

**The effect of dietary lipid saturation and antioxidant
sources on performance and meat quality of lambs**

by

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Bloemfontein

10 June 2012

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A handwritten signature in black ink that reads "Käte Booyens". The signature is written in a cursive style and is positioned above a horizontal line.

Käte Erna Booyens

Bloemfontein

June 2012

Dedicated to my husband and family

To my husband Daniël and my family, thank you for all the guidance, love and opportunities you gave me in life. Thank you for the interest, encouragement and support throughout my life.

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Acronyms and Abbreviations

α	Alpha
β	Beta
AA	Arachidonic acid
ADF	Acid detergent fibre
ADG	Average daily gain
AOAC	Association of Official Analytical Chemists
ATP	Adenosine Triphosphate
B	Natural Antioxidant
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
2-BHA	2-tert-butyl-4-hydroxyanisole
3-BHA	3-tert-butyl-4-hydroxyanisole
C	Synthetic antioxidant
C1	Blank bag correction
C4:0	Butyric acid
C8:0	Caprylic acid
C10:0	Capric acid
C12:0	Lauric acid
C14:0	Myristic acid
C16:0	Palmitic acid
C18:0	Stearic acid
C20:0	Arachidic acid
C16:1	Palmitoleic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	α -linolenic acid
C20:3	Dihomo-gamma-linolenic acid
C20:4	Arachidonic acid
C20:5	Eicosapentaenoic acid

C22:6	Docosahexaenoic acid
CH ₄	Methane gas
CLA	Conjugated linoleic acid
cm	Centimetre
CO ₂	Carbon dioxide
CP	Crude Protein
CV	Coefficient of variance
DE	Digestible energy
DHA	Docosahexaenoic acid
DM	Dry Matter
DMI	Dry Matter Intake
DMI/lamb	Dry Matter Intake per lamb
EE	Ether extract
EPA	Eicosapentaenoic acid
FAMES	Fatty acid methyl esters
FCR	Feed conversion ratio
FFA	Free fatty acid
FFAs	Free fatty acids
g	Gram
g/animal	Gram per animal
g DM	Gram Dry Matter
g/kg	Gram per kilogram
g/lamb/day	Gram per lamb per day
g NDF/kg DM	Gram Neutral detergent fibre per kilogram dry matter
g/sheep/day	Gram per sheep per day
g/ton	Gram per ton
GE	Gross Energy
h	Hour
H ₂	Hydrogen gas
HDL	High-density lipoprotein
kg	Kilogram

kg/lamb/day	Kilogram per lamb per day
km	Kilometre
KOH	Potassium hydroxide
Kpa	Kilo Pascal
LDL	Low-density lipoprotein
LDL:HDL	Low-density lipoprotein:high-density lipoprotein
MEI	Metabolizable energy Intake
M	Molar
m	Metre
mm	Millimetre
MMG	Metmyoglobin
mm ²	Millimetre squared (area)
m ²	Metre squared (area)
ME	Metabolizable energy
mg	Milligram
mg/kg	Milligram per kilogram
mg/animal/day	Milligram per animal per day
min	Minutes
ml/bag	Millilitre per bag
ml	Millilitre
MJ/kg	Mega joules per kilogram
MJ/kg DM	Mega joules per kilogram dry matter
MJ GE/kg DM	Mega joules Gross Energy per kilogram dry matter
MJ/lamb/day	Mega joules per lamb per day
MUFA	Monounsaturated fatty acid
MUFAs	Monounsaturated fatty acids
n	Number
<i>n</i> -6	Omega-6
<i>n</i> -3	Omega-3
<i>n</i> -6: <i>n</i> -3	Linoleic acid:α-linolenic acid ratio
N ₂	Nitrogen gas

NBV	Neutrale bestande vesel
ND	Neutral detergent
NDF	Neutral detergent fibre
NRC	National Research Council
OM	Organic Matter
<i>P</i>	Significance
P/S	Polyunsaturated fatty acids: Saturated fatty acids ratio
PUFA	Polyunsaturated fatty acid
PUFAs	Polyunsaturated fatty acids
PUFA:SFA	Polyunsaturated:saturated fatty acid ratio
PVC	Polyvinyl chloride
TBHQ	Tert-butylhydroquinone
TBARS	Thiobarbituric acid reactive substances
pH	Hydrogen ion concentration
ROS	Reactive oxygen species
RP	Ruproteïen
S	Soyabean oil
SAMM	South African Mutton Merino
SAS	Statistical Analysis System
SC	Unsaturated soyabean oil with synthetic antioxidant
SB	Unsaturated soyabean oil with natural antioxidant
SD	Standard deviation
SFA	Saturated fatty acid
SFAs	Saturated fatty acids
T	Beef Tallow
TC	Saturated beef tallow with synthetic antioxidant
TB	Saturated beef tallow with natural antioxidant
UFA	Unsaturated fatty acid
UFAs	Unsaturated fatty acids
VFAs	Volatile fatty acid
W1	Bag tare weight

W2	Sample weight
W3	Dried weight of bag with fibre after extraction process
w/w	Weight/weight
µm	Micro millimetre
µl	Micro litre
°C	Degrees Celsius
°	Degree
"/ ‘	Inch
%	Percentage

Chapter 1

General Introduction

Meat is considered as a very nutritive food, since it provides humans with high quality proteins, fat, vitamins and minerals (Simitzis & Deligeorgis, 2010). History has proven that for a period of at least two million years the human ancestral line had been consuming increasing quantities of meat. During that time, evolutionary selection adapted our genetic makeup and physiological features to a diet high in lean meat, low in saturated fat and relatively rich in poly-unsaturated fatty acids (PUFAs) (Li *et al.*, 2005). Most saturated fatty acids (SFAs) are assumed to be bad for human health because they are connected to various diseases: such as cardiovascular disease and cancer (Wood *et al.*, 2003). On the other hand, unsaturated fatty acids (especially PUFAs) are perceived to be beneficial for human health because their consumption is associated with a lower risk of coronary heart disease, hypertension, type 2 diabetes, renal disease, ulcerative colitis, chronic obstructive pulmonary disease and Crohn's disease (Wood *et al.*, 2003). Therefore, consumers are increasingly focused on the quality and nutritional characteristics of meat and meat products.

Of all mammal species, ruminants have the most differentiated, specialized and complex stomachs, which are influenced by many dietary, environmental and host factors. Most ruminants are herbivorous and consume plant material that is high in structural carbohydrates. Consequently, ruminants have evolved a specially adapted digestive system to enable them to break down these feedstuffs into smaller pieces (Aluwong *et al.*, 2010).

Feed lipids are an excellent source to provide dietary metabolisable energy to the animal as it contains 2.25 times more energy than carbohydrates (McDonald *et al.*, 2002). A further important benefit from an energy utilization point of view, is reduced methane (CH₄) emissions (McDonald *et al.*, 2002; Beauchemin *et al.*, 2007), potentially decreasing its negative effect on climate change. Dietary supplementation with fat is the most promising dietary strategy to mitigate enteric CH₄ emissions from ruminants (Grainger & Beauchemin, 2011).

Too much fat in the ruminant diet may affect nutrient digestibility, and highly digestible unsaturated fats could negatively alter voluntary feed intake (if oxidized) and fibre digestibility (McDonald *et al.*, 2002). The presence of saturated- or unsaturated long chain fatty acids in the rumen may alter microbial populations, hence affecting fermentation and digestibility (Harris, 2003). Unsaturated fatty acids appear to have more of an adverse effect on rumen fermentation than do saturated fats, because of their antimicrobial properties (Bauman *et al.*, 2003). Physical coating of the fibre with added fat may also decrease fibre digestibility (Harris, 2003) by inhibiting microbes fermenting fibre (Jenkins & Lundy, 2001).

Although the fatty acid composition of ruminant tissues is affected to some degree by factors such as animal sex (Clemens *et al.*, 1973), breed (Smith *et al.*, 2009) and animal age (Oka *et al.*, 2002), dietary affects (Smith *et al.*, 2009) seems to be the most pronounced. The micro flora in the rumen converts the majority of the dietary unsaturated fatty acids to SFAs through the process of microbial biohydrogenation (Felton & Kerley, 2004). Unlike their short chain counterparts, long chain fatty acids are not absorbed directly from the rumen. When they reach the small intestine, they are mainly saturated and incorporated in body tissues. Despite lipid biohydrogenation, a proportion of dietary PUFAs bypasses the rumen intact and is available for absorption and subsequently deposition in muscle and adipose tissue (Wood *et al.*, 2008; Kott *et al.*, 2010).

Unsaturated fatty acids in diets can easily undergo oxidation. Hence, antioxidants are added to rations to help prevent this process (Smith *et al.*, 2007). A diet supplemented with antioxidants also enables these substances to enter the circulation system of the ruminant and be distributed and retained in body tissues (Simitzis & Deligeorgis, 2010). Dietary supplementation with antioxidants may effectively control the loss of desirable meat colour, lipid oxidation and accumulation of metmyoglobin of beef meat (Morrissey *et al.*, 1994; Brannegan, 2000; Velasco & Williams, 2011).

The increasing preference for natural food products has obliged the food industry to include more natural substances to improve its dietary quality and nutritional value. By adding natural antioxidants (i.e. Vitamin E and flavonoids) and replacing synthetic antioxidants in various

products and feeds is a more natural approach to delay nutrient oxidative degradation (Velasco & Williams, 2011). Synthetic antioxidants can inhibit lipid oxidation in feed, but they exhibit toxic properties like carcinogenicity, resulting in strict regulations over their use in foods (Haak *et al.*, 2006). These findings, together with increased resistance to the use of synthetic additives, have increased the interest in the properties of naturally occurring antioxidants (Haak *et al.*, 2006; Simitzis & Deligeorgis, 2010).

The discoloration rate of meat is believed to be related to the effectiveness of oxidative processes and enzymatic reducing systems in controlling metmyoglobin levels in meat (Morrissey *et al.*, 1994). Unsaturated fatty acids make lipids susceptible to oxygen attack with negative implications on meat quality and consumer health due to lipid peroxidation (Simitzis & Deligeorgis, 2010). Apart from the reduction of PUFAs, fat-soluble vitamins and pigments, oxidation also leads to off-flavours and odours, the accumulation of peroxides and aldehydes (which may be toxic), ultimately lowering consumer acceptability (Morrissey *et al.*, 1994, Simitzis & Deligeorgis, 2010). The oxidative stability of meat depends on the balance of antioxidants and the composition of oxidative substrates (PUFAs, cholesterol, proteins and pigments) (Haak *et al.*, 2006).

With the increasing raw material price and demand for meat, it is thus even more important to focus on the oxidative status of lipids and maintain high lipid meat quality. Research regarding the effect of dietary lipid saturation together with a natural antioxidant source on animal production, carcass fatty acid quality and meat stability of lambs seems to be limiting. Therefore, the aim of this study was to investigate the influence of a natural bioflavonoid antioxidant and fatty acid saturation in a standard feedlot diet on the production performance, oxidative stability and fatty acid composition of muscle and lipid tissue of lamb.

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 dietary lipid saturation and antioxidant sources on diet digestibility is 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 repetition was inevitable.

Chapter 2

Literature review

2.1 Introduction

In the last few decades there has been increasing interest in adding supplementary lipid sources to ruminant diets in order to increase the energy density of these diets and also improve dietetic quality of the carcass and other ruminant products (Bauchart *et al.*, 1996). Accordingly there has been an increased interest to find suitable and natural ways to positively manipulate the fatty acid composition of red meat (Wood *et al.*, 2003).

Lipid oxidation is one of the most important quality deterioration processes in lipid containing foods (Liu *et al.*, 1995; Guillén & Cabo, 2002; Smith *et al.*, 2007; López-Duarte & Vidal-Quintanar, 2009; Waraho *et al.*, 2009). Jacobsen *et al.* (2008) mentioned that this process can be retarded by antioxidants, which may occur as natural constituents of foods, or which may be intentionally added. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ethoxyquin and propyl gallate have been extensively used as antioxidants in the feed industry (Haak *et al.*, 2006; Jacobsen *et al.*, 2008; Velasco & Williams, 2011). However, there is an increasing industrial interest in replacing synthetic antioxidants with natural compounds with anti-oxidative activities because of the worldwide trend to avoid or minimize the use of synthetic food additives (Jacobsen *et al.*, 2008; Velasco & Williams, 2011). Natural antioxidants include carotenoids, flavonoids and phenolic acids (Robards & Antolovich, 1997; Miranda & Buhler, 2000; Jacobsen *et al.*, 2008) and have been used in diets fed to ruminants.

The application of antioxidants for protection against oxidative flavour deterioration is particularly important in foods enriched with long chain polyunsaturated fatty acids (PUFAs) (Jacobsen *et al.*, 2008). This is because PUFAs, due to their unsaturated nature, are more susceptible to lipid oxidation than less unsaturated lipids (Jacobsen *et al.*, 2008).

This literature review was an extensive study on all aspects of supplementary lipid sources in ruminant diets and the effect of synthetic and natural antioxidants in such diets.

2.2 Lipids

Lipids (more commonly known as fats) are a class of non-polar (not soluble in water) organic substances (Enig & Fallon, 1999; Vander *et al.*, 2001; Zamora, 2005). Lipids can be divided into four subclasses: fatty acids, 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 incorporated as structural components of the brain and cell membranes (Zamora, 2005).

2.2.1 Triacylglycerols

Triacylglycerols (also known as triglycerides) constitute the majority of the lipids in the body, and it is these molecules that are generally referred to as “fat” (McDonald *et al.*, 2002).

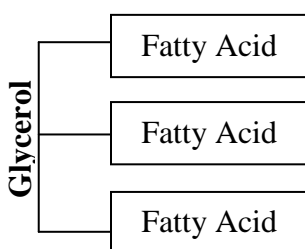


Figure 2.1 Graphical illustration of the structure of a triglyceride.

Triglycerides are the main constituents of vegetable oils and animal fats. Triglycerides have lower densities than water and at normal room temperatures they may be in a solid or liquid state, depending on the level of fatty acid saturation (Zamora, 2005). When solid, they are called "fats" and when liquid they are called "oils" (Zamora, 2005). Triacylglycerols are formed by the linking together of one glycerol molecule (a three-carbon carbohydrate) with three fatty acids as illustrated in Figure 2.1. Each of the three hydroxyl groups in glycerol is linked to the carboxyl group of a fatty acid by the removal of one molecule of water (Garrett & Grisham, 1999; Vander *et al.*, 2001).

The three fatty acids in one molecule of triacylglycerol need not be identical. Therefore, a variety of fats can be formed with fatty acids of different chain lengths and degrees of saturation (Vander *et al.*, 2001).

2.2.2 Fatty acids

As illustrated in Figure 2.2 a fatty acid is composed of a hydrocarbon chain and a terminal carboxyl group (or “head”). Most naturally occurring fatty acids have a chain of an even number of carbon atoms, ranging from 4 to 28 (Garrett & Grisham, 1999; Vander *et al.*, 2001). Fatty acids are usually derived from triglycerides or phospholipids. When they are not attached to other molecules, they are known as free fatty acids (FFAs). Fatty acids are important sources of fuel for body cells because their metabolism yields large quantities of energy in the form of Adenosine Triphosphate (ATP). Many cell types can use either glucose or fatty acids for this purpose. In particular, heart and skeletal muscle prefer fatty acids (Zamora, 2005).

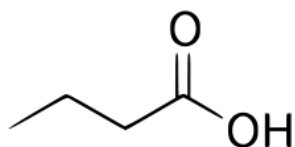


Figure 2.2 Chemical structure of the terminal carboxyl group of a fatty acid.

In Table 2.1 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).

Table 2.1 Basic information and dietary source of most commonly found fatty acids in nature (Zamora, 2005; Enig & 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 acid
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.

2.2.2.1 Saturated fatty acids

A fatty acid is termed saturated when all available carbon bonds are occupied by a hydrogen (H₂) atom. They are highly stable because all the carbon-atom linkages are filled (or saturated) with hydrogen atoms. This means that they do not normally go rancid (or oxidize easily), even when heated for cooking purposes. They are straight in form, pack together easily, and form a solid or semi-solid fat at room temperature (Enig & Fallon, 1999; Garrett & Grisham, 1999; Hickman *et al.*, 2001; Vander *et al.*, 2001). The body can produce its own saturated fatty acids from carbohydrates.

2.2.2.2 Monounsaturated fatty acids

Monounsaturated fatty acids (MUFAs) have one double bond between two carbon atoms and, therefore, lack two hydrogen atoms (Enig & Fallon, 1999; Garrett & Grisham, 1999; Hickman *et al.*, 2001; Vander *et al.*, 2001). Monounsaturated fats have a kink or bend at the position of the double bond so that they do not pack together as easily as saturated fats and, therefore, tend to be a liquid at room temperature. Like saturated fats, they are relatively stable. They do not go rancid easily and can be used for cooking purposes. According to Table 2.2 MUFAs most commonly found in food is oleic acid (the main component of olive oil), as well as the oils from almonds, pecans, cashews, peanuts and avocados (Enig & Fallon, 1999).

2.2.2.3 Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFAs) have two or more pairs of double bonds between each pair of carbon atoms and, therefore, lack four or more hydrogen atoms (Enig & Fallon, 1999; Garrett & Grisham, 1999; Hickman *et al.*, 2001; Vander *et al.*, 2001). The two most commonly found PUFAs in food products are linoleic acid [also called omega-6 (*n*-6); Figure 2.3; Table 2.1] and α -linolenic acid [also called omega-3 (*n*-3); Figure 2.4; Table 2.1].

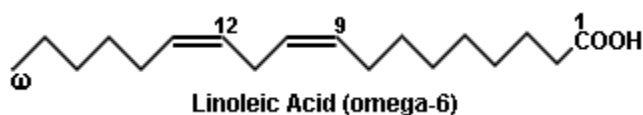


Figure 2.3 Chemical structure of an omega-6 (*n*-6) fatty acid.

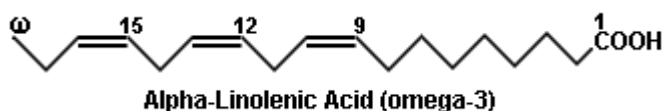


Figure 2.4 Chemical structure of an omega-3 (*n*-3) fatty acid.

Mammals cannot produce PUFAs and hence are termed essential fatty acids (Enig & Fallon, 1999; Zamora, 2005). 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, and must be treated with care. In nature, PUFAs are usually found in the *cis*- form (Enig & Fallon, 1999).

All fats and oils, whether of vegetable or animal origin, are composed of a combination of saturated, mono- and polyunsaturated fatty acids. In general, animal fats such as butter, lard and tallow contains about 40-60% saturated fats (Enig & Fallon, 1999). Table 2.2 presents the fatty acid composition of some commonly found fats and oils.

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

Oil or Fat (% of total fatty acid content)	Unsaturated/Saturated (ratio)	Saturated					Mono-unsaturated	Poly-unsaturated	
		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 (C18:2n-6)	α -Linolenic acid (C18:3n-3)
Beef Tallow	0.9	-	-	3	24	19	43	3	1
Butterfat (cow)	0.5	3	3	11	27	12	29	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 are used.

2.2.2.4 Classification of fatty acids according to carbon chain length

Fatty acids are not classified only according to their degree of saturation but also by their carbon chain length:

Short-chain fatty acids contain four to six carbon atoms. These fatty acids are always saturated. Examples of these fatty acids are shown in Table 2.1. These fatty acids contain antimicrobial properties (Enig & Fallon, 1999).

Short-chain fatty acids are the products of anaerobic microbial fermentation of complex carbohydrates in the fore-stomach of ruminant species and large intestine (McDonald *et al.*, 2002). They are formed principally from polysaccharide, oligosaccharide, protein, peptide and glycoprotein precursors by anaerobic micro-organisms. In quantitative terms carbohydrates are the most important short-chain fatty acid progenitors (Macfarlane & Macfarlane, 2003; Aluwong *et al.*, 2010). In ruminants short chain fatty acids can be absorbed very rapidly from the lumen of the intestine directly into the portal blood stream (McDonald *et al.*, 2002). Ruminants depend on short chain fatty acids for up to 80% of their maintenance energy requirements (Aluwong *et al.*, 2010).

Medium-chain fatty acids have 8 to 12 carbon atoms and are found mostly in butterfat and tropical oils (Table 2.1). Like short-chain fatty acids these fats have antimicrobial properties, are absorbed directly into the portal circulation and transported to the liver for rapid oxidation (quick energy), and may contribute to the health of the immune system (Enig & Fallon, 1999; St-Onge & Jones, 2002).

Long-chain fatty acids contains from 14 up to 18 carbon atoms and can either be saturated, mono- or poly-unsaturated. Stearic acid (C18:0) is an 18-carbon saturated fatty acid found mainly in beef and mutton tallow's (Table 2.1) and is the primary determinant of fat hardness (Wood *et al.*, 2003; Smith *et al.*, 2009). Long-chain fatty acids are transported via chylomicrons into the lymphatic system, allowing for extensive uptake into adipose tissue (St-Onge & Jones, 2002).

Oleic acid is an 18-carbon (C18:1) MUFA which is the main fatty acid of olive oil. Oleic acid is primarily responsible for soft fat in meat and it is positively correlated with palatability of beef (Smith *et al.*, 2009). Smith *et al.* (2009) found that approximately 5% of the total fatty acids in beef are comprised of PUFAs, by far the most abundant of which is linoleic acid (C18:2). Conjugated linoleic acid (CLA) is also present in meat and milk from ruminant animals and is formed through the isomerization of linoleic acid (C18:2) by ruminal bacteria (Enig & Fallon, 1999; Smith *et al.*, 2009). Enig & Fallon (1999) reported that CLA have strong anticancer properties, encourages the build-up of muscle and prevents weight gain. Another important fatty acid is palmitoleic acid (C16:1) which has strong antimicrobial properties and is found almost exclusively in animal fats.

Very-long-chain fatty acids contain 20 to 24 carbon atoms. They tend to be highly unsaturated, with four, five or six double bonds (Enig & Fallon, 1999). Some of the most important very-long-chain fatty acids are dihomo-gamma-linolenic acid (C20:3), arachidonic acid (AA; C20:4), eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6). All of these, except DHA, are used in the production of prostaglandins. In addition, AA and DHA play an important role in the function of the nervous system (Enig & Fallon, 1999).

2.2.3 Phospholipids

Phospholipids are important components of the molecular organization of tissue, especially membranes (Hickman *et al.*, 2001). They resemble triglycerides in structure, except that one of the three fatty acids is replaced by phosphoric acid and an organic base (Garrett & Grisham, 1999; Hickman *et al.*, 2001; Vander *et al.*, 2001).

They are found in all plants and animals and include such substances as lecithin, cephalin and sphingomyelin. Lecithin, also called phosphatidylcholine, is a significant constituent of brain and nervous tissue consisting of a mixture of stearic-, palmitic-, and oleic acids linked to the choline ester of phosphoric acid. The chemical structure of dipalmitoyl lecithin is typical of the phosphatides found in the brain, lung, and spleen tissue (Zamora, 2005).

