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The effect of Karoo browse and veldt feeding on long-chain and volatile fatty acid components in lamb

by

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ABSTRACT

THE EFFECT OF KAROO BROWSE AND VELDT FEEDING ON LONG-CHAIN FATTY ACID AND VOLATILE FATTY ACID COMPONENTS IN LAMB

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Keywords: C*is* fatty acid to *trans* fatty acid ratio, comprehensive gas chromatography time of flight mass spectrometry, Dorper, gas chromatography, Karoo browse veldt, Karoo grass veldt, Karoo meat of origin, meat provenance, palmitic acid, oleic acid, origin of production, saturated fatty acid to unsaturated fatty acid ratio traceability, stearic acid

A uniform group of typical Dorper lambs were obtained from a breeding farm in Upington in the Northern Cape Province of the Republic of South Africa. The sheep were randomly allocated into two groups; one herd was raised on Karoo browse veldt, and the other group grazed Karoo grass veldt. Both groups consisted of 5 ewes and 5 wethers. Lambs were allowed to graze for a period of ninety days at an initial live mass of approximately 27 kg in order to reach an average target live mass of 40 kg.

Fat percentage and subcutaneous fat thickness of lambs did not differ significantly between those from Karoo browse veldt or Karoo grass veldt. Subcutaneous adipose tissue samples from lambs from Karoo grass veldt contained significantly higher molar concentrations of oleic acid (C18:1(n-9) *cis*) and significantly lower molar concentrations of myristic acid (C14:0) and linoleic acid (C18:2(n-6)) compared to subcutaneous fat tissue samples from those from Karoo browse veldt. The subcutaneous fat of lambs from Karoo browse veldt contained higher gravimetric concentrations of lauric acid (C12:0) compared to the subcutaneous fat of lambs reared on Karoo grass veldt. The subcutaneous fat from lambs from Karoo browse veldt displayed marginally higher saturated fatty acid to unsaturated fatty acid ratios compared to subcutaneous adipose tissue samples from those from Karoo grass veldt. Subcutaneous fat samples of lambs from Karoo browse veldt contained

significantly lower concentrations of monounsaturated fatty acids and significantly higher saturated fatty acid concentrations in comparison to the subcutaneous adipose tissue of lambs from Karoo grass veldt ($P < 0.05$). Subcutaneous fat of lambs from Karoo grass veldt displayed numerically higher c*is* fatty acid to *trans* fatty acid ratios.

Interesting differences in longer-chain fatty acids (> C20) were discovered between dietary treatments. Gondoic acid (C20:1) was present at significantly higher gravimetric proportions in the fat of ewes from both Karoo browse veldt and Karoo grass veldt compared to wethers and was significantly higher in adipose tissue samples of animals reared on Karoo grass veldt ($P < 0.05$). The subcutaneous fat of lambs from Karoo grass veldt contained significantly higher molar proportions of behenic acid (C22:0) compared to the subcutaneous fat samples of lambs from Karoo browse veldt ($P < 0.05$).

Erucic acid (C22:1(n-9)) was significantly higher in the oesophageal samples obtained from Karoo grass veldt compared to oesophageal samples from lambs that had grazed Karoo browse veldt ($P < 0.05$). Lignoceric acid (C24:0) was present in oesophageal samples, while it was absent in the samples from subcutaneous adipose tissue. The rate at which one fatty acid is deposited from the diet into subcutaneous adipose tissue compared to the rate at which a fatty acid is synthesized *de novo* can explain differences in fatty acid metabolism between different diets. The relation between dietary and tissue fatty acids displayed significant interactions between diet and sex for the ratio of C18:3(n-3)/ C16:0 ($P < 0.05$). Animal fatty acid metabolism did not differ significantly between Karoo browse veldt and Karoo grass veldt in this study ($P < 0.05$).

The application of comprehensive gas chromatography time of flight mass spectrometry identified 3-methylthio-2-butanone as a potential marker for Karoo lamb in raw meat samples. Caryophyllene was present in three of the five Karoo grass-fed lamb samples. β-Caryophyllene may serve as a biomarker in meat to designate a grass diet (Priolo *et al.*, 2004). Cymene was present in each of the Karoo browse and Karoo grass-fed lamb samples and may serve as a potential marker.

“I know what I’m doing. I have it all planned out – plans to take care of you, not abandon you, plans to give you a future to hope for.”

Jeremiah 29:11

DECLARATION

I declare that this dissertation for the degree M.Sc. Agric. Animal Science (Production Animal Physiology and Product Quality) at the University of Pretoria, has not been submitted by me for a degree at any other university.

ETHICS STATEMENT

The author, whose name appears on the title page of this dissertation/thesis, has obtained, for the research described in this work, the applicable research ethics approval.

The author declares that s/he has observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research.

Signature: E.M. 31/08/2019

Erna Mostert

August 2019

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LIST OF ABBREVIATIONS

CLA – conjugated linoleic acid

DFD – Dark, firm and dry meat

GC x GC-TOFMS – comprehensive gas chromatography time of flight mass spectrometry

GC-C-IRMS – gas chromatography-combustion-carbon isotope mass spectrometry

IRMS – isotope ratio mass spectrometry

KDF – Karoo Development Fund

KMOO – Karoo Meat of Origin

MFA/ MUFA – monounsaturated fatty acid

MIRS – mid infra-red spectroscopy

NIRS – near infra-red spectroscopy

NMR – nuclear magnetic resonance

P:S – polyunsaturated fatty acid to saturated fatty acid ratio

PUFA – polyunsaturated fatty acid

RDP – rumen-degradable protein

RT – relative trampling

RUP – rumen-undegradable protein

SAMIC – South African meat industry company

SAMM – South African mutton merino

SCF – subcutaneous fat

SDE – steam distillation-extraction

SFA – saturated fatty acid

SFA:UFA – saturated to unsaturated fatty acid ratio

SIRA – stable isotope ratio analysis

SPME – solid phase microextraction

UFA – unsaturated fatty acid

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1. INTRODUCTION

Human population growth and the emphasis on health and longevity has led to an increase in the demand for quality animal protein by consumers (Webb & Erasmus, 2013). A variety of different livestock production systems are established on the diverse veldt types of South Africa. Sheep and goat production systems utilize natural resources and vary in terms of exploitation of genetic make-up and the implementation of farming methods. Nutrition and environment influence the quality and characteristics of lamb meat. The composition and quality of an animal product depends on agroclimatic environment, nutrition, management, endemic disease, parasites, reproduction, genetic potential, species and breed (Zervas & Tsiplakou, 2011).

Grassland production systems are of a lower nutritional quality during winter as they mainly consist of sour veldt. Livestock are reared by extensive grazing practices or fed on cultivated pastures. A small proportion of lambs are kept in feedlots during the fattening phase. In South Africa, the majority of lambs are raised and marketed from extensive grazing production systems (Webb & Erasmus, 2013).

Karoo farms incorporate extensive grazing systems based on sweet veldt. The Karoo biome is semi-arid and consists of annual grasses and succulent shrubs. Livestock farming, with special emphasis on small stock, is a sustainable farming method that can be employed in the Karoo. Small ruminants are farmed extensively in the Karoo as they have the ability to convert plant material of a low nutritive value to animal products of high quality. The extensive arid environment of the Karoo does not allow for crop production. Small ruminants play an essential role in developing countries as they can be kept in areas that are inappropriate for agronomy, such as rocky terrain or arid landscapes. Some environments prove to be too challenging or impractical to keep cattle (Zervas & Tsiplakou, 2011).

The Karoo Development Foundation established the Karoo Meat of Origin initiative to enable the community to prosper from business generated from the distinctive Karoo sheep meat taste and flavour. The unique taste and sensory characteristics associated with Karoo lamb is linked to free-range production and the grazing by sheep herds of indigenous Karoo vegetation. The possibility arises that meat products can carry false claims of origin from the Karoo without a proper means of authentication. The Karoo Meat of Origin certification ensures that flocks have been reared exclusively on Karoo veldt (‘Karoo Meat of Origin’, 2019).

Grazing activity affects meat quality and characteristics. The selective behaviour of sheep that prefer Karoo vegetation will produce meat that is characteristic of Karoo meat flavour. Dorpers display grazing behaviour that favours woody-type vegetation. They tend to choose Karoo bush species and prefer browse such as shrubs and trees compared to Merino-type breeds (Brand, 2000).

Biohydrogenation in the rumen will affect meat characteristics and quality as it alters the composition of plant material consumed by the animal. Biohydrogenation of fatty acids in the rumen leads to a fatty acid profile that is more saturated. The microbial population of the rumen degrades and metabolizes diet substrates anaerobically to products of fermentation. End products of fermentation are digested and absorbed by the gastrointestinal tract of the animal (Leek, 1993). Therefore, biohydrogenation pathways alter diet material to such an extent that they influence the chemical composition of compounds deposited into muscle.

Factors such as diet and environment influence meat quality and flavour, carcass characteristics and the fatty acid content of meat. Linolenic acid, docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), and eicosapentaenoic acid (EPA) are present at higher concentrations in the meat of livestock reared on grass-based systems. Concentrate-based feeds are associated with high levels of linoleic acid and arachidonic acid in meat (Wood *et al*., 2008). The saturation level of meat fatty acids affects fat firmness (Webb & O'Neill, 2008). Meat fatty acid composition influences shelf life since rancidity is caused by the oxidation of unsaturated fatty acids (Wood *et al*., 2003). Meat pH is affected by diet in that higher meat pH values are linked to grass-based systems. Concentrate diets display a meat pH that is lower compared to meat from animals reared on grass-based systems (Priolo *et al.*, 2002). Meat texture is also affected by the type of feed an animal consumes. High shear force values in cattle are linked to pasture systems (Nuernberg *et al*., 2005). Animal diet can influence meat flavour directly or indirectly (Resconi *et al*., 2009). Chemical compounds in the diet can be transferred directly to meat or serve as precursors to the development of flavour.

Techniques to ascertain the environment and production system from which a meat product originates are of economic importance (Priolo, 2004). Specific chemical compounds can be linked to animal diet and production system. Carotenoids are present at increased concentrations in the meat of animals reared on grass-based systems. Grass-based diets also lead to high levels of skatole (3-methylindole) in meat (Webb & Erasmus, 2013). Grass diets can also be identified by the presence of 2,3-octanedione in meat.

