to more effective fibre to stimulate mastication and saliva production (Wilcox and Van Horn, 1992). The digestibility of the

roughage component of a feed source is likely to be reduced by feeding it in combination with a concentrate (McDonald *et al.*, 2011). One of the key concerns when supplementing an unsaturated lipid source in ruminant diets is the negative effect it has on fibre digestion because of the physical coating effect that prevents the attachment of bacterial cellulolytic enzymes to fibre (Jenkins, 1993). A direct inhibition of rumen microbial activity due to unsaturated fatty acids (UFA) may also decrease fibre digestibility (McDonald *et al.,* 2011). This was however not the case in the present study as the total lipid inclusion did not exceed the maximum tolerable level (6 to 7% DM basis; NRC, 2001) (see Table 4.1) which probably also explains the lack of any significant effect (P >0.05) on NDF digestibility (Table 4.2).

Smith (2008) stated that any reduction in NDF digestibility may be attributed to associative effects of feeds due to the increase of easily fermentable carbohydrates (starch in maize). Starch (carbohydrate) may have a negative influence on roughage digestibility. Under these conditions, fast fermentation of starch to volatile fatty acids (VFA) depressed the rumen pH to 6 or less. The low pH then reduced fibre digestibility by inhibiting cellulolytic micro-organism (MO) activity. There are MOs that ferment both cellulose and starch but prefer to ferment starch by choice ('carbohydrate effect'). The decrease in cellulolysis observed in high-starch diets may be only moderately reduced by the addition of buffering agents such as sodium bicarbonate through the ruminant's saliva or included as an additive.

Acid-detergent fibre and EE digestibility, as well as ash solubility were also affected (P <0.05) by increasing lucerne hay inclusion (Table 4.2). Van Soest (1965) explained that the lignin, ADF and cell-wall constituents of plants are a better measurement of feed digestibility and are therefore more negatively correlated. The reduction in lipid digestibility could be attributed to the negative association between diet lipids (Jenkins, 1993) and UFA content in relation to NDF (McDonald *et al*., 2011).

It has to be mentioned that the digestible nutrient composition of each treatment diet is a function of the respective nutrient and feed intake, as well as its digestibility. Therefore, it is inevitable that associations could be drawn between a nutrient's digestibility, as well as its digestible content within each respective treatment diet. The significant effect (P <.0001)

of the incremental increase of NDF on the digestible OM content of the experimental diets (Table 4.2) serves as such an example.

The digestible NDF and ADF content increased (P <0.05) following lucerne hay inclusion (treatment T15 to T75, Table 4.2), even though neither a positive nor negative affect were recorded between NDF and ADF digestibility. As expected and in contrast, the digestible NSC content significantly decreased (P <.0001) probably due to a higher and less favourable ruminal pH to support efficient NSC fermentation (McDonald *et al*., 2011). NDF digestibility was the only chemical constituent of the three mentioned here not significantly affected (P >0.05) by roughage inclusion (Table 4.2). Therefore, the significant response (P <0.05) regarding the digestible NDF, ADF and NSC dietary content could be attributed to its altering dietary content (see Table 4.1), similar feed intake (apart from T15) (Table 4.2) and effect (or lack thereof) on nutrient digestibility (only ADF and NSC) (Table 4.2).

The ME content of the treatment diets decreased significantly (P <.0001) following the increased incremental inclusion of lucerne hay (NDF and ADF) from T15 to T75 (Table 4.2). As explained, the TDN content of a feed can be calculated as the combined weight in 100 kg of feed of digestible CP and digestible carbohydrate (CF plus NFC), plus 2.25 times the weight of digestible EE. Therefore, the significant effect on CP, ADF, NSC and EE digestibility (P <0.05) (Table 4.2), or even small difference in lipid content (Table 4.1) in the current study could be associated with the ME content of the diet. McDonald *et al*. (2011) stated that the low NE of mature roughage is not just because of a low OM digestibility, but it is also associated with a high concentration of cellulose. The digestion of this polysaccharide in the rumen and the metabolism of its end products give rise to a high HI, hence loss of energy. Also, during rumen fermentation of OM, hydrogen is produced and methanogenic bacteria use this with carbon dioxide to produce methane and water with a further loss of energy occurring which could influence animal production and carcass characteristics (McDonald *et al*., 2011). This could be the reason why the ME content declined (P <0.05) as lucerne hay was incrementally increased from T15 to T75, respectively. Methane production following increased roughage inclusion could have been higher and the ME content of the diet was maybe over-estimated, as 0.8 was used to calculate ME from DE. High energy dense diets (e.g. high maize inclusion in treatment T15) could also increase the energy content of ruminant diets due to its higher digestibility compared to other feedstuffs (McDonald *et al*., 2011). In addition, it is known that methane emissions are reduced when ruminant diets

are supplemented with PUFA rich lipid sources (Beauchemin *et al*., 2007). Free hydrogen molecules are used during the hydrolysis and BH of UFAs and reduce the amount of methane produced by MOs (Rasmussen and Harrison, 2011). In theory, this probably could result in a higher ME content in the treatment diets containing higher levels of fibre supplemented with an unsaturated fat source. The possible increased energy available due to more BH and capturing of free hydrogen molecules in higher fibre diets (cellulolytic bacteria being the main hydrogenating bacteria) (Martin and Jenkins, 2002) are probably offset by more concentrated diets higher in maize meal which produces less hydrogen when fermented in the rumen (McDonald *et al*., 2011). However, methane production was not measured and therefore not reflected in the ME values presented in Table 4.2.

Even though no association could be drawn between EE digestibility and digestible EE content (P <.0001) (Table 4.2), the significant decrease in digestible EE content supported DM digestibility.

4.4 Conclusion

The formulated and chemical compositions of the experimental diets compared well with each other. From the results of the present study it is apparent that by incrementally increasing the NDF content (as well as ADF) of the experimental diets, the feed nutrient digestibility as well as digestible nutrient content of the diets were significantly affected. Dietary NDF level seems to be, at least primarily, negatively correlated with feed ME content, feed digestibility and digestible nutrient content, which is comparable to most literature and could influence animal production and carcass characteristics. It was strange, however, that DMI response to dietary NDF level was limited. It is clear from the data presented that lamb finishing diets are more digestible and contains more digestible nutrients when formulated for low NDF content. Regression equations should be used to establish the optimum digestibility response between NDF increments and warrants further research.

Even though NDF digestibility was not affected by dietary NDF content, the null hypothesis is accepted due to a significant decrease in DM digestibility and ME content of the treatment diets following an increasing incremental inclusion of lucerne hay.

CHAPTER 5

THE EFFECT OF NEUTRAL-DETERGENT FIBRE CONTENT IN FINISHING DIETS ON THE PRODUCTION PERFORMANCE AND CARCASS CHARACTERISTICS OF LAMBS

5.1 Introduction

Ruminant diets generally contain low levels of lipid, usually in the order of 2% (Scott and Ashes, 1993). These lipids are an efficient energy supplement providing approximately 2.25 times the digestible energy (DE) than that of carbohydrates (McDonald *et al.,* 2011). Therefore, the incorporation of lipids in ruminant diets are commonly practiced due to their high energy value (Chilliard, 1993; Bauman *et al.,* 2003) and potential improvement of ruminant carcass quality (Bauchart *et al.,* 1996). Unfortunately, the capacity of ruminal micro-organisms (MO) to digest lipids is limited and only relatively low levels of dietary lipid can be metabolized (McDonald *et al.,* 2011). The general recommendation is that total dietary lipids should not exceed 6 to 7% of dietary dry matter (DM) (Jenkins, 1993; Doreau *et al*., 1997; NRC, 2001). Higher levels of lipid may disturb the rumen environment and adversely affect ruminal functions (Manso *et al.,* 2005), consequently affecting animal performance parameters such as live weight gain and feed conversion (Raes *et al.,* 2004). Some researchers (McAllister *et al*., 1996; Rasmussen and Harrison, 2011) stated that when cattle feed was supplemented with lipid within normal safety bounds [especially rich in polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA)], methane (CH_4) emissions were significantly reduced with a resultant positive effect on animal production.

Ruminants have evolved to efficiently utilize roughage primarily as an energy source due to the symbiotic relationship with the MOs present in the reticulo-rumen. Roughage, with special reference to its provision of fibre [neutral-detergent fibre (NDF)] to the diet, is also essential to maintain rumen function and health (McDonald *et al*., 2011). Fibre, and therefore NDF, plays an essential part in the long term health and productivity of the animal. Metabolic disturbances are often the result of fibre requirements that are not sustained (McDonald *et al*., 2011). The quality of a roughage source also affects animal production to some extent. For example, roughage with a high NDF digestibility (or low

maturity) results in a higher dry matter intake (DMI) compared to roughages with a lower NDF digestibility (increased maturity) due to a shorter rumen retention time (Arelovich *et al*., 2008). Roughage palatability (voluntary intake), digestibility (mentioned earlier), nutrient content and anti-nutritional factors are also considered as important quality aspects thereof (Lemus, 2009). Still the chemical composition of roughage is a very good indication of its quality (Van Soest, 1965). In addition, the "fill" effect (Dado and Allen, 1995) of a roughage source may also impair feed intake when increased in a diet. However it is important to note that roughage quality is a function of both animal and plant factors (McDonald *et al*., 2011).

The roughage:concentrate ratio of a ruminant's dietary composition is commonly used. For producing dairy cows, this ratio is normally accepted as 40:60 to still maintain normal rumen function (Wilcox and Van Horn, 1992). This ratio may however differ between species fed, age of the animal and the required outcome. The roughage:concentrate ratio of ruminant feeds may affect DMI (Garrett *et al*., 1999; Allen, 2000), feed digestibility as well as ruminal fermentation (Colucci *et al*., 1982; Rode *et al*., 1985; Yang and Beauchemin, 2006), and ultimately animal performance (Allen, 1997; Whitney and Lupton, 2010). Non-fibrous carbohydrates (NFC), for example starches and simple sugars, are found commonly in low roughage:concentrate diets. These NFC ferment at a faster rate in the rumen and as a result the energy density of a diet increases. Non-fibrous carbohydrates do not stimulate rumination or saliva production and may even impair fibre fermentation and could cause ruminal acidosis (McDonald *et al*., 2011), ultimately lowering production (Whitney and Lupton, 2010).

In Chapter 4, it was found that the incremental inclusion of roughage (lucerne hay) by replacing maize meal in the finishing diets of lambs decreased feed digestibility and metabolizable energy (ME) content. Therefore some effect on animal performance and carcass characteristics could be expected. However, DMI, methane production and heat increment (HI) of fermentation could influence animal production and carcass characteristics and needs further investigation. The objective of this study was therefore to investigate the effect of increasing incremental dietary NDF levels on the production performance and carcass characteristics of finishing lambs.

Even though the main purpose of increasing the NDF increments in lamb diets were to determine the effect thereof on biohydrogenation (BH) and carcass fatty acid (FA) content, its effect on animal performance and carcass characteristics remain of interest.