2.2.4 Steroids

Steroids are complex alcohols. Although they are structurally unlike fats, they have fatlike properties. Four interconnected rings of carbon atoms form the skeleton of all steroids (Garrett & Grisham, 1999; Hickman *et al.*, 2001; Vander *et al.*, 2001). Figure 2.5 indicate examples of the chemical structure of steroids found, i.e. cholesterol, Vitamin D, many adrenocortical hormones and the sex hormones (Hickman *et al.*, 2001; Vander *et al.*, 2001).

Sterols of vegetable origin are called phytosterols. They have the same basic structure as cholesterol, but differ in the side chains attached to carbon 17. Phytosterols, such as stigmasterol (Figure 2.5) from soybean oil, are of current interest because they lower blood cholesterol levels. Sterols that are fully saturated are called stanols (Zamora, 2005).

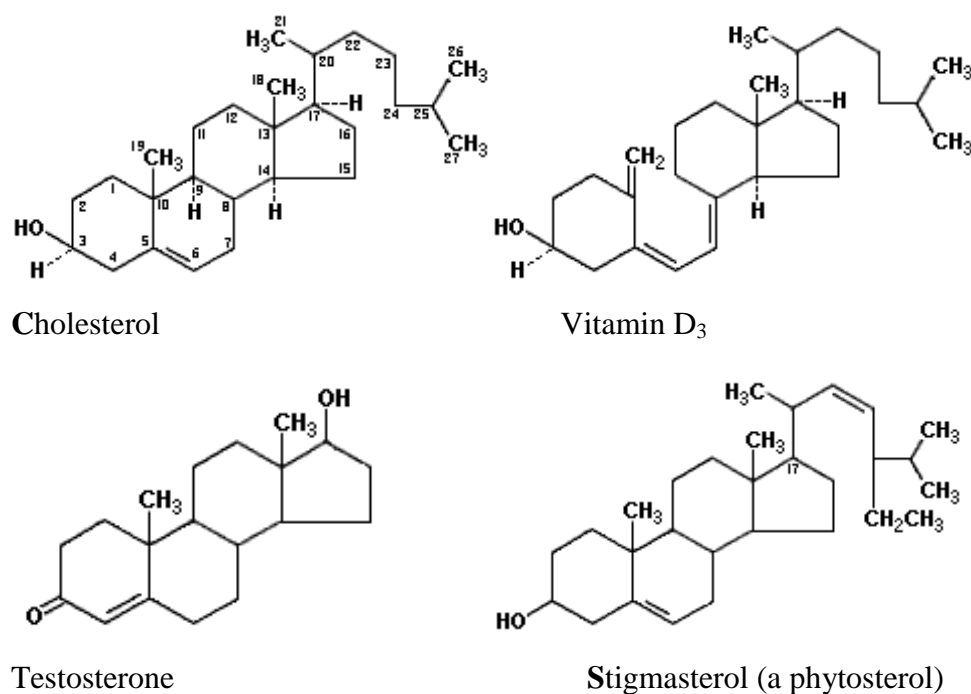


Figure 2.5 Chemical structures of some of the most important steroids.

2.2.5 Properties of lipids

2.2.5.1 Hydrolysis

Lipids may be hydrolysed by boiling with alkalis to give glycerol and soap (sodium- and potassium salts of the fatty acids). This process (lipolysis) may take place naturally under the influence of enzymes known as lipase (McDonald *et al.*, 2002). Under natural conditions the products of lipolysis are usually a mixture of mono- and di-acylglycerol with FFAs. Most of these fatty acids are odourless and tasteless, but some of them (i.e. butyric- and caproic acid) have extreme taste and smells. The lipases are mostly derived from bacteria and moulds, which are chiefly responsible for spoilage (rancidity) of fat (McDonald *et al.*, 2002).

2.2.5.2 Lipid Oxidation

Oxidation of fat is the process where fatty acids are broken down by oxygen to give shorter chain products, including free radicals which attack other fatty acids 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 (McDonald *et al.*, 2002). The products of oxidation include shorter chain fatty acids, fatty acid 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.*, 2002).

Previous reports (Liu *et al.*, 1995; Guillén & Cabo, 2002; Smith *et al.*, 2007; López-Duarte & Vidal-Quintanar, 2009; Waraho *et al.*, 2009) have shown that lipid oxidation may lead to a loss in sensory quality (rancid flavours, change of colour and texture), and nutritional quality (essential fatty acids and vitamins) of food, which eventually could result in health risks (toxic compounds, growth retardation and heart disease). Lipid oxidation also destroys the membrane structure, disturbs transport processes and causes loss in the function of the cell organelles. Red muscles are more susceptible to oxidative deterioration because the lipid content of red fibres is appreciably higher than that of white fibres (Simitzis & Deligeorgis, 2010).

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 & Cabo, 2002; López-Duarte & Vidal-Quintanar, 2009; Waraho *et al.*, 2009). Buckley & 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 polyunsaturated fatty acid (PUFA) present in the particular muscle system. MUFA is more resistant to oxidative modification than PUFA (Bonanome *et al.*, 1992; Frémont *et al.*, 1998). It is now generally accepted that the phospholipids present in the sub-cellular membranes (microsomes and mitochondria), rather than the triacylglycerols, are responsible for the initial development of oxidized flavours in raw and cooked meat during storage (Buckley & Morrissey, 1992). Fat oxidation is temperature-sensitive and concentration of intermediates can be raised by lowering or increasing sample temperature (López-Duarte & Vidal-Quintanar, 2009).

2.2.5.3 Antioxidant content of lipids

Fats possess a certain degree of resistance to oxidation, owing to the presence of compounds called antioxidants (McDonald *et al.*, 2002). Antioxidants prevent oxidation of unsaturated fat until they themselves have been transformed into inert products. The most important natural antioxidant is fat-soluble vitamin E, which protects fat by preferential acceptance of free radicals (McDonald *et al.*, 2002).

2.2.5.4 Hydrogenation

The industrial hydrogenation process comprises of an isomerisation reaction, where H₂ is added to the double bond of the unsaturated acid of a fat, thereby converting them to their saturated (single bond) analogues (Bauman *et al.*, 1999; Enig & Fallon, 1999; McDonald *et al.*, 2002). This process is known as hardening, and is an important process for producing firm and hard lipids from vegetable and fish oils in the manufacturing of margarine (McDonald *et al.*, 2002).

The lipid composition of forages consists largely of glycolipids and phospholipids, and the major fatty acids are the unsaturated fatty acids α -linolenic- and linoleic acid. The lipid composition of seed oils used in concentrate feedstuffs is predominantly triglycerides containing linoleic- and oleic acid as the predominant fatty acids (Bauman *et al.*, 1999). Dietary lipids consumed by ruminants first undergo hydrolysis in the rumen and this is followed by progressive

hydrogenation of unsaturated free fatty acids (mainly linoleic- and α -linolenic acid) to stearic acid (Bauman *et al.*, 1999; Jenkins & Lundy, 2001; McDonald *et al.*, 2002).

Rumen hydrogenation (or biohydrogenation) of dietary lipids is responsible for the production of *trans*- fatty acids as well as high levels of SFAs in body fat of ruminants (Bauman *et al.*, 1999; Enig & Fallon, 1999; McDonald *et al.*, 2002), a feature considered undesirable for human health (Bauman *et al.*, 1999). Consumption of hydrogenated fats is associated with a host of other serious diseases, not only cancer but also atherosclerosis, diabetes, obesity, immune system dysfunction, low-birth-weight babies, birth defects, decreased visual acuity, sterility, difficulty in lactation and problems with bones and tendons (Enig & Fallon, 1999; McDonald *et al.*, 2002).

Healthy CLA is produced by different bacterial species in the rumen through the isomerisation of linoleic acid, but also through endogenous synthesis from vaccenic acid via $\Delta 9$ -desaturase enzymes (Bauman *et al.*, 1999; Radunz *et al.*, 2009). After the isomerisation of linoleic acid, CLA isomers are rapidly hydrogenated to vaccenic acid, then less rapidly to stearic acid, thus resulting in an increase in ruminal vaccenic acid (Bauman *et al.*, 1999). CLA-isomers such as *cis*-9,*trans*-11-CLA are commonly formed in the hydrogenation process of linoleic acid while isomers such as *cis*-12,*trans*-10-CLA are also formed (Fellner *et al.*, 1997), but are less common and usually formed as a result of changes in the rumen environment after feeding high concentrate diets (Bauman *et al.*, 1999).

2.2.6 Evaluation of the nutritional value of lipid in animal tissue

Fatty acid composition and cholesterol levels in meat play an important role in human health and meat product quality. Apart from the type of fatty acid present in the edible tissues, the ratios of PUFA:SFA (P/S) and *n*-6:*n*-3 are also widely used to evaluate the nutritional value of fat and deemed to be important (Orellana *et al.*, 2009). From a consumer health viewpoint the recommended ratio of *n*-6:*n*-3 is below 4.0 in the muscle tissue (Wood *et al.*, 2003), while the recommended value for the P/S ratio is 0.4 or higher (De la Fuente *et al.*, 2009).

Wood *et al.* (2008) compared the fatty acid composition and total fatty acid content of subcutaneous adipose tissue with *M. longissimus* muscle from loin chops or steaks of pigs, sheep

and cattle purchased at retail stores (Table 2.3). Table 2.3 indicates that pigs have much higher proportions ($P < 0.05$) of favourable linoleic acid in both adipose and muscle tissues than cattle and sheep, but much less of the unfavourable myristic- and stearic fatty acids. Sheep muscle contains significant proportions of α -linolenic acid compared to that of pigs and cattle muscle (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)]

Fatty acid	Symbol	Adipose tissue (g/100g)			Muscle (g/100g)		
		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	C16:1 <i>cis</i>	2.4 ^a	2.4 ^a	6.2 ^b	2.7 ^b	2.2 ^a	4.5 ^c
Oleic acid	C18:1 <i>cis</i> -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 -linolenic acid	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		65.3	70.6	70	2.2	4.9	3.8

^{a,b,c} Means with different superscripts in the same row differ significantly ($P < 0.05$).

* Polyunsaturated:saturated fatty acid ratio.

It is evident from the fatty acid comparisons in Table 2.3 that mutton and beef contains more of the unfavourable saturated fatty acid (SFA; especially myristic acid), less of the more favourable unsaturated fatty acids (linoleic- and α -linolenic acid), and close to the same MUFA content of pork muscle- and adipose tissue.

2.2.6.1 Fatty acids important for human and animal health

Most SFAs are assumed to be bad for human health because they are connected to various diseases such as cardiovascular disease and cancer (Wood *et al.*, 2003). Apart from the negative

assumptions associated with human health regarding SFA, Enig & Fallon (1999) mentioned that SFAs constitute at least 50% of cell membranes (giving cells the necessary stiffness and integrity), play a vital role in the health of bones (for calcium to be effectively incorporated into the skeletal structure, at least 50% of the dietary fats should be saturated). Most SFAs lower lipoprotein(a) levels (a substance in the blood that indicates proneness to heart disease), protect the liver from alcohol and other toxins, enhance the immune system, are needed for the proper utilization of essential fatty acids (elongated *n*-3 fatty acids are better retained in the tissues when the diet is rich in saturated fats), are the preferred energy substrate for the heart, and some short- and medium chain SFAs have important antimicrobial properties (Enig & Fallon, 1999).

On the other hand, unsaturated fatty acids (especially PUFAs) are perceived to be beneficial for human health because their consumption is associated with a lower risk of coronary heart disease, hypertension, type 2 diabetes, renal disease, ulcerative colitis, chronic obstructive pulmonary disease and Crohn's disease (Wood *et al.*, 2003). When the favourable fatty acid content (MUFAs and PUFAs) of animal diets is increased, it could possess the same beneficial properties as in the case in human health.

2.2.7 Lipid inclusion in ruminant diets

As mentioned before, lipid is an important component in increasing the energy density of animal diets (Bauman *et al.*, 2003), but it must be restricted to 6 to 7% of dietary dry matter in ruminant rations (Bock *et al.*, 1991; NRC, 2001; Cruywagen *et al.*, 2003; Wistuba *et al.*, 2006). Wistuba *et al.* (2006) reported that a diet containing 3% added oil (a total dietary fat content of 7.1%) had no effect on the apparent total tract digestibility of dry matter (DM), acid-detergent fibre (ADF), organic matter (OM) and nitrogen (N₂).

Jenkins & Lundy (2001) classified fat supplementation within ruminant diets based on their expected rumen response, i.e. rumen-inert, rumen active and protected fats. The term "rumen-inert" has been assigned to fats that were specifically designed to have little, if any, negative effect on feed digestibility when fed to dairy cattle. Rumen-inert fats are often dry fats, easily transported and can be mixed into diets without the need for specialized equipment. Rumen-inert

fats are often high in calcium salts of fatty acids, SFAs, or hydrogenated fats. Fats in this category have also been referred to as “bypass” fats.

Rumen-active fats include fats of animal origin (e.g. tallow and grease), plant oils (e.g. soyabean oil and canola oil), oilseeds (e.g. cottonseeds and soyabeans), and high fat by-products such as residues from food processing plants (Jenkins & Lundy, 2001).

Protected fat is most applicable to fat sources specifically designed to resist biohydrogenation by ruminal microbes and modify the fatty acid profile of body tissues and milk lipids (Jenkins & Lundy, 2001), by being made available in the lower digestive tract of ruminants. The mechanism used to protect these fats is based on surrounding unsaturated fatty acids by a protective capsule (such as formaldehyde-treated proteins) that act to shield the internal fatty acids from biohydrogenation by microorganisms. Another strategy for protection is chemical modification of unsaturated fatty acids to chemical forms that resist biohydrogenation, such as calcium-salts of fatty acids or fatty amides (mentioned above).

2.2.8 Influence of dietary lipids on animal performance

2.2.8.1 Influence of lipids on diet digestibility

Feeding fat to ruminants can cause palatability problems which in turn may affect apparent DM digestibility (Johnson & McClure, 1973) in a negative manner, and could increase crude protein (CP) apparent digestibility (Cruywagen *et al.*, 2003). The primary effect on digestibility may be ascribed to the presence of either saturated- or unsaturated long chain fatty acids in the rumen which may modify microbial populations and alter rumen fermentation and digestibility (Harris, 2003). Unsaturated fatty acids appear to have more adverse effects on rumen fermentation than do saturated fats, because they are antimicrobial probably because of a toxic effect of long-chain fatty acids on ruminal bacteria (Bock *et al.*, 1991; Bauman *et al.*, 2003). Physical coating of the fibre with added fat has also been proposed as a possible theory for the sometimes observed depressed fibre digestibility (Firkins & Eastridge, 1994; Harris, 2003) by inhibiting microbial fibre fermentation (Jenkins & Lundy, 2001).

Rumen inert fat sources are usually included in ruminant diets to avoid a decreased fibre digestibility (Cruywagen *et al.*, 2003). Rumen inert lipid sources are not excluded from being hydrolysed or hydrogenated in the rumen. Rumen inertness, therefore, simply means that the lipid or fatty acid source does not alter or affect rumen fermentation (Cruywagen *et al.*, 2003).

Past research has indicated that dietary calcium levels should be increased when added fat is fed to ruminants to help alleviate the negative effects on fibre digestion in high-forage diets (>40% forage) (Bock *et al.*, 1991). In addition, the chemical and physical form of lipid sources may affect diet digestibility. For hydrogenated fats, the higher the iodine value and the ratio of palmitic:stearic fatty acids, the better is the fatty acid digestibility (Firkins & Eastridge, 1994; Jenkins & Lundy, 2001).

2.2.8.2 Influence of fats on animal production

Feeding fats differing in origin and degree of saturation to ruminants has resulted in a variety of responses in ruminant production. Most commonly added lipid sources may improve the average daily gain (ADG) and feed efficiency (due to increased energy content; McDonald *et al.*, 2002), which could also result in an increased fat deposition of animal tissues in comparison to non-supplemented diets (Brandt & Anderson, 1990).

Jenkins & Lundy (2001) reported that adding additional fat to dairy rations can affect productive efficiency of dairy cows through a combination of caloric and non-caloric effects. Caloric effects are attributable to greater energy content and energetic efficiency for lipids compared to carbohydrate or protein with the overall benefit being increased milk production (Jenkins & Lundy (2001). Non-caloric effects are caused by benefits from added lipid that are not directly attributable to its energy content or increased milk production. Examples of proposed non-caloric effects include improved reproductive performance, and altered fatty acid profile of milk (Jenkins & Lundy, 2001).

Methane (CH₄) production is depended on the volatile fatty acids (VFAs) produced from carbohydrate fermentation in the rumen (McAllister *et al.*, 1996; McDonald *et al.*, 2002). Some researchers (McAllister *et al.*, 1996; Rasmussen & Harrison, 2011) mentioned that when cattle

feed was supplemented with fat (especially rich in PUFAs and MUFAs) CH₄ emission was significantly reduced. This could be explained by the fact that PUFAs has an inhibitory effect on CH₄ production through a direct use of hydrogen by hydrolyzing bacteria in the rumen (McAllister *et al.*, 1996; Rasmussen & Harrison 2011).

Hence, not only does additional lipid increase the useable energy density of the diet (lipids contain 2.25 times more energy than carbohydrates; McDonald *et al.*, 2002), but the reduced production of ruminal CH₄ may also help improve animal performance.

2.2.9 Factors affecting carcass fatty acid composition

Meat quality depends not only on the degree of marbling, but also on its fatty acid composition (Oka *et al.*, 2002). Variation in fatty acid composition, in particular variation in saturation, affects firmness of fat, which in turn affects the economics of meat processing and consumer acceptance of meat (Perry *et al.*, 1998). Beef with the most desirable flavour has lower percentages of SFAs and PUFAs, and higher percentages of MUFAs present in the carcass fat (Westerling & Hedrick, 1979; Melton *et al.*, 1982; Oka *et al.*, 2002). Fatty acid composition of ruminant tissues is affected to some degree by factors such as animal sex (Waldman *et al.*, 1968; Clemens *et al.*, 1973), breed (Huerta-Leidenz *et al.*, 1996; Perry *et al.*, 1998; Smith *et al.*, 2009), diet (Melton *et al.*, 1982; Smith *et al.*, 2009), and animal age (Huerta-Leidenz *et al.*, 1996; Oka *et al.*, 2002).

Clemens *et al.* (1973) found that the fatty acid composition (especially myristic-, palmitoleic-, stearic- and linoleic acid) of ruminant (bulls and steers) adipose tissue differed with respect to the age of the animal. Waldman *et al.* (1968) found that steer fat depots had higher concentrations of SFAs, where heifers contained higher amounts of unsaturated acids.

Breed types differ in their ability to accumulate certain fatty acids (especially MUFAs) in their adipose tissues (Smith *et al.*, 2009). For instance, Huerta-Leidenz *et al.* (1996) reported that subcutaneous adipose tissue from Brahman cows and steers contains a greater proportion of MUFAs (especially oleic acid) and less SFAs (especially palmitic acid), than adipose tissue from Hereford steers when the cattle are raised under identical production systems.

Huerta-Leidenz *et al.* (1996) explained that the carcass percentages of oleic- and linoleic acid increased with animal age and weight, whereas a decrease was observed for the percentage of α -linolenic acid. Although more of an indirect effect, as animals grow older the MUFA content of carcass fat may increase markedly, the PUFA content slightly (due to the increase in linoleic acid), and the SFA content declines in the carcass (Huerta-Leidenz *et al.*, 1996). The age and 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 fatty acids (Smith *et al.*, 2009).

The carcass fatty acid composition may also differ depending on the deposit sites (Oka *et al.*, 2002). Oka *et al.* (2002) found that the subcutaneous adipose tissue lipid content of steers had higher percentages of myristoleic-, palmitoleic-, and oleic acids, a higher oleic:stearic acid ratio, and lower percentages of stearic acid than lipid from other sites.

The use of supplemental fat (or rumen inert fat like calcium-salts; Scollan *et al.*, 2003) may also affect the composition of depot fat in ruminants, depending on the degree of hydrogenation (Brandt & Anderson, 1990). Some examples of these added fats which could positively affect the ruminant's carcass fatty acid composition include supplementary oils (Wistuba *et al.*, 2006; Dikeman, 2007) (like saturated tallow, and more unsaturated soyabean- and sunflower oils), or by adding any specific fatty acid to the diet (i.e. a CLA concentrate; Dikeman, 2007).

Grasses and pasture plants contains different concentrations of PUFAs which may increase the carcass PUFAs content when fed to ruminants (Wood *et al.*, 2008). Manipulating (preventing) rumen PUFAs from biohydrogenation is another way of increasing the availability of these fatty acids in the lower gastrointestinal tract. One way of manipulating the biohydrogenation process in the rumen is by the use of antibiotic additives. As the production of H₂ decreases in the rumen as certain bacteria are inhibited by ionophore action, it is possible that ionophores can interfere with bacterial species responsible for lipid biohydrogenation (Fellner *et al.*, 1997). However there is little information regarding the effects of ionophores on rumen lipid metabolism.

2.3 Antioxidants

Antioxidants are chemical substances that can prevent damage to cells induced by free radicals and other oxidative processes (Percival, 1998; Flora, 2009). Antioxidants are found in a variety of foods, especially in brightly coloured fruits and vegetables. Vitamins, such as vitamin E and C, are also regarded as antioxidants (Flora, 2009; Velasco & Williams, 2011).

These substances are capable of stabilizing or deactivating free radicals before they attack cells and are absolutely critical for maintaining optimal cellular and systemic health and well-being (Percival, 1998). Antioxidants commonly used in the feed industry are characterised as chemical or biological antioxidants.

2.3.1 Chemical antioxidants

Artificial- or chemical antioxidants are added to commercial food products and feedstuffs produced for animals, primarily to inhibit nutrient oxidation (Brannegan, 2000). Hundreds of chemicals have been tested, only a few are suitable for use in preventing undesirable oxidations in feedstuffs, finished feeds, and in the guts and carcasses of animals. Ramsey (1980) has reported that for an antioxidant to be useful in animal feeding it must be effective in preserving animal and vegetable fats from oxidative destruction, must be non-toxic to man and farm animals, should be effective at very low concentrations and must be economically practical to include in animal diets.

2.3.1.1 Chemical antioxidants in animal nutrition

In the past chemical antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and ethoxyquin and gallates were extensively used. These antioxidants were intended to delay, retard or prevent the negative effects of lipid peroxidation by scavenging chain-carrying peroxy radicals or diminishing the formation of initiating lipid radicals (Simitzis & Deligeorgis, 2010).

Sodium citrate (monosodium-, disodium- and trisodium citrate) is a well-known antioxidant used in food, as well as to improve the effects of other antioxidants. It is commonly found in gelatine products, jam, sweets, ice cream, carbonated beverages, milk powder, wine and processed

cheeses (ANON, 2011a). Table 2.4 contains some of the commonly used chemical antioxidants that are considered safe to use in animal feeds.

Ethoxyquin (1,2 dihydro-6-ethoxy-2,2,4- trimethy quinoline) (Figure 2.6) is a common primary antioxidant used to protect a variety of unsaturated hydrocarbon systems (Ramsey, 1980; Brannegan, 2000). The most common feeds where ethoxyquin is included are in fishmeal and fish oils, but it is also found in other oils, fats, and meat meals. Brannegan (2000) stated that the purpose of ethoxyquin is to protect lipids and preserve carotene and vitamins A and E, and may also prevent the spontaneous combustion of stored food products by inhibiting the heat production caused by oxidation of lipids.