Dynamic headspace analysis can identify compounds that are responsible for meat flavour and aroma. The application of gas chromatography measures meat volatile compounds that may be linked to diet and production system. Stable isotope ratio analysis can be applied to measure the isotopic ratios of meat. Information on geographic origin and diet can be gained by analysis of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ meat ratios (Monahan *et al*., 2018). The aim of this study was to identify compounds responsible for the unique flavour of Karoo lamb. Methods of traceability for the purpose of authenticating the origin of production of lamb meat were also explored.

2. LITERATURE REVIEW

2.1. Veldt types of Southern Africa and corresponding production systems

The South African landscape is characterized by different veldt types that are composed of specific plant species which belong to the same topographic environment. Little seasonal and vegetative variability occur within a certain veldt environment. The natural resources of a biome can be utilized for farming purposes. Sheep farming is practised extensively in the Kalahari, Karoo and grassland biomes of South Africa.

2.1.1. Characterisation of veldt types

The different veldt types of South Africa can be classified according to the nutritive value of the vegetation and can be grouped into sour veldt, sweet veldt and mixed veldt. Sour veldt sustains animal production requirements exclusively during the growing season. Forage contains increasingly more fibre with an increase in maturity of the vegetation. Mature plant material is of a lower digestibility and also contains less nutrients (Tainton *et al.*, 1993). Animals also ingest less mature forage as it is not as palatable as immature plant matter. High levels of rainfall and sandy soils with a lower plane of nutrients lead to the formation of sour veldt. The eastern parts of South Africa harbour sour veldt, which can further be classified into grassland or bush veldt (Tainton *et al.*, 1993).

Browse that can sustain animal production for longer periods of time is classified as sweet veldt. Sweet veldt that grows during the summer can support animal production during the following winter season in a summer rainfall area (Tainton *et al.*, 1993). Vegetation of the sweet veldt type retains palatability and its ability to meet livestock's nutritional production requirements throughout the year. Mature sweet veldt does not contain excessive amounts of fibre and contains sufficient energy and mineral levels (Tainton *et al.*, 1993). Animals can therefore continue browsing on veldt in late summer and autumn. Forage will continue to grow during the year in areas that receive year-round rainfall, which will have the potential to support production throughout the year. Sweet veldt is established in areas that contain clay-type soils and it occupies arid and semi-arid environments. Mixed veldt contains attributes of both sweet veldt and sour veldt. An environment that consists of sweet-mixed veldt can sustain animal production for 9 – 11 months, whereas sour-mixed veldt can support grazing for a shorter period of 6 – 9 months (Tainton *et al*., 1993). Figures 2.1.1 and 2.1.2 show the distribution of the different veldt types of South Africa.

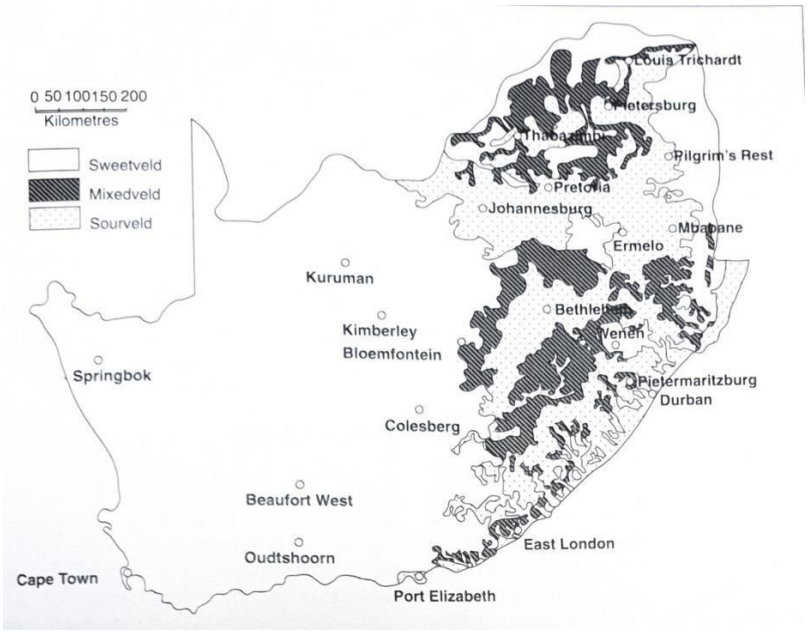

Figure 2.1.1. The distribution of sweet, mixed and sour veldt types in South Africa (Tainton *et al.*, 1993)

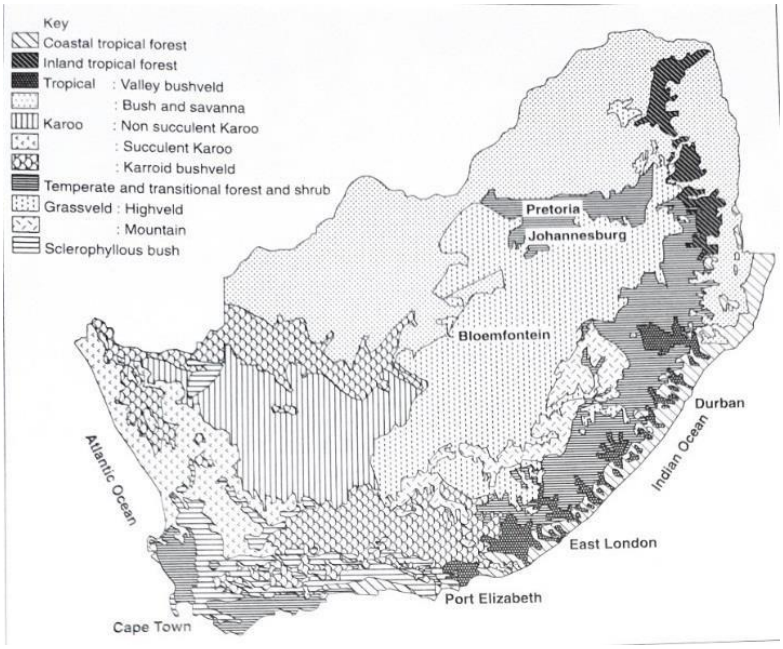

Figure 2.1.2. A map of the vegetation types of South Africa (Tainton *et al.*, 1993)

2.1.2. Karoo production systems

Extensive grazing systems established in the Karoo are subject to semi-arid environments with low annual rainfall (125 – 375mm). Plant distribution is limited and is xerophytic in nature. Karoo veldt contains annual grasses and succulent shrubs (Van Niekerk & Schoeman, 1993). The type of vegetation found in the Karoo is sweet veldt. Sweet veldt has a lower carrying capacity and is sensitive to overstocking and overgrazing (Van Niekerk & Schoeman, 1993). It is high in nutrients and keeps its nutritive value throughout the year. Camps and sheep flocks in the Karoo are large due to low stocking rates and the sensitive character of sweet veldt. The application of low stocking rates ensures that veldt remains in good condition. Vegetation must be rested from grazing to enable optimum growth and production. Veldt that is in a healthy state safeguards sustainable production. The trampling of veldt can be avoided by distributing water troughs and supplements equally (Van Niekerk & Schoeman,1993).

The Karoo biome can be divided into three veldt types; the succulent Karoo; the non-succulent Karoo and the Karroid bush veldt. The Karoo veldt type covers the environment that contains Karoo bushes, which can either be succulent or non-succulent. Soils are lime-based or sandy. The Great Karoo is associated with bush veldt, whereas the northern regions of the Karoo contain grasses such as *Stipagrostis* species. *Stipagrostis* grasses are an important source of grazing in arid environments (Tainton *et al*., 1993).

Grass types that are present in the Karoo biome range from temperate grasses in the west to sub-tropical species in the east. Sub-tropical grasses include *Eragrostis, Aristida, Panicum, Digitaria, Sporobolus and Stipagrostis* species (Tainton *et al*., 1993). *Ehrharta, Pentaschistis, Chaetobromus* and *Stipa* species are examples of temperate grasses (Tainton *et al*., 1993). The Karroid environment contains a variety of different veldt types. The western section of this region contains more succulent species compared to the eastern part (Tainton *et al*., 1993).

The rate at which Karoo vegetation grows during winter and summer is moderate, whereas bush grows vigorously during the autumn season. The speed at which Karoo shrubs recover from grazing is slower compared to grasses. Regrowth of grazed material is depended on storage carbohydrates, which is replenished at a slower rate in Karoo bush compared to grass species (Tainton *et al*., 1993). Veldt management must therefore be implemented to prevent overstocking and overgrazing in order to guarantee a sustainable source of nourishment for livestock (Tainton *et al*., 1993).

Cattle herds are farmed with sheep and goats in extensive areas that possess a climate which is more moderate in character. Cattle graze different plant species compared to small ruminants – they are bulk grazers, whereas sheep and goats graze selectively. Small ruminants have the ability to convert low quality herbage into good quality animal products. Cattle do not flourish in semi-arid areas due to limited vegetation and low rainfall. Sheep and goats have the ability to select plant material that is higher in nutrients and can therefore survive in the semi-arid Karoo (Tainton *et al*., 1993).

Sheep have the ability to consume saline water, which enables them to survive in semi-arid environments due to borehole water serving as the only water source on some farms (Van Niekerk & Schoeman, 1993). Drought, predators and damage to veldt are some of the obstacles encountered in extensive sheep farming practices of the Karoo. Merino, Merino types and Dorper sheep are kept for the purposes of meat production. Merino breeds are also kept for wool production (Van Niekerk, Schoeman, 1993). Other types of small ruminants in the Karoo include Karakul sheep (pelt production); Boer goats (meat production) and Angora goats which are kept for the purposes of mohair production (Tainton *et al*., 1993).