5.2 Materials and Methods

The materials and methods used for the production study and measuring of carcass characteristics have been described in Chapter 3 and were briefly as follows:

The production study was conducted over a period of 61 days (including a ten-day adaptation period) to investigate the influence of increasing NDF increments on performance parameters [DMI, average daily gain (ADG) and feed conversion ratio (FCR)] and carcass characteristics of finishing lambs. Sixty lambs (mean 29.3±1.8 kg live weight) were randomly allocated to five dietary treatments (n=12 lambs/treatment). The five dietary treatments were formulated to contain a similar nutrient composition, differing only in respect to the NDF content as the primary parameter. The NDF content increased from low roughage (primarily lucerne hay) inclusion to a high inclusion rate representing a dose-response trial in the following order: 12.76% (T15), 17.69% (T30), 22.53% (T45), 27.48% (T60) and 32.40% (T75) NDF/kg DM, respectively. The lambs were housed in individual pens.

At the onset of the production study (day 0) the lambs were subjected to a ten-day adaptation period. After dietary adaptation the animals were fed the respective experimental diets on an *ad lib*. basis for the remainder of the experimental period (61 days) until all the lambs attained an average live weight of ±50 kg prior to slaughter. Live weight and feed intake were recorded on a weekly basis. At termination of the production study all lambs (mean 48.11±2.94 kg live weight) were slaughtered. Carcass characteristics (carcass weight, dressing percentage, subcutaneous fat thickness, carcass length, shoulder and buttock circumference, *Longissimus* muscle area, width and depth) were measured.

The data was subjected to analysis of variance (PROC ANOVA) of the SAS program, version 9.2 (SAS, 2008). Tukey's honest significant difference (HSD) test was used to identify significant differences (P <0.05) between treatments.

The null hypothesis for this trial was that an increase in NDF percentage would result in a decrease in feed intake and metabolizable energy intake (MEI), as well as animal performance. The null hypothesis will be accepted with P <0.05 and influenced by these mentioned parameters.

5.3 Results and Discussion

5.3.1 Intake and production performance

The effect of incremental dietary NDF content on the mean intake and production performance of S.A. Mutton Merino lambs are presented in Table 5.1. Sustained voluntary feed intake is necessary for increased animal productivity (Whitney and Lupton, 2010; McDonald *et al*., 2011). Dietary treatment did effect DMI significantly (P =0.0011) (Table 5.1). The DMI of T15 was lower compared to that of T45 and T60, but in essence comparable between all treatments (Table 5.1). Beauchemin (1991) reported that when the roughage (NDF) content of a diet increased, the time spent ruminating would increase and negatively affect DMI. In other words, roughage with a low NDF content results in a higher DMI than roughages with a high NDF content due to a shorter rumen retention time (Arelovich *et al*., 2008). Van Soest (1965) explained that roughage NDF content is more closely related to DMI of sheep compared to other diet composition measurements. This was however not the case in the present study and difficult to explain. Neutral-detergent fibre may also limit DMI of ruminants due to its "bulkiness" it provides to a diet (Dado and Allen, 1995; McDonald *et al.,* 2011).

As discussed in Chapter 4, due to a possible lower ruminal pH as a result of feeding higher energy (grain based) diets and its negative effect on ruminants' voluntary feed intake, (McDonald *et al*., 2011) this does not hold true in the current data set. Rumen pH is positively related to the NDF level of roughage as well as the total NDF content of the diet of a ruminant (Galyean and Defoor, 2003). When diets high in fermentable concentrates or low in physically effective fibre are fed, it causes a build-up of volatile fatty acids (VFA) (especially lactic and propionic acid) which results in a decreased ruminal pH (Yang and Beauchemin, 2006). Fibre, and therefore NDF, plays an essential part in long term health and productivity of the animal.

Table 5.1 The effect of dietary NDF content on the DM intake and production performance of S.A. Mutton Merino lambs (mean±SD)

Parameter	Treatment diets*					Significance (*P*-value)	CV[#]
	T15	T30	T45	T60	T75		
Intake:							
DM* feed intake (g/sheep/day)	1.31[b]±0.08	1.41[ab]±0.07	1.47[a]±0.15	1.51[a]±0.11	1.43[ab]±0.11	0.0011	7.70
ME* intake (MJ/sheep/day)	14.06[a]±0.83	14.62[a]±0.81	14.10[a]±1.55	14.17[a]±1.07	12.56[b]±1.03	0.0004	7.86
Production performance:							
Initial weight (day 0)*	29.35±2.17	29.30±1.7	29.27±1.96	29.43±1.58	29.40±1.92	0.9995	6.40
End weight (day 61)*	48.24[a,b]±1.76	49.57[a]±1.65	49.30[a]±3.78	48.13[a,b]±2.66	45.33[b]±2.51	0.0017	5.41
Average daily gain (ADG; g/sheep/day)	310[a]±35	332[a]±31	328[a]±45	307[a]±41	261[b]±25	<.0001	11. 83
Feed conversion ratio (FCR)*	4.28[c]±0.36	4.28[c]±0.35	4.51[bc]±0.31	4.98[b]±0.46	5.51[a]±0.54	<.0001	8.73
ME* intake/kg live weight gain	45.77[ab]±3.76	44.25[ab]±3.55	43.15[b]±2.79	46.63[ab]±4.23	48.38[a]±4.74	0.0164	8.49

[a,b,c] Mean values with different superscripts differ significantly ($P<0.05$).

[#] CV = Coefficient of variation (%).

* Treatment diets: Increments of neutral-detergent fibre (NDF) depicted as percentage roughage included; Initial and end weight = Empty stomach weight (kg); DM = Dry matter; ME = Metabolisable energy; Feed conversion ratio = kg DM feed intake/kg live weight gain.

Metabolic disturbances are often the result of fibre requirements that are not met or sustained (McDonald *et al*., 2011). The NDF content [not less than 15% DM for finishing lambs as recommended by Smith (2008)] of all treatments (except T15) probably contained enough NDF and effective fibre to stimulate rumination (Wilcox and Van Horn, 1992).

In contrast, the average MEI of T75 were significantly lower (P =0.004) compared to the other treatments (Table 5.1). It has to be remembered that DMI and ME content of the diets are used to calculate the MEI. Therefore, the similar DMI of T75 compared to the other treatments (Table 5.1) and significant lower ME content of T75 (Table 4.2; Chapter 4) could have resulted in this effect.

The ADG and FCR of the lambs receiving treatment T75 were negatively affected (P <0.001) by NDF inclusion, compared to the other treatments (Table 5.1). Similarly, Whitney and Lupton (2010) found that ADG decreased with increased concentrations of roughage in high-concentrate lamb diets. As the MEI/kg weight gain decrease, the FCR increases (McDonald *et al*., 2011). This result may relate to the MEI (P =0.0004) of the same lambs. A more specific reason could probably be accounted to a decreased (P <0.05) DM digestibility (see Chapter 4; Table 4.2), and proposed higher HI and loss of methane gas resulting from high fibre inclusion. During the rumen fermentation of organic matter (OM), hydrogen (H^+) is produced and methanogenic bacteria use this with carbon dioxide (CO_2) to produce methane and water (H_2O) and a further loss of energy occurs (McDonald *et al*., 2011). Methane is produced by the fermentation of feeds in the gut by microbes, particularly in ruminants. The activity of methanogenic bacteria, and thus the amount of methane produced, is reduced with increased rates of passage and digestibility of feed, increased levels of feeding, reduced rumen pH and the fermentation of starchy feeds (low roughage:concentrate diets), all of which favour the channelling of carbon (C) and hydrogen into propionate production (McDonald *et al*., 2011). Methane production is dependent on the VFAs produced from carbohydrate fermentation in the rumen (McAllister *et al*., 1996; McDonald *et al*., 2011). It is important to note that even though the DMI of T15 was low (1.31 kg DM/day; Table 5.1), the ADG of the sheep in the same treatment, compared well with the rest (P >0.05) – except for T75 – which resulted in a good and comparable FCR (compared only to T30 and T45).

The empty stomach end weight of the lambs fed treatment T75 were significantly lower (*P* =0.0017), only compared to treatments T30 and T45 (Table 5.1). The amount of energy [mega joule (MJ) ME intake/kg live weight gain] consumed and converted to live weight gain were also affected significantly (*P* =0.0164) by dietary treatment. Even though comparable with the other treatments (T15, T30 and T60), feed energy used by the lambs in T45 for weight gain purposes were more efficient (*P* <0.05) compared to T75 (Table 5.1). As explained in Chapter 4, the low DM digestibility, high HI and loss of methane gas resulting from mature and increased roughage could be acknowledged for this effect (T75) (McDonald *et al*., 2011).

As described in Chapter 4, it could be speculated that methane production following increased roughage inclusion could have been higher (McDonald *et al*., 2011), and probably over-estimated the ME content of the diet. This suggests that the MEI and resultant ME efficiency for lamb growth fed treatment T75 could have been even worse.

5.3.2 Carcass characteristics

The effect of incrementally increasing dietary NDF content on the carcass characteristics of S.A. Mutton Merino (SAMM) lambs are presented in Table 5.2. It is evident that dietary NDF content had no significant effect (*P* >0.05) on the external carcass length (EL), eye muscle width, as well as fat thickness measured 45 mm and 110 mm from the mid dorsal line between chop 12 and 13 on the left side of the carcass. This is difficult to explain due to the significant effect of dietary treatment on dietary ME content (Table 4.2, Chapter 4), MEI and the growth performance of the lambs (Table 5.1).

However, dietary NDF content significantly affected (*P* <0.05) the carcass weight as well as lamb dressing percentage (Table 5.2). Diaz *et al*. (2001) found that the carcass dressing percentage was lower for lambs raised on pasture (consuming a diet containing 45.7% NDF on average) compared to those raised on concentrates (consuming 15.96% NDF on average). Priolo *et al*. (2001) also reiterated the effect that the consumption of grass (59.2% NDF) or concentrate (24.7% NDF) based diets had on lamb carcass and meat quality. It was found that the carcass weight of the grass-fed lambs was lower, whereas the digestive tract weight expressed as a percentage of live weight was higher in grass-fed lambs.

Table 5.2 The effect of dietary NDF content on the carcass characteristics of S.A. Mutton Merino lambs (mean±SD)

	Treatment diets*						
Parameter	**T15**	**T30**	**T45**	**T60**	**T75**	**Significance (*P*-value)**	**CV[#]**
Cold carcass weight (kg)	22.40^{a,b}± 1.65	23.16^{a,b}±0.93	23.69^{a}±2.10	21.92^{b}±1.17	20.21^{c}±1.25	<.0001	6.64
Dressing percentage (%)	47.0^{a,c}±0.02	47.0^{a,c}±0.02	48.0^{a}±0.02	46.0^{b,c}±0.02	45.0^{b}±0.01	<.0001	3.33
Shoulder circumference (cm)	77.92^{a,b}±1.55	78.25^{a} ±1.27	78.54^{a}±2.73	77.50^{a,b}±1.09	76.08^{b}±1.26	0.0073	2.17
Buttock circumference (cm)	67.50^{b}± 2.30	66.13^{ab}±1.42	67.04^{ab}±3.09	66.21^{ab}±1.99	64.50^{a}±2.59	0.0351	3.55
Carcass length (cm)	62.88±7.99	62.08±1.51	61.46±1.88	61.38±1.46	60.25±1.71	0.5597	6.27
Eye muscle width (mm)	59.76± 5.61	62.32±5.45	60.61±3.37	58.59±4.61	59.50±4.49	0.3934	7.94
Eye muscle depth (mm)	30.99^{a,b}± 3.73	31.52^{a,b}±3.21	33.48^{a}±2.80	29.55^{b}±2.86	28.98^{b}±2.65	0.0065	9.95
Area of eye muscle (mm^2)	1806^{a}±338	1794^{a}±299	1714^{a,b}±202	1154^{a,b}±191	1492^{b}±145	0.0062	14.69
Fat thickness 45* (mm)	3.69±1.40	4.89±2.10	4.24±1.33	3.73±0.94	3.98±1.18	0.2468	35.12
Fat thickness 110* (mm)	9.93±2.39	9.93±3.44	9.27±2.97	9.22±3.08	9.01±2.89	0.9075	31.39

[a,b,c] Mean values with different superscripts differ significantly (P <0.05).