Table 2.4 Commonly used chemical antioxidants that are generally regarded safe to use in animal feed (Ramsey, 1980)

Ascorbic acid	Propionic acid
Ascorbyl palmitate	Propyl gallate
Benzoic acid	Propyl paraben
BHA	Resin guaiac
BHT	Sodium ascorbate
Calcium ascorbate	Sodium benzoate
Calcium propionate	Sodium bisulphite
Calcium sorbate	Sodium metabisulphite
Citrate acid	Sodium nitrite
Dilauryl thiodipropionate	Sodium propionate
Distearyl thiodipropionate	Sodium sorbate
Erythorbic acid	Sodium sulphite
Ethoxyquin	Sorbic acid
Formic acid	Stannous chloride
Methylparaben	Sulphur dioxide
Potassium bisulphite	THBP - Trihydroxy-butyrophenone
Potassium metabisulphite	TBHQ - Tertiary-buthylhydroquinone
Potassium sorbate	Tocopherols

Ethoxyquin is also used to preserve the red colours of many spices such as paprika and chilli powder, and as a post-harvest dip for apples and pears to inhibit brown spots (Brannegan, 2000).

Ethoxyquin, however, has been demonstrated to be the most efficacious, followed closely by BHT and BHA in animal diets.

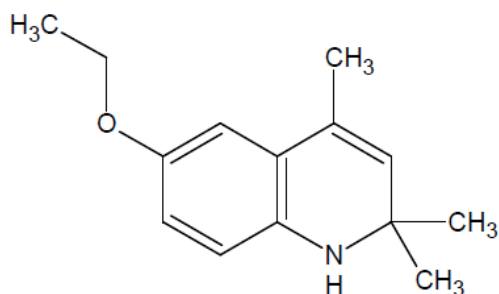


Figure 2.6 Chemical structure of Ethoxyquin (Brannegan, 2000).

BHA and BHT are perhaps the most extensively used antioxidants in the food industry. BHA is used in fats and oils, fat-containing foods, confectioneries, essential oils, food-coating materials, and waxes. It is used in low-fat foods, fish products, packaging materials, paraffin, and mineral oils. BHT is also widely used in combination with other antioxidants such as BHA, propyl gallate, and citric acid for the stabilization of oils and high-fat foods (Madhavi *et al.*, 1996). BHA is a mixture of two isomers, 2-tert-butyl-4-hydroxyanisole (2-BHA) and 3-tert-butyl-4-hydroxyanisole (3-BHA), with the commercial compound containing 90% of the 3-isomer. Ingested BHA is easily absorbed from the gastrointestinal tract, rapidly metabolized and completely excreted (Madhavi *et al.*, 1996). Grinstead (2005) reported that BHT is also readily absorbed through the gastrointestinal tract and slightly through intact skin. It is then metabolized and excreted primarily in the urine and to a lesser degree in the faeces. BHT can accumulate in adipose tissue if animals are exposed to it for a prolonged period (Grinstead, 2005).

Many commercially available antioxidants are composed of mixtures of BHA, BHT, ethoxyquin and trisodium citrate and are suitable to be used in animal feeds and pet foods (Hilton, 1989). As mentioned above, the primary objective for the inclusion of these commercial products in animal nutrition is to stabilize and extend the shelf life of feed fats and vitamins, prevent rancidity and odours, and chelating with some metal ions to still maintain the availability of the micro-elements for livestock and pets (Hilton, 1989).

The inclusion rate of these commercial products is determined by the fat content and -quality in the animal feed that is being formulated. Therefore, the inclusion rate recommended within many product data sheets may vary from 125 g/ton (diets with <10% fat) to 300 g/ton (diets with >25% fat content). In short, Ramsey (1980) mentioned that the inclusion levels of chemical antioxidants in finished feed may vary from 150 g/ton (ethoxyquin) to 200 g/ton (BHT and BHA).

2.3.2 Biological antioxidants

Natural- or biological antioxidants are various substances with different chemical characteristics, which are widely present in plants. As with chemical antioxidants, these natural antioxidants retard or inhibit oxidation of other substances by inhibiting the initiation or propagation of oxidizing chain reactions (Karami *et al.*, 2010a; Velasco & Williams, 2011). Consequently, natural antioxidants can protect the biologically important cellular components from oxidative processes caused by reactive oxygen species (ROS) (Velasco & Williams, 2011). The total antioxidant capacity of fruit and vegetable extracts depends on the concentrations of ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), beta-carotene (a vitamin A precursor), various flavonoids, and other phenolic compounds (Flora, 2009; Velasco & Williams, 2011).

Flavonoids are widely distributed in the plant kingdom (Jaakola, 2003; Prior *et al.*, 2006). Flavonoids act in the plant as an antioxidant, serving the role of antimicrobials, photoreceptors (Middleton *et al.*, 2000; Jaakola, 2003), visual attractors, feeding repellents, light screening (Robards & Antolovich, 1997; Middleton *et al.*, 2000; Karami *et al.*, 2010a) and are also involved in many biological interactions (Middleton *et al.*, 2000; Jaakola, 2003).

The basic structure of flavonoids is shared by tocopherols (vitamin E). More than 5 000 flavonoids have already been identified and described, many of them being common in higher plants (Robards & Antolovich, 1997; Middleton *et al.*, 2000; Jaakola, 2003; Prior *et al.*, 2006).

2.3.2.1 The role of flavonoids in the (animal) body

Flavonoids serve as important micro-nutrients in human and animal diets (Robards & Antolovich, 1997; Winkel-Shirley, 2001). According to Middleton *et al.* (2000) these flavonoids

have the property of decreasing capillary permeability (and fragility) and reducing signs of hypovitaminosis C in animals. There is nonetheless a strong indication that flavonoids have a potent antioxidant and vitamin C sparing activity in the animal body.

After flavonoids has been absorbed from the gastrointestinal tract of animals they are excreted either unchanged or as flavonoid metabolites in the urine and faeces (Robards & Antolovich, 1997; Havsteen, 2002). Hollman (2004) reported that only 0.1% to 3.6% of the ingested dietary quercetin was excreted as quercetin conjugates in urine. No literature could be found that indicate the levels of flavonoids that are absorbed and stored in the animal body.

2.3.2.1.1 Inhibition of nutrient oxidation

Several researchers (Robards & Antolovich 1997; Frémont *et al.*, 1998; Miranda & Buhler, 2000) have reported that flavonoids protect low-density lipoprotein (LDL) (LDL prevents atherosclerotic plaque formation) from oxidation in the living animal. Flavonoids also have the ability to scavenge reactive oxygen and nitrogen species *in vitro*, chelate metal ions, inhibit redox-sensitive transcription factors (e.g. nuclear factor-*n*B) and inhibits the expression of free radical-generating enzymes (e.g. inducible nitric oxide synthase), hence, functioning as strong antioxidants (Frank *et al.*, 2007). The intake of PUFAs at higher levels than those required to prevent a deficiency may have beneficial effects (see paragraph 2.2.6.1) on risk factors associated with coronary heart disease (Goodnight *et al.*, 1982; Frémont *et al.*, 1998), but this high intake of PUFAs may lead to increased oxidative degradation of these fatty acids within body cells (Bonanome *et al.*, 1992) and can lead to the formation of modified atherogenic LDL (Frémont *et al.*, 1998). In plasma, circulating LDL is protected from the effects of lipid peroxidation by several antioxidants solubilised in their aqueous environment (Thomas *et al.*, 1995). Other than antioxidants, another dietary means of reducing the susceptibility of LDL to lipid peroxidation is the partial substitution of dietary PUFAs by MUFAs. MUFAs are more resistant to oxidative modification than PUFAs (see paragraph 2.2.5) (Bonanome *et al.*, 1992; Frémont *et al.*, 1998). Flavonoids are also shown to protect organ tissues from oxidative substances within animal bodies (Boyer & Liu, 2004).

2.3.2.1.2 Cholesterol effects

Ingestion of flavonoids added to certain feed products fed to animals (especially citrus fruits or juices) has shown to decrease levels of plasma and liver cholesterol, plasma triglycerides, as well as the circulating low-density lipoprotein:high-density lipoprotein (LDL:HDL) ratio in animals (Morin *et al.*, 2008).

2.3.2.1.3 Phytoestrogens

Isoflavones (a phytoestrogen; Lampe, 2003), although boasting antioxidant activity (Yousef *et al.*, 2004; Jiang *et al.*, 2007), are ubiquitous, non-steroidal, plant-derived compounds exhibiting both oestrogen agonist and antagonist activities and are found primarily in members of the *Leguminosae* family and foods such as soyabeans, lentils, beans and chickpeas (Vera *et al.*, 2005). However, soyabeans are one of the few foods that contain appreciable amounts of isoflavones (Yeung & Yu, 2003; Vera *et al.*, 2005) primarily in the form of various β -glycoside conjugates.

An infertility syndrome of sheep is recognized to be caused by ingestion of certain species of clover containing phytoestrogens (isoflavonoids), formononetin and biochanin, which is transformed by gut micro flora to equol (Middleton *et al.*, 2000). Equol, a nonsteroidal oestrogen, has oestrogenic properties and is absorbed into the circulation and it antagonizes estradiol-17- β binding to cytoplasmic oestrogen receptors resulting in decreased fertility (Rüfer *et al.*, 2005).

2.3.2.1.4 Vitamins

Flavonoids improve the absorption and utilization of Vitamin C and protect the vitamin molecule from oxidation (ANON, 2010). The bioavailability of vitamin C is enhanced when adequate amounts of flavonoids are present, and in turn, the bioavailability of flavonoids are enhanced when adequate vitamin C is present (ANON, 2010). Flavonoids also assist vitamin C in keeping collagen in a healthy condition.

2.3.2.2 Inclusion of flavonoids in animal (ruminant) diets

The increasing preference for natural food products has obliged the food industry to start with the inclusion of more natural substances to try and improve its dietary quality and nutritional value. By adding natural antioxidants and replacing synthetic antioxidants in various products and feeds is a more natural approach to delay nutrient oxidative degradation (Velasco & Williams, 2011). Synthetic antioxidants can inhibit lipid oxidation in feed but they exhibit toxic properties (carcinogenicity) resulting in strict regulations over their use in foods (Haak *et al.*, 2006; Velasco & Williams, 2011). These findings, together with increased resistance to the use of synthetic additives, have increased interest in the antioxidant properties of naturally occurring substances (Haak *et al.*, 2006; Simitzis & Deligeorgis, 2010).

The benefit of supplying antioxidants in human and animal health has been covered in this review and by other researchers (Percival, 1998; Velasco & Williams, 2011). From the available literature only a few studies were conducted on animals (mostly on lab animals) to conclude if flavonoids have any beneficial effect on animal performance or products (Robards & Antolovich, 1997; Frémont *et al.*, 1998; Benito *et al.*, 2004).

As mentioned earlier these compounds are fed mainly to improve the animal's total antioxidant status, or for their resistance properties to oxidative degradation within feed products and added beneficial properties in the animal body (Middleton *et al.*, 2000).

2.3.3 Antioxidants and meat lipid oxidation

Dietary supplementation of antioxidants appears to be a more effective way of retarding lipid oxidation of meat products and controlling its stability compared to post mortem addition of antioxidants during processing (Buckley *et al.*, 1995; Gray *et al.*, 1996; Simitzis & Deligeorgis, 2010). An animal diet supplemented with antioxidants enables these substances to enter the circulation system and be distributed and retained in tissues. Simitzis & Deligeorgis (2010) reported that the available hydrogen atoms from phenol and allylic groups react with lipid and hydroxyl radicals and convert them into stable products, protecting meat products against the primary oxidative process. At the same time, no negative implications on meat quality properties such as pH, colour, water holding capacity, and tenderness have been observed. Ramsey (1980)

reported that an antioxidant can block this peroxidation by supplying hydrogen in the first free radical formed, thereby reconvertng it to the original fatty acid. If the hydroperoxides are allowed to form, they continue to decompose by breaking down into a variety of aldehydes and ketones.

Vitamin E is the primary lipid-soluble antioxidant in biological systems and breaks the chain of lipid peroxidation in cell membranes and prevents the formation of lipid hydroperoxides (Buckley *et al.*, 1995; Gray *et al.*, 1996).

Natural antioxidants appear to be an alternative to synthetic additives in the meat industry. However, all natural antioxidants aren't as equally effective (Payne *et al.*, 2001).

2.3.3.1 Influence of dietary fat on meat lipid oxidation

Lipid oxidation is a major deterioration reaction which results in loss of meat quality (Buckley *et al.*, 1995). The susceptibility of muscle tissue to lipid oxidation depends on a number of factors, the most important being the level of PUFA present in the particular muscle system (Buckley *et al.*, 1995). Simitzis & Deligeorgis (2010) reported that the unsaturation of fatty acids makes lipids susceptible to oxygen attack with negative implications on meat quality and consumer health due to lipid peroxidation. Phospholipids are more susceptible to oxidation than triglycerides and cholesterol esters because of their high content of PUFA (Buckley *et al.*, 1995; Smith *et al.*, 2007).

Oxidative deterioration leads to the production of hydroperoxides, which are susceptible to further oxidation or decomposition to secondary reaction products such as short-chain aldehydes, ketones and other oxygenated compounds. These may adversely affect lipids, pigments, proteins, carbohydrates, vitamins and the overall quality of meat by causing loss of colour and nutritive value, therefore limiting shelf-life (Simitzis & Deligeorgis, 2010).

2.4 Meat quality

Meat quality cannot be defined specifically, because the concept of quality refers to the acceptability of meat and purpose for which it is intended, either for the table or for processing (Maree & Casey, 1993). Generally, meat quality refers to palatability, but may also include elements of nutritional value and food safety. In these terms Maree & Casey (1993) stated that the quality 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 or adding these compounds onto the meat surface (Velasco & Williams, 2011). Velasco & Williams (2011) and Karami *et al.* (2010b) mentioned that among the positive effects of natural antioxidants on meat characteristics is the delay of meat lipid oxidation and even microbial growth. Meat colour has been reported as the most important factor when consumers assess meat quality since they relate colour to freshness. However, colour does not correspond to differences in eating satisfaction (Carpenter *et al.*, 2001).

2.4.1 Nutritional qualities of lamb meat

Lamb is an excellent source of high quality protein and essential vitamins and minerals (Maree & Casey, 1993; ANON, 2011b). Red meat provides an ideal source of copper, manganese, selenium, iron, 45% of the daily requirement of zinc, it's a great source of B vitamins (especially thiamine) and essential fatty acids where half is unsaturated (ANON, 2011b) (see paragraph 2.2.6).

Many people are concerned with the saturated fat and cholesterol content in meat, which may result in reduced consumption (Maree & Casey, 1993). However, the inclusion of specific lipid sources and antioxidants in ruminant diets may result in healthier lamb carcasses for human consumption. One third of the fatty acids in lamb are saturated, where the remainder are in the healthier forms of MUFA and PUFA (ANON, 2011b; ANON, 2011c). Compared to other meats, lamb contains very little marbling (fat in the meat). Since lamb fat is concentrated on the edges

of the meat, it is easily trimmed off, which means fewer calories (ANON, 2011b; ANON, 2011c; Seaside, 2011).

2.4.2 Consumer preferences in terms of meat quality

The three sensory properties by which consumers most readily judge meat quality are appearance, texture, and flavour (Liu *et al.*, 1995). The most important of these is product visual appearance (colour) because it strongly influences the consumer's purchase decision (Liu *et al.*, 1995). In most parts of the world, leanness is an important criterion when consumers purchase meat (Strydom *et al.*, 2009). While a small amount of fat is desirable to sustain palatability, increase tenderness and decrease the risk of the meat drying out, too much fat is perceived as being unhealthy (Strydom *et al.*, 2009). Consumers discriminate against meat cuts that have lost their fresh appearance (oxidized nutrients, especially unsaturated fatty acids) and meat that becomes discoloured is often ground and marketed in a reduced value form (Gray *et al.*, 1996). The keeping quality of meat seems to be very important to ensure healthy and desirable cuts for the consumer.

2.4.3 Stability of meat

The oxidative stability of meat depends upon the balance of anti- and pro-oxidants, and the composition of oxidative substrates including PUFAs, cholesterol, proteins and pigments (Haak *et al.*, 2006). Jensen *et al.* (1997) mentioned factors that could have an additional influence on oxidative stability of meat, these being the degree of processing (mincing, heating) and storage conditions (time, temperature, and packaging). These degradation processes have an influence on the characteristic of meat quality like meat colour, taste and shelf life.

2.4.3.1 Thiobarbituric acid reactive substances (TBARS)

Plasma concentrations of thiobarbituric acid reactive substances (TBARS) can be used to determine the lipid peroxidation and oxidative stress within meat (Jensen *et al.*, 1997; Hugo *et al.*, 2009; Karami *et al.*, 2010a). Chemical and natural antioxidants can be used to improve oxidative stability in meat (Buckley *et al.*, 1995), ultimately leading to decreased TBARS values.

As mentioned earlier, increasing the PUFA content of meat may lead to an increased oxidation of these fatty acids leading to a rejected product by the consumer. Hugo *et al.* (2009) showed that thighs and breasts from broilers receiving fish oil had higher TBARS values compared to thighs and breasts from broilers receiving tallow, high-oleic sunflower oil or normal sunflower oil. The oxidative stability of meat could be increased by adding antioxidants to animal diets or directly to meat cuts (Karami *et al.*, 2010b).

One of the major advantages of the TBARS method is that its results are highly negatively correlated with sensory evaluation scores (Hugo *et al.*, 2009). The only TBARS standard found in literature was for raw pork (Hugo *et al.*, 2009). In terms of a rancid taste for raw pork, Buckley & Connolly (1980) found a TBARS value of 1.0 mg malonaldehyde per kilogram (kg) meat to be a good cut-off point. Jensen *et al.* (1997) has shown that only fatty acids with three or more double bonds are believed to cause faster than normal malonaldehyde formation, and although breast meat has a higher percentage of PUFAs (especially linoleic acid; C18:2) in the fat, the absolute amount of these fatty acids in thigh meat is approximately five times that of breast meat.

2.4.3.2 Meat colour

Meat colour is an important parameter in meat quality and is the main factor affecting meat product acceptability at the time of consumer purchase (Gray *et al.*, 1996; Jensen *et al.*, 1997; Guillén & Cabo, 2002; López-Duarte & Vidal-Quintanar, 2009; Karami *et al.*, 2010b). The measurement of colour, depending on the scale used, is usually presented as the resultant of three different components – such as L*, a*, b* on the CIELAB scale or L*, C*, h° on the CIELCh scale (Karamucki *et al.*, 2011). Each colour parameter has a certain relationship with quality traits, such as the contents of basic chemical components in the meat, pH, and water holding capacity (Karamucki *et al.*, 2011). The colour of meat depends on different factors such as the number of haemic 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 to metmyoglobin (MMG), which gives meat an unattractive brown colour (Karami *et al.*, 2010b;

Velasco & Williams, 2011). At a high pH, muscle has a closed structure, appears dark and the meat tends to be tough. To maintain acceptable fresh meat colour over prolonged periods of time, it is necessary to delay pigment oxidation and/or enhance reduction of oxidized myoglobin (Buckley *et al.*, 1995; Gray *et al.*, 1996). The presence of chemical- (Karami *et al.*, 2010b) or natural (Morrissey *et al.*, 1994; Gray *et al.*, 1996; Velasco & Williams, 2011) antioxidants within meat can retard meat colour loss by reducing the oxidation process, extending the red colour (a^* -values) of meat, delaying MMG formation, ultimately improving retail shelf-life (Dunshea *et al.*, 2005).

In contrast, some researchers (Carpenter *et al.*, 2001) found no advantage to adding compounds containing natural antioxidants (grape seed and bearberry extracts) on the colour parameters (lightness L^* , b^* , and a^*) of raw pork patties. The same results were obtained for fresh chicken breast meat (Chouliara *et al.*, 2007).

2.4.3.3 Meat shelf life

López-Duarte & Vidal-Quintanar (2009) defined the shelf life of foods as the time from manufacture to consumption, where a food product remains safe and wholesome under recommended conditions. The oxidative stability of oils is an important indicator of the performance and shelf life of meat (Jensen *et al.*, 1997; Guillén & Cabo, 2002; Smith, 2008), where the oxidation of unsaturated fatty acids adversely affects the colour, texture, nutritive value and safety of meat (Buckley & Morrissey, 1992).

As described earlier, the presence of chemical- (Dunshea *et al.*, 2005; Akil *et al.*, 2011) or natural (α -Tocopherylacetate; Haak *et al.*, 2006) antioxidants have been shown to be highly effective in extending the shelf-life of foods from animal origin. Some authors reported that plant extracts with antimicrobial properties can also be used to increase meat shelf life (Carpenter *et al.*, 2001).

2.4.3.4 Meat pH

The biochemical changes that accompany post-slaughter metabolism and post-mortem aging in the conversion of muscle to meat give rise to conditions whereby the process of lipid oxidation is no longer tightly controlled and the balance of pro-oxidative factors/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 energy metabolism in the muscle (Maree & Casey, 1993). Glycogen is 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 & Casey, 1993; Morrissey *et al.*, 1994).

The propensity of meat and meat products to undergo oxidation 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 is related both directly and indirectly to the ultimate pH. The ultimate pH in its turn is related to the time of onset of *rigor mortis* and the related cooling of the carcass and individual muscles, thereby to the extent of myofibrillar contraction (Maree & Casey, 1993). Muscles with high glycogen content at slaughter proceed slowly into *rigor* and have a low ultimate pH (5.4). The latter thus have a greater chance of cold shortening (Maree & Casey, 1993) resulting in tender meat.

2.5 Conclusions

It seems from the available literature that different lipid sources can alter the meat fatty acid profile. With the inclusion of antioxidants in animal feeds the effect of lipid oxidation can be slowed down to a large extent, probably further increasing the fatty acid profile of ruminants and other meat quality properties.

Chemical antioxidants are being used less due to their cost of manufacturing and the negative effect they may have on human and animal health. Natural antioxidants possess the same characteristics as chemical antioxidants, except for showing no health risk when they are used in animal diets.

From the literature cited, it is evident that much research has been conducted to show the positive effects chemical (and natural; vitamin C) antioxidants have on the nutritional and keeping quality of meat. There is, however, no research available assessing the use of natural antioxidants (bioflavonoids) for the same purposes at the same inclusion levels. Therefore, improving lamb fatty acid composition by adding a favourable lipid source and a natural antioxidant to ruminant diets warrants further research.

Chapter 3

General Material and Methods

3.1 Introduction

A production study (from March to May 2010) and digestibility study (May 2010) with lambs 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 and at 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 summer, and the onset of the autumn season. The average minimum and maximum temperatures recorded during the duration of the study were between 13.7°C and 23.2°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. 04/2010).

3.2 Experimental animals

Eighty-four (84) South African Mutton Merino (SAMM) lambs were used for the production study. The lambs, approximately three months of age and with an initial live weight of 27.64 ± 1.72 kg, were randomly allocated to four dietary treatments (21 lambs per treatment) and placed in pens in a closed but well ventilated building.

Fourteen lambs from each treatment were randomly selected at the end of the production study. Seven were used for the digestibility study, while the remaining seven were slaughtered and carcass characteristics and meat quality evaluated as later discussed.