2.1.3. Karoo Meat of Origin (KMOO)

The Karoo Development Foundation (KDF)

The Karoo Development Foundation (KDF) was created in 2009 as an *inter vivos* trust (nr IT1498/2009) under Section 6 (1) of the Trust Property Control Act (Act 57 of 1988). The KDF acts as a guardian for the intellectual rights associated with the "Karoo" name. It aspires to boost and develop the Karoo community from profits gained by the use of the Karoo title. The Karoo is notorious for its beautiful landscapes, tourism and sheep products and the foundation aims to promote these features to enable the local people of the Karoo to prosper. The aim of the KDF is to track, record, protect and celebrate the rich inheritance of the Karoo region, to conserve the natural ecology of the Karoo, to encourage socio-economic development, to strengthen relationships and empower the Karoo community ('Karoo Meat of Origin', 2019).

The certification scheme involves the use of the "Karoo Meat of Origin" – mark that confirms the origin of sheep meat. Producers, abattoirs, butcheries, restaurants and other establishments involved in the production chain or trade of Karoo sheep meat are eligible to apply for certification. The scheme allows for third party certification wherein producers and marketers of Karoo sheep meat can speculate on the rates consumers are prepared to pay for a genuine, high-value product. Third party certification also provides consumers with the opportunity to verify the authenticity of meat. The scheme promotes economic growth for the Karoo region.

The KDF acknowledges the financial contribution of the Western Cape Department of Agriculture; the Northern Cape Department of Agriculture, Land reform and Rural Development and Intellectual Property Services (IPS) of REMGRO Pty Ltd in the process of establishing the Certification Scheme for Karoo Meat of Origin. The KDF also acknowledges contributions from Brand Tree, the University of Pretoria and The University of the Free State ('Karoo Meat of Origin', 2019).

On-farm production

The Karoo Meat of Origin (KMOO) certification guarantees that lambs have been reared extensively on indigenous veldt of the Karoo. Sheep must be born and reared in the Karoo to qualify for the Karoo Meat of Origin certification. The distinctive taste and sensory characteristics of Karoo lamb is associated with free range production and the grazing by herds of local Karoo vegetation ('Karoo Meat of Origin', 2019).

The characteristic taste and flavour associated with meat from lambs raised in the Karoo is derived from vegetation that occurs naturally in the environment. These plant species include:

1. *Plinthus karrooicus* ("Silverkaroo")
2. *Pentzia spinescens* ("Skaapbossie")
3. *Eriocephalus ericoides* ("Kapokbossie")
4. *Salsola glabrescens* ("Rivierganna");
5. *Pentzia incana* ("Ankerkaroo"), and
6. *Pteronia glauca* and *Rosenia humilis* ("Perdebos")

Karoo Meat of Origin is practised in the region where the above plant types are prevalent. Areas that fall outside of this region can also qualify for Karoo Meat of Origin certification when two of the above plant species occur on a specific farm and comprise more than 60% of that farm's natural vegetation. Farmers qualify for certification by providing proof that their farm is situated within the Karoo district and by rearing flocks on indigenous veldt. The map in Figure 2.1.3 demarcates the area wherein Karoo lamb can be produced.

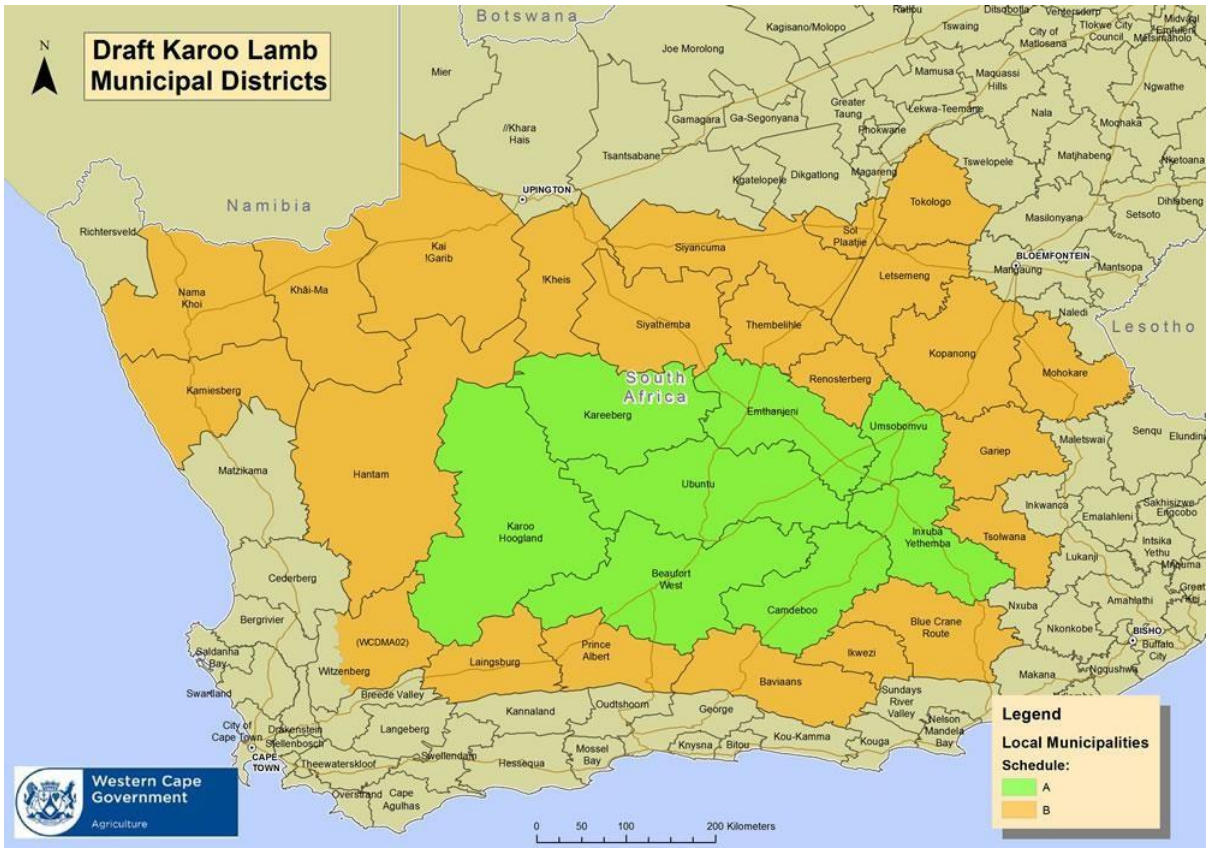

Figure 2.1.3. Karoo Meat of Origin area of production ('Karoo Meat of Origin', 2019)

In order to qualify for the KMOO brand, flocks are not allowed to graze on permanent pasture. Feedlot systems where lambs are fed higher levels of concentrate are prohibited. Supplementary feeding can be given to animals during periods where the natural veldt is sparse or of lower nutritional quality. Supplements can also be considered to aid in reproduction when natural veldt does not meet requirements. Supplements must be given to animals while grazing on veldt and supplementary feeding is limited to 30% of total daily intake. Supplements can consist of added minerals, grains, silage, urea, good quality hay or any type of plant material. Exclusively registered supplementary feeds are to be given to herds and the application of supplements must be documented thoroughly.

The use of antibiotics and growth-promoting substances to boost animal performance is forbidden. Anti-microbial drugs are only to be used for the treatment of disease. Animals are only allowed to be taken from Karoo veldt when handled, such as when being sheared, receiving tags, being processed, receiving medical treatment or during threatening weather conditions. It is important that flocks have access to clean water at all times. Grazing management is essential so as to ensure that veldt is not overgrazed or overstocked beyond carrying capacity. Veldt should be rested to allow for regrowth.

Animal welfare is of the utmost importance at all times. Sheep must be handled carefully during processing and transportation in order to prevent injuries. Trucks are not to be overloaded and animals may not travel further than 250 km between the farm and the abattoir. The keeping of documentation during transport procedures is of utmost importance in that it confirms that animals were reared according to the Karoo Meat of Origin standards. Karoo Meat of Origin sheep must be held in separate pens upon arrival at the abattoir. Certified Karoo lamb contains a Karoo Meat of Origin mark on the packaging. Carcasses are traceable to the farm of origin ('Karoo Meat of Origin, 2019).

Abattoirs and slaughter facilities

Traceability and food safety specifications are applicable to abattoirs that aim to qualify for the Karoo Meat of Origin certification. Abattoirs must be registered with the Red Meat Abattoir Association of South Africa and have to comply with a minimum hygiene and safety (HAS; Neethling, 2019) rating of 75%. Short distances travelled from farm to abattoir relate to animal health procedures and animal welfare requirements. The stress level experienced by animals is minimized when transport time is limited. Abattoirs must adhere to the rules of the Meat Safety Act. A traceability system that has been permitted by SAMIC confirms the provenance of meat products and carcasses. Abattoirs apply a meat stamp and a roller mark to carcasses that comply with the Karoo Meat of Origin certification. Registered abattoirs provide copies of the HAS and KMOO audit reports to the KDF.

The Karoo lamb/mutton Meat of Origin certification system requires that carcasses meet the specifications of the South African meat classification system (source: Meat Classification regulations No R863, published in the RSA Government Gazette of 1 September 2006), as depicted in Table 2.1.1.

Table 2.1.1. The South African meat classification system and Karoo Meat of Origin (KMOO) carcass requirements (adapted from 'Karoo Meat of Origin', 2019)

	Classification	Class	Description	KMOO Requirement
1	Age	A	0 Teeth	All age classes are included.
		AB	1 – 2 Teeth	
		B	1-6 Teeth	
		C	> 6 Teeth	
2	Carcass Mass	A	> 14 kg, but < 25 kg	Carcass mass as described by A, AB, B and C classes are included.
		AB	> 14 kg, but < 29 kg	
		B	> 14 kg, but < 31 kg	
		C	> 14 kg, but < 31 kg	
3	Fatness	0	No fat	Fatness level according to classes 1 – 6 is allowed.
		1	Very lean	
		2	Lean	
		3	Medium	
		4	Fat	
		5	Slightly overfat	
		6	Excessively overflat	
4	Conformation	1	Very Flat	Conformation scores allowed as described by classes 3, 4 and 5.
		2	Flat	
		3	Medium	
		4	Round	
		5	Very round	
5	Damage	1	Slight	Only slight damage to the carcass is permitted (class 1).
		2	Moderate	
		3	Severe	
6	Breed	Ideally meat breeds with even fat distribution and good bone:muscle ratio.		