[#] CV = Coefficient of variation (%).

* Treatment diets: Increments of neutral-detergent fibre (NDF) depicted as percentage roughage included.

* Measured 45 mm and 110 mm from the mid dorsal line between the 12th and 13th thoracic vertebra, respectively.

Similarly, in the present study, a high roughage inclusion (T75) seemed to decrease carcass weight (P <.0001) (Table 5.2) and could be the result of the lower (P <0.05) MEI (hence energy retention) (Table 5.1), crude protein (CP) digestibility (P <0.05; Table 4.2, Chapter 4), as well as ADG and FCR (Table 5.1). The negative effect (P <0.05) of 75% roughage inclusion (T75) in finishing diets on lamb growth performance was also evident in the shoulder circumference (compared to T30 and T45), buttock circumference (BC) (compared to T15), eye muscle area (compared to T15 and T30), as well as eye muscle depth (compared to T45) (Table 5.2).

5.4 Conclusion

It would seem from the results of the present study that the effect of increasing incremental NDF levels in the diet of finishing lambs had a significant effect on the MEI, which accordingly resulted in a significant negative effect on animal performance and carcass characteristics. However, even though significant affects were recorded, some results related to specific treatments within a parameter makes it difficult to explain the results, as well as to propose conclusive recommendations. It seems clear however that a high roughage inclusion (T75) negatively affected most production and carcass characteristics. Therefore, the null hypothesis is accepted due to a decreased MEI and lamb performance with special reference to ADG and FCR.

Regression equations are necessary to establish the preferred lucerne hay inclusion (hence NDF%/kg DM) of lamb finishing diets to optimally benefit animal production parameters and warrants further investigation. Smith (2008) did however propose a 15 to 20% NDF inclusion in a diet to be ideal for the finishing of lambs.

CHAPTER 6

THE EFFECT OF NEUTRAL-DETERGENT FIBRE CONTENT IN FINISHING DIETS ON THE MEAT QUALITY OF LAMBS

6.1 Introduction

Ruminant products have been criticized for the possible undesirable effects of saturated fatty acids (SFA) on human health which has contributed to a decline in its consumption (Dewhurst *et al*., 2003). For example, consumption of SFAs have been associated with increased serum low-density lipoprotein (LDL) cholesterol concentrations, a risk factor for coronary heart disease (Keys, 1970). On the other hand, monounsaturated fatty acids (MUFA) and some polyunsaturated fatty acids (PUFA) are anti-thrombogenic (Ulbricht and Southgate, 1991). In addition, polyunsaturated conjugated linoleic acid (CLA: C18:2*cis*-9,*trans*-11) has been revealed to decrease the possibility of cancer, cardiovascular disease, diabetes, improvement of the immune system, renal disease, type 2 diabetes, chronic obstructive pulmonary disease, ulcerative colitis, Crohn's disease and bone strength (Wood *et al*., 2003). There is also growing appreciation for the well-being benefits by regular consumption of PUFAs - especially those exerted by omega-3 (*n*-3) fatty acids (FA) and different CLA isomers (McDonald *et al*., 2011). In contrast to popular belief, SFAs comprise more than 50% of the cell membranes that provides cells with the needed rigidness and durability, contribute to the strength of bones by assimilating calcium effectively into the skeletal structure - at least 50% of the dietary fats should be saturated. Most SFAs lower lipoprotein levels, provide liver protection due to alcohol and toxins, improve the immune system, provide proper utilization of essential FAs (long chain *n*-3 FAs are better retained in tissue with a rich saturated fat diet), are the suitable energy substrate for the heart, and some short and medium chain SFAs have important antimicrobial properties (Enig and Fallon, 1999).

Recently there has been more interest in finding different techniques to manipulate the FA composition of red meat (McDonald *et al*., 2011). The effect of nutrition on FA composition of muscle and adipose tissue is mostly accredited to the FA composition of the diet fed (Whitney and Lupton, 2010) as well as its chemical composition (Cooper *et al.,* 2004). Cooper *et al.* (2004) stated that when lambs are fed concentrated diets, the FA

content of the meat can be improved to more closely resemble what is recommended for the human diet as a whole. Research has also confirmed that there are differences between carcass FA composition of ruminants raised on the field and those finished on concentrate diets. Those raised on natural pasture have a more favourable FA composition than intensively finished ruminants, primarily because of the fact that grass is usually rich in *n*-3 PUFAs, even though it has low lipid contents (Webb and O'Neill, 2008). Therefore, "grass-fed" beef and lamb have natural higher levels of *n*-3 and long chain *n*-3 PUFAs. Grazing also provides antioxidants including vitamin E which maintain PUFA levels in meat and prevent quality deterioration during the processing and display (Wood *et al*., 2008). Some research have been published focusing on total roughage inclusion and its effect on ruminal FA composition and outflow (Kucuk *et al*., 2001), but no data exists with special reference to the NDF content of lamb (and all ruminant) diets and its effect on carcass FA composition. The influence of roughage and concentrate inclusion in the diet on the FA composition of muscle and adipose tissue remains in abundant attentiveness (Aurousseau *et al.,* 2004) and there seems to be a lack of published studies (Helander, 2014). As a result, the effect of dietary lipid composition on the FA composition of muscle and adipose tissue, as well as the feeding system in question remains of great interest.

Ruminant fat has a higher SFA and a lower polyunsaturated:saturated fatty acid (PUFA:SFA) ratio than non-ruminant fat due to the hydrogenation of dietary unsaturated fatty acids (UFA) in the rumen. However, ruminant fats are amongst the richest natural sources of CLA isomers, in particular the *cis*-9,*trans*-11 isomer which arises from the microbial hydrogenation of dietary linoleic acid (C18:2*c*9,12; *n*-6) in the rumen (French *et al*., 2000). Therefore, the type of FA present in meat is used to evaluate the tissues. However, the ratios of PUFA:SFA and omega-6 (*n*-6):*n*-3 are also commonly used for the evaluation of the nutritional value of animal tissue and are considered important (McDonald *et al*., 2011). From a consumer's health point of view the suggested ratio of *n*-6:*n*-3 is below 4.0 in muscle tissue (Wood *et al*., 2003), while the suggested value for the PUFA:SFA ratio is 0.4 or higher (Wood *et al*., 2003; Webb and O'Neill, 2008). Another familiar suggestion is that fat should supply no more than 30% of total energy intake of sheep, and that this fat should be divided equally among SFAs, MUFAs and PUFAs (i.e. each supplying 10% of energy intake). Only plant lipids come close to this recommendation. Fats of ruminants have predominance for SFAs, as where one-third of the FAs in lamb meat are saturated fat. The rest are in the healthier forms of MUFAs and PUFAs (ANON, 2011).

Strategies that lead to an increase in the PUFA:SFA ratio in animal fat, hence all beneficial FAs, would improve the healthiness of beef from a consumer perspective (French *et al*., 2000). The strategies which alter the FA composition (e.g. increasing the PUFA content) of the lipid and muscle fractions of meat could however influence several aspects of meat quality and may include the firmness, colour and stability of lipid tissue, as well as meat colour and flavour. These parameters are influenced by meat FA proportion (Wood *et al.,* 2003), and as the degree of unsaturation increases in muscle membranes, it reduces the oxidative stability of muscle tissue (Morrissey *et al.,* 1998). Therefore by increasing the PUFA content and the PUFA:SFA ratio of ruminant meat may inadvertently lead to a higher susceptibility to oxidative breakdown, decreased colour stability (Ponnampalam *et al.,* 2001; Moloney *et al*., 2006; Whitney and Lupton, 2010) and a reduced shelf-life (Webb and O'Neill, 2008).

From the available literature it seems possible to alter the FA composition of ruminant meat via dietary means. However, there seems to be no data available where neutral-detergent fibre (NDF) (apart from total roughage/fibre intake), as a measure of roughage content, were used to test its effect on the finishing lamb carcass FA content and meat quality aspects. From a dietary point of view, a high roughage diet fed to ruminants normally results in greater BH activities, affecting the carcass FA composition (Kucuk *et al*., 2001). Therefore, the aim of this study was to determine the effect of dietary incremental NDF levels of finishing lamb diets on the FA composition of intramuscular and subcutaneous tissues, as well as its effect on subsequent oxidative and colour stability of meat.

6.2 Material and Methods

The materials and methods used for meat quality evaluation have been described in Chapter 3 and were briefly as follow:

At termination of the production study (61 days, see Chapter 5) all lambs (mean 48.11±2.94 kg live weight) were slaughtered. As mentioned in Chapters 4 and 5, the five dietary treatments were formulated to contain a similar nutrient composition, differing only in respect to the NDF content as the primary parameter. The NDF content increased incrementally from low roughage (primarily lucerne hay) inclusion to a high inclusion rate representing a dose-response trial in the following order: 12.76% (T15), 17.69% (T30), 22.53% (T45), 27.48% (T60) and 32.40% (T75) NDF/kg DM, respectively.

To assess the effect of dietary NDF content on colour and lipid stability of fresh meat, one loin chop from each carcass was overwrapped with oxygen-permeable polyvinyl chloride (PVC) meat stretch wrap in polystyrene trays and stored for seven days at 4°C under fluorescent light. Meat colour (a*-, b*- and L*-values) was determined on days 0 and 7 using a Minolta chromometer. A second loin chop was vacuum sealed and stored for 90 days at -18°C in the dark for frozen storage stability studies. A 5 g sample of lean meat was then removed from the middle of each loin chop on days 0, 7 (stored at 4°C) and 90 (stored at -18°C) to determine the thiobarbituric acid reactive substance (TBARS) content. Total lipid from muscle, subcutaneous fat and feed samples were quantitatively extracted and stored in a polytop (glass vial, with a push-in top) and frozen at -20°C under a blanket of nitrogen pending FA analyses. For meat tenderness the *Musculus longissimus lumborum* (LL) (11^{th} to 13^{th} rib) was removed from one side of the carcass, prepared according to an oven-broiling method using direct heat and sheared perpendicular thereafter using a Warner Bratzler shear device mounted on an Universal Instron apparatus.

The data was subjected to analysis of variance (PROC ANOVA) of the SAS program, version 9.2 (SAS, 2008). Tukey's honest significant difference (HSD) test was used to identify significant differences (P <0.05) between treatments.

The null hypothesis for this trial was that an increase in NDF percentage would result in an increased total SFA and decreased PUFA content of lamb meat with its resultant effect on the PUFA:SFA and *n*-6:*n*-3 ratios, which could influence meat stability and visual aspects. The null hypothesis would be accepted at P <0.05 and influenced by these mentioned parameters.

6.3 Results and Discussion

6.3.1 Lipid content and fatty acid composition of experimental diets

The mean lipid content and FA composition of the different experimental diets are presented in Table 6.1. Only the major FAs of ruminant meat, as well as those mentioned in literature, were reported in this study. Even though no statistical analysis was possible, a few important observations were made:

The lipid content of feed samples taken to analyse for FA content did not only compare well with each other (Table 6.1), but also compared well with the analysed feed samples used in the digestibility study (see Chapter 4, Table 4.1).