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 practiced in the commercial feedlot sector of South Africa. The vaccine used was a 7-in-1 Clostridial plus Pasteurella vaccine (Reg. No. G3694; Act 36 of 1947) to aid in the build up of active immunisation. This vaccine also controls lamb dysentery, pulpy kidney, tetanus, blackleg, clostridial metritis (malignant oedema of the uterus), blood gut and infections caused by *Clostridium novyi* type-B. All the animals were weaned about four weeks prior to entering the trial.

All lambs were dosed against tapeworm (Reg. No. G1546; Act 36/1947), and a broad spectrum parasite remedy (Reg. No. G3548; Act 36/1947) was used against round worm, liver fluke and nasal worm. Animals were also injected with a trace mineral optimizer (Reg. No. G1852; Act 36/1947).

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 daily gain and feed efficiency 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 of the day. Facilities to weigh the lambs are shown in Figure 3.1.



Figure 3.1 Scale used to weigh the lambs.

3.3 Housing

The 21 lambs in each of the four treatments were randomly allocated to pens (n=3 lambs per pen; 2.808 m²) 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 production vary a lot, but is mostly accepted to fall within the margins of 0.6 to 1.1 m² (Færevik *et al*, 2005). This method of random allocation of the treatments reduced the probability that all animals of the same treatment were penned next to each other and that a specific treatment may have been affected either positively or negatively by environmental factors due to pen location and/or stall conditions. The elevated slatted floor allowed urine and faeces to accumulate on a concrete floor below. The adjacent constructed pens allowed animals to have visual contact with each other. However, the construction of the pens prevented access to the feed troughs by adjacent animals. The pens were separated from each other by partitions constructed of steel pipes. All pens were clearly marked with a number (Figure 3.3). The pens were properly washed and disinfected with a quaternary ammonium compound (Glutabac Plus, GNR 592/30268) before the onset of the study. Each pen was also cleaned daily to ensure and maintain a good hygienic environment.



Figure 3.2 Pens 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 single extended feed trough in order to provide feedlot comparable feeding space (27.7 cm per lamb) for each animal (Figure 3.4). Petherick & Phillips (2009) proposed that the minimum length of trough space per animal (L in meters) required for feeding and drinking can be determined from $L = 0.064W^{0.33}$, with the number of animals required to feed/drink simultaneously taken into account, together with any requirement to minimise competition. The feed troughs were designed to limit feed wastage to a minimum and ensure more accurate results. The feed troughs were placed along the common centre partitioning of each pen and the centre partitioning was reinforced to prevent animals from reaching feed from the adjacent pen (Figure 3.5). Each water bucket was located at the opposite end of each pen's feed trough to minimize the water being contaminated with feed (Figure 3.4). These buckets were fixed to the side of each pen and cleaned and refilled on a daily basis.



Figure 3.4 Feed troughs and water buckets used.



Figure 3.5 Reinforced partitioning between the feed troughs of adjacent pens.

3.5 Experimental diets

The complete experimental diets were prepared at a local commercial feed company (Table 3.1) and fed in a pelleted form.

3.5.1 Physical and chemical composition of the experimental diets

The calculated physical, dry matter (DM) and chemical composition of the experimental diets are presented in Table 3.1. The four dietary treatments consisted of the same iso-nitrogenous and iso-caloric basal diet differing only in respect to the lipid source [i.e. 30 g/kg of either saturated beef tallow (T); or unsaturated soyabean oil (S)] and type of antioxidant included [125g/ton of

either a synthetic antioxidant (C) or a natural antioxidant (B)]. The natural antioxidant was included at 125 g/ton to match the level of the synthetic antioxidant for a direct comparison due to a lack of information regarding its proposed inclusion in lamb diets. The synthetic antioxidant contained a combination of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin, and trisodium citrate. The natural antioxidant (Reg. No. V20924; Act 36 of 1947) is a polyphenolic plant extract containing bioflavonoids (catechin and gallo-catchecin) and proanthocyanidins (which generate the anthocyanidins delphinidin, robinetidin and fisetidin). Accordingly the experimental diet was formulated to obtain maximum live weight gain. The NRC (1985) requirements for finishing lambs in a feedlot were used as a guideline.

After proper mixing the treatment diets were pelleted and fed to the lambs in this form to minimize feed selection that could affect the results of the study. After mixing of a specific experimental diet the whole mixing system was flushed with wheat bran to prevent contamination of the different additives (lipid and/or antioxidant sources).

Feed samples were taken at the start (day 0) and at the end of the production study (day 49) and stored at -15°C until the fatty acid profile, as well as the free fatty acid (FFA) content for the four treatment diets could be determined. The FFA value was used as an indicator of how much the feed oxidized over time during the trial period (especially important for the unsaturated soyabean oil treatments).

Table 3.1 Mean calculated physical and chemical composition of the experimental diets containing different lipid and antioxidant sources

Parameter	%
Physical composition (as fed):	
Yellow maize meal	5
Maize chop	27
Lucerne hay	35.5
Soyabean oilcake	7
Sunflower oilcake	4
Wheat bran	12
Sunflower husks	3
Lipid source*	3
Premix [#]	0.7
Feedlime	1.6
Fine salt	0.8
Other additives	0.4
Chemical composition (dry matter basis):	
Dry matter	90.98
Ash	10.27
Crude Protein	18.65
Non-Protein Nitrogen	0.20
Non-Fibrous Carbohydrates	29.40
Neutral Detergent Fibre	35.46
Ether extract	7.08
Calcium	1.10
Metabolizable energy (MJ/kg)	10.81

* Lipid sources were mixed into the basal diet according to dietary treatment as follows: Saturated beef tallow (T; included at 30 g/kg feed); Unsaturated soyabean oil (S; included at 30 g/kg feed).

[#] Antioxidant sources were mixed into the premix before included in the basal diet according to dietary treatment as follows: Synthetic antioxidant (C; included at 125 g/ton feed); Natural antioxidant (B; included at 125 g/ton feed).

Note: The feeding value of feeds used to calculate chemical composition of diets was according to a commercial feed manufacturer's data.

3.6 Production study

The production study was conducted over a period of 49 days (including an eight-day adaptation period) to investigate the influence of a bioflavonoid antioxidant and fatty acid saturation in a standard feedlot diet on the dry matter intake (DMI), average daily gain (ADG) and feed conversion ratio (FCR) of finishing lambs.

3.6.1 Experimental design

The production study was compiled according to a 2x2 factorial design which represents a saturated (tallow) and unsaturated (soyabean oil) lipid source, combined with a synthetic- or natural antioxidant source, culminating into four dietary treatments (n=21 lambs per treatment) (see paragraph 3.5.1), subdivided into seven replicates per treatment (n=3 lambs per replicate).

3.6.2 Adaptation of the lambs

Before the onset of the study all animals (84 lambs) had free access to a hay mixture. Good quality lucerne hay was mixed with tef hay in a ratio of 1:1 and fed to all lambs from the first day. The lucerne- and tef hay was milled by means of a hammer mill through a 20 mm sieve. At the onset of the production studies (day 1) the lambs were subjected to an eight-day adaptation period procedure (Figure 3.6) as describe by Smith (2008). The experimental diets within each treatment were increased incrementally with 250 g/animal every second day and fed on top of the hay mixture to ensure intake thereof. Accordingly the lucerne/tef hay mixture was incrementally reduced with the same amount that the experimental diets increased (250 g/animal every second day), resulting in an average total feed intake of 1 kg/lamb/day. Total feed intake was recorded during the adaptation period (trial feed including the hay mixture). 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.

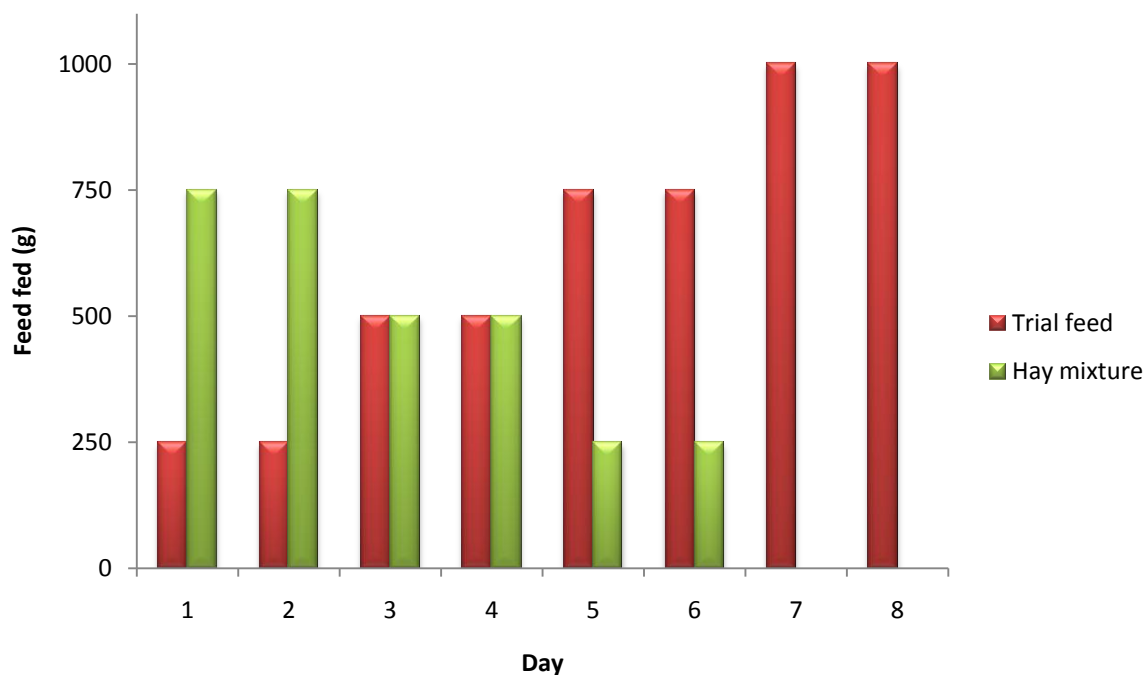


Figure 3.6 Dietary adaptation of the experimental animals in the production study during an eight-day period.

3.6.3 Feeding the lambs

After dietary adaptation the hay mixture was removed and the animals were fed the respective experimental diets on an *ad lib.* basis for the remainder of the experimental period (41 days) until all the lambs attained an average live weight of ± 48 kg prior to slaughter. The animals were fed twice daily, at 07h15 and at 15h45, not only to reduce feed wastage, but also to increase feeding frequency and hence the DMI of the animals. The feeding periods was followed precisely to accommodate the lambs as creatures of habit and 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. 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 an consecutive eight-day collection period) to investigate the influence of antioxidant source and fatty acid saturation in a standard lamb feedlot diet on the apparent digestibility coefficients and digestible nutrient content of each diet.

Seven lambs (45.11 ± 2.99 kg; total of twenty-eight) were randomly selected within each treatment after completion of the production study to be used in the digestibility study. 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 3.7). The pens remained in exactly the same manner as for the production study. The only difference was that the pen space for each lamb during the digestibility study was more than during the production study.



Figure 3.7 Lamb housed individually in pen.

3.7.1 Experimental design

The digestibility study was compiled according to the same 2x2 factorial design and experimental treatments as discussed in paragraph 3.6.1, culminating into four dietary treatments (n=7 lambs per treatment), subdivided into seven replicates per treatment (n=1 lamb per replicate).

3.7.2 Adaptation of the lambs

No feed adaptation was needed due to the fact that the lambs were already accustomed to the treatment diets during the production study. As already mentioned the lambs were allowed four days to adapt to the faecal bags before the collection period commenced.

3.7.3 Feeding and feed refusals

The lambs were offered the same experimental diets 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 food at all times to each animal. If a particular lamb ate more feed than that presented for the 24-hour cycle, more feed was weighed, recorded and provided to that lamb.

This method differed from that explained by Blaxter *et al.* (1961). It consisted of feeding each animal 1.15 times its mean consumption on the preceding two days. Adjustments of food offered were made only if the amount of food refused was less than 15% of that offered to the same animal. If so, the food offered was increased to 1.15 times of that consumed during the previous day.

The lambs were fed twice daily (at 07h15 and 15h45). In order to calculate actual intake and feed digestibility, the refusals was collected every morning just before the 07h15 feeding time. Only half of the calculated feed required for every lamb was provided at 07h15 and the rest at the 15h45 feeding period. As already mentioned, if a particular lamb ate more feed than that presented for the 24-hour cycle; more feed was weighed, recorded and provided to that lamb.

Daily feed refusals by each animal was collected and 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 four 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 in the same way for chemical analysis. Representative samples of feed and feed refusals were obtained by means of the quartering method (McDonald *et al.*, 2002).

3.7.4 Faeces collection

All lambs were fitted with a harness and faecal collecting bags (Figure 3.8), four days prior to the collection period as already mentioned. The faeces voided were collected twice daily after each feeding to avoid over accumulation of faeces inside the bag. The first collection of faeces occurred 24 hours after the first feeding, and collection continued 24 hours after the last feed was allocated to the lambs. 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.



Figure 3.8 Lamb fitted with faecal bag and harness.

After weighing, smaller representative samples of the dried faeces from each lamb were obtained by the quartering method (McDonald *et al.*, 2002), 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 refilled daily at 8h00.

3.9 Chemical analysis

Milled feed, refusal and faecal samples were analysed for DM, crude protein (CP), neutral detergent fibre (NDF), gross energy (GE), ash, organic matter (OM) and ether extract (EE). Any analysis was repeated if the value between each duplicate differed more than 3%.

3.9.1 Dry matter (DM)

DM content of the feed samples was analysed according to the AOAC (Association of Official Analytical Chemists) 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 follows:

$$\% \text{ Moisture} = [\text{Weight loss after drying (g)} / \text{Weight of test sample (g)}] \times 100$$

$$\% \text{ Dry matter} = 100 - \% \text{ Moisture}$$

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 Crude protein (CP)

The CP content of the feed, feed refusal and faecal samples were analysed 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 continuously. The principle of the Dumas method is that nitrogen (N₂), freed by pyrolysis and subsequent combustion, and is swept by carbon dioxide

(CO₂), as carrier into the nitro meter. The CO₂ is absorbed in potassium hydroxide (KOH) and the residual N₂ volume, measured. The N₂ content is then converted to the protein equivalent by multiplying the percentage N₂ with the factor of 6.25. Protein values were recorded on a computer, which was connected to the scale, as well as the analysing instrument. The protein equivalent was calculated by the computer program from the numerical factor obtained as described above.

3.9.3 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 weigh the filter bag (W1) and zero the balance. Approximately 0.45 to 0.55 g of the prepared sample (W2) is weighed directly into the filter bag. By using a heat sealer, the upper edge of the filter bag is completely sealed, within 4 mm of the top. Weigh one blank bag and include in the run, to determine the blank bag correction. Insert the bag suspender with bags into the fibre analyser vessel and place a weight on top - to keep it submerged. Add 100 ml/bag of neutral detergent (ND) (use minimum of 1500 ml to ensure bag suspender is submerged). Add 20 g (0.5 g per 50 ml of ND solution) of sodium sulphite and 4.0 ml of alpha-amylase to the solution in the vessel. Turn, agitate and heat, setting the timer for 75 min, and close the lid. At end of extraction, turn off the heat and agitator. Open the drain valve (slowly at first), and empty hot solution before opening the lid. After the solution has been drained, close the exhaust valve and open the lid. Add 1900 ml rinse water (70 - 90°C) and 4.0 ml alpha-amylase to the first and second rinses. Turn, stir and rinse for 5 min. The lid may be sealed with the heat on, or left open with the heat off. Repeat the hot water rinses for a total of three times. When the rinsing process is complete, remove the samples and gently press out excess water from the bags. Place bags in a 250 ml beaker and add enough acetone to cover the bags and soak for 3 - 5 min. Remove bags from acetone and place on a wire screen to air-dry. Completely dry the bags in an oven at $102 \pm 2^\circ\text{C}$ for 4h, until a constant weight. Remove bags from oven, place directly into a collapsible desiccant pouch and flatten to remove the air. Cool to ambient temperature and weigh bags (W3).

$$\% \text{ NDF (as-received basis)} = \frac{[W3 - (W1 \times C1)]}{W2} \times 100$$

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)

3.9.4 Gross energy (GE)

Gross energy (GE) content of feed, feed refusals and faecal samples were determined using a LECO AC500 Isoperibol Calori-meter (Leco Corp., St. Joseph, MI) following ASTM standard D5865 (Cantrell *et al.*, 2010). Approximately 0.2 g (on a DM-basis) of each sample (feed, feed refusal and faeces) was weighed accurately to the 4th decimal and placed in a steel crucible. A platinum wire (5 cm) was connected to the electrodes of the bomb and the steel crucible containing the sample were 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 were in contact with the sample and too 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. A Windows operating system records the temperature every six seconds accurately to 0.0001°C using an electronic thermometer. The gross energy is expressed as megajoules per kilogram dry matter (MJ/kg DM).

3.9.5 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. Porcelain crucibles containing samples were then placed in the cold muffle furnace and pre-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 until the oven could be opened and samples handled. The samples were then transferred

to desiccators to cool and immediately weighed afterwards. The ash content was calculated as follows:

$$\% \text{ Ash} = [\text{Weight of ash (g DM)} / \text{Weight of test sample (g DM)}] \times 100$$

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.

3.9.6 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.

$$\% \text{ Organic matter (OM)} = 100 - \% \text{ Ash}$$

3.9.7 Ether extract (EE)

The EE content of the feed, feed refusal and faecal samples were measured according to the AOAC official method 920.39 for chemical procedures (AOAC, 2000).

The EE fraction of samples was calculated as follows:

$$\% \text{ EE} = [\text{Weight of EE (g DM)} / \text{Weight of test sample (g DM)}] \times 100$$

3.10 Apparent digestibility of feed nutrients

The apparent digestibility of feed or nutrients 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 (McDonald *et al.*, 2002).

The following formula was used to calculate apparent digestibility (McDonald *et al.*, 2002):

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

Metabolisable energy was calculated from digestible energy (DE) values by multiplying the DE by a factor of 0.8 (McDonald *et al.*, 2002) to compensate for energy losses in the urine and methane gas (CH₄).

The apparent digestible nutrients of a food is the specific nutrient content of a unit weight of the food less the specific nutrient content of the faeces (digestion study) resulting from the consumption of any unit weight of that food. The digestible nutrient content of the diet was calculated as follows (McDonald *et al.*, 2002):

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

3.11 Carcass evaluation

With completion of the 49 day production study, seven lambs per treatment (45.11 ± 2.99 kg) (total of 28) were randomly selected from the 56 remaining lambs not selected for the digestibility study. These lambs were slaughtered at a commercial abattoir. Cold carcass weights were recorded 24 hours after refrigeration at 2-4 °C according to the methods described by Fisher & De Boer (1993). The cold carcass weight was then used to determine the dressing percentage:

$$\text{Dressing percentage (\%)} = [\text{Cold carcass weight (kg)} / \text{Live weight (kg)}] \times 100$$

Carcass evaluation was performed on the left side of each carcass. Carcasses were split between the 12th and 13th rib (thoracic vertebra) and fat depth measured with a calliper (Electronic digital caliper; Omni-Tech), 35 and 110 mm from the mid dorsal line (Carson *et al.*, 1999) (Figure 3.9). To measure the area of the longissimus muscle (*Musculus longissimus dorsi*) between the 12th and 13th rib, the longissimus muscle was traced directly off onto transparent film (Edwards *et al.*,

1989) (Figure 3.10). 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 analyzing system was calibrated with the scale bar.

The external length, shoulder circumference and buttock circumference of each carcass were also measured (Figure 3.11). *Longissimus* muscle pH was measured between the 12th and 13th ribs at 45 minutes (pH₁) and 24 hours (pH₂) post-mortem using a portable HI9625 pH meter (Hanna Instruments, Padova, Italy) (Figure 3.12).



a)



b)

Figure 3.9 Fat thickness measured between the 12th and 13th rib at 35 mm (a) and 110 mm (b) from the mid dorsal line.



Figure 3.10 Tracing of the eye muscle on to transparent paper.



Figure 3.11 Measuring the external length (a), shoulder- (b) and buttock circumference (c).



Figure 3.12 Measuring meat pH between the 12th and 13th rib.

3.12 Meat quality evaluation

Three loin chops (from 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, butylated hydroxytoluene 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 and the extracts were dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as a moisture adsorbent.

The extracted fat from feed, muscle and subcutaneous fat was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at -20°C pending fatty acid analyses.

A lipid aliquot (20 mg) of feed, muscle and subcutaneous lipid were converted to methyl esters by base-catalysed transesterification, in order to avoid conjugated linoleic acid (CLA) isomerisation, with sodium methoxide (0.5 M solution in anhydrous methanol) during 2 h at 30 °C, as proposed by Park *et al.* (2001), Kramer *et al.* (2002) and Alfaia *et al.* (2007). Fatty acid methyl esters (FAMES) from feed and muscle were quantified using a Varian GX 3400 flame ionization 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 minutes). Thereafter, temperature was increased at a rate of 4°C per minute to 230°C. Finally an isothermic period of 230°C for 10 minutes followed. FAMES n-hexane (1µl) was injected into the column using a Varian 8200 CX Auto sampler. 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 Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Conjugated linoleic acid (CLA) standards were obtained from Matreya Inc. (Pleasant Gap, Unites States). These standards included: cis-9, trans-11; cis-9, cis-11, trans-9, trans-11 and trans-10, cis-12-18:2 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 fatty acid to the total of all fatty acids present in the sample. The following fatty acid combinations were calculated: omega-3 (*n*-3) fatty acids, omega-6 (*n*-6) fatty acids, total saturated fatty acids (SAFs), total monounsaturated fatty acids (MUAFs), poly-unsaturated fatty acids (PUAFs), PUFA/SFA ratio (P/S) 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 were individually placed in polystyrene trays containing absorbent pads, overwrapped with oxygen-permeable polyvinyl chloride (PVC) meat stretch wrap (Figure 3.13) and stored for seven days at 4°C under fluorescent light for fresh meat stability studies. The third loin chop (chop 3) was vacuum sealed (Figure 3.14) and stored for 90 days at -18°C in the dark for frozen storage stability studies.



Figure 3.13 Chop 2 placed in polystyrene trays containing absorbent pads, overwrapped with PVC meat stretch wrap.



Figure 3.14 Vacuum sealing third loin chop.

3.12.3 Meat colour

The colour (a^* -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 chroma meter, a technique described by Denoyelle & Berny (1999).

3.12.4 Thiobarbituric acid reactive substance (TBARS) determination

A 5 g sample of lean 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°C) and day 90 (chop 3 stored at -18°C) to determine the malonaldehyde content per 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 Free fatty acid values of feed fats

Free fatty acids (FFA) and acid value (AOAC official method 940.28 for chemical procedures) of feed fats was determined according to AOAC (1990).

3.13 Statistical analysis

The data was subjected to PROC ANOVA and analyzed according to a 2x2 factorial arrangement of treatments (effects of two dietary lipid sources and two antioxidant sources) and tested for significant differences using the General Linear Model procedures of the SAS program (SAS, 1999). Tukey's honest significant difference test was used to identify significant differences ($P < 0.05$) between treatments.