Moderate and severe damage to carcasses is not accepted. Carcasses from rams that indicate late signs of castration (marked with the MD stamp) will not be accepted for KMOO certification. Animals will be admitted by the abattoir upon receipt of a document that verifies the origin of production. Administration of a carcass number to each animal allows the animal to be tracked to the farm of origin. Each carcass receives a CERT KMOO mark and a Certified Karoo Meat of Origin stamp. Documentation that provides proof of compliance by the abattoir is transported with carcasses. Karoo Meat of Origin carcasses are stored separately from other carcasses during refrigeration.

The following certification marks (Figures 2.1.4, 2.1.5 & 2.1.6) illustrate the CERT KMOO mark and a Certified Karoo Meat of Origin stamp applied to KMOO products. The Karoo Development Foundation (KDF) is the owner of these certification marks ('Karoo Meat of Origin', 2019).

Figure 2.1.4. Certified Karoo Meat of Origin (colour label)

Figure 2.1.5. Certified Karoo Meat of Origin (label)

Figure 2.1.6. Certified Karoo Meat of Origin (stamp)

Processors and packers

Processing units must adhere to Food Premises Regulation R962 requirements. Hygiene, food safety and product quality are aspects of extreme importance when carcasses are managed. Legal specifications such as acts, regulations and industry standards apply to establishments that handle, process, store and pack meat. Traceability standards guarantee the tracking of carcasses to farm of origin, abattoirs and processing facilities. Processing units operate under the Karoo Meat of Origin title following proof of compliance by the abattoir. Meat product packaging is to display the certified Karoo Meat of Origin mark clearly. Figure 2.1.7 displays an example of the product label applied by the processing facility (‘Karoo Meat of Origin’, 2019).

Figure 2.1.7. An example of the product label applied by the processing facility ('Karoo Meat of Origin', 2019)

Butcheries

The KMOO certification only accepts carcasses from recognised KMOO abattoirs, processing facilities and distributors. Auditors may implement audits at random to confirm that butcheries respect KMOO regulations. It is the responsibility of the butchery to trace meat products and carcasses back to the farm of origin, abattoir and processing units. The butchery can add the KMOO certification mark as a separate label or as part of their private label. Figure 2.1.8 shows examples of product labels butcheries can apply to meat products. The change of the tracking number on the certification label to the abattoir number allows the consumer to find the abattoir on the KMOO website from which the meat originated ('Karoo Meat of Origin', 2019).

Figure 2.1.8. Examples of product labels butcheries can apply to meat products ('Karoo Meat of Origin', 2019)

2.1.4. Kalahari production systems

The Kalahari is an environment that consists of a variety of different plant species and topographic features. The majority of the Kalahari consists of sandy soils, with some areas containing limey soils. Rainfall is approximately 100 mm per annum, and it is classified as a semi-arid environment. *Acacia* species are prevalent in the north-western area of the Kalahari thorn veldt. This region also contains Karoo shrubs and desert-type grasses. The bush veldt that occupies the southern scope of the Kalahari experiences higher levels of rainfall with corresponding sour veldt vegetation. *Digitaria eriantha, Heteropogon contortus* and *Panicum maximum* are examples of grass species associated with this veldt type (Tainton *et al*., 1993).

By contrast, the north-eastern component of the Valley bush veldt harbours more grassy vegetation and is less dense, whereas the vegetation towards the south-west is denser and contains more succulent species and dwarf shrubs. Shrub vegetation such as *Portulacaria affra* serve as an important source of browse as grazing is sparse in the Valley bush veldt. Sandy soils and high rainfall contribute to the formation of sour veldt as minerals leach out in response to the poor holding capacity of soils.

2.1.5. Grassland production systems

The grassland environment is distributed within the central region of South Africa at lower altitudes. It encompasses a wide variety of soil types, including sandy and clay-type soils. The presence of *Themeda triandra* among vegetation is an indication that the veldt is in a healthy state. *T. trianda, Tristachya leucotrix, Trachypogon spicatus, Heteropogon contortus* and *Digitaria tricholaenoides* are grass species that are indigenous to the north-eastern parts of South Africa. Mixed grass veldt contains a combination of temperate and sub-tropical grass species. The north-eastern Cape and Free State consist of grass species such as *T. trianda, H. contortus* and *Setaria flabellata*. Overstocking and overgrazing lead to an increase in less desirable grass species such as *Eragrostis obtusa* and *Aristida congesta,* accompanied by a degradation in the quality of veldt. The western region of the grassland biome is arid, and overgrazing will lead to an increase in browse species (Tainton *et al*., 1993).

Production systems utilize resources from the environment. Livestock feed on cultivated pastures or are reared by extensive grazing practices. The addition of supplements is limited, and supplement type is restricted to energy, protein or mineral (Webb & Erasmus, 2013). No hormonal implants are allowed in organic sheep production systems and the use of antibiotics is restricted therapeutic use. Natural veldt is less effective in terms of production efficiency as compared to intensive systems where cereal concentrates are fed. However, animal products are produced at lower input costs in natural grazing systems as they are less intensive.

Rangelands can also be utilized in organic farming systems. A rangeland is composed of particular plant species that occur in the same topographic environment. Rangelands include grasslands, shrublands and forest ranges (Zervas & Tsiplakou, 2011). Grasslands are grazed throughout the year and the quality of the grass is dependent on the season in which it is grazed. Grasslands are of lower nutritional quality during the dry season (Zervas & Tsiplakou, 2011).

Forage that occurs in xerothermic environments consist of a mixture of different plant types that contain bioflavonoids and phytosterols. Bioflavonoids and phytosterols are contained within forage and have antioxidant and anti-inflammatory properties. The meat and milk from small ruminants that feed on forage may contain these bioactive molecules (Zervas & Tsiplakou, 2011).

Sourveldt displays lower digestibility and is lower in nutritional value during winter in summer rainfall areas (Tainton *et al*., 1993). The chemical composition of grass veldt during the winter months is characterized by a decrease in concentration of phosphorous and protein (Tainton *et al*., 1993). Lower nutritional quality in veldt is associated with a decrease in animal performance. Supplements can be given during the time of year when veldt does not succeed in fulfilling the nutritional requirements of production animals. Energy supplementation can be given to sustain maintenance requirements of animals during the dry season, to supplement ewes during the lambing period, for the purposes of flush feeding or to finish animals on veldt.

Sheep are highly selective grazers. They select the greener parts of plants that contain higher levels of protein and phosphorous (Van Niekerk & Schoeman, 1993). Sheep also select grazing of a higher digestibility (Van Niekerk & Schoeman, 1993). Selection habits depends on the veldt type and the availability of plant material. Protein supplements on veldt are provided during late summer and early winter when grass contains insufficient crude protein to meet requirements and is of a lower digestibility. Nitrogen supplementation stimulates growth of the microbial population in the rumen to improve digestibility and intake. Sour veldt is also lower in phosphorous content during winter and supplementation is important in ewes during late pregnancy and lactation.

A small proportion of lambs are kept in feedlots during the fattening phase. The majority of lambs in South Africa are fattened and marketed from systems that are based on extensive grazing (Webb & Erasmus, 2013). Meat from lambs that were raised on grass-based systems is prone to be less tender when compared to lambs that received a concentrate ration (Webb & Erasmus, 2013).

2.1.6. The characterisation of grass species

The species of grass that manifest within an environment is largely dependent on temperature and rainfall. Temperate grasses grow in areas that experience low temperatures and frequent rains, whereas tropical and sub-tropical grasses exist in warm environments. Tropical climates are distinguished by separate wet and dry seasons, wherein herbage grows during the humid summer season. Grass growth stops during the dry season and plant material matures and dies off. High soil temperatures allow for the growth of herbage during the year in tropical regions, but plant growth is limited by a shortage of water (McDonald *et al*., 2011).

Temperate grasses are grazed at an early stage of growth since they mature at a slower rate compared to tropical grasses. Younger herbage is more nutritious and contains higher levels of minerals, proteins and energy compared to aged herbage. Structural carbohydrates are of lower nutritional quality and are present at higher concentrations in mature plant material (McDonald *et al*., 2011). Grasses that exist in tropical climates mature at a quicker rate, leading to high levels of fibre and low quantities of protein and phosphorous compared to slower-maturing temperate grasses (McDonald *et al*., 2011). Mature herbage is grazed as "standing hay" in drier climates, whereas grazing that prevails in humid environments is often lush and contains high levels of fibre.

The mesophyll cells of tropical grasses are more condensed compared to that of temperate grasses. The space between cells in the leaves of tropical grasses is therefore less than that of temperate species of grass. Tropical grasses also have more lignin due to higher amounts of vascular bundles and thick-walled bundle sheaths. Temperate grasses are degraded more effectively in the rumen because the plant material is of lower tensile strength. Thus, temperate grasses allow for higher levels of voluntary dry matter intake and a higher degree of digestibility compared to tropical grasses (McDonald *et al*., 2011).

The pathway of photosynthesis differs between temperate and tropical grasses. Tropical grasses follow the C_4 pathway of photosynthesis, wherein the four-carbon compound oxaloacetate is fixated with carbon dioxide. C_4 metabolism is an adaption of tropical plants that allows for the survival of herbage in environments that contain soils of low mineral status. The low levels of protein found in tropical grasses are attributed to C_4 metabolism. Temperate grasses follow the C_3 route of photosynthesis that involves the fixation of carbon dioxide with a three-carbon compound phosphoglycerate (McDonald *et al*., 2011).

Temperate and tropical grass species differ in storage carbohydrate type. Tropical grasses store starches in their leaves, whereas temperate grasses store fructans in their stems. Grass types within a species display small differences in nutritional value when compared at the same stage of maturity. However, the difference in nutritional value between grass types of different species are more notable (McDonald *et al*., 2011).