Table 6.1 Mean lipid and fatty acid composition of experimental diets

	Treatment diets*				
Parameter (% of total fatty acids)	**T15**	**T30**	**T45**	**T60**	**T75**
Proximate analysis:					
Feed lipid content (% DM)	5.09	5.34	5.05	5.61	5.17
Saturated fatty acids:					
Myristic (C14:0)	0.08	0.12	0.14	0.14	0.16
Palmitic (C16:0)	11.00	10.79	10.96	10.72	11.16
Stearic (C18:0)	3.37	3.38	3.31	3.37	3.43
Monounsaturated fatty acids:					
Oleic (C18:1c9; *n*-9)	24.25	24.20	24.48	24.27	23.25
Vaccenic (C18:1t11)	0.96	2.09	2.16	2.26	2.17
Polyunsaturated fatty acids:					
Linoleic (C18:2c9,12; *n*-6)	53.15	51.28	50.35	50.06	49.89
α-Linolenic (C18:3c9,12,15; *n*-3)	5.90	6.65	6.94	7.41	8.02
Total fatty acids:					
SFA*	15.29	15.23	15.43	15.32	15.89
MUFA*	25.46	26.55	26.93	26.83	25.77
PUFA*	59.25	58.22	57.64	57.85	58.34
n-6*	53.16	51.29	50.37	50.08	49.91
n-3*	6.09	6.93	7.27	7.78	8.44
Fatty acid ratios:					
n-6:*n*-3	8.72	7.40	6.93	6.44	5.92
PUFA:SFA	3.87	3.82	3.74	3.78	3.67

* Treatment diets: Increments of neutral-detergent fibre (NDF) depicted as percentage roughage included; SFA = Total saturated fatty acids; MUFA = Total monounsaturated fatty acids; PUFA = Total polyunsaturated fatty acids; *n*-6 = Total omega-6 fatty acids; *n*-3 = Total omega-3 fatty acids.

In Chapter 3 (3.5.1) it was explained that, due to the fact that maize inclusion decreased with increasing incremental lucerne hay inclusion (from T15 to T75), maize germ oil was added using the difference (maize lipid contribution to experimental diets subtracted by the standard 3% soybean oil inclusion) (Table 3.1) to keep the total lipid content for all treatments similar, as well as similar FA compositions. This is clearly portrayed in Table 6.1, where all treatment FA contents compared well (very similar) with each other. Hence, it was therefore possible to include soya oil at 3% in all the treatment diets (as is), eliminating the possibility that different dietary lipid contents between treatments, i.e. FA content, could possibly influence treatment affects (differing NDF increments). However, the small increase in polyunsaturated α-linolenic acid and resultant total *n*-3 dietary content could probably be ascribed to the higher *n*-3 content of lucerne hay contributing more as its inclusion increased from T15 to T75 (Table 6.1) (Mitchell *et al*., 1991). Even though lucerne hay's lipid content was low (1.61% on a DM basis) (McDonald *et al*., 2011), including a high amount in any diet (e.g. T75) could affect certain dietary FAs to a limited extent. Therefore, similar to the current study, Mitchell *et al*. (1991) reported a decrease in the feed *n*-6:*n*-3 ratio affected by an increase of grass in the diet, as grass is also high in *n*-3 FAs. It is nevertheless important to note that dietary treatments contained no trace of any CLA isomers.

Although a chemical antioxidant was included within all treatment diets, it is likely that oxidation may still occur as antioxidants do not completely stop oxidation, but only slows down the process to some extent (McDonald *et al*., 2011). Lipids containing PUFAs are highly sensitive to oxidative reactions during storage and are likely to turn rancid at high environmental temperatures (Waheed *et al*., 2004; Moloney *et al*., 2006; Whitney and Lupton, 2010). It is important to reiterate that MUFAs are more resistant to oxidative breakdown than PUFAs (McDonald *et al*., 2011).

6.3.2 Muscle fatty acid composition of lamb meat

The effect of incremental dietary NDF content on the muscle FA composition of S.A. Mutton Merino lamb meat is illustrated in Table 6.2.

Table 6.2 The effect of incremental dietary NDF content on the muscle fatty acid composition of S.A. Mutton Merino lamb meat (mean±SD)

	Treatment diets*						
Fatty acid (% of total fatty acids)	**T15**	**T30**	**T45**	**T60**	**T75**	**Significant (*P*-value)**	**CV[#]**
Meat lipid content (%)	3.62±0.77	3.44±0.80	3.74±0.88	3.68±0.90	3.66±1.00	0.9402	24.11
Saturated fatty acids:							
Myristic (C14:0)	2.95±0.62	2.99±0.60	2.86±0.50	2.61±0.41	2.95±0.84	0.5611	21.27
Palmitic (C16:0)	28.47±2.03	28.46±1.32	27.99±2.08	27.58±1.74	26.92±1.71	0.1898	6.44
Stearic (C18:0)	15.62^{a}±1.97	15.51^{a}±0.94	17.70^{b}±1.94	17.90^{b}±1.20	18.90^{b}±1.72	<.0001	9.40
Monounsaturated fatty acids:							
Oleic (C18:1c9; *n*-9)	35.88^{b}±2.97	36.23^{b}±2.61	$38.22^{a,b}$±1.95	39.89^{a}± 3.06	$38.01^{a,b}$±3.81	0.0102	7.82
Vaccenic (C18:1t11)	2.70^{a}±0.29	2.67^{a}±0.15	1.85^{b}±0.92	1.29^{b}±0.95	1.36^{b}±0.77	<.0001	35.47
Palmitoleic (C16:1c9)	$1.44^{a,b}$±0.26	1.54^{a}±0.12	$1.33^{a,b,c}$±0.22	$1.21^{b,c}$±0.16	1.14^{c}±0.23	<.0001	15.40
Polyunsaturated fatty acids:							
Linoleic (C18:2c9,12; *n*-6)	7.18^{a}±2.51	$6.87^{a,b}$±1.88	$5.06^{a,b}$±1.42	4.58^{b}±1.97	$5.13^{a,b}$±2.07	0.0054	34.72
α-Linolenic (C18:3c9,12,15; *n*-3)	0.62±0.14	0.71±0.11	0.67±0.12	0.66±0.16	0.77±0.18	0.1176	20.97
CLA*(C18:2*c*9*t*11; *n*-6)	$0.55^{a,b}$±0.20	$0.69^{a,b}$±0.16	0.51^{b}±0.21	$0.66^{a,b}$±0.14	0.75^{a}±0.21	0.0126	29.40

Table 6.2 (Cont.)

Parameter (% of total fatty acids)	Treatment diets*					Significance (*P*-value)	CV[#]
	T15	T30	T45	T60	T75		
Total fatty acids:							
SFA*	48.53±2.11	48.34±1.92	49.95±3.12	49.46±2.27	50.28±2.29	0.2011	4.82
MUFA*	40.71±3.15	41.13±2.56	42.08±1.94	42.87±2.70	41.10±3.27	0.3141	6.65
PUFA*	10.76[a]±3.53	10.53[a]±2.78	7.97[b]±2.30	7.67[b]±3.28	8.62[a,b]±3.17	0.0398	33.39
n-6*	9.53[a]±3.19	9.25[a,b]±2.50	6.81[c]±2.01	6.52[c]±2.84	7.29[b,c]±2.72	0.0167	34.00
n-3*	1.22±0.39	1.28±0.31	1.16±0.33	1.15±0.45	1.34±0.46	0.7363	31.73
Fatty acid ratios:							
n-6:*n*-3	7.79[a]±1.17	7.20[a]±0.81	5.94[b]±0.87	5.61[b]±0.49	5.42[b]±0.53	<.0001	12.75
PUFA:SFA	0.22[a]±0.08	0.22[a]±0.06	0.16[b]±0.06	0.16[b]±0.07	0.17[a,b]±0.07	0.0424	36.24

[a,b,c] Mean values with different superscripts differ significantly ($P < 0.05$).

[#] CV = Coefficient of variation (%).

* Treatment diets: Increments of neutral-detergent fibre (NDF) depicted as percentage roughage included; CLA = Conjugated linoleic acid; SFA = Total saturated fatty acids; MUFA = Total monounsaturated fatty acids; PUFA = Total polyunsaturated fatty acids; *n*-6 = Total omega-6 fatty acids; *n*-3 = Total omega-3 fatty acids.

It is clear from the data set that dietary NDF content had no significant (*P* >0.05) effect on the lipid content of muscle tissue, as well as saturated myristic (C14:0), palmitic (C16:0), polyunsaturated α-linolenic acid (C18:3*cis*9,12,15; *n*-3), and total FAs (SFA, MUFA, and *n*-3). The lack of a significant affect (*P* >0.05), especially with regard to the total FAs mentioned, are difficult to explain due to the fact that the major FAs, i.e. unsaturated stearic (C18:0), monounsaturated oleic (C18:1c9; *n*-3), vaccenic (C18:1t11) and palmitoleic (C16:1c9) acid, polyunsaturated linoleic acid, as well as to a lesser extent CLA (C18:2*c*9*t*11; *n*-6) were significantly affected by NDF content (*P* <0.05) (Table 6.2) and primarily used to calculate the total FAs.

It is however important to mention that the slight increase in polyunsaturated α-linolenic acid and resultant total *n*-3 content of the diets following lucerne hay inclusion (from T15 to T75; Table 6.1) due to its higher *n*-3 content (Mitchell *et al*., 1991) did not affect (*P* >0.05) these mentioned parameters of muscle tissue. The dietary α-linolenic acid and total *n*-3 content, hence intake, was probably too low to have any meaningful affect. In addition, greater hydrogenation of polyunsaturated α-linolenic acid and a long rumen transit time for high roughage diets therefore limits the amount available for tissue uptake and could also pose as a possible explanation (Wood *et al*., 2008).

As stated, dietary treatment however significantly increased (*P* <.0001) muscle saturated stearic acid content of T45, T60 and T75, compared to T15 and T30. Whereas the monounsaturated vaccenic and palmitoleic acid significantly decreased (*P* <0.05) with increasing NDF percentage inclusion in the finishing diets of lambs (Table 6.2). Even though dietary treatment significantly affected the monounsaturated oleic acid, polyunsaturated linoleic acid and CLA content of ruminant meat (*P* <0.05), there seems to be no additional benefit or treatment effect with regards to T15 vs. T75 for the mentioned parameters and an intermediate treatment (NDF content) seems to be preferred. Hence, there was a significant increase (*P* =0.0102) in muscle monounsaturated oleic acid content following T60, compared to T15 and T30, as well as CLA content (*P* =0.0126, T75 compared to T45) (Table 6.2). In contrast, the polyunsaturated linoleic acid content of lamb muscle decreased (*P* =0.0054) following T60, compared to treatment T15 (Table 6.2). With reference to total FAs, increasing NDF percentage seems to decrease (*P*<0.05) the PUFA, *n*-6, as well as *n*-6:*n*-3

and PUFA:SFA ratios. The *n*-6:*n*-3 (P <.0001) and PUFA:SFA ratio (P =0.0424) (Table 6.2) of ruminant muscle tissue thus seems to be affected by dietary NDF content (Table 6.2).