Chapter 4

The effect of lipid saturation and antioxidant source on the digestibility of finishing diets for lambs

4.1 Introduction

Although primary feed sources for ruminants such as forages and concentrates contain lipids, the dietary lipid content can be increased by adding fat supplements. Forage leaf tissue typically contains 4 to 6% lipid on a dry matter (DM) basis, of which the major lipid class is glycolipids (McDonald *et al.*, 2002; Bauman *et al.*, 2003). The lipid content of concentrates is usually higher than that of forages, and the majority is present in the form of triglycerides (Bauman *et al.*, 2003).

Fat is an important energy component in the diet of ruminants (Firkins & Eastridge, 1994; Bauchart *et al.*, 1996; Bauman *et al.*, 2003) and contains on average up to 2.25 times more energy as carbohydrates (McDonald *et al.*, 2002). Nevertheless, fats included in ruminant diets may negatively affect voluntary feed intake and nutrient digestibility, especially unsaturated fats that are highly digestible (Bock *et al.*, 1991; Jenkins & Lundy, 2001; NRC, 2001; Manso *et al.*, 2005). For example dietary fat may modify the ruminal microbial population, which is responsible for cellulose digestion, but has few effects on other propionate-producing organisms (Manso *et al.*, 2005). Supplementing more than 6% fat (on a DM-basis) in ruminant diets may decrease fibre digestion and methane (CH₄) production *in vitro* (Firkins & Eastridge, 1994; Nelson *et al.*, 2001; Harris, 2003). This can be explained by physically coating the fibres with oil, and inhibiting (modification of microbial population) rumen microbial activity through intoxication (Jenkins & Lundy, 2001). On the other hand, saturated fat has less of an influence on fibre digestion in the rumen, but digestibility of the fat may be inferior depending on the level of saturation (NRC, 2001). Tallow is an economical source of additional energy in cattle diets and is high in saturated fatty acids (SFAs) (LaBrune *et al.*, 2008). As the amount of SFAs increase in dietary fat, ruminal lipolysis decreases, hence resulting in a decreased total tract digestibility and an increase in the flow of triglycerides to the duodenum (LaBrune *et al.*, 2008).

According to Manso *et al.* (2005) studies conducted to assess the effects of fats on nutrient digestibility of ruminants have not yielded consistent results. This could be due to a variety of factors such as the level and/or source of dietary fat and the nature of the basal diet, which in turn influences the effects of fat on ruminal microbes.

Due to the vulnerability of unsaturated fatty acids to undergo oxidation, antioxidants are added to diets to help prevent this process (Smith *et al.*, 2007). McDonald *et al.* (2002) explained that fats possess a certain degree of resistance to oxidation, owing to the presence of compounds called natural antioxidants. Antioxidants prevent oxidation of unsaturated fat until they themselves have been transformed into inert products.

According to Ramsey (1980) lipid oxidation in a diet occurs when the unsaturated fatty acid loses a hydrogen molecule, resulting in the formation of a free radical at the site of unsaturation. This free radical is quickly converted to a fatty acid peroxide free radical and finally to a fatty acid hydroperoxide. An antioxidant (natural or chemical) can block this oxidation by supplying a hydrogen molecule in the first free radical formed, thereby reconvert it to the original fatty acid. If the hydroperoxides are allowed to form, they continue to decompose by breaking down into a variety of aldehydes and ketones. Apart from synthetic antioxidants [normally a combination between butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin, or trisodium citrate], one of the most important naturally occurring antioxidants is fat soluble vitamin E, which protects fat by preferential acceptance of free radicals (McDonald *et al.*, 2002).

No information regarding the effect of dietary lipid saturation level together with a natural antioxidant source on diet digestibility of lambs fed finishing diets could be detected in the available literature. Therefore, the aim of this study was to determine if different lipid saturation (saturated and unsaturated fat) and antioxidant (synthetic and natural) sources could have an influence on nutrient digestibility and the 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 over a period of 12 days (4-day adaptation to the faecal bags followed by an 8-day collection period). Twenty-eight lambs (45.11 ± 2.99 kg) were randomly selected from the four dietary treatments (n=7 lambs per treatment). The four dietary treatments consisted of the same basal diet (187 g CP- and 355 g NDF/kg DM) differing in the lipid source (30 g/kg of either saturated beef tallow or unsaturated soyabean oil) and type of antioxidant included (125 g/ton of either a synthetic or natural antioxidant). The lambs were housed individually in pens and each fitted with a harness and faecal collecting bag, four days prior to the faecal collection period. The faeces voided were collected twice daily. A sequential method of feed allocation was followed by providing each animal with 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. Daily composite feed and feed refusals of each animal were also collected. Collected feed, refusal and faecal samples were analysed for DM, crude protein (CP), neutral detergent fibre (NDF), gross energy (GE), ash, organic matter (OM) and ether extract (EE). Apparent digestibility calculations were made accordingly.

4.3 Results and Discussion

4.3.1 Chemical composition of experimental diets

The actual chemical composition of the four experimental diets is presented in Table 4.1. No chemical analysis of the individual feed ingredients was conducted before formulating the experimental diets and hence some nutrient variation between formulated (Table 3.1) and actual values (Table 4.1) was expected. It is however evident from these tables that the actual DM, CP, EE and ash content of the various experimental diets compared well with that of the calculated values (Table 3.1).

Table 4.1 Mean chemical composition of the four experimental diets used during the digestible study

Parameter	Treatment diets*			
	TC	TB	SC	SB
Chemical composition (dry matter basis):				
Dry matter (%)	92.00	91.70	91.80	91.00
Organic matter (%)	89.31	89.94	89.71	88.74
Crude protein (%)	19.12	18.28	18.65	19.08
Neutral detergent fibre (%)	33.50	35.61	32.80	33.30
Ether extract (%)	7.54	7.05	6.95	7.06
Gross energy (MJ/kg DM)	18.54	18.66	18.21	17.98

* Treatments: T = Saturated beef tallow (30 g/kg feed); S = Unsaturated soyabean oil (30 g/kg feed); C = Synthetic antioxidant (125 g/ton feed); B = Natural antioxidant (125 g/ton feed).

Roughage sources contribute most to nutrient variation as there are large differences found in nutrient densities within the same roughage due to various factors such as locality, climate, soil and production practices (Smith, 2008). Due to the accepted variation in roughage nutrient composition it was expected that a 35.5% lucerne hay inclusion in the experimental diets could affect actual diet nutrient composition. In the present study the NDF content of the formulated diet was slightly higher (4.9%) than the analysed values. Accordingly, a small variation in the NDF content of the experimental diets between treatments occurred ($SD \pm 1.24$). The fact that the four experimental diets contained the same feed ingredients and only differed in the additives included, could contribute to the small variation in nutrient composition.

The average GE content of the experimental diets (on average 18.35 MJ/kg DM) agreed with McDonald *et al.* (2002) statement that most common foods contain about 18.5 MJ GE/kg DM.

Thorough mixing and efficient sampling probably ensured that the chemical composition of the different diets compared well between each other.

4.3.2 Apparent digestibility and digestible nutrients

Dry matter intake (DMI), apparent digestibility and digestible nutrient content of experimental diets are presented in Table 4.2.

Table 4.2 Dry matter intake, apparent digestibility and digestible nutrient content of experimental diets containing different dietary antioxidant and lipid sources (mean values)

Parameter	Antioxidant (125 g/ton)	Fat source (30 g/kg)			Significance (<i>P</i> -value)			CV [#]
		Saturated	Unsaturated	Mean	Fat source	Antioxidant	Interaction	
Dry matter intake (g/lamb/day)	Synthetic	1717	1718	1717	0.9101	0.8662	0.9005	12.25
	Natural	1713	1694	1704				
	Mean	1715	1706					
Apparent digestibility coefficients (%):								
Dry matter	Synthetic	69.35	65.26	67.31	0.2039	0.3811	0.1028	5.31
	Natural	68.26	68.79	68.52				
	Mean	68.80	67.02					
Organic matter	Synthetic	71.74	67.31	69.53	0.2771	0.2198	0.0388	5.06
	Natural	70.50	71.94	71.22				
	Mean	71.12	69.63					
Crude protein	Chemical	81.38	79.17	80.27	0.3473	0.4625	0.0888	2.66
	Natural	80.54	81.21	80.88				
	Mean	80.96	80.19					
Neutral detergent Fibre	Synthetic	46.53	38.25	42.39	0.0548	0.0672	0.2556	15.35
	Natural	48.49	46.27	47.38				
	Mean	47.51	42.26					
Ether extract	Synthetic	91.11	90.70	90.88	0.5646	0.8153	0.2887	2.08
	Natural	90.12	91.31	90.71				
	Mean	90.59	91.01					
Gross energy	Synthetic	72.80	68.26	70.53	0.1117	0.2755	0.0379	4.39
	Natural	71.53	72.17	71.85				
	Mean	72.17	70.22					

Table 4.2 (Cont.)

Parameter	Antioxidant (125 g/ton)	Fat source (30 g/kg)			Significance (<i>P</i> -value)			CV [#]
		Saturated	Unsaturated	Mean	Fat source	Antioxidant	Interaction	
Apparent digestible nutrients (%):								
Organic matter	Synthetic	64.04	60.34	62.19	0.1891	0.2481	0.0974	5.06
	Natural	63.39	63.84	63.62				
	Mean	63.72	62.09					
Crude protein	Synthetic	15.56	14.82	15.19	0.6561	0.5804	<.0001	2.74
	Natural	14.66	15.54	15.10				
	Mean	15.11	15.18					
Neutral detergent Fibre	Synthetic	15.65	12.51	14.08 ¹	0.0098	0.0159	0.4961	15.58
	Natural	17.35	15.46	16.41 ²				
	Mean	16.50 ^b	13.98 ^a					
Ether extract	Synthetic	6.88 ^{a1}	6.34 ^b		0.0002	0.0016	<.0001	2.23
	Natural	6.38 ²	6.45					
ME* (MJ/kg DM)	Synthetic	10.81	9.93	10.37	0.0022	0.3431	0.1269	4.42
	Natural	10.70	10.38	10.54				
	Mean	10.75 ^b	10.15 ^a					

^{a,b} Row means with different superscripts differ significantly ($P < 0.05$) within parameter means.

^{1,2} Column means with different superscripts differ significantly ($P < 0.05$) within parameter means.

[#] Coefficient of variation (%).

* Metabolizable energy = digestible energy \times 0.8 (McDonald *et al.*, 2002).

The DMI of the lambs did not differ significantly ($P > 0.05$) between the various experimental diets. Therefore, feeding level was not a factor that could influence apparent digestibility of the diets. This similar feed intake was expected due to the fact that the diet nutrient composition and especially NDF content was the same. Differences in NDF content could affect diet digestibility and rumen fill. These are two important factors influencing feed intake (McDonald *et al.*, 2002). DMI results over a relative short period of eight days is however less accurate and should be interpreted with caution.

From the results in Table 4.2 it seems that the saturation level of lipid source had in general no significant ($P > 0.05$) influence on the apparent digestibility of nutrients in the experimental diets. The results of Wistuba *et al.* (2006) illustrated that a total dietary fat content of 7.1 % had no

effect on the apparent total tract digestibility of DM, ADF, OM, and N₂. This level corresponds with those included in the present study (Table 4.1). However the inclusion of an unsaturated lipid source in the form of soyabean oil in the diet reduced ($P = 0.0548$) the apparent digestibility of NDF in the diet. A possible explanation for the lack of a significant response at the $P = 0.05$ significance level for NDF digestibility could be the high coefficient of variation ($CV = 15.35\%$). It is known that lipid sources with a high unsaturated fatty acid content have a negative effect on fibre digestibility due to its antimicrobial (Jenkins & Lundy, 2001) and coating effect on feed fibres (McDonald *et al.*, 2002). This inhibits growth and function (Jensen *et al.*, 1997) of the ruminal microbes. In accordance with the current study, Beauchemin *et al.* (2007) found that dietary lipid source (unsaturated sunflower oil and saturated tallow) had no effect ($P > 0.05$) on the DM-, CP-, GE- and NDF digestibility. Macleod & Buchanan-Smith (1972) also found that protein digestibility was not affected by the addition of saturated tallow or unsaturated soyabean oil in sheep diets. On the other hand apparent fibre digestibility was significantly ($P < 0.05$) lowered by the inclusion of unsaturated soyabean oil compared to saturated tallow in the diet (Macleod & Buchanan-Smith, 1972). Studies conducted to assess the effects of lipids on nutrient digestibility within ruminants have not yielded consistent results (Manso *et al.*, 2005). This could be due to a variety of factors such as the level and/or source of dietary lipid and the nature of the basal diet, which in turn influences the effects of fat on ruminal microbes. Beauchemin *et al.* (2007) explained that the potential harmful effects of an unsaturated lipid source on fibre digestion can be minimized by feeding a high-concentrate diet low in fibre. Hence, fibre content of the diets could pose as an explanation for inconsistent results within literature.

In contrast with the results of the present study, Macleod & Buchanan-Smith (1972) found that the EE digestibility of a hydrogenated tallow diet fed to lambs were significantly ($P < 0.01$) lower than that of a soybean oil supplemented diet. The hydrogenated tallow used in the research of Macleod & Buchanan-Smith (1972) was more than 99% saturated. These researchers are of opinion that the degree of saturation of dietary lipid appears to be negatively correlated with its digestibility by ruminants. Therefore an unsaturated fatty acid deficiency can partially account for a low digestibility of highly saturated hydrogenated tallow (Macleod & Buchanan-Smith, 1972). Supporting this theory Roberts & McKirdy (1964) fed tallow to lambs with less than 50%

of the fatty acids saturated. No significant ($P > 0.05$) influence on the apparent EE digestibility of tallow, rapeseed oil and sunflower oil containing diets respectively could be detected.

It is further evident from Table 4.2 that the inclusion of a synthetic compared to a natural antioxidant in the diet also decreased ($P = 0.0672$) the apparent digestibility of NDF. The detrimental effect of the unsaturated lipid source ($P = 0.0548$) and synthetic antioxidant on the apparent digestibility of NDF were associated with a significantly lower ($P < 0.05$) digestible NDF content in the experimental diet. Accordingly the unsaturated lipid source, soyabean oil, resulted a significantly ($P = 0.0022$) lower metabolizable energy (ME) content in the diet. When ruminant feed is supplemented with fat rich in PUFAs and MUFAs, CH_4 emission is reduced (Rasmussen & Harrison, 2011). This can be due to the fact that PUFAs has an inhibitory effect on CH_4 production through a direct use of hydrogen by hydrolysing bacteria in the rumen (McAllister *et al.*, 1996). Therefore, the factor of 0.8 representing CH_4 and urine losses and used to calculate the ME content of the diet from that of the digestible energy (DE) (Table 4.2), (McDonald *et al.*, 2002), probably over-estimated the production of CH_4 where unsaturated soyabean oil was included in the diet. This could contribute to the significant decrease in the ME content of the soyabean oil compared to the tallow diet.

The source of antioxidant however did not influence ($P = 0.3431$) the ME content of the finishing diet. No results in the available literature could be found regarding the influence of a synthetic or natural antioxidant in ruminant diets on the nutrient digestibility and ME content.

From Table 4.2, and Figure 4.1 and 4.2, it seems that a lipid x antioxidant interaction ($P < 0.0001$) occurred for the digestible EE content. In contrast with the natural antioxidant, the inclusion of unsaturated soyabean oil in the diet containing a synthetic antioxidant resulted in a lower ($P = 0.0002$) digestible EE content (Figure 4.1). Similarly, a combination of saturated beef tallow and a natural antioxidant was characterised with a lower ($P = 0.0016$) digestible EE content (Figure 4.2). These results are the combined effects of EE digested and DMI and are difficult to explain as the latter was not significantly ($P > 0.05$) influenced by dietary treatments.

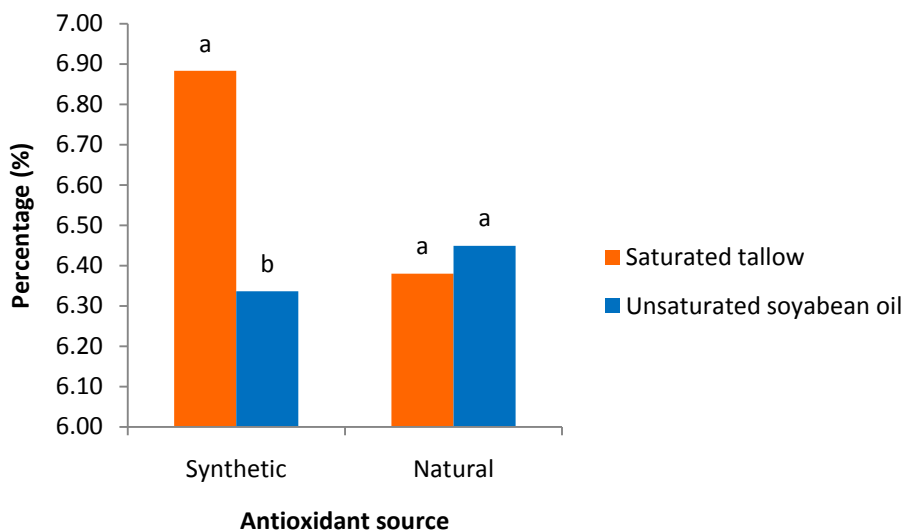


Figure 4.1 Dietary lipid x antioxidant source interaction for apparent digestible ether extract.

^{a,b} Chart bars with different superscripts within antioxidant source differ significantly ($P < 0.05$).

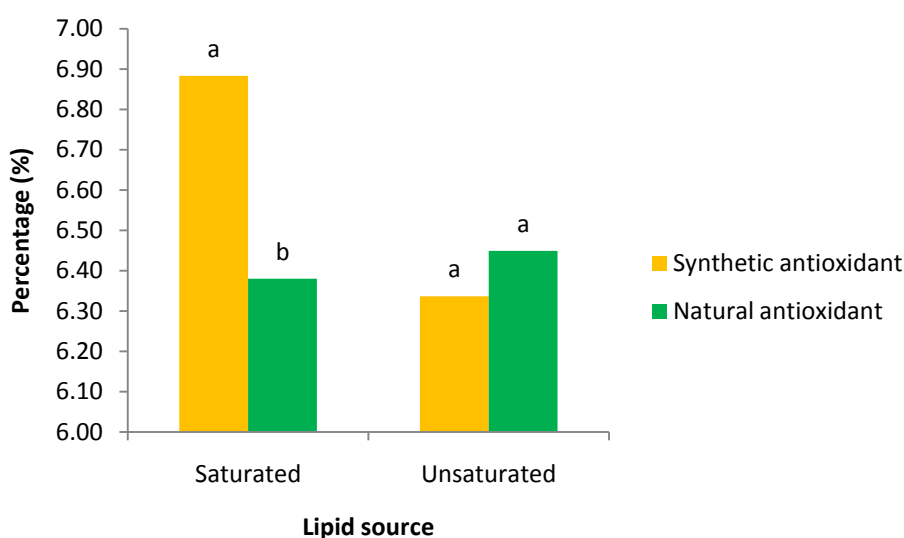


Figure 4.2 Dietary lipid x antioxidant source interaction for apparent digestible ether extract.

^{a,b} Chart bars with different superscripts within lipid source differ significantly ($P < 0.05$).

4.4 Conclusions

From the results of the present study it seems that the adding of unsaturated soyabean oil compared to saturated beef tallow in finishing diets for lambs resulted in a lower apparent NDF digestibility and ME content. However the inclusion of a synthetic compared to a natural antioxidant in finishing diets for lambs did not influence the ME content. Accordingly these findings should be reflected in the production performance of lambs in the feedlot and needs further investigation.

Chapter 5

The effect of dietary lipid saturation and antioxidant source on the production performance and carcass characteristics of lambs

5.1 Introduction

Fat is an important energy component in the diet of ruminants (Firkins & Eastridge, 1994; Bauchart *et al.*, 1996; Doreau & Chilliard, 1997; Bauman *et al.*, 2003) and contains on average up to 2.25 times more energy than carbohydrates (McDonald *et al.*, 2002). Feeding lipids differing in origin and degree of saturation to ruminants may result in a variety of responses in ruminant production (Felton & Kerley, 2004), either by negatively affecting voluntary feed intake and fibre digestibility (Manso *et al.*, 2005), or by not yielding consistent performance results (Manso *et al.*, 2005). On the other hand various researchers is of opinion that added fat does not only increase the energy content of finishing diets, but may also increase daily gain and feed efficiency of animals (Zinn, 1989; Brandt & Anderson, 1990). Various lipid sources have shown either an improvement in feed efficiency or to have no effect (Brandt & Anderson, 1990). Generally it is recommended that total lipid should not exceed 6 to 7% of the dietary dry matter (DM) content otherwise a decrease in feed intake can occur, negating the advantages of increased energy density of the diet (Beauchemin *et al.*, 2007).

Unsaturated fatty acids are more susceptible to lipid oxidation than saturated fatty acids (Kelley *et al.*, 2007; Kott *et al.*, 2010). Therefore, it may be favourable to supplement animal diets with additional antioxidant sources (natural or chemical), not only to prevent feed flavour deterioration (which could influence voluntary feed intake), but also prevent a decrease in the favourable mono- and poly-unsaturated fatty acid content of the animal diets due to lipid oxidation.

In Chapter 4 a negative influence of unsaturated soyabean oil on fibre digestion and ME content of the diet was detected. No information could be found in literature, regarding the effect of dietary lipid saturation combined with either a natural or chemical antioxidant, on the performance and carcass characteristics of lambs. Therefore, the objective of this study was to

investigate the effect of dietary lipid saturation (saturated and unsaturated lipid source) and antioxidant source (chemical and natural) on the production performance and carcass characteristics of lambs fed a finishing diet.

5.2 Materials and Methods

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

The production study was conducted over a period of 49 days. Eighty-four lambs (27.64 ± 1.72 kg) were randomly allocated to the four dietary treatments (n=21 lambs per treatment), subdivided into seven replicates per treatment (n=3 lambs per replicate). The four dietary treatments consisted of the same basal diet differing in the lipid source (30 g/kg of either saturated beef tallow or unsaturated soyabean oil) and type of antioxidant included (125 g/ton of either a synthetic or natural antioxidant). At the onset of the production study the lambs were subjected to an eight-day adaptation period using a stair-step adaptation procedure. After dietary adaptation the animals were fed the respective experimental diets on an *ad lib.* basis for the remainder of the experimental period (41 days) until all the lambs attained an average live weight of approximately 48 kg prior to slaughter. Live weight and feed intake was recorded on a weekly basis. At termination of the production study seven lambs per treatment (45.11 ± 2.99 kg) were randomly selected and slaughtered. Carcass characteristics (carcass weight, dressing percentage, subcutaneous fat thickness, intramuscular fat content, carcass length, shoulder- and buttock circumference, area of eye muscle and *Longissimus* muscle pH) was measured accordingly.

5.3 Results and Discussions

5.3.1 Feed intake and production performance

The mean intake and production performance of lambs fed finishing diets containing different dietary antioxidant and lipid sources are presented in Table 5.1.

From the results in Table 5.1 it seems that lipid saturation and antioxidant type had no-significant ($P > 0.05$) influence on the dry matter intake (DMI) of the lambs. This similar feed intake between the treatments was expected due to the fact that the diet nutrient composition and especially neutral detergent fibre (NDF) content was the same as discussed in Chapter 4.