2.2. Differences in the grazing behaviour of livestock

The grazing behaviour of livestock influences the type and amount of plant material ingested by an animal. Diet affects meat quality (Webb & O'Neill, 2008). Therefore, the meat characteristics of a ruminant will be influenced by the type of grazing behaviour it expresses. The distinctive taste and flavour characteristics of meat from lambs reared in the Karoo are associated with certain plant species ('Karoo Meat of Origin', 2019). The selective behaviour of lambs that favour these plant types will produce meat that is more characteristic of Karoo flavour attributes compared to the meat of lambs that are less selective toward plant types that are linked to the Karoo flavour. Differences in grazing behaviour occur within a species and between species. Dorper and Merino breeds differ in their grazing behavior (Brand, 2000). Boer goats, cattle and sheep also display differences in grazing behaviour.

The Dorper is a non-woolled breed of sheep that is adapted to arid environments where small stock is farmed extensively. It is hardy, adaptable and possesses exceptional meat characteristics (Brand, 2000). Dorpers display grazing behaviour that is less selective and are prone to focus on woody-type vegetation. They tend to select high amounts of Karoo bush species and prefer browse such as shrubs and trees in comparison to Merino-type breeds.

Dorpers devote less time on grazing veldt in contrast to Merinos. They also display less individual grazing periods than Merino sheep. Roux (1992) found that Dorper sheep do not walk as far as Merino sheep in search of food and that the relative trampling (RT) factor is less for Dorpers than Merinos. The RT-factor takes factors such as body mass, stride length and the size of the hoof into account (Brand, 2000). Veldt is more likely to be affected by trampling when an animal displays a higher RT-factor. Dorper sheep tend to creep through fences from one paddock to another when grazing is limited. The Dorper consumes lower levels of plant material per metabolic size compared to Merinos (Brand, 2000). However, the differences are offset when intake per sheep is considered as the Dorper is heavier in body mass than Merino.

Plant types ingested by Dorpers or Merinos are alike when ample grazing is present for selection. Yet the Dorpers have the habit of selecting vegetation of lower nutritional quality and digestibility in situations of limited grazing when compared to Merinos (Brand, 2000). Merinos are more selective in their grazing behaviour and prefer to ingest the soft, herbaceous parts of plants. They tend to focus on greener vegetation such as grasses and Karoo shrub leaves. The selective grazing behaviour of Merino sheep enables them to harvest plant material with a higher plane of nutrition. Merinos graze for longer periods than Dorpers and also partake in more separate grazing events than Dorpers (Brand, 2000).

The difference in grazing behaviour within a species relates to the Dorper sheep consuming more browse than Merinos. However, the Dorper grazes more grass in comparison to Boer goats. Boer goats prefer woody plant material (Brand, 2000). Cattle are bulk grazers that ingest larger amounts of grass in contrast to small ruminants, which express selective grazing behaviour. Brand (2000) also found that small ruminants focus more on seasonal annual grasses than cattle. Small ruminants browse more shrubs than cattle do. Cattle also browse less shrubs compared to small ruminants.

The grazing behaviour of an animal influences veldt condition. Brand (2000) described the result that grazing patterns of different livestock species had on the state of veldt. The number of palatable shrubs increased after a period of grazing by cattle and Boer goats. The grazing of veldt by cattle was also associated with an increase in grass. The grazing of veldt by Dorper sheep lead to an increase in the grass section of the veldt. An increase in unpalatable shrub species and a decrease in palatable shrubs was also associated with the grazing of veldt by Dorpers. Selective grazing behaviour of Merino sheep was correlated with a decrease in the plant material of veldt. Boer goats harmed the browse component of veldt.

2.3. Fermentation processes of the rumen

Plant material that is ingested by the ruminant is subjected to fermentation processes within the rumen. Microorganisms alter diet substrates anaerobically to form products of fermentation, which are then absorbed by the gastrointestinal tract of the animal. Biohydrogenation pathways alter diet material significantly. Plant carbohydrates are transformed into volatile fatty acids by bacteria. Bacteria also hydrolyze unsaturated dietary lipids to saturated fatty acids. Dietary proteins are degraded to form part of bacterial proteins. The rumen environment affects the type and amount of fermentation end products formed. Biohydrogenation processes within the rumen will therefore influence meat characteristics and quality as they alter the composition of plant material ingested by the animal (Leek, 1993).

2.3.1. Dietary substrates of fermentation

The majority of the carbohydrate fraction is of the non-structural type. Starches, fructosans and simple sugars are stored as energy in the intracellular component of grains. Amylose is a type of starch that consists of glucose sugars that are linearly coupled via α–1, 4 glucose linkages. The structure of amylopectin is branched and consists of glucose components connected via α–1, 6 glucose linkages. Fructosans are composed of fructose units that are connected via β–1,2 linkages (Leek, 1993).

Most of the carbohydrates in roughages are embedded in the plant cell wall and play an important structural role. Pectins are structural carbohydrates that are based in the intracellular component of plant cells. The plant cell wall contains cellulose and hemicellulose. Cellulose consists of glucose units, whereas hemicellulose is a polysaccharide consisting of xylose components. The individual sugars in cellulose and hemicellulose are connected via β–1,4 linkages. Hemicellulose is replaced by lignin as plant cell walls age. Lignin proves to be difficult to degrade by the ruminant (Leek, 1993).

Amylases occur in the gastrointestinal tract of the majority of animals and are responsible for hydrolysing α–1 linkages. Animals are incapable of breaking down β–1 linkages. Microorganisms and plants contain enzymes that are able to hydrolyze β–1 linkages. These enzymes include cellulase, hemicellulase, pectin lyase and fructanase. The microbial population in the rumen therefore performs the beneficial function of degrading structural carbohydrates that would otherwise be indigestible by the gastrointestinal system of mammals (Leek, 1993).

2.3.2. The microbial population of the rumen

The microbial population of the rumen consists of bacteria, fungi and protozoa which thrive in an anaerobic setting. Fermentation involves hydrolysis and anaerobic oxidation. *Primary bacteria* degrade dietary plant material. The chewing of feed and cud increases the contact area between ingested plant material and microbes, which enables fermentation to occur (Leek, 1993). Primary bacteria can either be cellulolytic or amylolytic. Cellulolytic bacteria prefer to degrade cellulose, while amylolytic bacteria degrade amylose. *Secondary bacteria* degrade the end-products of fermentation processes governed by primary bacteria. An example of secondary bacteria is methanogenic bacteria which use hydrogen to produce methane gas. Propionate bacteria utilise lactate to produce propionate (Leek, 1993).

Protozoa feed on bacteria, starch and polyunsaturated fatty acids. Protozoa are scattered in the undigested material of the rumen and serve as a source of microbial protein in times of food shortage (Leek, 1993). They also act as a buffer that envelops starch particles when higher levels of grains are consumed in order to prevent rapid escalation of the amylolytic bacterial population. Protozoa are digested in the gastrointestinal tract along with small amounts of starches and unsaturated fatty acids that escaped ruminal degradation (Leek, 1993).

Ruminal pH has an important effect on the microbial population. Amylolytic bacteria function in a ruminal environment which is more acidic – at a pH of around 5.8. Lactic acid–producing bacteria flourish in acidic environments where the pH is lower than 5.8. A pH of 6.2 and higher is ideal for primary cellulolytic bacteria, most secondary bacteria and protozoa to thrive in. A highly acidic ruminal environment is unsuitable for the microbial population. Grain-based rations that are rich in starch lead to a rapid increase in amylolytic bacteria. Modifications in diet should be implemented slowly since it takes approximately two weeks for microbes to adjust to a change in diet (Leek, 1993).

The undigested material within the rumen is composed of a layer of fibrous material and microbes that continuously ferment plant material. The rumen also contains a fluid portion that consists of end products of fermentation, salivary secretions, reswallowed cud, ingested food and water. Generally, the fluid portion is sampled in order to gain a representation of the ruminal environment. The fluid segment does not give an accurate depiction of the microbial composition and fermentation processes within the rumen. Exchanges arise between blood and ruminal fluid within the membrane of the ruminoreticulum (Leek, 1993).

2.3.3. Pathways of fermentation

The pH of the rumen affects the state wherein the volatile fatty acid occurs, for example, propionate vs. propionic acid. The process of fermentation can be divided into different phases. The first stage entails the breakdown of plant polysaccharides into separate monosaccharides. Starch and cellulose are broken down to form glucose, whereas hemicellulose and pectin form xylose as a result of breakdown processes. Fructose–1,6–biphosphate is formed from glucose, xylose and fructosans. Fructose–1,6–biphosphate is oxidated anaerobically to phosphoenolpyruvate in the second stage of fermentation. Phosphoenolpyruvate is then converted to pyruvate, formate and butyrate (Leek, 1993).

The third stage involves the conversion of pyruvate to butyrate, with β–OH–butyrate as intermediate. Pyruvate is also converted to propionate (oxaloacetate and succinate serve as intermediary products). Some propionate is also produced from lactate and acrylate. The production of methane and propionate is essential as these two substances function to reoxidize reduced coenzymes. The reoxidation of coenzymes makes them accessible for processes of rehydrogenation. Microbial synthesis entails the utilisation of the intermediate and end products of the above fermentation reactions (Leek, 1993).

2.3.4. Fermentation of cellulose

Primary cellulolytic bacteria degrade β–1 linkages in pectin, fructosans, cellulose and hemicellulose. The breakdown of cellulose is a slow process. Adjustment in the microbial population of cellulolytic bacteria occurs at a gradual rate as they have a slow metabolism. Cellulolytic bacteria utilise ammonia and stage 2 and 3 intermediates in order to synthesize microbial protein. They flourish in a ruminal pH range of 6.2 to 6.8, which is characteristic of a ration with high levels of roughage. The secondary bacteria that produce methane also thrive at a pH of 6.2. Cellulolytic and methanogenic microbes produce carbon dioxide, methane gas and volatile fatty acids. The relative volatile fatty acid proportions produced from the fermentation of cellulose is 70:15:10 for acetate, propionate and butyrate, respectively (Leek, 1993).

2.3.5. Fermentation of starch

Primary amylolytic bacteria are responsible for the breakdown of α–1 linkages of amylopectin and amylose, as well as basic sugars such as sucrose and maltose. They thrive at pH levels of 5.5 to 6.6. A ruminal environment that is more acidic in pH corresponds to diets that are higher in concentrates (contain higher levels of starch). Amylolytic bacteria proliferate at faster rates than cellulolytic bacteria and the rate at which they ferment substrates is also quicker. Diets that are rich in concentrates produce higher levels of propionate. The relative volatile fatty acid proportions produced from the fermentation of grain-based diets is 55:25:15 for acetate, propionate and butyrate, respectively. Amylolytic bacteria utilise ammonia and amino acids for the synthesis of proteins (Leek, 1993).