This mentioned significant effect on the muscle CLA content of lamb meat occurred despite the fact that dietary treatment contained no detectable CLA isomers (see Table 6.1). As such, tissue *cis*-9*,trans*-11-CLA content could probably not have originated directly from any diet. Conjugated linoleic acid is produced by different bacterial species in the rumen through the isomerisation of polyunsaturated linoleic acid, but also through the endogenous synthesis from monounsaturated vaccenic acid via Δ9-desaturase enzymes (Mulvihill, 2001; Radunz *et al*., 2009; Woods and Fearon, 2009). After the isomerisation of linoleic acid in the rumen, the *cis*-9,*trans*-11-CLA isomers are rapidly hydrogenated to monounsaturated vaccenic acid, and less rapidly to saturated stearic acid, resulting in an increase in vaccenic acid (Bauman *et al.,* 1999). This could result in the accumulation of vaccenic acid and CLA isomers in the rumen digesta (Demeyer and Doreau, 1999: Booyens, 2012). The available monounsaturated vaccenic acid content within muscle tissue could also be desaturated to the *cis*-9*,trans*-11-CLA isomer (Schmid *et al.,* 2006), thereby possibly lowering the vaccenic acid content within the same tissues. Ruminal biohydrogenation (BH) could therefore explain the significant effect (P <0.05) of NDF increments on muscle lipid monounsaturated vaccenic acid, polyunsaturated linoleic acid and CLA content of lamb meat.

The rumen is a site of powerful microbial lipid metabolism where dietary UFAs are hydrolyzed and hydrogenated to a large extent as a defence mechanism to detoxify FAs. This is because PUFAs are toxic to micro-organisms (MO) if present in high concentration by manifesting bactericidal and bacteriostatic effects (Maia *et al*., 2007). Several factors are known to affect the BH pattern in the rumen, including roughage to concentrate ratio (Whitney and Lupton, 2010). Greater hydrogenation of polyunsaturated α-linolenic acid and a long rumen transit time for high roughage diets therefore limits the amount available for tissue uptake (Wood *et al*., 2008). Although there are only a few experimental results, it is accepted that feeding diets with high roughage content increases the lipolysis of triacylglycerols (TAG) and BH of unsaturated long chain FAs in the rumen. Changing the diet of cows from a low to a high roughage type increased the number of lipolytic bacteria in the rumen, while the *in vitro* lipolytic and BH activity was also increased (Van Nevel and Demeyer, 1995). This could probably be the case in the present study with special reference

to the decreasing PUFA and *n*-6 content of lamb meat, hence affecting the PUFA:SFA and *n*-6:*n*-3 ratios (P <0.05) (Table 6.2).

The FA composition of muscle tissue is less influenced by the diet than that of adipose tissue (Schollan *et al*., 2006), as most of the FAs are located in the phospholipids and cellular membranes (Wood *et al*., 2008). Lamb meat contain very little marbling (fat within the meat) (ANON, 2011), compared to other meats (McDonald *et al*., 2011). Their FA composition is maintained fairly constant in order to ensure normal membrane function and thus cellular metabolism (Lee *et al*., 2008). Due to this, the capacity to increase PUFAs in phospholipids are limited (Wood *et al*., 2008). This ass evident in some of the total FAs measured (SFA, MUFA and *n*-3; P >0.05) in the present study (Table 6.2), but could however not serve as a definitive similar result.

From a consumer's health point of view the suggested ratio of *n*-6:*n*-3 is below 4.0 in muscle tissue (Wood *et al*., 2003), while the suggested value for the PUFA:SFA ratio is 0.4 or higher (Wood *et al*., 2003; Webb and O'Neill, 2008). A less extreme proposal is that the ratio of PUFA:SFA should be 0.5–0.8 (McDonald *et al*., 2011). The PUFA:SFA ratio is low (0.1) for lamb and beef (Schollan *et al*., 2006). In the present study the PUFA:SFA ratio of lamb meat varied between 0.16 and 0.22 (Table 6.2) which is still not ideal, but higher than the above mentioned average values. Treatment T15 and T30 seems to be closer to the 0.4 preferred value. Looking at the *n*-6:*n*-3 ratio of lamb and beef meat, a value of 2.11 for beef and 1.32 for lamb would be satisfactory (Wood *et al*., 2003; Webb and O'Neill, 2008). As stated, a desirable and recommended ratio in a diet of *n*-6:*n*-3 is 4:1 (McDonald *et al*., 2011). Whitney and Lupton (2010) found that lambs fed lucerne hay showed a lower ratio of *n*-6:*n*-3. With an increasing NDF level the *n*-6:*n*-3 ratio varied between 7.79 and 5.42 (P <.0001) (Table 6.2) which is higher than the preferred ratio. Treatment T75 came the closest to the preferred value (Table 6.2). Therefore, it was again found that lucerne fed lambs showed a lower *n*-6:*n*-3 ratio (Whitney and Lupton, 2010).

In contrast to the present study, French *et al*. (2000) found that steers that were fed a combination of grazed grass, grass silage, or concentrates, without any fat supplementation demonstrated that high grass intake resulted in a higher PUFA:SFA ratio. However, a similar and lower *n*-6:*n*-3 PUFA ratio in intramuscular fat of steers than in that of similar steers fed

concentrates. The favourable FA content of the roughage sources used was probably the reason for the mentioned significant effects.

According to Demeyer and Doreau (1999) and Aurousseau *et al*. (2004) the FA composition of muscle and adipose tissue is mainly influenced by the FA composition of the diet fed to ruminants, as well as the rumen metabolism or BH. As mentioned, BH decreases the UFA and increases the SFA content of rumen digesta. Therefore, ingested PUFAs are extensively hydrogenated in the rumen, resulting in the formation of stearic acid as the major end product (Lee *et al*., 2008). Furthermore, free fatty acids (FFA) are more quickly and easily hydrogenated to SFAs than those in an esterified form because lipolysis of triglycerides is required prior to the occurrence of BH (Bauman *et al*., 1999). Van Nevel and Demeyer (1995) however stated that lipolysis can be inhibited at low pH values, hence with high inclusion of concentrates in ruminant diets. It was further explained that it seemed that the lipolytic activity in the rumen was more sensitive to pH changes than BH. This means that the inhibition of BH in the rumen due to low pH values after the feeding of high concentrate diets was the consequence of an inhibited lipolysis. It has been noted that the growth of *Anaerovibrio lipolytica*, a lipolytic rumen bacterium, was lowered at pH 5.7 and completely inhibited at pH 5.3 (Hobson, 1965).

In accordance to the present study, Van Nevel and Demeyer (1995) stated that, based on their results, it can be speculated that the establishment of low pH values in the rumen through the feeding of high-concentrate diets could be a strategy for protecting unsaturated lipid supplements (oils) against BH. However, a negative aspect of such a strategy is the fact that at pH values lower than 6.2, fibre digestion in the rumen is depressed (Mould *et al.*, 1983).

Feed intake and animal age (see Chapter 5), as well as lipid content (Table 6.1; Table 4.1, Chapter 4) was consistent and therefore could not influence the FA composition.

6.3.3 Adipose tissue fatty acid composition of lamb meat

The effect of incremental dietary NDF content on the subcutaneous fatty acid composition of S.A. Mutton Merino lamb meat is presented in Table 6.3.

Table 6.3 The effect of incremental dietary NDF content on the lipid content and subcutaneous fatty acid composition of S.A. Mutton Merino lamb meat (mean±SD)

	Treatment diets*						
Fatty acids (% of total fatty acids)	**T15**	**T30**	**T45**	**T60**	**T75**	**Significance (*P*-value)**	**CV#**
Lipid content (%)	76.96^{b}±4.32	80.24^{a,b}±2.39	80.70^{a,b}±2.65	80.22^{a,b}±4.18	81.02^{a}±3.51	0.0437	4.38
Saturated fatty acids:							
Myristic acid [C14:0]	3.25±0.39	3.95±1.13	3.41±0.48	3.38±0.33	4.17±2.13	0.2036	30.99
Palmitic acid [C16:0]	28.42±1.14	28.70±1.62	27.93±1.99	28.56±1.12	27.67±2.00	0.4867	5.74
Stearic acid [C18:0]	20.80^{c}±4.09	24.01^{b,c}±2.79	27.71^{a,b}±3.61	27.64^{a,b}±2.78	30.39^{a}±4.63	<.0001	14.00
Monounsaturated fatty acids:							
Oleic acid [C18:1c9; *n*-9]	34.44^{a}±2.59	30.97^{b}±2.77	31.63^{a,b}±3.43	31.67^{a,b}±2.92	28.71^{b}±2.79	0.0005	9.26
Vaccenic acid [C18:1t11]	2.48^{a}±0.32	1.99^{b}±0.45	0.92^{c}±0.44	1.05^{c}±0.43	0.99^{c}±0.37	<.0001	27.28
Palmitoleic (C16:1c9)	0.95 ± 0.18	0.89 ± 0.18	0.77 ± 0.14	0.79 ± 0.11	0.76 ± 0.29	0.0709	22.88
Polyunsaturated fatty acids:							
Linoleic acid [C18:2c9,12 (*n*-6)]	3.94^{a}±0.83	4.27^{a}±0.92	2.87^{b}±0.46	2.40^{b}±0.42	2.59^{b}±0.73	<.0001	21.85
α-Linolenic acid [C18:3c9,12,15 (*n*-3)]	0.63±0.37	0.72±0.12	0.57±0.10	0.54±0.10	0.64±0.17	0.2306	32.54
CLA* (C18:2*c*9*t*11; *n*-6)	0.79±0.36	0.86±0.20	0.63±0.18	0.77±0.22	0.80±0.25	0.2504	32.29

Table 6.3 (Cont.)

Fatty acids (% of total fatty acids)	Treatment diets*					Significance (*P*-value)	CV[#]
	T15	T30	T45	T60	T75		
Total fatty acids:							
SFA	55.84^{c}±3.71	59.50^{b}±2.45	61.85^{a,b}±3.69	62.09^{a,b}±2.89	64.88^{a}±2.57	<.0001	5.11
MUFA	38.68^{a}±2.97	34.57^{b}±2.92	34.03^{b}±3.72	34.15^{b}±3.09	31.02^{b}±2.91	<.0001	9.09
PUFA	5.47^{a}±1.24	5.94^{a}±1.17	4.12^{b}±0.60	3.76^{b}±0.64	4.10^{b}±1.03	<.0001	20.78
n-6	4.83^{a}±1.10	5.21^{a}±1.06	3.55^{b}±0.52	3.23^{b}±0.55	3.44^{b}±0.85	<.0001	21.07
n-3	0.64±0.40	0.73±0.12	0.57±0.10	0.54±0.10	0.65±0.19	0.2492	34.23
Fatty acid ratios:							
n-6/*n*-3 ratio	8.57^{a}±2.28	7.14^{b}±0.66	6.27^{b,c}±0.85	6.03^{b,c}±0.59	5.35^{c}±0.59	<.0001	17.82
PUFA:SFA ratio	0.10^{a}±0.03	0.10^{a}±0.02	0.07^{b}±0.01	0.06^{b}±0.01	0.06^{b}±0.20	<.0001	23.13

^{a,b,c} Mean values with different superscripts differ significantly (P <0.05).

$^{\#}$ CV = Coefficient of variation (%).