It is evident from Table 5.1 and Figure 5.1 that a lipid x antioxidant interaction ($P < 0.0001$) occurred for the metabolizable energy intake (MEI) of lambs. In contrast with the natural antioxidant the inclusion of unsaturated soyabean oil in the diet containing a synthetic antioxidant resulted in a lower ($P = 0.0014$) MEI of the lambs. This lower energy intake of the lambs could probably be explained by the negative influence of an unsaturated lipid source on fibre digestion and the ME content of the experimental diets as observed and discussed in Chapter 4. The statistical non-significant ($P > 0.05$) influence of the unsaturated lipid on MEI of lambs when a natural antioxidant was included in the diet is difficult to explain. No significant ($P > 0.05$) influence of antioxidant type on MEI could be detected.

Table 5.1 Intake and production performance of lambs fed diets containing different dietary antioxidant and lipid sources (mean values)

Parameter	Antioxidant (125 g/ton)	Fat source (30 g/kg)			Significance (<i>P</i> -value)			CV [#]
		Saturated	Unsaturated	Mean	Fat source	Antioxidant	Interaction	
Intake:								
Dry matter intake (g/lamb/day)	Synthetic	1641	1605	1623	0.5626	0.1408	0.05911	5.29
	Natural	1575	1573	1574				
	Mean	1608	1589					
MEI ¹ (MJ/lamb/day)	Synthetic	16.68 ^a	14.96 ^b		0.0014	0.6308	0.0438	5.04
	Natural	15.89	15.45					
	Mean							
Production performance:								
Initial weight ² (day 0)	Synthetic	27.68	27.69	27.68	0.9605	0.8235	0.9703	6.35
	Natural	27.58	27.61	27.60				
	Mean	27.63	27.65					
End weight ² (day 49)	Chemical	45.53	45.51	45.52	0.5648	0.2133	0.5454	6.65
	Natural	44.31	45.09	44.70				
	Mean	44.92	45.30					
Average daily gain (g/sheep/day)	Synthetic	364	364	364	0.4946	0.1642	0.4666	13.75
	Natural	341	357	349				
	Mean	353	360					
Feed conversion ratio ³	Synthetic	4.51	4.42	4.47	0.1307	0.5159	0.5267	5.73
	Natural	4.64	4.42	4.53				
	Mean	4.58	4.42					
MJ ME ⁴ /kg live weight gain	Synthetic	45.86	41.21	43.54	0.0003	0.1118	0.5140	5.75
	Natural	46.82	43.44	45.13				
	Mean	46.34 ^b	42.33 ^a					

^{a,b} Row means with different superscripts differ significantly ($P < 0.05$) within parameter means.

[#] Coefficient of variation (%).

¹ Metabolizable energy intake.

² Empty stomach weight (kg).

³ kg DM feed intake/kg live weight gain.

⁴ Metabolizable energy.

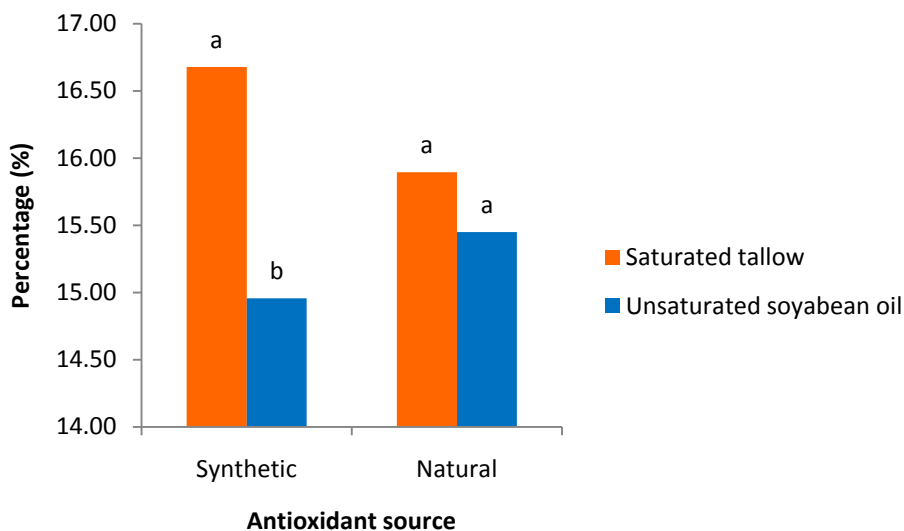


Figure 5.1 Dietary lipid x antioxidant source interaction for the metabolizable energy intake (MEI) of lambs.

^{a,b} Chart bars with different superscripts within antioxidant source differ significantly ($P < 0.05$).

From the results in Table 5.1 it is clear that the saturation level of lipid source and type of antioxidant included had no significant ($P > 0.05$) influence on the final live weight, average daily gain (ADG) and feed conversion ratio (FCR) of the lambs. These results did not support the lower MEI of lambs fed the diet containing the synthetic antioxidant and unsaturated soyabean oil. It could be speculated that the heat increment and the methane (CH_4) production of this diet was lower and resulted eventually in the same net energy intake. Micro-organisms in the rumen of ruminants generate heat through the fermentation of nutrients. McDonald *et al.* (2002) explained that the heat increment of foods varies considerably, according to the nature of the food, the type of animal consuming it and the processes for which nutrients are used. This heat of fermentation is especially generated from the fibre content in the diet. As the inclusion of an unsaturated lipid source has a negative effect on fibre digestibility due to its antimicrobial and coating effect, it could result in less energy lost as heat in the rumen resulting in more energy available for productive purposes by the animal. The reduced production of ruminal CH_4 within the unsaturated treatment as discussed in Chapter 4, could also contribute to the non-significant differences observed. Therefore, both the heat increment of fermentation as well as CH_4

production was probably reduced by the unsaturated lipid source resulting in the non-significant differences.

In contrast with DMI, ADG and FCR, the inclusion of an unsaturated lipid source in the form of soyabean oil resulted in a significantly ($P = 0.0003$) better utilisation of ME in the experimental diet (Table 5.1). This could be attributed to the lower ($P < 0.05$) ME content of the unsaturated soyabean oil diet as determined in the digestibility study and no-significant ($P > 0.05$) differences in DMI and ADG between lipid sources.

From the results in Table 5.1 it is clear that the source of antioxidant did not influence ($P = 0.1118$) the efficiency of ME utilisation by the experimental lambs.

The findings of the present study supported those of Zinn (1989) and Bock *et al.* (1991) who reported that feeding soyabean soap stock, or a blend of soyabean oil and tallow did not affect the DMI, ADG, or feed efficiency of steers compared with tallow or yellow grease. Accordingly Beauchemin *et al.* (2007) found no effect ($P > 0.05$) on the DMI and ADG of Angus heifers fed diets containing 3.4% of either saturated tallow or more unsaturated sunflower oil.

Studies conducted to assess effects of lipids on performance of ruminants have not yielded consistent results (Doreau & Chilliard, 1997; Manso *et al.*, 2005). Manso *et al.* (2005) is of opinion that this could be due to a variety of factors, such as the level and/or source of dietary fat and the nature of the basal diet, which can determine the effects of added lipids on ruminal microbes. Research regarding the effects of added lipid source (level of fatty acid saturation) and type of antioxidant on the feed intake and production performance of lambs seems to be limiting.

5.3.2 Carcass characteristics

The effect of dietary antioxidant and lipid saturation on the carcass characteristics of S.A. Mutton Merino lambs is presented in Table 5.2.

It is clear from the results in Table 5.2, that with the exception of pH, lipid saturation and antioxidant source had no significant ($P > 0.05$) influence on any of the carcass characteristics

tested. In accordance with the results of the present study Roberts & McKirdy (1964) found that steers fed a diet containing 5% of either saturated tallow, unsaturated rapeseed and -sunflower oil, had no influence ($P > 0.05$) on the dressing percentage and carcass lipid content.

Adding different antioxidants to the finisher diets of lambs seems to affect ($P < 0.05$) the muscle pH tested at 45 minutes and 24 hours after slaughter (Table 5.2). The meat pH measured 45 minutes after slaughter was significantly ($P = 0.009$) lower when a synthetic antioxidant was added to the diet, compared to that of a natural antioxidant. In contrast, meat pH measured at 24 hours post slaughter was lower ($P = 0.0433$) when a natural antioxidant was added to the diet, compared to that of a synthetic antioxidant. Therefore, it seems that a significantly slower decline in meat pH occurred during the first 45 minutes where a natural antioxidant was included in the diet.

Meat from animals receiving the natural antioxidant reached a significantly lower pH twenty four hours post mortem. Both can be considered as positive since a rapid decrease in pH within the first 45 minutes post mortem, while the temperature of the meat is still high, may lead to protein denaturation resulting in pale colour and water loss. A high final pH may result in dark colour of the meat and reduced microbiological shelf life (Olaoye & Ntuen, 2011).

Table 5.2 The effect of dietary antioxidant and lipid source on the carcass characteristics of S.A. Mutton Merino lambs (mean values)

Parameter	Antioxidant (125 g/ton)	Fat source (30 g/kg)			Significance (<i>P</i> -value)			CV [#]
		Saturated	Unsaturated	Mean	Fat source	Antioxidant	Interaction	
Cold carcass weight (kg)	Synthetic	21.52	21.07	21.29	0.3088	0.4616	0.0708	6.63
	Natural	20.12	21.67	20.90				
	Mean	20.82	21.37					
Dressing %	Synthetic	47.06	46.93	47.00	0.1237	0.1977	0.0716	2.6
	Natural	45.59	47.19	46.39				
	Mean	46.33	47.06					
Fat thickness 35* (mm)	Synthetic	2.66	2.65	2.65	0.9733	0.7047	0.9896	50.22
	Natural	2.48	2.46	2.47				
	Mean	2.57	2.55					
Fat thickness 110* (mm)	Synthetic	8.15	9.07	8.61	0.5934	0.1454	0.5580	26.71
	Natural	7.42	7.37	7.39				
	Mean	7.78	8.22					
Intramuscular fat content (%)	Synthetic	3.88	3.79	3.83	0.7668	0.1948	0.5526	21.30
	Natural	3.31	3.58	3.44				
	Mean	3.60	3.68					
Carcass length (cm)	Synthetic	58.43	57.61	58.02	0.2679	0.9043	0.9840	3.21
	Natural	58.50	57.71	58.11				
	Mean	58.46	57.66					
Shoulder circumference (cm)	Synthetic	75.21	74.71	74.96	0.6645	0.6645	0.2728	2.59
	Natural	74.07	75.21	74.64				
	Mean	74.64	74.96					
Buttock circumference (cm)	Synthetic	64.57	64.93	64.75	0.0723	0.4116	0.1872	2.81
	Natural	63.07	65.29	64.18				
	Mean	63.82	65.11					
Area of eye muscle (mm ²)	Synthetic	1573	1646	1609	0.3580	0.0984	0.8889	11.52
	Natural	1466	1520	1493				
	Mean	1519	1583					
pH after 45 min.	Synthetic	6.30	6.38	6.34 ¹	0.9060	0.0090	0.2632	2.46
	Natural	6.54	6.48	6.51 ²				
	Mean	6.42	6.43					
pH after 24 hours	Synthetic	5.64	5.73	5.69 ¹	0.1026	0.0433	0.5400	1.99
	Natural	5.57	5.62	5.59 ²				
	Mean	5.60	5.68					

^{1,2} Column means with different superscripts differ significantly ($P < 0.05$) within parameter means.

[#] Coefficient of variation.

* Measured 35 and 110 mm from the mid dorsal line between the 12th and 13th thoracic vertebra.

Normal metabolism and cellular integrity cease when an animal is slaughtered and the metabolism is maintained only by residual metabolites. These metabolites are then gradually used by energy metabolism in the muscle (Maree & Casey, 1993). Maree & Casey (1993) explained that glycogen is converted to lactic acid, which accumulates in the muscle due to the absence of a circulatory system. This accumulated lactic acid causes the meat pH to decline as observed especially for the animals fed the natural antioxidant diet.

Lipid oxidation is also a major deterioration reaction which results in loss of meat quality like meat colour, pH and shelf life (Buckley *et al.*, 1995). By adding antioxidants onto meat or formulated into the ruminant diets may help to delay lipid oxidation of meat (Karami *et al.*, 2010a,b; Velasco & Williams, 2011) and improve meat quality. This aspect warrants further research.

Animals that are not stressed or suffer pre-slaughtered exhaustion usually have a lower ultimate meat pH value around 5.4 (Maree & Casey, 1993; Morrissey *et al.*, 1994). Stressful conditions before slaughter could however not have affected the ultimate muscle pH of the present study as all the animals were slaughtered at random following the same procedures. Muscles with high glycogen content at slaughter proceed slowly into *rigor* and have a low ultimate pH (5.4). The post-mortem softening process of muscle seems to be more efficient the lower the meat pH (must remain below 6) (Hwang *et al.*, 2003). According to the results of the present study the inclusion of the natural antioxidant in the diet is more beneficial in this regard.

5.4 Conclusions

It seems from the results of the present study that dietary lipid saturation in finishing diets of lambs had no influence on DMI, ADG, FCR and carcass characteristics. However, a more efficient utilisation of ME in the finishing diet containing unsaturated soyabean oil compared to saturated beef tallow occurred.

It further seems from the results of the present study that the inclusion of a synthetic or natural antioxidant in finishing diets of lambs had no effect on DMI, ADG, FCR and efficiency of ME utilisation. However, the inclusion of a natural antioxidant in the diet resulted in a higher and lower meat pH, 45 minutes and 24 hours after slaughter, respectively. These influences on meat pH could influence meat quality positively and needs further investigation.

Chapter 6

The effect of dietary lipid saturation and antioxidant source on the meat quality of lambs

6.1 Introduction

During the last few decades there has been increasing interest in adding supplementary lipid sources to ruminant diets, not only in order to increase the energy density of these diets, but also to improve the nutritional quality of the carcass and other ruminant products (Bauchart *et al.*, 1996). As a result of this, there has been an increased interest to find suitable and natural ways to manipulate the fatty acid composition of red meat in a positive way (Wood *et al.*, 2003).

In most parts of the world, carcass leanness is an important criterion when consumers purchase meat (Carpenter, 2001; Strydom *et al.*, 2009). However, a small amount of fat is desirable to sustain palatability, increase tenderness and decrease the risk of the meat drying out. Too much animal fat is perceived as being unhealthy (Strydom *et al.*, 2009). Tallow contains about 40-60% of saturated fatty acids (SFAs) (Enig & Fallon, 1999). Most SFAs are considered as bad for human health because they are associated with diseases such as cardiovascular disease and cancer (Wood *et al.*, 2003). On the other hand, unsaturated fatty acids (UFAs) (especially polyunsaturated fatty acids; i.e. PUFAs) are perceived to be beneficial for human health because their consumption is associated with a lower risk of coronary heart disease, hypertension, type 2 diabetes, renal disease, ulcerative colitis, chronic obstructive pulmonary disease and Crohn's disease (Wood *et al.*, 2003). Mammals cannot produce essential PUFAs (Enig & Fallon, 1999; Zamora, 2005). The two most commonly found PUFAs in food products are linoleic acid (omega-6, i.e. *n*-6) and α -linolenic acid (omega-3, i.e. *n*-3). Excessive amounts of *n*-6 fatty acids in the diet and intake of fats with a very high *n*-6:*n*-3 ratio have been linked with a prevalence to cardiovascular disease, cancer, inflammatory and autoimmune diseases (Zamora, 2005). The ratio of *n*-6:*n*-3 in modern human diets is approximately 15:1, whereas ratios of 2:1 to 4:1 have been associated with reduced mortality from cardiovascular disease, suppressed inflammation in patients with rheumatoid arthritis, and decreased risk of breast cancer (Zamora, 2005).

The micro flora in the rumen converts the majority of dietary UFAs to SFAs through the process of biohydrogenation (Roberts & McKirdy, 1964; Felton & Kerley, 2004). Unlike their short chain counterparts, long chain fatty acids are not absorbed directly from the rumen. When they reach the small intestine, they are mainly saturated and incorporated into body tissues. Despite lipid biohydrogenation, a proportion of dietary PUFAs bypasses the rumen intact and is available for absorption and subsequently deposition in muscle and adipose tissue (Wood *et al.*, 2008; Kott *et al.*, 2010). According to Demeyer & Doreau (1999) ruminant lipid composition reflects the rumen metabolism of dietary fatty acids. As a consequence of this, the fatty acid composition of ruminant meat is mainly saturated and monounsaturated (Wood *et al.*, 2008). This fatty acid composition of muscle and adipose tissue of ruminants is also influenced by the fatty acid composition of the feed (Aurousseau *et al.*, 2004).

The susceptibility of muscle tissue to lipid oxidation depends on a number of factors, the most important being the level of PUFAs present in the particular muscle system (Buckley *et al.*, 1995) and antioxidant levels (Jensen *et al.*, 1997). In meat, monounsaturated fatty acids (MUFAs) are more resistant to oxidative modification than PUFAs (Frémont *et al.*, 1998).

The increasing preference for natural foods has obliged the food industry to include natural antioxidants in various products, not only to delay oxidative degradation of lipids and improve the quality and nutritional value of foods, but to replace synthetic antioxidants (Velasco & Williams, 2011). Synthetic antioxidants [like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin, and trisodium citrate] may exhibit toxic properties like carcinogenicity, resulting in strict regulations over their use in foods (Haak *et al.*, 2006). Flavonoids are secondary plant metabolites derived from phenylalanine and acetyl co-enzyme A (Winkel-Shirley, 2001) and acts as a natural antioxidant (Ross & Kasum., 2007). The use of these naturally occurring bioflavonoids in animal diets is one of the most promising steps in improving meat quality because of its antioxidant properties.

The colour of meat is an important consumer factor (Karami *et al.*, 2010a) indicating meat quality. One way of improving meat colour and fat stability post slaughter is by adding antioxidants directly to the final product (Karami *et al.*, 2010a,b). Another alternative could be

by supplementing antioxidants (also natural antioxidants) in animal diets (Velasco & Williams, 2011). Antioxidants are easily absorbed into the animal circulatory system after ingestion, and are distributed throughout muscle (and other) tissues (Velasco & Williams, 2011). Results in Chapter 5 indicated that the inclusion of a natural- compared to a synthetic antioxidant in a finishing diet for lambs influenced meat pH and probably quality favourably.

No information regarding the effect of dietary lipid saturation combined with a natural antioxidant source on carcass fatty acid quality and meat stability of lambs fed finishing diets could be detected in the available literature. The aim of this study was therefore to investigate the influence of lipid saturation level and antioxidant source in a standard finishing diet on oxidative stability of meat and fatty acid composition of intramuscular- and subcutaneous fat of lamb.

6.2 Material and Methods

The materials and methods used in the present study have been described in Chapter 3 and were briefly as follows:

As mentioned in Chapters 4 and 5, the four dietary treatments consisted of the same basal diet (187 g CP- and 355 g NDF/kg DM) differing in the lipid source (30 g/kg of either saturated beef tallow or unsaturated soyabean oil) and type of antioxidant included (125 g/ton of either a synthetic or natural antioxidant). At termination of the production study (Chapter 5) seven lambs per treatment (45.11 ± 2.99 kg) were randomly selected and slaughtered. To assess the effect of dietary lipid saturation and type of antioxidant 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^* -values) was determined on days 0 and 7 using a Minolta chromo meter. 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 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 fatty acid analyses.

6.3 Results and Discussions

6.3.1 Fatty acid composition and oxidative quality of experimental diets

The mean fatty acid composition and free fatty acid (FFA) content of the four experimental diets are presented in Table 6.1. Day 0 and day 49 depict representative feed samples taken at the start and end of the study period, respectively. Only those fatty acids present in the feed at more than 0.5% of total fatty acid content are reported in Table 6.1.

The influence of saturated beef tallow and unsaturated soybean oil on the higher SFA and UFA (especially PUFA) content of the diet respectively can clearly be observed in Table 6.1. This fatty acid profile was also reflected in the total fatty acids and fatty acid ratios (total SFA, MUFA, PUFA, *n*-6, *n*-3, PUFA:SFA and *n*-6:*n*-3) between the diets. In other words, the increased favourable total PUFA and *n*-3 fatty acid content of the treatments resulting from soyabean oil inclusion (unsaturated soyabean oil with synthetic antioxidant [SC], and unsaturated soyabean oil with natural antioxidant [SB]) improved the PUFA:SFA (P/S) (higher) and *n*-6:*n*-3 (lower) ratios (Table 6.1). Although statistical analysis of the data was not possible, the following observations are of interest:

The FFA content of all the experimental diets tested was higher on day 49 compared to day 0. This primary oxidation of the experimental diets occurred when stored during the study for a period of 49 days at relative moderate average minimum and maximum temperatures of 13.7°C and 23.2°C , respectively. The highest oxidation (40.3%) during the 49 day experimental period occurred in the saturated diet containing a synthetic antioxidant (TC). On the other hand the lowest oxidation (14.3%) was recorded in the saturated diet with a natural antioxidant (TB). This beneficial effect of the natural antioxidant was not supported in the unsaturated diets. The increased FFA content in these diets containing a synthetic or natural antioxidant was 25.9% and 23.7%, respectively. The increased FFA content from day 0 to day 49 is only an indication of primary oxidation, whereas only secondary oxidation affects the fatty acid content of feedstuffs.

Therefore, these results indicate no occurrence of lipid oxidation. According to Buckley *et al.* (1995), Kelley *et al.* (2007) and Kott *et al.* (2010) unsaturated diets are more susceptible to lipid oxidation than more saturated diets, and adding antioxidants to protect these favourable fatty acids from oxidation are of utmost importance. The results of the present study could not support these findings and warrants further research.

Table 6.1 Mean fatty acid composition and free fatty acid (FFA) content of the four experimental diets used during the experimental period

Parameter	Treatment diets* (Day 0)				Treatment diets* (Day 49)			
	TC	TB	SC	SB	TC	TB	SC	SB
Proximate analysis:								
Free fatty acids (g oleic acid per 100g fatty acid)	33.95	35.39	38.33	41.90	47.64	40.44	48.27	51.83
Fatty acid (% of total fatty acids):								
Saturated fatty acids:								
Myristic (C14:0)	1.83	1.91	0.10	0.09	1.90	1.86	0.09	0.05
Palmitic (C16:0)	21.00	21.30	13.63	13.81	20.71	20.42	13.45	13.51
Stearic (C18:0)	11.90	12.62	3.36	3.41	12.65	12.36	3.34	3.35
Monounsaturated fatty acids:								
Palmitoleic (C16:1c9)	0.63	0.68	0.11	0.11	0.68	0.68	0.11	0.11
Oleic (C18:1c9; <i>n</i> -9)	27.63	28.41	24.58	24.70	28.47	28.28	24.62	24.56
Vaccenic (C18:1t11)	1.08	1.03	1.03	1.01	1.05	1.05	1.03	1.00
Polyunsaturated fatty acids:								
Linoleic (C18:2c9,12; <i>n</i> -6)	31.07	29.48	50.33	49.80	29.82	30.55	50.30	50.22
α -Linolenic (C18:3c9,12,15; <i>n</i> -3)	3.01	2.73	5.81	5.89	2.83	2.98	5.95	6.06
Total fatty acids:								
SFA ¹	36.12	37.25	17.91	18.13	36.71	36.03	17.72	17.65
MUFA ²	29.67	30.38	25.82	25.96	30.49	30.30	25.84	25.87
PUFA ³	34.21	32.35	56.31	55.88	32.79	33.67	56.44	56.45
<i>n</i> -6 ⁴	31.07	29.48	50.33	49.80	29.82	30.55	50.30	50.22
<i>n</i> -3 ⁵	3.14	2.87	5.98	6.08	2.97	3.11	6.14	6.23
Fatty acid ratios:								
PUFA:SFA	0.95	0.87	3.14	3.08	0.89	0.93	3.19	3.20
<i>n</i> -6: <i>n</i> -3	9.90	10.28	8.42	8.19	10.05	9.82	8.19	8.06

* Treatments: T = Saturated beef tallow (30 g/kg feed); S = Unsaturated soyabean oil (30 g/kg feed); C = Synthetic antioxidant (125 g/ton feed); B = Natural antioxidant (125 g/ton feed). Day 0 and day 49 depict representative feed samples taken at the start and end of the production study, respectively.