Secondary bacteria are responsible for the production of propionate and methane and function optimally at a pH range of 6.2 to 6.8. This range of pH is higher than the level of pH required for amylolytic bacteria. Secondary bacteria need amino acids to synthesize microbial protein and multiply at a slower rate than amylolytic bacteria. An abrupt change in diet from roughage to concentrate will cause an increased rate of fermentation of starches by amylolytic bacteria,

accompanied by an increase in the amylolytic bacterial population. The result is increased concentrations of volatile fatty acids and lactic acid. The ruminal pH will lower in response to an increase in amylolytic bacterial activity. Secondary bacteria do not function in acidic environments. Higher levels of starch in the ration cause an increase in protozoa numbers and activity. Therefore, protozoa act as a buffer to decrease the amount of starch that is degraded by amylolytic bacteria, which leads to a decrease in pH. Protozoal activity is only effective above a pH of 5.5. Protozoa die off below a pH of 5.5, but amylolytic bacteria remain active and continue to ferment starches (Leek, 1993).

2.3.6. The fermentation of dietary protein

Dietary protein is grouped into rumen-degradable protein (RDP) and rumen-undegradable protein (RUP). Protein that has been subjected to the application of formaldehyde or that has undergone heat treatment escape degradation by ruminal microbes and travel to the gastrointestinal tract to be hydrolysed by proteolytic enzymes. The breakdown of proteins by ruminal bacteria produce peptides and amino acids which are taken up by other microbes. Deamination processes by microbes lead to the formation of ammonia and metabolic acids, which are fermented to form volatile fatty acids. Ammonia is also formed by the transformation of dietary protein and non- protein nitrogen in the rumen (Leek, 1993).

Urease is responsible for the conversion of urea to ammonia. Ammonia and volatile fatty acids serve as a substrate for the production of microbial protein. Volatile fatty acids function as a carbon skeleton to which amino groups can be attached to. Therefore, rations must be balanced in such a way to provide sufficient readily fermentable carbohydrates and NPN to guarantee sufficient substrates for microbial protein synthesis. Excessive levels of dietary protein are uneconomical as it causes overproduction of ammonia and unnecessarily uses energy to transform ammonia to urea in the liver. High levels of ammonia bear the added threat of ammonia toxicity. Microbes and unfermented dietary protein pass to the abomasum and small intestine and are of a better-quality protein, containing more essential amino acids compared to dietary plant proteins (Leek, 1993).

2.3.7. The fermentation of dietary lipids

Dietary lipids exist within oilseeds as storage lipids. They are also found within the leaves of plants where they serve a structural function. Most of the total lipids within the plant are in the form of phospholipids. Palmitic, linoleic and linolenic acids are the major fatty acids present in plants. Unsaturated fatty acids accept hydrogen atoms during fermentation processes. The hydrolyzation of unsaturated fatty acids by bacteria leads to the production of stearic acid. Microbial unsaturated

fatty acids are produced from volatile fatty acids and occur in the *trans*-form. The majority of dietary plant unsaturated fatty acids exist in the *cis*-configuration. Excessive inclusion of lipid in the ration negatively affects consistency and palatability of the feed, as well as inhibiting cellulolytic bacterial activity in the rumen (Leek, 1993).

2.3.8. Diet and the fatty acid profile of subcutaneous tissue

Webb *et al*. (1994) illustrated the effect of diet on the end products of rumen fermentation. Two diets, one with a medium level of energy (10.2 MJ ME/ kg DM (M)) and one with a high plane of energy (11.8 MJ ME/kg DM (H)), were fed to wethers of South African Mutton Merino and Dorper breeds. The high energy concentrate diet was associated with a decline in rumen pH, which led to an increase in the lactate-utilizing bacterial population of the rumen. An increase in lactate-utilizing bacteria affected the end-products of rumen fermentation by decreasing the acetate to propionate ratio. The limited ability of the liver to metabolize high levels of propionate and methylmalonate lead to the synthesis of odd-numbered and branched and/or unsaturated fatty acids in subcutaneous fat (Webb *et al*., 1994) Therefore, high levels of maize significantly affected the subcutaneous fatty acid profile in the study of Webb *et al*.,1994.

2.4. Meat quality and composition

Production system and diet affects meat characteristics and consumer acceptability of the meat product. The environment in which an animal was raised will inevitably influence meat quality and flavour, carcass characteristics and the fatty acid content of meat (Webb & O'Neill, 2008).

2.4.1. An overview of lipids

Steroids, oils, fats and waxes are all classified as lipids (Campbell, 1995). Triacylglycerol contains a glycerol bound to three fatty acid molecules and is considered to be a neutral lipid. A phospholipid consists of a mono or diester phosphoric acid molecule (IUPAC, 1978). Fatty acids are composed of a hydrophobic hydrocarbon chain and a hydrophilic carboxyl group. Their chemical makeup gives them an amphiphilic character. Saturated fatty acids only contain single bonds, whereas unsaturated fatty acids consist of carbon–carbon double bonds. Oleic acid (C18:1) is monounsaturated because it contains one double bond. Polyunsaturated fatty acids have more than one double bond and are typically associated with vegetable oils. Lipid consistency is influenced by the amount and type of double bonds a lipid contains, as well as the main fatty acid chain length (Campbell, 1995). Long- chain fatty acids are characterised by twelve or more carbon atoms and are distinctive to fats of animal origin.

The location of the ethylenic bond is denoted by the position of the carboxyl carbon, and the parent hydrocarbon is responsible for the name of the straight-chain fatty acid (IUPAC, 1978). A *cis* or a *trans* configuration relates to the conformation of the ethylenic bond. The specific structure of a fatty acid is indicated by its systemic name. However, we mostly refer to fatty acids by their trivial names (Webb & O'Neill, 2008).

Non-essential fatty acids are ingested via the diet or produced by the animal from acetyl CoA. Animals introduce double bonds at locations nearer to the carboxyl group or at the n-9 position (Smith, 2007). Sphingolipids and glycerophospholipids are the predominant non-essential fatty acids and include palmitic, stearic and oleic acids (Webb & O'Neill, 2008). Linoleic acid serves as the precursor of the n-6 class of essential fatty acids.

Essential fatty acids are sourced from the diet because they are not produced *de novo* by monogastric animals. Linoleic acid (C18:2n-6) and Linolenic acid (C18:3n-3) are examples of essential fatty acids. The n-3 and n-6 fatty acids maintain immunological processes and store fat-soluble vitamins (Webb & O'Neill, 2008). Eicosapentaenoic acid (C20:5n-3) and arachidonic acid (C20:4n-6) are two significant essential fatty acids that consist of twenty carbons each. Eicosapentaenoic acid is formed from α-linoleic acid, while arachidonic acid is produced from the elongation and desaturation of linoleic acid (Smith, 2007). Meat and fish are the main sources of arachidonic acid and eicosapentaenoic acid. *Cis*-9, *trans*-11 is the most significant conjugated linoleic acid (CLA) isomer and is beneficial to human health. Ruminant products such as meat and milk are adequate sources of *cis*-9, *trans*-11 CLA.

Tables 2.4.1. – 2.4.3. display fatty acid nomenclature and chemical formulas (Christie, 1982).

Table 2.4.1. Saturated fatty acids of general formula $CH_3(CH_2)nCOOH$ after Christie (1982)

Systemic name	Trivial name	Shorthand designation
Ethanoic	Acetic	C2:0
Propanoic	Propionic	C3:0
Butanoic	Butyric	C4:0
Pentanoic	Valeric	C5:0
Hexanoic	Caproic	C6:0
Heptanoic	Enanthic	C7:0
Octanoic	Caprylic	C8:0
Nonanoic	Pelargonic	C9:0
Decanoic	Capric	C10:0
Hendecanoic	Undecylic	C11:0
Dodecanoic	Lauric	C12:0
Tridecanoic	Tridecylic	C13:0
Tetradecanoic	Myristic	C14:0
Pentadecanoic	Pentadecyclic	C15:0
Hexadecanoic	Palmitic	C16:0
Heptadecanoic	Margaric	C17:0
Octadecanoic	Stearic	C18:0
Nonadecanoic	Nonadecyclic	C19:0
Eicosanoic	Arachidic	C20:0
Heneicosanoic	-	C21:0
Docosanoic	Behenic	C22:0
Tetracosanoic	Lignoceric	C24:0

Table 2.4.2. Monoenoic fatty acids $[CH_3(CH_2)_mCH=CH(CH_2)nCOOH]$ after Christie (1982)

Systemic name	Trivial name	Shorthand designation
Cis-9-dodeecenoic	Lauroleic	C12:1(n-3)
Cis-9-tetradecenoic	Myristoleic	C14:1(n-5)
Trans-3-hexadecenoic	-	C16:1[1]
Cis-9-hexadecenoic	Palmitoleic	C16:1(n-7)
Cis-6-octadecenoic	Petroselinic	C18:1(n-12)
Cis-9-octadecenoic	Oleic	C18:1(n-9)
Trans-9-octadecenoic	Elaidic	C18:1[1]
Cis-11-octadecenoic	*Cis*-vaccenic	C18:1(n-7)
Trans-11-octadecenoic	*Trans*-vaccenic	C18:1[1]
Cis-9-eicosenoic	Gadoleic	C20:1(n-11)
Cis-11-eicosenoic	Gondoic	C20:1(n-9)
Cis-13-docosenoic	Erucic	C22:1(n-9)
Cis-15-tetracosenoic	Nervonic	C24:1(n-9)

Table 2.4.3. The important non-conjugated polyunsaturated fatty acids [$(CH=CHCH_2)_m(CH_2)_x(CH_2)_nCOOH$] after Christie (1982)

Systemic name	Trivial name	Shorthand designation
9,12-octadecadienoic*	Linoleic	C18:2(n-6)
6,9,12-octadecatrienoic	δ-Linoleic	C18:3(n-6)
8,11,14-eicosatrienoic	Homo- δ-linolenic	C20:3(n-6)
5,8,11,14-eicosatetraenoic	Arachidonic	C20:4(n-6)
4,7,10,13,16-docosapentanoic	-	C20:5(n-6)
9,12,15-octadecatrienoic	α-Linolenic	C18:3(n-3)
5,8,11,14,17-eicosapentaenoic	-	C20:5(n-3)
4,7,10,13,16,19-docosahexaenoic	-	C22:6(n-3)
5,8,11-eicosatrienoic	-	C20:3(n-9)

*The double-bond configuration in each instance is cis.