* Treatment diets: Increments of neutral-detergent fibre (NDF) depicted as percentage roughage included; CLA = Conjugated linoleic acid; SFA = Total saturated fatty acids; MUFA = Total monounsaturated fatty acids; PUFA = Total polyunsaturated fatty acids; *n*-6 = Total omega-6 fatty acids; *n*-3 = Total omega-3 fatty acids.

The effect of dietary NDF content on the FA composition of subcutaneous adipose tissue was in essence similar to that observed in muscle tissue (Table 6.2), apart from the total lipid, monounsaturated palmitoleic acid, polyunsaturated CLA as well as total SFA and MUFA content of adipose tissue (Table 6.3). This however does not confirm beyond any doubt the fact that, as explained in 6.3.2, the FA composition of muscle tissue is less effected by the diet than that of adipose tissue (Schollan *et al*., 2006).

It is clear from the data presented in Table 6.3 that dietary NDF content had a significant (P =0.0437) effect on the lipid content of adipose tissue (T15 was lower compared to T75). The saturated palmitic and myristic acid, monounsaturated palmitoleic acid, polyunsaturated α-linolenic acid and CLA, as well as total *n*-3 FA's of subcutaneous lamb adipose tissue were however not effected (P >0.05) by dietary treatment (Table 6.3). The same argument is proposed to explain the lack of adipose tissue response, with special reference to α-linolenic acid and total *n*-3 content, despite a dietary increase in these mentioned FA's due to lucerne hay inclusion, as well as perceived greater hydrogenation of α-linolenic acid and reduced rumen transit time for animals fed high roughage diets (Wood *et al*., 2008). As NDF percentage increased, subcutaneous saturated stearic acid increased (P <.0001) (T75, compared to T15 and T30) (Table 6.3). In contrast, monounsaturated vaccenic acid and polyunsaturated linoleic acid decreased (P <.0001) following increased lucerne hay inclusion (T45, T60 and T75 compared to T15 and T30; Table 6.3). The monounsaturated oleic acid content of T15 supplemented with 15% lucerne hay were higher (P =0.0005), compared to T30 and T75 (Table 6.3).

As expected, from Table 6.3 it is evident that the total SFA content of subcutaneous lamb adipose tissue increased (P <.0001) following increasing increments of dietary NDF, whereas the total MUFA, PUFA and *n*-6 content decreased (P <.0001). As mentioned, this does not strengthen the fact that subcutaneous adipose tissue generally seems to be more responsive to changes in dietary FA supplementation and ruminal metabolism (Schollan *et al*., 2006; Wood *et al*., 2008), with special reference, but not confined to total adipose SFA and MUFA content. Differences in tissue response (adipose vs. muscle tissue) due to lipid supplementation are not uncommon and FA composition has been shown to differ depending on the deposit site (Oka *et al*., 2002). Demirel *et al*. (2004) observed that there appears to be tissue-specific effects following lipid supplementation. It was furthermore

stated that many metabolic processes are involved in determining the tissue level of FAs and thus it may not reflect the pattern of FA absorption from the intestine. Changes in the rumen environment lead to changes in the microbial population and activity, which affects the rumen lipid digestion and as a result the end products of BH (Martin and Jenkins, 2002). The significant (P <0.05) effect on the abovementioned parameters could therefore be attributed to dietary roughage content and its effect on lipolysis of TAG and BH of unsaturated long chain FAs in the rumen (Van Nevel and Demeyer, 1995).

As with muscle tissue (Table 6.2), even though dietary treatment also effected the *n*-6:*n*-3 and PUFA:SFA ratios of subcutaneous adipose tissue (P <0.05) (Table 6.3), the recommended ratios proposed by Wood *et al*. (2003) and Webb and O'Neill (2008) still leaves a lot of room for improvement. As previously stated, greater BH of *n*-3 and an extended rumen transit time for high roughage diets also limits the amount available for tissue uptake, compared with *n*-6 from concentrate diets. As a result low NDF content favourably increased (P <.0001) the PUFA:SFA ratio and unfavourably increased the *n*-6:*n*-3 (P <0.0001) ratio of adipose tissue as well. A positive feature of grass feeding is that levels of the nutritionally important long chain *n*-3 PUFA are increased e.g. eicosopentaenoic acid (EPA) (20:5*n*-3) and docosohexaenoic (DHA) (22:6*n*-3) (Wood *et al*., 2008). It is clear, as mentioned earlier and explained in the previous section (see 6.3.2), that the ideal *n*-6:*n*-3 as well as PUFA:SFA ratios were still not acceptable and presents opportunity for improvement. Therefore it is proposed, to increase the total UFA content (MUFA, PUFA and *n*-6) and PUFA:SFA ratio of lamb meat, having a low roughage diet containing lucerne hay as main fibre source (15 to 20 %NDF: T15 and T30, Table 4.1) (Smith, 2008) should be supplemented with a lipid source high in the preferred UFA in question by not exceeding total dietary lipid content of 6-7% DM (Jenkins, 1993; Doreau *et al*., 1997; NRC, 2001). To decrease the *n*-6:*n*-3 ratio and ensure an increased total *n*-3 content of ruminant tissue, a low roughage diet can still be fed by just including a dietary lipid source containing higher levels of total *n*-3.

For practical purposes and to limit repetition, dietary effects on lipid lipolysis and FA BH serves as the main reason (Van Nevel and Demeyer, 1995) for significant effects (P <0.05) in subcutaneous FA composition of lamb meat.

6.3.4 Oxidative stability of lamb muscle tissue

The effect of incremental increasing dietary NDF content on the oxidative stability of S.A. Mutton Merino lamb meat is presented in Table 6.4. No significant (P >0.05) effects occurred for any of the parameters tested, e.g. fresh lamb meat (day 0), either stored for 7 days at 4°C or at -18°C for 90 days. The TBARS can generally be used to determine the lipid peroxidation and oxidative tension within meat (Jensen *et al*., 1997). Jensen *et al*. (1997) also revealed that only FAs with three or more double bonds are believed to induce faster malonaldehyde formation. A malonaldehyde level of 0.5 mg/kg and above is considered to be the turning point from where lipid oxidation causes rancid odour and taste of meat as detected by the consumers (Wood *et al*., 2008). A lower TBARS value indicates lower susceptibility of meat to oxidation (Buckley *et al*., 1995). The malonaldehyde content for all the cuts of lamb and dietary treatments in the present study are all well below this critical threshold except treatment T15 (0.74), T30 (0.57) and T75 (0.54) measured 7 days after slaughter (Table 6.4). This could be attributed to the fact that the higher level of total PUFAs (P =0.0398) present in the muscle tissue (Table 6.2) increased its susceptibility to lipid oxidation (Buckley *et al*., 1995). As mentioned before, MUFAs are more resistant to oxidative modification than PUFAs (McDonald *et al*., 2011).

According to Hugo *et al*. (2009) the TBARS content of meat is highly negatively correlated with the sensory evaluation scores. The propensity of meats and meat products to undergo oxidation not only depends on the PUFA content, but several other factors including pre-slaughter events such as stress, and post-slaughter events such as early post-mortem pH, carcass temperature, cold shortening, and techniques such as electrical stimulation play a role (Buckley *et al*., 1995; Gray *et al*., 1996). All treatment carcasses were treated similar and could not have influenced any of the results.

Although an antioxidant was added to all the dietary treatments, Buckley *et al*. (1995) concluded that it is highly unlikely that the armoury of the antioxidant defensive systems (superoxide dismutase, glutathione peroxidase, ceruloplasmin and transferrin) available to the cell in the live animal still function during the post slaughter phase, because of quantitative changes in several metabolites and physical properties.

Table 6.4 The effect of incremental dietary NDF content on the malonaldehyde content of S.A. Mutton Merino lamb muscle tissue (mean±SD)

	Treatment diets*						
Parameter	**T15**	**T30**	**T45**	**T60**	**T75**	**Significance (*P*-value)**	**CV[#]**
Thiobarbituric acid reactive substances (mg malonaldehyde/kg meat):							
Day 0 (fresh product)	0.1026±0.10	0.0554±0.03	0.0649±0.03	0.0914±0.06	0.0476±0.02	0.0819	76.44
Day 7 (refrigerated storage at 4°C)	0.7427±0.66	0.5716±0.64	0.4555±0.30	0.434±0.34	0.5400±0.58	0.6306	96.11
Day 90 (frozen storage at -18°C)	0.1010±0.06	0.0871±0.04	0.0907±0.03	0.0766±0.02	0.1026±0.03	0.4791	42.78

[a,b,c] Mean values with different superscripts differ significantly (P <0.05).

[#] CV = Coefficient of variation (%).

* Treatment diets: Increments of neutral-detergent fibre (NDF) depicted as percentage roughage included.

Schollan *et al.* (2006) mentioned that the lipid and colour stability of the meat are expected to be more related to its FA composition rather than its antioxidant concentration. This consequently means that a more UFA profile of meat would be more susceptible to lipid oxidation (Buckley *et al*., 1995; Hur *et al.,* 2004). Therefore, significant differences in oxidative stability were expected for muscle tissue, but were not the case in the present study (Table 6.4).

6.3.5 Colour stability of lamb meat

The effect of incremental dietary NDF percentages on meat colour stability of S.A. Mutton Merino lamb muscle tissue are illustrated in Table 6.5. Any changes in meat colour was primarily attributed to the oxidation of red oxymyoglobin (OMG) to metmyoglobin (MMG), which gives meat an unattractive brown colour (Karami *et al*., 2010; Velasco and Williams, 2011). As previously mentioned, the main cause affecting meat product acceptability at the time of consumer purchase is the colour of meat (Gray *et al*., 1996; Jensen *et al*., 1997; Karami *et al*., 2010; Karamucki *et al*., 2011).

From Table 6.5 it is clear that, apart from the subjective redness (a*-values), dietary NDF content only affected (P <0.05) the colour stability of lamb muscle stored for seven days at 4^{o}C. When dietary NDF percentage were increased, the lightness (L*-values; T45 and T60 compared to T15), yellowness (b*-values; T45, T60 and T75 compared to T15), Hue angle (T75 compared to T15) and Chroma (saturation index; T60 compared to T15) of lamb muscle tissue were increased (P <0.05). Although there was no significant effect on a*-values as mentioned above, they were higher in relation to the b*-values (Table 6.5), indicating an increased colour stability. A meat b*-value of 22 or more is discriminated against (Priolo *et al*., 2001) and none of the treatments were even close to the mentioned value. Hue angle, being a function of a* and b*, gives a more realistic perspective on meat browning than any other single parameter (Luciano *et al*., 2009). The closer the Hue angle is to 90, the more the colour corresponds to yellow (Priolo *et al*., 2001). According to Priolo *et al*. (2001), the subcutaneous caudal fat was more yellow (b*-value), had a wider Hue angle and tended to have a higher Chroma for grass-fed lambs. The researchers then explained that they were almost certain that this was due to the presence of carotenoids in the carcasses of the grass-fed lambs, which were not tested for in the present study.