¹ Total saturated fatty acids; ² Total monounsaturated fatty acids; ³ Total polyunsaturated fatty acids; ⁴ Total omega-6 fatty acids; ⁵ Total omega-3 fatty acids.

From Table 6.1 it is clear that the SFA, MUFA and PUFA content of the experimental diets sampled at day 0 compared well with that sampled on day 49. With the absence of a diet containing no antioxidant, the inclusion of an antioxidant in the diet on the SFA and UFA content as affected by oxidation over time, could however not be detected in this study. According to Frémont *et al.* (1998) MUFAs are more resistant to oxidative modification than PUFAs. Accordingly the MUFA content as a percentage (%) of total fatty acids did not change over 49 days in the current study.

6.3.2 Muscle fatty acid composition

The effect of dietary antioxidant and lipid source on the fatty acid composition of intramuscular fat of S.A. Mutton Merino lambs is shown in Table 6.2.

Table 6.2 The effect of dietary antioxidant and lipid source on the muscle fatty acid composition of S.A. Mutton Merino lamb meat (means)

Fatty acid (% of total fatty acids)	Antioxidant (125 g/ton)	Fat source (30 g/kg)			Significance (<i>P</i> -value)			CV [#]
		Saturated	Unsaturated	Mean	Fat source	Antioxidant	Interaction	
Saturated fatty acids:								
Myristic (C14:0)	Synthetic	2.88	2.84	2.86	0.7406	0.9540	0.9582	13.26
	Natural	2.89	2.84	2.87				
	Mean	2.89	2.84					
Palmitic (C16:0)	Synthetic	26.12	24.68	25.40 ¹	0.2593	0.0414	0.1535	5.56
	Natural	26.49	26.67	26.58 ²				
	Mean	26.31	25.68					
Stearic acid (C18:0)	Synthetic	18.21	19.48	18.85	0.7399	0.0777	0.0448	7.44
	Natural	18.35	17.43	17.89				
	Mean	18.28	18.46					
Monounsaturated fatty acids:								
Palmitoleic (C16:1c9)	Chemical	2.13	1.71	1.92	<.0001	0.6344	0.5244	10.19
	Natural	2.05	1.72	1.89				
	Mean	2.09 ^b	1.71 ^a					

Table 6.2 (Cont.)

Fatty acid (% of total fatty acids)	Antioxidant (125 g/ton)	Fat source (30 g/kg)			Significance (<i>P</i> -value)			CV [#]
		Saturated	Unsaturated	Mean	Fat source	Antioxidant	Interaction	
Oleic (C18:1c9; <i>n</i> -9)	Synthetic	39.24	36.79	38.02	0.0269	0.1294	0.2783	4.98
	Natural	37.35	36.47	36.91				
	Mean	38.29 ^b	36.63 ^a					
Vaccenic (C18:1t11)	Synthetic	1.02	1.03 ¹		0.0021	0.0117	0.0041	7.17
	Natural	1.01 ^a	1.20 ^{b2}					
Polyunsaturated fatty acids:								
Linoleic (C18:2c9,12; <i>n</i> -6)	Synthetic	4.81	7.15	5.98	<.0001	0.4059	0.5084	16.29
	Natural	5.38	7.22	6.30				
	Mean	5.09 ^a	7.19 ^b					
CLA* (C18:2c9t11; <i>n</i> -6)	Synthetic	0.75	1.00	0.88	0.0007	0.2962	0.1007	31.94
	Natural	0.68	1.31	1.00				
	Mean	0.72 ^a	1.16 ^b					
α -Linolenic (C18:3c9,12,15; <i>n</i> -3)	Synthetic	0.58	0.73	0.66	0.0006	0.1696	0.6816	12.98
	Natural	0.65	0.76	0.70				
	Mean	0.61 ^a	0.75 ^b					
Total fatty acids:								
SFA*	Synthetic	49.02	48.77	48.90	0.2452	0.9250	0.5020	2.58
	Natural	49.39	48.49	48.94				
	Mean	49.20	48.63					
MUFA*	Synthetic	43.12	40.32	41.72	0.0112	0.1738	0.1715	4.32
	Natural	41.23	40.33	40.78				
	Mean	42.17 ^a	40.32 ^b					
PUFA*	Synthetic	7.86	10.90	9.38	0.0001	0.2560	0.4995	15.74
	Natural	8.93	11.18	10.06				
	Mean	8.40 ^b	11.04 ^a					
<i>n</i> -6*	Synthetic	6.50	9.33	7.91	<.0001	0.2034	0.7492	15.35
	Natural	7.28	9.80	8.54				
	Mean	6.89 ^a	9.56 ^b					
<i>n</i> -3*	Synthetic	1.36	1.58	1.47	0.3145	0.0943	0.2818	17.35
	Natural	1.65	1.64	1.65				
	Mean	1.51	1.61					

Table 6.2 (Cont.)

Fatty acid (% of total fatty acids)	Antioxidant (125 g/ton)	Fat source (30 g/kg)			Significance (<i>P</i> -value)			CV [#]
		Saturated	Unsaturated	Mean	Fat source	Antioxidant	Interaction	
Fatty acid ratios:								
PUFA:SFA	Synthetic	0.16	0.22	0.19	<.0001	0.1466	0.9162	15.58
	Natural	0.18	0.24	0.21				
	Mean	0.17 ^b	0.23 ^a					
<i>n</i> -6: <i>n</i> -3	Synthetic	4.82	5.98	5.40	<.0001	0.8154	0.3327	13.92
	Natural	4.47	6.20	5.34				
	Mean	4.64 ^a	6.09 ^b					

^{a,b} Row means with different superscripts differ significantly ($P < 0.05$) within parameter means.

^{1,2} Column means with different superscripts differ significantly ($P < 0.05$) within parameter means.

[#] Coefficient of variation (%).

* Fatty Acids: 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 Table 6.2 that lipid saturation in the diet had no significant effect ($P > 0.05$) on the SFA content of lamb intramuscular fat. However the MUFA and PUFA content of lamb intramuscular fat were significantly ($P < 0.05$) influenced by lipid saturation in the diet. In the case of MUFAs, palmitoleic- and oleic acid were significantly ($P < 0.05$) decreased in muscle by the inclusion of unsaturated lipid in the diet. As indicated in Table 6.2 and Figure 6.1 a significant ($P = 0.0041$) lipid x antioxidant source interaction occurred for vaccenic acid. This fatty acid was increased ($P = 0.0021$) in muscle fat when unsaturated soyabean oil was included in a diet with a natural antioxidant.

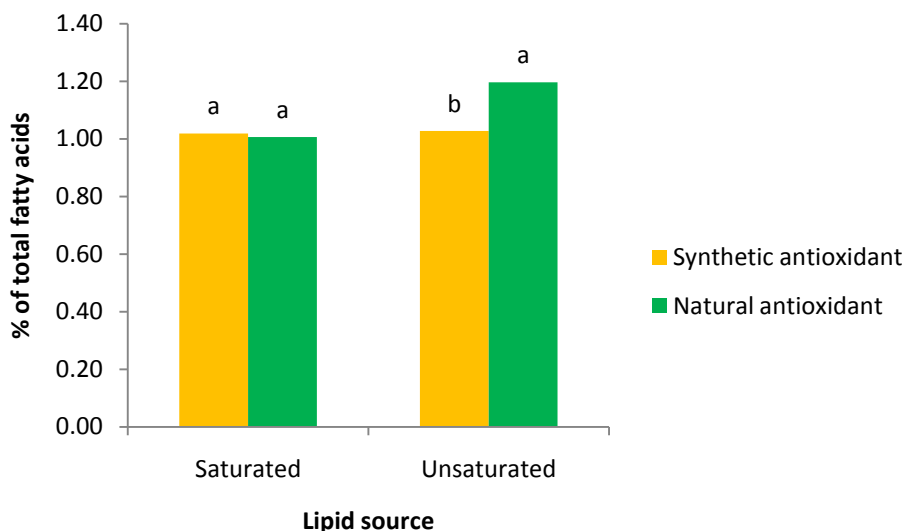


Figure 6.1 Dietary lipid x antioxidant source interaction for muscle monounsaturated vaccenic acid (C18:1t11) content.

^{a,b} Chart bars with different superscripts within a lipid source differ significantly ($P < 0.05$).

In contrast with MUFAs (palmitoleic and oleic acids), the PUFA [linoleic acid, conjugated linoleic acid (CLA) and α -linolenic acid] content of lamb muscle increased ($P < 0.05$) following the more unsaturated soyabean oil inclusion in the diet (Table 6.2). Accordingly Demeyer & Doreau (1999) and Aurousseau *et al.* (2004) explained that the fatty acid composition of muscle and adipose tissue is mainly influenced by the fatty acid composition and rumen metabolism of the diet fed to ruminants. As mentioned, the favourable CLA content of the muscle tissue of lambs were also positively influenced ($P = 0.0007$) by unsaturated soyabean oil inclusion, despite the fact that the dietary treatments did not contain any CLA (Table 6.1). Several researchers (Mulvihill, 2001; Radunz *et al.*, 2009; Woods & Fearon, 2009) is of opinion that CLA 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 $\Delta 9$ -desaturase enzymes. After the isomerisation of linoleic acid in the rumen, the *cis-9,trans-11*CLA isomers are rapidly hydrogenated to vaccenic acid, then less rapidly to stearic acid, resulting in an increase in vaccenic acid (Bauman *et al.*, 1999) and could result in the accumulation of vaccenic acid and CLA-isomers in the rumen digesta (Demeyer & Doreau,

1999). Hence, the formation of CLA was to be expected since the unsaturated soyabean oil diets contained much more linoleic acid (Table 6.1; SC and SB) compared to the saturated tallow diets (Table 6.1; TC and TB).

As a result of the increase in total PUFA and total *n*-6 fatty acid content of intramuscular fat in animals receiving the more unsaturated soyabean oil, the P/S and *n*-6:*n*-3 ratios also demonstrated a significant ($P < 0.05$) increase. Total SFA and total *n*-3 content of the muscle tissue were not significantly ($P > 0.05$) influenced by dietary treatment. The lack of dietary effect on total SFA and total *n*-3 content of muscle can be explained by the fact that the increase in total PUFA and total *n*-6 in muscle of animals receiving the unsaturated soyabean oil was balanced by a decrease ($P < 0.05$) in individual (palmitoleic- and oleic acid) and total MUFA.

With regard to the nutritional indices studied, apart from the type of fatty acid present in the edible tissues, the ratios of P/S and *n*-6:*n*-3 are widely used to evaluate the nutritional quality of fat and deemed to be important (Orellana *et al.*, 2009). The recommended value for the P/S ratio is 0.4 or higher (De la Fuente *et al.*, 2009). Although the P/S ratio was improved by replacing SFAs (tallow) with UFAs (soyabean oil) in the diet, the average ratio (0.23) was still lower than the recommended value. It seems that the P/S ratio (3.1) in the experimental unsaturated diet was too low to achieve the desirable ratio in the muscle of lambs (Table 6.1).

According to Wood *et al.* (2003) the recommended ratio of *n*-6:*n*-3 fatty acids is below 4.0 in muscle from a consumer-health viewpoint. From Table 6.2 it is evident that this ratio was higher in intramuscular fat, especially where the diet with unsaturated soybean oil was fed to lambs. The less favourable results of the unsaturated soyabean oil diet were unexpected. These results were related to the non-significant ($P = 0.3145$) influence of dietary unsaturated soyabean inclusion on total *n*-3, and on the other hand significant ($P < 0.0001$) increase of total *n*-6 in intramuscular fat. Furthermore these results occurred despite the fact that muscle from animals receiving the soyabean diet had a significantly ($P < 0.05$) higher content of an *n*-3 fatty acid in the form of α -linolenic acid compared to animals receiving the tallow supplemented diet. This can be ascribed to a relative higher increase of linoleic acid (linoleic and CLA, both *n*-6 fatty acids) in the muscle fat of the animals receiving the soyabean oil supplemented diet (Table 6.2). In an

effort to manipulate the *n*-6:*n*-3 ratio of lamb meat the focus should either be to increase the *n*-3 and lower the *n*-6 content of the diet and eventually the muscle tissue. By merely replacing saturated tallow with unsaturated soyabean oil in the diet, the desirable *n*-6:*n*-3 fatty acid ratio in lamb muscle could not be achieved.

From the results in Table 6.2 it further seems that, with the exception of palmitic-, stearic- and vaccenic acid, antioxidant type in the diet did not influence the fatty acid content and ratios in lamb muscle. Adding a natural compared to a synthetic antioxidant to finishing diets of lambs, increased ($P = 0.0414$) the saturated palmitic- and decreased ($P = 0.0777$) the stearic acid content of lamb muscle. The lipid x antioxidant interaction ($P = 0.0041$) that occurred for the monounsaturated vaccenic acid content in lamb intramuscular fat has already been discussed. The inclusion of a natural antioxidant in the diet containing unsaturated soyabean oil resulted in a higher vaccenic acid content in muscle fat. As explained above, after the isomerisation of polyunsaturated linoleic acid in the rumen to CLA (Radunz *et al.*, 2009; Woods & Fearon, 2009), these isomers are rapidly hydrogenated to vaccenic acid, hence accumulating in the rumen digesta (Demeyer & Doreau, 1999). This increased production of vaccenic acid in the rumen could be due to the higher linoleic acid content of the more unsaturated soyabean oil diets (SC and SB) (Table 6.1) compared to the more saturated tallow diets (TC and TB). The natural antioxidant seems to enhance this process.

6.3.3 Subcutaneous fatty acid composition

The fatty acid composition of subcutaneous lipid tissue of S.A. Mutton Merino lambs as affected by dietary antioxidant and lipid source is presented in Table 6.3.

The influence of lipid source on intramuscular- and subcutaneous fat fatty acid composition was in general the same. The most important difference was that the total subcutaneous *n*-3 fatty acid content was significantly ($P < 0.0001$) increased by the inclusion of unsaturated soybean oil in the diet. Therefore the higher ($P < 0.05$) *n*-6:*n*-3 ratio observed for intramuscular fat of lambs in the UFA treatment, did not occur in the case of subcutaneous lipid tissue.

Table 6.3 The effect of dietary antioxidant and lipid source on the subcutaneous fatty acid composition of S.A. Mutton Merino lamb meat (means)

Fatty acid (% of total fatty acids)	Antioxidant (125 g/ton)	Fat source (30 g/kg)			Significance (<i>P</i> -value)			CV [#]
		Saturated	Unsaturated	Mean	Fat source	Antioxidant	Interaction	
Saturated fatty acids:								
Myristic (C14:0)	Synthetic	3.27	3.13	3.20	0.8487	0.1533	0.5297	13.24
	Natural	3.41	3.48	3.44				
	Mean	3.34	3.30					
Palmitic (C16:0)	Synthetic	25.56	24.22	24.89 ¹	0.1738	0.0441	0.6539	7.44
	Natural	26.76	26.08	26.42 ²				
	Mean	26.16	25.15					
Stearic acid (C18:0)	Synthetic	24.46	25.87	25.16 ²	0.8149	0.0121	0.2630	11.36
	Natural	22.86	21.93	22.39 ¹				
	Mean	23.66	23.90					
Monounsaturated fatty acids:								
Palmitoleic (C16:1c9)	Synthetic	2.14	1.67	1.91 ¹	<.0001	0.0056	0.4472	7.6
	Natural	2.27	1.89	2.08 ²				
	Mean	2.21 ^b	1.78 ^a					
Oleic (C18:1c9; <i>n</i> -9)	Synthetic	34.49	33.31	33.90	0.5049	0.7406	0.4843	6.68
	Natural	33.60	33.63	33.61				
	Mean	34.04	33.47					
Vaccenic (C18:1t11)	Synthetic	0.90	0.92 ¹		0.0006	0.0388	0.0060	7.63
	Natural	0.87 ^a	1.06 ^{b2}					
Polyunsaturated fatty acids:								
Linoleic (C18:2c9,12; <i>n</i> -6)	Synthetic	3.06	5.00	4.03	<.0001	0.3087	0.9512	14.05
	Natural	2.84	4.80	3.82				
	Mean	2.95 ^a	4.90 ^b					
CLA* (C18:2c9t11; <i>n</i> -6)	Synthetic	0.94	1.25	1.09	0.0514	0.4479	0.8684	37.54
	Natural	1.04	1.40	1.22				
	Mean	0.99	1.33					
α -Linolenic (C18:3c9,12,15; <i>n</i> -3)	Synthetic	0.46	0.64	0.55	<.0001	0.8853	0.1661	12.96
	Natural	0.42	0.67	0.54				
	Mean	0.44 ^a	0.65 ^b					

Table 6.3 (Cont.)

Fatty acid (% of total fatty acids)	Antioxidant (125 g/ton)	Fat source (30 g/kg)			Significance (<i>P</i> -value)			CV [#]
		Saturated	Unsaturated	Mean	Fat source	Antioxidant	Interaction	
Total fatty acids:								
SFA [*]	Synthetic	57.20	56.28	56.74	0.1808	0.5031	0.5815	5.25
	Natural	57.06	54.90	55.98				
	Mean	57.13	55.59					
MUFA [*]	Synthetic	38.28	36.72	37.50	0.5344	0.8090	0.2766	6.31
	Natural	37.50	37.94	37.72				
	Mean	37.89	37.33					
PUFA [*]	Synthetic	4.52	7.00	5.76	<.0001	0.3446	0.7896	11.62
	Natural	4.35	6.70	5.52				
	Mean	4.44 ^a	6.85 ^b					
<i>n</i> -6 [*]	Synthetic	4.06	6.37	5.21	<.0001	0.3554	0.7037	12.08
	Natural	3.93	6.06	4.99				
	Mean	4.00 ^a	6.21 ^b					
<i>n</i> -3 [*]	Synthetic	0.46	0.64	0.55	<.0001	0.8853	0.1661	12.96
	Natural	0.42	0.67	0.54				
	Mean	0.44 ^a	0.65 ^b					
Fatty acid ratios:								
PUFA:SFA ratio	Synthetic	0.08	0.12	0.10	<.0001	0.6772	0.9805	14.7
	Natural	0.08	0.12	0.10				
	Mean	0.08 ^a	0.12 ^b					
<i>n</i> -6: <i>n</i> -3 ratio	Synthetic	8.89	10.01	9.45	0.1254	0.6409	0.1555	10.13
	Natural	9.60	9.65	9.62				
	Mean	9.25	9.83					

^{a,b} Row means with different superscripts differ significantly ($P < 0.05$) within parameter means.

^{1,2} Column means with different superscripts differ significantly ($P < 0.05$) within parameter means.

[#] Coefficient of variation (%).

^{*} Fatty Acids: CLA = Conjugated linoleic acid; SFA = Total saturated fatty acids; MUFA = Total monounsaturated fatty acids; PUFA = Total poly-unsaturated fatty acids; *n*-6 = Total omega-6 fatty acids; *n*-3 = Total omega-3 fatty acids.

These results confirmed the findings of Demeyer & Doreau (1999) and Aurousseau *et al.* (2004) who also observed that lipid source influenced the fatty acid profile of lamb subcutaneous lipid

tissue. Variation in fatty acid content between body tissues of lambs (Enser *et al.*, 1996; Wood *et al.*, 2008) could offer a possible explanation to the observation that some of the fatty acids and fatty acid ratios within intramuscular fat and subcutaneous lipid tissue were differently affected by lipid source. Oka *et al.* (2002) found that the fatty acid content of subcutaneous adipose tissue of steers had higher percentages of monounsaturated palmitoleic- and oleic acid, and a lower content of saturated stearic acid than lipid from other deposit sites. Therefore carcass fatty acid composition may differ depending on the adipose tissue deposit sites (Oka *et al.*, 2002). Huerta-Leidenz *et al.* (1996) reported that the fatty acid content of subcutaneous adipose tissue from Brahman cows and steers differed from the same tissue of Hereford steers, even when raised under identical production systems. A breed effect on the fatty acid content of meat lipid tissue is therefore also possible.

From the results in Tables 6.2 and 6.3 is it clear that the influence of antioxidant type on fatty acid composition was more or less similar in intramuscular fat and subcutaneous lipid tissue. An exception was the monounsaturated palmitoleic acid which was increased ($P < 0.05$) by the inclusion of the natural antioxidant in the diet.

6.3.4 Colour and oxidative stability of lamb meat

The effect of dietary antioxidant and lipid source on the oxidative- and colour (a*-values) stability of S.A. Mutton Merino lamb muscle tissue are presented in Table 6.4.

Table 6.4 The effect of dietary antioxidant and lipid source on the malonaldehyde content and colour (a*-values) stability of S.A. Mutton Merino lamb muscle tissue (mean values)

Parameter	Antioxidant (125 g/ton)	Fat source (30 g/kg)			Significance (<i>P</i> -value)			CV [#]
		Saturated	Unsaturated	Mean	Fat source	Antioxidant	Interaction	
Thiobarbituric acid reactive substance (mg malonaldehyde per kg meat):								
Day 0 (fresh product)	Synthetic	0.12	0.13	0.13	0.038	0.297	0.669	16.79
	Natural	0.11	0.13	0.12				
	Mean	0.11 ^a	0.13 ^b					
Day 7 (refrigerated storage at 4°C)	Synthetic	0.22	0.17	0.19	0.882	0.245	0.231	48.17
	Natural	0.22	0.26	0.24				
	Mean	0.22	0.21					
Day 90 (frozen storage at -18 °C)	Synthetic	0.13	0.16	0.14	0.046	0.129	0.564	28.61
	Natural	0.15	0.19	0.17				
	Mean	0.14 ^a	0.17 ^b					
Meat colour (a*-values):								
Day 0 (fresh product)	Chemical	10.06	10.73	10.39	0.906	0.187	0.139	11.68
	Natural	11.43	10.65	11.04				
	Mean	10.74	10.69					
Day 7 (refrigerated storage at 4°C)	Synthetic	8.95	8.95	8.95	0.685	0.576	0.683	18.33
	Natural	8.86	8.36	8.61				
	Mean	8.90	8.66					

^{a,b} Row means with different superscripts differ significantly ($P < 0.05$) within parameter means.

[#] Coefficient of variation (%).