2.4.2. The fatty acid composition of meat

Linoleic acid (18:2n-6) is an important fatty acid in concentrate-based rations. A limited amount (10%) of dietary linoleic acid is transferred to tissue lipids since linoleic acid is biohydrogenated to monosaturated and saturated fatty acids in the rumen. Grass-based systems contain high levels of linolenic acid (18:3n-3). More linolenic acid is degraded in the rumen compared to linoleic acid, leading to less linolenic acid being transferred into muscle lipids. Both linoleic and linolenic acids are stored in the phospholipid fraction of muscle in ruminants, whereas these two fatty acids are incorporated into the neutral lipid of adipose tissue in monogastric animals (Wood *et al*., 2008).

The action of Δ5 and Δ6 desaturase and elongase enzymes converts linoleic and linolenic acids to long-chain (C20 – C22) polyunsaturated fatty acids. Increased concentrations of these long-chain polyunsaturated fatty acids are therefore found in phospholipids due to the action of these enzymes. Phospholipids are the main class of lipid found in muscles. Cell membranes within muscle contain polyunsaturated fatty acids and, with an increase in fatness, the level of fatty acids within the phospholipid class will remain constant. Neutral lipid or triacylglyceride is the major lipid class within adipose tissue. Higher levels of monounsaturated and saturated fatty acids are found in neutral lipids. An increase in total fat would therefore be caused by an increase in the amount of fatty acids in the neutral lipid fraction as the phospholipid component would remain constant (Wood *et al*., 2008).

Linoleic acid, an important fatty acid in grain-based diets of plants and seeds, serves as the precursor to arachidonic acid (20: 4n-6). Linoleic acid also acts as a precursor to conjugated linoleic acid (18:2*cis*-9, *trans*-11), with 18:1 *trans*-vaccenic acid as intermediate product. The conversion of linoleic acid to conjugated linoleic acid is mediated via the enzyme stearoyl Co–A desaturase in the neutral lipid of adipose tissue. Conjugated linoleic acid is formed by microbial processes in the rumen. However, the

majority of conjugated linoleic acid (CLA) found in tissue is from the conversion of this fatty acid in adipose tissue as discussed. Ruminant products contain the highest levels of CLA *cis*-9, *trans*-11 (Wood *et al*., 2008).

Linolenic acid serves as a precursor to the formation of docosahexaenoic acid (22:6n-3), docosapentaenoic acid (22:5n-3) and eicosapentaenoic acid (20:5n-3) in phospholipids. Pasture systems and grass-based products contain high levels of linolenic acid. Wood *et al*. (2008) documented higher tissue levels of linolenic acid, 18:1 *trans*-vaccenic acid and CLA in freshly grazed grass when compared to the meat of animals that had received grass silage, indicating that there is a difference in the rumen degradation processes of fresh vs. harvested grass. Pasture management, time of harvesting, growing season, wilting and conservation would therefore also play a role in the level of meat fatty acids in grazing systems. Supplements such as linseed oil also contain higher concentrations of linolenic acid.

Oleic acid (18:1 *cis*-9) is the predominant fatty acid found in meat (Wood *et al*., 2008). Oleic acid is converted from stearic acid (18:0) in adipose tissue via the enzyme stearoyl Co–A desaturase. In cattle, an increase in age is accompanied by an increase in carcass fat in animals that receive sufficient nutrition. The function of stearoyl Co-A desaturase is proven by increasing levels of CLA, 18:1 *trans*-vaccenic acid and oleic acid with an increase in age. An increase in age is also accompanied by decreasing levels of linoleic acid, stearic acid and palmitic acid. Low concentrations of oleic acid and high levels of linoleic acid are found in the fat of young animals and animals that receive a diet low in energy.

2.4.3. Meat quality

Fatty acid composition and type affects the mechanical aspects of fat. Fat that is more solid melts at a higher temperature and is of a whiter colour, whereas liquid fat is less white in appearance and melts at a lower melting point (Wood *et al*., 2003). Firmness of fat is affected by the level of saturation of fatty acids. An increase in unsaturation would lead to a decrease in melting point. The type of double bond in an unsaturated fatty acid also affects melting point; with double bonds of the *cis* type having higher melting points than double bonds of the *trans* type. A *cis*-type double bond is characterised by H-atoms that point in the same direction when attached to carbon atoms in a fatty acid chain (Wood *et al*., 2008). Biohydrogenation processes in the rumen lead to the production of double bonds of the *trans* type that melt at lower temperatures. Fatty acids that have a straight- chain structure melt at higher temperatures than branched-chain fatty acids with the same number of carbon atoms (Wood *et al*., 2003).

The process of biohydrogenation affects the amount and type of fatty acids that are incorporated into tissue. Biohydrogenation, in turn, is affected by the level of feed intake, diet composition, type and source of diet carbohydrates, the degree to which feed fatty acids are saturated, forage-to-grain ratio and the nitrogen content of the diet (Nuernberg *et al.,* 2005). Biohydrogenation of fatty acids in the rumen leads to a fatty acid profile that is more saturated in nature. The higher levels of saturated fatty acids in ruminant tissue cause tissues to be firmer than in monogastric animals. Very fat cattle contain fat that is soft and oily in texture due to higher lipid concentrations of 18:1 and less 18:0 and 16:0 (Wood *et al*., 2008). The average melting point of subcutaneous fat is 39.5 ◦C. Lamb fat is of a harder texture and appears sticky when chewed, because of the saturated nature of the fatty acid profile. The majority of fatty acids in lamb meat therefore do not melt in the mouth when eaten.

Branched chain fatty acids are effective predictors of lamb fat firmness. Fat of a softer texture is caused by concentrate-based diets when high levels of grains are fed. Lower concentrations of 18:0 and higher levels of medium- to long-chain branched fatty acids (C10 – 17) are incorporated into tissues when a diet which is rich in concentrates is fed. Medium- to long-chain branched fatty acids are formed from methylmalonate, which is in turn formed by propionate. Diets based on grain products give rise to increased propionate concentrations in the rumen (Wood *et al*., 2003).

Shelf life is affected by the composition of meat fatty acids. The oxidation of lipid unsaturated fatty acids leads to rancidity. Consumers prefer meat of a bright red colour. Brown metmyoglobin is produced by the oxidation of red oxymyoglobin, which has a negative impact on consumer perception of the meat product. Products of lipid oxidation can stimulate the oxidation of pigments (Wood *et al*., 2003). Meat colour is retained for longer periods in the meat of animals that were raised on pasture. Grass contains high levels of Vitamin E, which serves as an antioxidant to postpone lipid and pigment oxidation.

The TBARS test measures the oxidative stability of lipids in food products in order to determine the level to which rancidity has occurred. It measures malondialdehyde, which is a product of oxidation. Consumers will perceive rancid colour and taste at a value higher than 0.5 (Wood *et al.,* 2008). Diets that are rich in soya bean or fish-oil supplements can lead to higher TBARS values due to increased polyunsaturated fatty acids prone to oxidation. Antioxidants such as Vitamin E can delay oxidation in these cases. Grass-rearing systems produce meat that is higher in α-tocopherol (Vitamin E). Vitamin E acts as an antioxidant that protects lipids and meat from oxidation during storage. Therefore, meat from lambs that were fed grass products tends to have a longer shelf life. Methods of processing can also affect the level to which fatty acids are oxidised. For example, pro-oxidants that allow fatty acid oxidation are released from muscle cells in minced products.

Although consumers may distinguish rancidity at a TBARS value less than 2.3, the meat flavour will still remain acceptable. At levels higher than 2.3, abnormal flavours will suppress meat flavour. Concentrate rations deliver higher TBARS values than grass-based diets because meat from grass-fed animals contains higher levels of Vitamin E. Oxidation of polyunsaturated fatty acids and pigments therefore occur more rapidly in animals that were raised on concentrates where the meat contains less Vitamin E. Haem compounds can also act as antioxidants in red meat with a low lipid to haem ratio by interacting with the peroxides of free radicals (Webb & Erasmus, 2013).

Production systems can influence the pH value of meat. Diets that are rich in grass products tend to exhibit higher meat pH values, whereas concentrate diets display a meat pH that is lower in value (Priolo *et al.*, 2002). Generally, concentrate diets rich in cereals give rise to sufficient glycogen reserves in muscle. Grass-based systems have the ability to produce muscle that is low in glycolytic potential. Sufficient muscle energy reserves are important in the conversion of muscle to meat. Muscle that is low in glycolytic potential tends to produce meat that is tough and dry in texture (dark, firm and dry meat) (Webb & Erasmus, 2013). The meat of poorly conditioned cattle or animals that experienced pre-slaughter stress also has the potential to display dark, firm and dry (DFD) characteristics. DFD meat exhibits higher shear force values, which is associated with less tenderness.

Ultimate pH affects the lightness of meat. A high meat pH value is associated with meat of a darker colour. Flavour attributes of meat are sensitive to pH. The meat of animals reared on pasture-based systems is associated with higher ultimate pH values and off flavours. High meat pH values are associated with strange odour and rancidity. Lamb meat odour and flavour is adversely affected by a meat pH value that is higher than 5.6 (Resconi *et al.*, 2009).

The temperature at which rigor mortis is reached also affects meat lightness (Priolo *et al*., 2002). Carcasses from grass-based systems are lower in carcass fatness. A lower degree of fatness would lead to muscle cooling down rapidly, which would lead to the attainment of rigor mortis at a lower temperature. pH decline tends to be slower in carcasses that attain rigor mortis at a lower temperature (Webb & Erasmus, 2013). A rapid decline in pH and sufficient glycogen reserves is beneficial for the effective conversion of muscle into meat. The formation of darker meat is linked to lower rigor mortis temperatures in carcasses from pasture-based systems.