Table 6.5 The effect of incremental dietary NDF content on meat colour stability of S.A. Mutton Merino lamb muscle tissue (mean±SD)

	Treatment diets*						
Parameter	**T15**	**T30**	**T45**	**T60**	**T75**	**Significance (*P*-value)**	**CV[#]**
Meat Colour Day 0 (fresh product):							
Lightness (L*-values)	37.22±1.04	36.88±1.13	38.56±2.01	37.62±2.86	38.07±1.71	0.2044	4.96
Redness (a*-values)	16.89±1.41	16.63±1.06	17.29±1.41	16.12±1.38	17.37±1.04	0.1170	7.53
Yellowness (b*-values)	5.67±0.71	5.50±0.82	6.23±0.71	5.90±1.18	6.22±0.78	0.1587	14.53
Hue Angle	18.54±1.56	18.27±2.33	19.80±1.20	19.99±3.14	19.65±1.63	0.1626	10.85
Chroma (Saturation Index)	17.82±1.50	17.53±1.14	18.38±1.53	17.19±1.56	18.45±1.18	0.1380	7.81
Meat Colour Day 7(refrigerated storage at 4°C):							
Lightness (L*-values)	42.19^{b}±2.77	43.46^{a,b}±1.49	45.83^{a}±1.92	45.55^{a}±2.10	44.41^{a,b}±2.16	0.0005	4.81
Redness (a*-values)	9.48±1.25	9.52±2.24	10.32±2.13	10.79±1.24	9.38±1.35	0.1828	17.18
Yellowness (b*-values)	6.49^{b}±1.81	7.86^{a,b}±1.31	8.41^{a}±1.02	8.89^{a}±1.13	8.45^{a}±1.10	0.0004	16.27
Hue Angle	34.16^{b}±8.73	39.96^{a,b}±7.03	39.63^{a,b}±3.97	39.50^{a,b}±4.87	42.16^{a}±5.39	0.0398	15.95
Chroma (Saturation Index)	11.61^{b}±1.31	12.43^{a,b}±2.15	13.34^{a,b}±2.18	14.02^{a}±1.21	12.68^{a,b}±1.27	0.0120	13.14

^{a,b,c} Mean values with different superscripts differ significantly (P <0.05).

$^{\#}$ CV = Coefficient of variation (%).

* Treatment diets: Increments of neutral-detergent fibre (NDF) depicted as percentage roughage included.

Meat from cattle raised on pasture is reported to be darker than meat from animals raised on concentrates if measured by objective (P <0.001) as well as subjective (P <0.05) methods. A higher pH is less beneficial due to a darker colour meat and a higher susceptibility to bacterial spoilage with a reduced flavour. Several factors are responsible for these different variations - including ultimate-pH and intramuscular fat content – last mentioned seems to play a major role (Velasco and Williams, 2011). In sheep, pastoral flavour is mostly determined by the branched-chain FAs and 3-methylindole. An important role seems to be played also by certain products of oxidation of polyunsaturated linolenic acid and its derivatives (Velasco and Williams, 2011). The post-mortem softening process of muscle is more efficient at a lower meat pH (ultimate meat pH value of 5.4) (Maree and Casey, 1993). However, meat pH was not determined in the present study.

The amount of chemical (Karami *et al*., 2010) or natural (*Gray et al*., 1996; Velasco and Williams, 2011) antioxidants within meat can delay meat colour loss by dropping the oxidation process, extending the red colour (a*-values) of meat and the lightness (L*, b*, and a*-values) of meat and delaying MMG formation, to finally improve retail shelf-life. However, antioxidants were added to all dietary treatments and no antioxidants were added directly onto the meat. As mentioned previously in 6.3.4, Buckley *et al*. (1995) found it highly unlikely for the armoury of antioxidants to still function post slaughter.

6.3.6 Tenderness of lamb meat

The effect of increasing incremental dietary NDF percentage on meat tenderness of S.A. Mutton Merino lamb muscle tissue is presented in Table 6.6. Neutral-detergent fibre had no significant effect (P >0.05) on meat tenderness. According to Priolo *et al*. (2001), meat from primarily concentrate-fed lambs was more tender and juicier than meat from grass-fed lambs as judged by a trained panel of assessors. The degree in fatness could affect meat tenderness either through a direct effect of the fat which is softer and/or by an indirect effect of reduced muscle fibre shortening (Priolo *et al*., 2001). This was probably the case in the present study due to the lack of dietary effect on carcass fatness (P >0.05) (Table 5.2, Chapter 5) and meat tenderness (Table 6.6). In addition, Diaz *et al*. (2001) presented that lambs raised on pasture are generally leaner compared to concentrate-raised lambs, possibly due to changes in metabolism of lambs maintained on pasture due to exercise.

These changes lead to increased mobilization of reserve lipids in order to form muscle tissue, with the subsequent reduction of carcass fatness, fundamentally at the cost of subcutaneous fat (Diaz *et al*., 2001).

Table 6.6 The effect of incremental dietary NDF content on meat tenderness of S.A. Mutton Merino lamb muscle tissue (mean±SD)

	Treatment diets*						
Parameter	**T15**	**T30**	**T45**	**T60**	**T75**	**Significance (*P*-value)**	**CV[#]**
Tenderness (kg)	2.40±0.48	2.45±0.55	2.46±0.71	2.54±0.75	2.50±0.48	0.9868	24.47

[a,b,c] Mean values with different superscripts differ significantly (P <0.05).

[#] CV = Coefficient of variation (%).

* Treatment diets: Increments of neutral-detergent fibre (NDF) depicted as percentage roughage included.

6.4 Conclusion

The research results indicate that the FA composition of intramuscular fat and subcutaneous adipose tissue of ruminants are influenced by the NDF content of the feed and that the FA profile of lamb meat can be manipulated (P <0.05) by dietary means with the main affect probably being attributed to ruminal BH. Therefore, it seems that by decreasing the NDF percentage in the finishing diets of lambs the total PUFA and *n*-6 content of both intramuscular and subcutaneous lipid tissue are increased, whereas the total SFA content of adipose tissue is decreased. These results are also pronounced in the related higher PUFA:SFA and *n*-6:*n*-3 ratios following a decreased NDF content. However, altering levels of dietary NDF from a human health point of view, did not result in the desirable PUFA:SFA and *n*-6:*n*-3 ratios in muscle or subcutaneous lipids. By just increasing the total *n*-3 content of lamb finishing diets and keeping the fibre content to a minimum, is

proposed as a viable option. The manipulation of the basal diet of finishing lambs to achieve the desirable FA ratios therefore warrants further investigation.

The presence of incremental NDF content in the diet had no effect on the oxidative stability (TBARS) and tenderness of lamb muscle tissue, but did significantly affect meat colour stability stored for 7 days at 4^0C.

In conclusion, the null hypothesis for this trial is accepted due to a significant increase in total SFA, and significant decrease in total PUFA content of lamb meat with its resultant effect on the PUFA:SFA and *n*-6:*n*-3 ratios (decreased) following increased incremental NDF content.

CHAPTER 7

GENERAL CONCLUSIONS

Meat by virtue is a valuable component of the human diet and essential for optimum nutrition. There is credit for human health benefits from the standard utilization of polyunsaturated fatty acids (PUFA), particularly those exerted by omega-3 (*n*-3) fatty acids (FA) and diverse conjugated linolenic acid (CLA) isomers. These benefits include reduced risk of cardiovascular disease and diabetes, lower risk of cancer, improved visual and nervous system development and maintenance, as well as having anti-inflammatory effects and improving the immune system and bone health. Due to the beneficial properties of unsaturated fatty acids (UFA) on human health there has been an increased interest in recent years to positively manipulate the FA composition of meat by increasing the essential monounsaturated fatty acid (MUFA), PUFA and in particular *n*-3 FA content of ruminant meat with a resultant decrease in the perceived less beneficial saturated fatty acid (SFA) content. However, there are still many questions left unanswered with some disparities when referring to the favourable FA content of meat on the health benefits of the consumer.

Feeding sheep mixed rations of high nutritional quality is not a common practice world-wide, mainly due to less affordable raw materials and the availability. Most attention in animal nutrition has been focused on increasing animal production and profitability, whereas improving product quality consumers preferred is also gaining momentum. The latter is reflected in the lack of published studies primarily focusing on diet and meat quality parameters. Meat quality affects consumer preference for this product, where visual and sensory aspects play a major role. However, hidden properties like FA content are just as important. Many factors do affect ruminant meat FA composition, whereas animal age, breed, gender and feed are some of the most important. According to literature, feed and the manipulation thereof are undoubtedly the most efficient method to affect the latter statement. Lipid sources and its inclusion on ruminant FA composition are profound. However, there seems to be a lack of knowledge how basal diet constituents affect ruminant FA tissue composition. One main concern for including healthy plant oils in ruminant diets to try and favourably increase meat lipid FA composition, is that its affect is not always consistent in most published research primarily due to the variation of dietary

composition. Dietary roughage content is one of the most important parameters assessed and plays a major role in the FA composition of ruminant meat, but most results are still inconsistent. The variation and influence of roughage (with special reference to neutral-detergent fibre - NDF) and concentrate ratio in the diet on the FA composition of muscle and adipose tissue of ruminants therefore remains of great interest. No literature evaluating the effect of specifically the NDF content of ruminant diets on animal production and meat FA composition was found and was the focus of this research.

From the results of the present study it is apparent that the incremental increase of NDF content in finishing diets of lambs significantly affected feed nutrient digestibility as well as digestible nutrient content (increasing lucerne hay inclusion from T15 to T75). The only chemical constituents' digestibility not affected by the incremental increased NDF content was ironically NDF itself, as well as acid-detergent fibre (ADF – though not negatively). This is however difficult to explain because literature states that by increasing the fibre content (decrease in easily fermentable carbohydrates) in the ruminant diet, rumination and rumen pH are increased, hence favouring NDF digestibility by promoting cellulolytic micro-organism (MO) activity. However, total tract OM digestibility usually decreases. The latter was consistent with literature. For all practical purposes the dry matter intake (DMI) was consistent between treatments. Dietary NDF level seems to be, at least primarily, negatively correlated with feed metabolizable energy (ME) content, also comparable with most literature. The most feasible explanation for differences in NDF digestibility in the literature is the negative effect of UFAs on the digestibility of fibre due to its antimicrobial and coating effect. In this experiment all treatments were however similar in this regard. There is a possibility that methane (CH_4) production is increased when feeds high in fibre are fed to ruminants. Hydrogen is produced and methanogenic bacteria use this with carbon dioxide (CO_2) to produce methane and water (H_2O) and a further loss of energy occurs. An increasing heat increment (HI) of fibre fermentation in the rumen further reduces the available energy content of high fibre diets.

Dry matter intake responses to dietary incremental NDF levels were limited in the present study. Treatment T15 was the lowest, but only significantly affected compared to T45 and T60. This is however strange as high fibre (NDF) diets affects dry matter (DM) feed intake negatively due to its fill effect, hence longer rumen retention time as a result of increased rumination. The low intake of T15 was accounted for by a probable lower ruminal

pH due to the minimum dietary NDF for finishing lambs not being met as proposed in literature (Smith, 2008). Increasing incremental NDF levels significantly affected the metabolizable energy intake (MEI) of lambs and decreased for only those fed the T75 treatment. This negative affect of high NDF content in T75 was evident and related to the same treatment's average daily gain (ADG) and feed conversion ratio (FCR). The efficiency of use of ME for weight gain purposes was less efficient for T75 compared to T45. The decreased feed digestibility, and possible increased HI and loss of methane gas resulting from high fibre inclusion were proposed as possible reasons. When looking at carcass characteristics, cold carcass weight and dressing percentage were also negatively affected by high roughage inclusion (treatment T75).