Unsaturated soyabean oil compared to saturated beef tallow seems to have a negative influence ($P < 0.05$) on the oxidative stability of lamb meat on day 0 of the fresh product as well as 90 days after storage at -18°C (Table 6.4). This could be attributed to the fact that the higher level of PUFAs ($P < 0.05$) present in the intramuscular fat (Table 6.2) increased its susceptibility to lipid oxidation (Buckley *et al.*, 1995). According to Hugo *et al.* (2009) the thiobarbituric acid reactive substance (TBARS) content of meat is highly negatively correlated with sensory evaluation scores. The propensity of meats and meat products to undergo oxidation not only depends on the PUFA content, but 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). These factors were similar for all treatments and could not have influenced the results.

From Table 6.4 it is clear that the oxidative stability of lamb meat were not influenced by antioxidant source ($P > 0.05$). Jensen *et al.* (1997) is of opinion that the oxidative susceptibility of muscle tissue decreases with the presence of antioxidants and their level in muscle tissue. According to Buckley *et al.* (1995) it is highly unlikely that the armory of the antioxidant defensive systems (superoxide dismutase, glutathione peroxidase, ceruloplasmin and transferrin) available to the cell in the live animal still function in the post slaughter phase, because of quantitative changes in several metabolites and physical properties. Therefore, adding antioxidants (natural and chemical) in the diets of lambs seems to be effective.

Dietary lipid- and antioxidant source did not affect ($P > 0.05$) lamb meat colour on days 0 and 7. In Chapter 5 it was speculated that the effect of a natural- compared to a synthetic antioxidant on pH could positively influence meat colour. These findings were however not supported by the results of the present study.

The data in Figure 6.2 represents the average meat colour (a^* -values) of lamb chops (Chop 2) stored for seven days at 4°C under florescent light. The graph clearly illustrates how the red colour of meat decreases over time under florescent light. This decrease in meat colour occurred despite the presence of the antioxidants in the experimental diets. The influence of an antioxidant in the diet on meat colour was however not investigated in the present study.

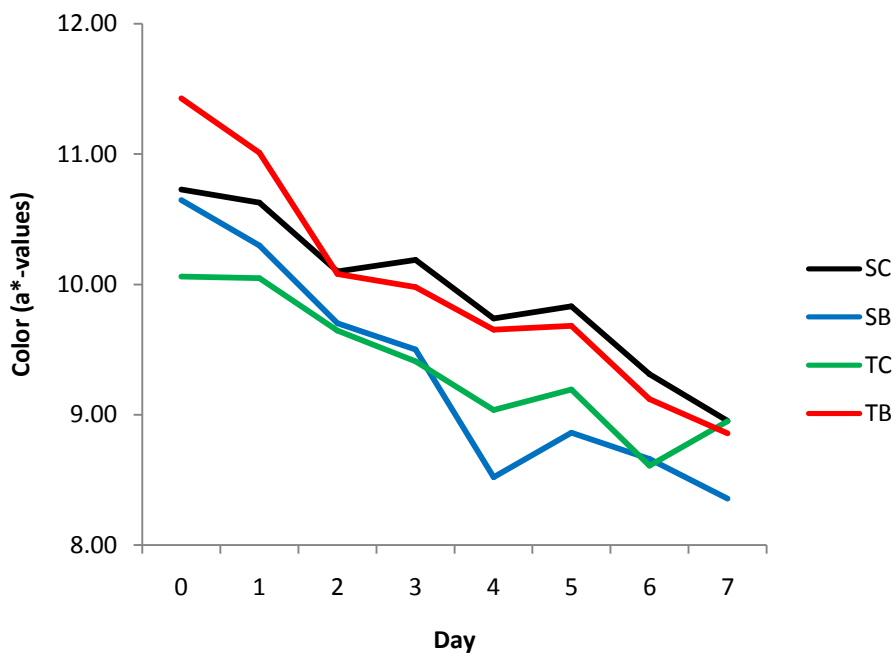


Figure 6.2 Average meat colour (a*-values) of lamb chops (Chop 2) stored for 7 days at 4°C under florescent light. Treatments: T = Saturated beef tallow (30 g/kg feed); S = Unsaturated soyabean oil (30 g/kg feed); C = Synthetic antioxidant (125 g/ton feed); B = Natural antioxidant (125 g/ton feed).

6.4 Conclusions

This research results clearly indicate that the fatty acid composition of intramuscular fat and subcutaneous adipose tissue of ruminants is influenced by the fatty acid composition of the feed and that the fatty acid profile of lamb meat can be manipulated ($P < 0.05$) by dietary lipid saturation level. In this regard it seems that the replacement of saturated beef tallow with unsaturated soyabean oil in finishing diets of lambs lowers the MUFA- (palmitoleic and/or oleic acid) and increases the PUFA content (linoleic acid, α -linolenic acid and CLA) of intramuscular fat and subcutaneous lipid tissue. These PUFA results were also related to a higher total PUFA as well as $n-6$ and/or $n-3$ content in intramuscular fat and subcutaneous lipid tissue. Accordingly the inclusion of unsaturated soyabean oil in the diet resulted in a higher PUFA:SFA ratio in both muscle fat and subcutaneous lipid tissues. In contrast with subcutaneous lipid a higher $n-6:n-3$ ratio occurred in intramuscular fat. However, the replacement of saturated beef tallow with unsaturated soyabean oil did not result, from a human health point of view, the desirable PUFA:SFA- and $n-6:n-3$ ratios in muscle fat and subcutaneous lipid. The manipulation of the fatty acid content of finishing diets of lambs to achieve the desirable ratios warrants further research.

The inclusion of unsaturated soyabean oil in the diet resulted in a decreased ($P < 0.05$) oxidative stability (TBARS) of lamb muscle tissue with no effect on colour stability ($P > 0.05$).

With the exception of the natural antioxidant that increased saturated palmitic- and monounsaturated palmitoleic acid, and decreased saturated stearic acid in muscle and/or subcutaneous fatty acids, dietary antioxidant type did not affect ($P > 0.05$) the fatty acid composition, colour and oxidative stability of lamb meat. It seems however that monounsaturated vaccenic acid was increased in muscle fat and subcutaneous lipid when unsaturated soyabean oil was included in a diet with a natural antioxidant.

Chapter 7

General Conclusions

Meat is by virtue a valuable animal product containing essential nutrients like high quality proteins (amino acids), fatty acids, vitamins and minerals. These nutrients form part of a daily healthy balanced diet for humans. As the fatty acids in ruminant tissue are mostly saturated, increasing the more healthy unsaturated fatty acids improves the dietetic quality of the product. Most saturated fatty acids (SFAs) contained in red meat are assumed to be bad for human health because they are connected to various diseases. On the other hand, unsaturated fatty acids (UFAs) are perceived to be beneficial for human health lowering the risk of coronary heart disease, hypertension and type-2 diabetes, to mention a few. Consuming UFAs from a young age help promote brain cognitive development, normal hormonal functioning and even maintaining a healthy body weight. Appropriate dietary lipid sources in lamb diets may aid in increasing the healthy fatty acid content of mutton for human consumption. Furthermore protecting these fatty acids from oxidation may ensure that these fatty acids remain available even if meat is stored for some time before it is purchased. Antioxidants are effectively used for this purpose. However the increasing preference for natural- compared to chemical antioxidants to delay nutrient oxidative degradation and maintain its dietary quality needs urgent attention. Hence the effects of a natural antioxidant and lipid saturation in a standard feedlot diet on nutrient digestibility, lamb performance, meat fatty acid composition, oxidative- and colour stability of lamb was addressed in this study.

From the results of the present study it seems that the apparent digestibility of NDF in the diet was reduced with the inclusion of unsaturated soyabean oil compared to saturated beef tallow. The most reasonable explanation for differences in NDF digestibility is the negative effect of UFAs on the digestibility of fibre due to its antimicrobial and coating effect. The detrimental effect of the unsaturated lipid source on the apparent digestibility of NDF was associated with a lower digestible NDF and ME content in the experimental diet. However important is the possibility that methane production is reduced when ruminant feed is supplemented with fat rich in PUFAs and MUFAs through a direct use of hydrogen by hydrolysing bacteria in the rumen. Therefore, calculating the ME content of the diet from that of the DE using a factor of 0.8, which

represents energy lost as methane gas and urine, probably over-estimated the production of methane where unsaturated soyabean oil was included in the diet. This could contribute to the decrease in the ME content of the soyabean oil compared to the tallow diet. Further research is required in this regard.

The inclusion of a synthetic compared to a natural antioxidant in the diet also decreased the apparent digestibility of NDF, which was also associated with a lower digestible NDF content of the experimental diet.

Dietary lipid source had no influence on the DMI of the lambs, irrespective the lower NDF digestibility of the unsaturated soyabean oil diet. Nevertheless the inclusion of unsaturated soyabean oil in the diet containing a synthetic antioxidant resulted in a lower MEI of the lambs in contrast with the natural antioxidant. This could probably be explained by the negative influence of an unsaturated lipid source on fibre digestion and the ME content of the experimental diets. Dietary saturation level had also no influence on the final live weight, ADG and FCR of the lambs. These results did not support the lower MEI of lambs fed the unsaturated soyabean oil diet containing the synthetic antioxidant. It could be speculated that the heat increment of fermentation and methane production of this diet was lower and resulted eventually in the same net energy intake. Heat of fermentation resulting from micro-organisms in the rumen of ruminants was therefore probably decreased by the unsaturated lipid source because of a negative effect on fibre digestibility due to its antimicrobial and coating effect. Hence, less energy was lost as heat in the rumen resulting in more energy available for productive purposes. In contrast, the inclusion of the same unsaturated lipid source in the form of soyabean oil in the diet resulted in a better utilisation of ME and could be attributed to the lower ME content of the unsaturated soyabean oil diet as determined in the digestibility study and no effect in DMI and ADG between lipid sources. The source of antioxidant did not influence any of these mentioned parameters.

Lipid saturation had no influence on any of the physical carcass characteristics tested. The meat pH measured 45 minutes after slaughter was lower when a synthetic antioxidant was added to the diet, compared to that of a natural antioxidant. In contrast, meat pH measured at 24 hours post

slaughter was lower when a natural antioxidant was added to the diet. The slower decline in meat pH during the first 45 minutes and lower ultimate pH 24 hours post mortem of animals supplemented a natural antioxidant could be considered beneficial since a rapid decrease in pH within the first 45 minutes post mortem, while the temperature of the meat is still high, may lead to protein denaturation resulting in pale colour and water loss.

It seems that replacing saturated beef tallow with unsaturated soyabean oil in the diets of lambs lowers the MUFA (palmitoleic and/or oleic acid) and increases the PUFA content (linoleic- and α -linolenic acid) of intramuscular fat and subcutaneous lipid tissue. Following the same trend as the individual fatty acids, the total PUFA, total *n*-6 fatty acid content as well as PUFA:SFA ratio of lamb meat increased with the inclusion of the more unsaturated soyabean oil in the diet. Only the total *n*-3 fatty acid content of subcutaneous fat was increased by the inclusion of unsaturated soybean oil in the diet. This may explain why the *n*-6:*n*-3 ratio of subcutaneous lipid tissue was left unaffected. The CLA content of both intramuscular fat and subcutaneous lipid tissue were also increased by unsaturated soyabean oil inclusion. CLA is produced by bacterial species in the rumen through the isomerisation of linoleic acid, or through the endogenous synthesis from vaccenic acid via $\Delta 9$ -desaturase enzymes. Due to the elevated level of linoleic acid in the soyabean oil treatments, this result was expected. A lipid x antioxidant source interaction occurred for vaccenic acid. Vaccenic acid was increased in lamb meat only when a natural antioxidant was supplemented in a diet containing unsaturated soyabean oil. CLA isomers are rapidly hydrogenated to vaccenic acid resulting in an accumulation of vaccenic acid in the rumen digesta, and could pose as a possible explanation. The desirable tissue P/S and *n*-6:*n*-3 fatty acid ratios (above 0.4 and below 4.0, respectively) could not be achieved by merely replacing saturated tallow with unsaturated soyabean oil in the diet. Variation in fatty acid content between adipose tissue deposit sites of lambs (with special reference to oleic acid, total MUFA, total *n*-3 and *n*-6:*n*-3 ratio), as well as animal sex and breed, could offer a possible explanation to the observation that some of the fatty acids and fatty acid ratios within intramuscular fat and subcutaneous lipid tissue were differently affected by lipid source.

The inclusion of unsaturated soyabean oil in the diet resulted in a decreased oxidative stability (TBARS) of lamb muscle tissue on day 0 of the fresh product as well as 90 days after storage at -

18°C. This could be attributed to the fact that the higher level of PUFAs present in the intramuscular fat increased its susceptibility to lipid oxidation.

With the exception of the natural antioxidant that increased palmitic- and palmitoleic acid and decreased stearic acid in muscle and/or subcutaneous fat, dietary antioxidant type did not affect the fatty acid composition, colour- or oxidative stability of lamb meat.

Considering the results of the present study, it seems that an unsaturated lipid source (i.e. soyabean oil) does affect the fatty acid composition of lamb meat favourable without effecting production performance. As antioxidant source did not affect diet DM digestibility, animal performance or meat quality in the current study to any meaningful extent, it can be speculated that the natural antioxidant was as effective as the synthetic antioxidant at the 125 g/ton inclusion level. Due to the lack of information regarding the proposed inclusion level of the natural antioxidant in lamb diets, and as it seems to have less of an effect on the apparent NDF digestibility, warrants further research.

The significant responses in terms of diet digestibility, production efficiency and carcass fatty acid composition are important considering the paucity of research concerning the effect of lipid saturation and antioxidant source on the mentioned parameters. As the individual fatty acid and fatty acid ratios of lamb meat were positively affected by unsaturated lipid in the diet, further research attempting to manipulate a specific fatty acid or fatty acid ratios of lamb meat to meet consumer demands from a human health point of view warrants further investigation.

Abstract

A study was conducted to investigate the influence of antioxidant source and fatty acid saturation in a standard finishing diet on apparent digestibility, production performance, fatty acid composition and oxidative stability of lamb. The four dietary treatments consisted of the same basal diet (187 g CP- and 355 g NDF/kg DM) differing in the lipid source (30 g/kg of either saturated beef tallow or unsaturated soyabean oil) and type of antioxidant included (125 g/ton of either a synthetic or natural antioxidant). Eighty-four S.A. Mutton Merino lambs (27.64 ± 1.72 kg) were randomly allocated to the four dietary treatments (n=21 lambs per treatment) and subdivided into seven replicates per treatment (n=3 lambs per replicate). After dietary adaptation of 8 days all lambs received the experimental diets for the remaining period (41 days). A digestibility study was conducted over a 12-day period (4-day adaptation to the faecal bags followed by an 8-day collection period). Seven lambs per treatment were randomly selected and slaughtered at completion of the production study. Physical carcass characteristics, muscle pH, muscle- and subcutaneous fatty acid composition, as well as meat oxidative- (malonaldehyde content) and colour stability was measured.

The apparent NDF digestibility was reduced ($P = 0.0548$) with the inclusion of unsaturated soyabean oil in the diet compared to saturated beef tallow. This was associated with a significant ($P < 0.05$) lower digestible NDF and ME content in the experimental diet. No significant ($P > 0.05$) differences in dry matter intake, daily gain and feed efficiency of lambs occurred. The addition of unsaturated soyabean oil significantly increased ($P = 0.0003$) the efficiency of ME utilisation in the diet. Lipid saturation level in finishing diets for lambs did not influence ($P > 0.05$) the physical carcass characteristics and meat pH of lambs.

Saturated beef tallow increased ($P < 0.05$) the monounsaturated palmitoleic- and oleic acid content of lamb subcutaneous and/or muscle tissue, whereas the more unsaturated soyabean oil increased ($P < 0.05$) the polyunsaturated linoleic acid, α -linolenic acid and CLA content of both muscle fat and subcutaneous lipid tissue. Monounsaturated vaccenic acid was increased in lamb meat when unsaturated soyabean oil was included in combination with the natural antioxidant. The total PUFA, total *n*-6 fatty acid and PUFA:SFA ratio of lamb meat increased ($P < 0.05$) with

the inclusion of the more unsaturated soyabean oil in the diet. A higher ($P < 0.0001$) $n-6:n-3$ ratio occurred in the intramuscular fat of lambs fed the unsaturated soyabean oil diet. Unsaturated soyabean oil negatively influenced ($P < 0.05$) the oxidative stability of lamb meat on days 0 (fresh) and 90 (frozen), compared to saturated tallow.

The inclusion of a synthetic compared to a natural antioxidant in the diet decreased ($P = 0.0672$) the apparent digestibility of NDF, which was also associated with a significantly ($P = 0.0159$) lower digestible NDF content of the experimental diet. The meat pH measured 45 minutes after slaughter was significantly ($P = 0.009$) decreased when a synthetic antioxidant was added to the diet. Meat pH measured at 24 hours post slaughter was lower ($P = 0.0433$) when a natural antioxidant was added to the diet. With the exception of the natural antioxidant that increased ($P < 0.05$) the saturated palmitic- and monounsaturated palmitoleic acid content of subcutaneous and/or muscle fat, and decreased ($P < 0.05$) the monounsaturated stearic acid content of intramuscular fat, dietary antioxidant type did not to effect ($P > 0.05$) the fatty acid composition of lamb meat, neither the colour- nor oxidative stability.

These results suggest that the fatty acid profile of lamb can be manipulated by the saturation level of the lipid source included in the diet. However, the replacement of saturated tallow with unsaturated soyabean oil did not result, from a human health point of view, in the desirable PUFA:SFA and $n-6:n-3$ ratios in muscle fat and subcutaneous lipid tissue. Therefore, the manipulation of the fatty acid content of finishing diets to achieve the desirable ratios within lamb meat, as well as the optimal inclusion level of a bioflavonoid antioxidant warrants further research.

Opsomming

‘n Studie is uitgevoer om die invloed van antioksidant tipe en lipiedversadiging binne ‘n standaard afrondingsdiëet op die skynbare verteerbaarheid van voedingstowwe, produksie van lammers, vetsuursamestelling asook oksidasiestabiliteit van lamsvleis te evalueer. Die vier proef diëte het uit dieselfde basale rantsoen bestaan (187 g RP- en 355 g NBV/kg DM) waarvan slegs die lipiedbron (30 g/kg versadigde beesvet of onversadigde sojaboonolie) en antioksidant tipe (125 g/ton sintetiese- of natuurlike antioksidant) verskil het. Vier-en-tagtig Suid Afrikaanse Vleismerino lammers (27.64 ± 1.72 kg) is ewekansig aan vier behandelings ($n=21$ lammers per behandeling) toegeken, waarna elke behandeling verder in sewe herhalings ($n=3$ lammers per herhaling) verdeel is. Na ‘n aanpassingsperiode van agt dae is die proefdiëte vir ‘n verdere periode van 41 dae verskaf. ‘n Verteringstudie is vir ‘n periode van 12 dae uitgevoer (vier dae aanpassing aan missakke, gevolg deur ‘n agt dae kolleksieperiode). Na voltooiing van die produksiestudie is 7 lammers per behandeling ewekansig geselekteer en geslag. Fisiese karkaseienskappe, spier pH, vetsuursamestelling van spierweefsel en onderhuidse vet, oksidasiestabiliteit (malonaldehydinhoud) en kleurstabiliteit van vleis is bepaal.

Die skynbare verteerbaarheid van neutraal-bestande vesel (NBV) binne die rantsoen het ($P = 0.0548$) met die insluiting van onversadigde sojaboonolie teenoor versadigde beesvet verlaag. Dienooreenkomstig het ‘n betekenisvolle verlaging ($P < 0.05$) in die skynbaar verteerbare NBV- en metaboliseerbare energie (ME) inhoud van die sojaboonoliedieet voorgekom. Geen betekenisvolle verskille ($P > 0.05$) in DMI, daaglikse toename en doeltreffendheid van voeromsetting van lammers het voorgekom nie. Die byvoeging van sojaboonolie het die doeltreffendheid van ME benutting in die diëet betekenisvol verbeter ($P = 0.0003$). Lipiedversadigheidsvlak in afrondingsdiëte vir lammers het geen invloed ($P > 0.05$) op die fisiese karkaseienskappe en pH van lamsvleis uitgeoefen nie.

Versadigde beesvet het die mono-onversadigde palmitoleïensuur- en oleïensuurinhoud van spierweefsel en/of onderhuidse vetweefsel verhoog ($P < 0.05$), terwyl die meer onversadigde sojaboonolie die poli-onversadigde linoleïensuur, α -linoleniese suur en gekonjugeerde

linoleïensuur inhoud van beide spierweefsel en onderhuidse vetweefsel verhoog het ($P < 0.05$). Mono-onversadige “vaccenic” suur is verhoog ($P < 0.05$) in lamsvleis wanneer onversadige sojaboonolie in kombinasie met ‘n natuurlike antioksidant ingesluit is. Die totale poli-onversadigde vetsuur, totale $n-6$ vetsuur en poli-onversadigde tot versadigde vetsuurverhouding van lamsvleis het met die insluiting van meer onversadigde sojaboonolie in die dieet verhoog ($P < 0.05$). ‘n Hoër ($P < 0.0001$) $n-6$ tot $n-3$ verhouding het voorgekom in die spierweefsel van lammers wat die sojaboonolie-dieet ontvang het. Onversadigde sojaboonolie het ‘n negatiewe invloed ($P < 0.05$) op die oksidasiestabiliteit van lamsvleis op dag 0 (vars) en dag 90 (geevries) gehad teenoor die van versadigde beesvet.

Die insluiting van ‘n sintetiese teenoor ‘n biologiese antioksidant in die dieet het die skynbare NBV verteerbaarheid verlaag ($P = 0.0672$). Die binnespiersse pH, 45 minute na slag was betekenisvol ($P = 0.009$) laer wanneer ‘n sintetiese antioksidant ingesluit is. Die binnespiersse pH, 24 uur na slag, was laer ($P = 0.0433$) wanneer ‘n natuurlike antioksidant in die dieet bygevoeg is. Met uitsondering van die natuurlike antioksidant wat die versadigde palmititiensuur en mono-onversadigde palmitoleïensuur binne subkutaniese- en/of spierweefsel verhoog ($P < 0.05$), en die versadige steariensuurinhoud van binnespiersse vet laat afneem ($P < 0.05$), blyk dit dat die antioksidant tipe geen effek ($P > 0.05$) op die vetsuursamestelling van lamsvleis, vleiskleur of oksidasiestabiliteit uitgeoefen het nie.

Die resultate toon dat die vetsuurprofiel van lamsvleis voordelig gemanipuleer kan word deur die insluiting van ‘n onversadigde lipiedbron in die dieet. Die vervanging van versadigde beesvet met onversadigde sojaboonolie in die dieet, het vanuit ‘n menslike gesondheidsoogpunt, nie die mees geskikte poli-onversadigde tot versadigde vetsuurverhouding en $n-6$ tot $n-3$ vetsuurverhouding in spierweefsel en onderhuidse lipiedweefsel tot gevolg gehad nie. Verdere navorsing rakende die manupilering van die vetsuurinhoud in afrondingsdiëte om die ideale verhoudings in lamsvleis te verkry, sowel as die optimale insluitingsvlak van ‘n bioflavonoid antioksidant is nodig.

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