The FA composition of intramuscular fat and subcutaneous adipose tissue of ruminants were significantly and negatively influenced by incremental NDF content in the diets. The saturated stearic acid content increased, whereas monounsaturated oleic and vaccenic acid, as well as polyunsaturated linoleic acid of both muscle and adipose tissue significantly decreased due to high NDF inclusion. The total FAs and their ratios could present a better understanding of the NDF's effect on carcass FA composition. Hence, increasing the NDF content of finishing diets fed to lambs increases the total SFA (of only adipose tissue), and decreases the total MUFA (also only adipose tissue), PUFA and omega-6 (*n*-6) of lamb unfavourably, with a resultant effect on the *n*-6:*n*-3 and PUFA:SFA ratios. The main effect of the diet could probably be attributed to ruminal biohydrogenation (BH), favoured by a high fibre diet resulting in an increased ruminal pH. Altering the NDF content of lamb finishing diets, from a human health point of view, did not induce the desirable PUFA:SFA and *n*-6:*n*-3 ratios (above 0.4 and below 4.0, respectively) in the muscle and subcutaneous lipids.

The inclusion of NDF in the diet had no effect on the oxidative stability (TBARS) of lamb muscle tissue, but significantly affected meat colour stability stored for 7 days at 4°C. Increasing the PUFA content and the PUFA:SFA ratio of ruminant meat may unintentionally lead to a higher vulnerability to oxidative breakdown, decreased colour stability and a restricted shelf-life.

Therefore it is proposed to favourably manipulate the total UFA and resultant ratios of lamb meat. A low roughage diet containing lucerne hay as main fibre source (probably between 15 to 20 %NDF) should be supplemented with a lipid source high in the preferred

UFA composition in question. Care must be taken not to exceed a total dietary lipid content of 6-7% DM, apart from feeding protected lipids of course. To decrease the *n*-6:*n*-3 ratio, hence to ensure an increased total *n*-3 content of ruminant tissue, a low roughage diet can still be fed, including a dietary lipid source, or a mixture thereof that contains higher levels of total *n*-3. To optimize the effect of a low roughage diet on lamb FA composition, regression equations should be used to establish the optimum and repeatable favourable response at a given NDF content. Further research attempting to manipulate a specific FA or FA ratios of lamb meat via dietary means to meet consumers' demands from a health point of view therefore warrants further attention.

ABSTRACT

A study was conducted to investigate the effect of incrementally increasing NDF levels of finishing diets on apparent digestibility, production performance, FA composition, oxidative stability and tenderness of lamb. The five dietary treatments were formulated to contain a similar nutrient composition, differing only in respect to the NDF content as the primary parameter. The NDF content increased from low roughage (primarily lucerne hay as fibre source) inclusion to a high roughage inclusion rate representing a dose-response trial in the following order: 12.76% (T15), 17.69% (T30), 22.53% (T45), 27.48% (T60) and 32.40% (T75) NDF/kg DM, respectively. No rumen modifiers or buffers were added to the diet. Sixty (60) South African Mutton Merino wether lambs (29.3±1.8 kg) were randomly allocated to the five dietary treatments (n=12 lambs per treatment) and further subdivided into 12 animals per replicate (n=1 lamb per replicate). After dietary adaptation of 10 days all lambs received the experimental diets for the remaining period (51 days). Live weight and feed intake were recorded on a weekly basis. A digestibility study was conducted over a 12-day period (4-day adaptation to the faecal bags followed by an 8-day collection period) where seven lambs (mean 48.11±2.94 kg live weight; total of 35 lambs) were randomly allocated to each treatment (n=7 lambs/treatment). At the end of the production study all lambs were slaughtered. Physical carcass characteristics, muscle and subcutaneous FA composition, meat oxidation (malonaldehyde content), colour stability, as well as meat tenderness were measured. The data was subjected to analysis of variance (PROC ANOVA) of the SAS program, version 9.2 (SAS, 2008). Tukey's honest significant difference (HSD) test was used to identify significant differences (P <0.05) between treatments.

From the results of the present study it is apparent that an incremental increase in the NDF content of lamb finishing diets presented a significant decrease (P <0.05) in DM, OM, NSC, GE, CP and EE digestibilities, as well as ash solubility. In addition, the significant (P <0.05) decrease in digestible OM, NSC and EE dietary content were associated with diet digestibility and resulted in a significant decrease (P <.0001) in ME content following an increased NDF incremental inclusion.

A high roughage inclusion in finishing diets for lambs (T75) resulted in a significant (P <0.05) reduction in MEI, ADG, FCR, and therefore cold carcass weight and dressing percentage.

Increased dietary NDF content significantly (P <0.05) increased saturated stearic acid, and significantly (P <0.05) decreased monounsaturated oleic and vaccenic acid, polyunsaturated linoleic acid, as well as total PUFA, *n*-6, *n*-6:*n*-3 and PUFA:SFA ratios of both lamb meat and adipose tissue. Apart from the NDF content significantly (P <0.05) affecting the monounsaturated palmitoleic acid (decreased) and polyunsaturated CLA (C18:2*c*9*t*11;*n*-6) content of muscle tissue, as well as total SFA (increased) and MUFA (decreased) content of only adipose tissue, the effect of dietary treatment between lamb deposit sites seem to be similar. Neutral-detergent fibre content did significantly (P <0.05) affect meat colour stability stored for 7 days at 4^{o}C. Neutral-detergent fibre content had no effect (P >0.05) on meat tenderness.

These results suggest that the FA profile of lamb can be manipulated by altering the NDF content of the finishing diet. This however did not result, from a human health point of view, in the desirable PUFA:SFA and *n*-6:*n*-3 ratios in muscle and subcutaneous lipid tissue. It is proposed that, to increase the total UFA content and its desirable effect on the mentioned ratios of lamb meat, regression equations should be used to establish the optimum response at a given NDF inclusion. Further research attempting to manipulate specific FAs (single or total) or FA ratios of lamb meat via dietary means to meet consumers' demands need further attention.

OPSOMMING

'n Studie is uitgevoer om die invloed van inkrementeel toenemende neutral bestande vesel vlakke binne afrondingsdiëte op die skynbare verteerbaarheid, produksie, vetsuursamestelling, oksidasie stabiliteit en sagtheid van lamsvleis te evalueer. Vyf afrondingsdiëte is geformuleer om 'n soortgelyke voersamestelling te bevat, maar het slegs ten opsigte van die neutral bestande vesel inhoud as die primêre eienskap verskil. Die neutral bestande vesel inhoud het toegeneem vanaf ‘n lae ruvoer- (lusernhooi as ruvoerbron) tot 'n hoë ruvoer insluiting om 'n dosis-respons studie voor te stel met die volgende intervalle: 12.76% (T15), 17.69% (T30), 22.53% (T45), 27.48% (T60) en 32.40% (T75) neutral bestande vessel per kg droë materiaal, onderskeidelik. Geen rumen modifiseerders of buffers was bygevoeg tot enige dieët nie. Sestig (60) Suid-Afrikaanse Vleismerino hamellammers (lewnendige gewig van 29.3±1.8 kg) was ewekansig aan die vyf diëte toegeken (n=12 lammers per behandeling) en verder verdeel in 12 herhalings per behandeling (n=1 lam per herhaling). Na ‘n aanpassingperiode van 10 dae is die proefdiëte aan die lammers gevoer vir die oorblywende tydperk (51 dae). Lewende massa en voerinname is op ‘n weeklikse basis gemeet. ‘n Verteringstudie is vir ‘n periode van 12 dae uitgevoer (4-dae vir aanpassing tot die missakke, gevolg deur 'n 8-dae kolleksieperiode) waar sewe lammers (lewendige gewig van 48.11±2.94 kg; ‘n totaal van 35) ewekansig toegeken was aan elke behandeling (n=7 lammers per behandeling). Al die lammers was aan die einde van die produksiestudie geslag. Fisiese karkaseienskappe, vetsuursamestelling van spierweefsel en onderhuidse vet, oksidasiestabiliteit (malonaldehiedinhoud), kleurstabiliteit asook vleissagtheid is gemeet. Die data was onderworpe aan ‘n variansie analise (PROC ANOVA) van die SAS-program, weergawe 9.2 (SAS, 2008). Tukey se HSD toets was gebruik om betekenisvolle verskille (P <0.05) tussen behandelings te identifiseer.

Uit die resultate van die huidige studie is dit duidelik dat 'n inkrementele toename in die NBV inhoud van ‘n lam afrondingsdieët 'n beduidende afname (P <0.05) in droë materiaal, organise materiaal, nie-strukturele koolhidrate, bruto energie, ru-proteien en eter ekstrak verteerbaarheid, sowel as oplosbare as het. Daarbenewens word die betekenisvolle (P <0.05) afname in verteerbare organise materiaal, nie-strukturele koolhidrate en eter ekstrak geassosieer met dieët verteerbaarheid wat gelei het tot 'n betekenisvolle afname (P <0.0001) in dieët ME inhoud soos NBV inkrementeel verhoog was.

'n Hoë ruvoer insluiting in die afrondingsdiëte vir lammers (T75) het gelei tot 'n betekenisvolle verlaging (P <0.05) in metabolise energie inname, gemiddelde daaglikse toename, voeromset verhouding, en daarom ook koue karkas gewig en uitslagpersentasie.

'n Verhoogde dieët NBV inhoud het versadigde steariensuur betekenisvol (P <0.05) laat toeneem en mono-onversadigde oleien-, "vaccenic"- en poli-onversadigde linoleïensuur, sowel as totale poli-onversadigde vetsure, *n*-6, *n*-6:*n*-3 en poli-onversadigde vetsuur tot versadigde vetsuur verhoudings van beide spierweefsel en onderhuidse vet het betekenisvol (P <0.05) verlaag. Afgesien van die neutral bestande velsel inhoud wat 'n betekenisvolle effek (P <0.05) op die mono-onversadigde palmitoleïen suur (afgeneem) en poli-onversadigde gekonjugeerde linoleiensuur (C18:2*c*9*t*11;*n*-6) inhoud van spierweefsel, asook die totale versadigde vetsure (toegeneem) en totale mono-onversadigde vetsure inhoud (afgeneem) van slegs onderhuidse vet gehad het, blyk die effek van dieët tussen die deponerings areas soortgelyk te wees. Neutraal-bestande veselinhoud het 'n betekenisvolle (P <0.05) effek op kleurstabiliteit van vleis gehad wat gestoor was vir 7 dae by 4°C. Neutraal-bestande veselinhoud het geen effek (P >0.05) op vleissagtheid gehad nie.

Hierdie resultate dui daarop dat die vetsuurprofiel van lamsvleis gemanipuleer kan word deur 'n verandering in die NBV inhoud van 'n afrondingsdieët. Die resultate het egter nie die wenslike poli-onversadigde vetsuur tot versadigde vetsuur en *n*-6:*n*-3 verhoudings in die spierweefsel en onderhuidse vet uit 'n menslike gesondheids oogpunt tot gevolg gehad nie. Daar word voorgestel dat, om die totale onversadigde vetsuur inhoud te verhoog met 'n wenslike effek op die genoemde verhoudings van lamsvleis, regressie vergelykings gebruik moet word om die optimum reaksie op 'n gegewe neutral bestande vesel inhoud te bevestig. Verdere navorsing om spesifieke vetsure van lamsvleis (enkel of totale) of verhoudings daarvan te probeer manipuleer deur die dieëtsamestelling om in verbruikers se behoeftes te voldoen, verdere aandag verg.

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