2.4.4. Meat flavour

Meat flavour is a complicated facet of meat quality and consumers may display bias due to cultural influence and prior experience. Meat sensory evaluation involves the assessment of tenderness, juiciness and flavour attributes. Animal diet can affect meat flavour directly or indirectly (Resconi *et al*., 2009). Specific chemical compounds in the diet can be transferred directly to meat to serve as flavour compounds or as precursors to the development of flavour. Compounds that are responsible for meat flavour are species-specific. Flavour components that contribute to the expression of sheep meat flavour are different from compounds that are responsible for beef flavour. Beef flavour is associated with more than fifty different compounds (Webb & Erasmus, 2013). Fatty acid composition, growth rate and age and live mass at slaughter affect the characteristics of meat in an indirect manner.

Amino acids and reducing sugars react during the cooking of meat to produce compounds that contribute to the development of meat odour and flavour. This phenomenon is known as the Maillard reaction. Maillard reaction products and volatile, odorous lipid oxidation products react during cooking to form products that are essential to meat flavour. Resconi *et al.* (2009) found a correlation between sweet and roast taste characteristics and lambs that were fed concentrates. The meat from lambs reared on concentrates contains higher levels of sugars and therefore possesses a greater ability to partake in reduction processes during the Maillard reaction, leading to an increase in Maillard reaction products. An increase in Maillard reaction products can be beneficial to the development of meat flavour.

The diet of ruminants can lead to the deposition of unique chemical compounds in meat responsible for meat flavour. Pentanal and hexanal are oxidation products formed from linoleic acid, which is associated with concentrate diets (Wood *et al.*, 2008). Linolenic acid is the main fatty acid in grass. A positive correlation exists between linolenic acid and meat flavour because of the creation of linolenic acid's oxidation products during cooking (Wood *et al*., 2003). Linolenic acid oxidation products include 1-penten-3-ol and cis-2-penten-1-ol. Aldehydes are products of oxidation that also contribute to meat flavour. The breakdown of chlorophyll in the rumen leads to the production of diterpenoids, which are present at increased concentrations in tissue when animals are reared on grass-based systems. Species-specific flavours can also be attributed to lipid content (Resconi *et al.,* 2009). Lipids can serve as precursors of aromatic compounds that are created during oxidation processes. The interaction of these aromatic compounds with other compounds leads to meat scents that are associated with a species. Lipids can also store fat-soluble volatile compounds or act as odorants, which in turn contribute to flavour and taste.

Grass-based diets exhibit high levels of skatole (3-methylindole) in meat. Linolenic acid serves as a precursor to skatole during oxidation processes. Skatole plays an important role in the development of flavour in sheep, but not in cattle (Webb & Erasmus, 2013). The compound known as 2,3-octanedione is present at high levels when animals consume pasture and serves as an indicator of grass consumption.

Medium length branched-chain fatty acids are important for lamb odour and flavour. Branched-chain fatty acids are produced when the liver's capacity to metabolise propionate is exceeded (Priolo *et al*., 2002). Propionate is present at higher levels when grain-based diets are fed. Compounds such as 4-methyloctanoic acid and 4-methylnonanoic acid are branched-chain fatty acids that contribute to meat flavour. Priolo *et al*. (2002) noted an increase in lamb and fatty flavours in the meat of lambs that were raised on a grain-based diet, associated with a decrease in livery flavour. In contrast, Wood et al (2003) reported metallic, bitter and rancid flavours associated with concentrate groups. Consumers that are familiar with a product from a specific production system tend to be subjective to taste and flavour preference. For example, British consumers prefer lamb that was raised on pasture, whereas Spanish and American consumers favour lamb that was finished on grain diets.

Lamb odour and flavour, together with tenderness, juiciness and overall liking are noted in the meat of lambs that were raised on pasture but finished on concentrates (Resconi *et al.*, 2009). Tenderness and organoleptic properties are therefore positively correlated with mixed diets. Consumers associate fat flavour with lambs that only receive concentrates in their diet due to a high level of marbling in meat. Strange odours and rancid flavours are associated with lambs that were raised on grass-based systems (Resconi *et al.*, 2009). High levels of linolenic acid from pasture influence lamb meat sensory quality in that the development of rancid flavours may overpower meat flavour characteristics associated with lamb. Forage contains high levels of condensed tannins which contribute to off flavours (Resconi *et al.*, 2009).

2.4.5. Carcass characteristics

The type of feeding system employed exerts an influence on growth rate, carcass mass, carcass fat, dressing percentage, carcass conformation, muscle-to-fat ratio, marbling, lipid composition, oxidative stability, meat colour and consumer acceptability (Zervas & Tsiplakou, 2011). Animals that were raised on pasture display lower growth performance due to grass being less dense in nutrients compared to concentrate diets. A diet based on grass consumption positively influences the fatty acid composition of meat, although this occurs at the expense of growth performance (Webb & Erasmus, 2013). A decrease in growth rate will produce meat that is less tender in texture. Lower levels of marbling are observed in the meat of animals that were raised on pasture systems. Fat is important

in the consumer perception of meat flavour and taste. Insufficient intramuscular fat may cause meat to be less tender and may influence consumer acceptability negatively. Muscle that is lower in glycogen reserves has the potential of developing DFD meat.

High shear force values are associated with pasture-based systems (Nuernberg *et al*., 2005). The age at which an animal is slaughtered affects meat tenderness and animals that are slaughtered at later stages exhibit meat that is less tender. Collagen increases with age and an older animal will possess more collagen tissue. Therefore, the meat of an older animal will be less tender compared to the meat of a younger animal, due to higher collagen content in the meat. Meat of lambs kept on pasture is darker in colour because of higher levels of myoglobin in muscle and an increase in oxidative fibres. These characteristics are associated with livestock that are more physically active on pasture as compared to intensive housing systems. The shortening of muscle fibres can also be explained by higher levels of physical activity on pasture. Muscle fibre shortening is associated with low meat tenderness.

The development of tender meat is facilitated via the breakdown of muscle fibres by enzymes during the ageing process. Differences in growth rate affect proteolytic processes and the structure of muscle. Ageing time of meat can occur during transportation to improve tenderness, although longer periods of ageing time may lead to the development of rancidity (Resconi *et al.*, 2009). Anoxic storage conditions can decrease the development of rancidity in meat due to lower levels of lipid oxidation.

Fat content affects muscle texture. The total lipid content of muscle consists of neutral lipid (triacylglyceride portion) and marbling fat (intramuscular fat). Intramuscular fat plays a role in the tenderness and juiciness of meat. Structurally, marbling fat is contained in perimysial connective tissue which is located between muscle fibres (Wood *et al.*, 2008). This characteristic aids in breaking down the structure of the muscle in order to express meat flavour when meat is chewed. Secretion of saliva promotes the perception of meat flavour. Neutral lipid is present as spots of adipose tissue in muscle. Adipose tissue affects the integrity of muscle tissue as well as the cohesion between lean tissue and fat.

Lamb tissue exhibits higher levels of yellow pigment in adipose tissue when reared on lush pastures. The texture of fat is also firmer when animals are reared on grass. The amount of pigment transferred to fat depots is dependent on the season in which grazing occurs. Less pigment is deposited during the dry season (Webb & Erasmus, 2013). Consumption of lucerne hay places higher concentrations of carotenoid pigments like xantophyll or lutein into fat reserves. Consumer perception plays a role in the perception of yellow fat. Consumers relate yellow fat to diseased livestock and animals that were slaughtered at a later stage in life. Eastern countries are fond of yellow fat in their meat.

Priolo *et al*. (2002) reported that housed lambs on a concentrate-based diet gave heavier carcasses with lower gastrointestinal weights compared to lambs kept on pasture that were slaughtered at the same live mass. Lambs that were reared on a grass-based system attained higher levels of dry matter intake in order to achieve the same growth rate as lambs that were fed concentrates. Higher intakes of grass products effectively lead to more developed digestive tracts. Therefore, the carcass mass of animals raised on pasture is less than that of lambs reared on concentrates in groups that are slaughtered at the same live mass because the gastrointestinal tract of animals fed grass is larger in size and thus weighs more. The fattening phase of animals can be controlled effectively in animals that are fed concentrates to produce carcasses of uniform conformation and quality (Webb & Erasmus, 2013). Consistent carcass characteristics are attainable in grass-based systems where effective management systems are employed and moderate levels of concentrate are provided.

2.4.6. The health benefits of meat

Recently, researchers have started placing more emphasis on the quality of fat consumed by humans rather than the amount of fat their diet consists of. Fatty acid type and the level of saturation of fatty acids in meat has an important effect on the value of meat from a consumer health perspective. Diseases associated with excessive fat consumption include cardiovascular conditions, obesity and diabetes. Excessive levels of saturated fatty acids are linked to heart disease and cancer. Inclusion of unsaturated fatty acids in the diet can be beneficial to human health. Linoleic acid is essential for cardiovascular health. Homogammalinolenic and arachidonic acids are formed from linolenic acid, while eicosapentaenoic acid is formed from α-linolenic acid. Eicosanoids are produced from eicosapentaenoic acid, while arachidonic acid forms thromboxane TXA2. Prostaglandin PGE1 prevents platelet aggregation and is derived from homogammalinolenic acid (Webb & O'Neill, 2008).

It is suggested that total energy intake consists of 30% total lipid intake. Lipid energy from saturated fatty acids should fall within the range of 10 – 30% (Webb & O'Neill, 2008). Decreased consumption of saturated fatty acids and *trans*-fatty acids is recommended. One must also take the fat composition of meat into account when considering its nutritional value to consumers instead of only considering the total amount of fat. It is beneficial to replace saturated fatty acids and *trans*- fatty acids with unsaturated fatty acids. Unsaturated fatty acids such as n-3 fatty acids are beneficial to human health (Nuernberg *et al.*, 2005). *Trans*-fatty acids contribute to the development of low- density lipoproteins (cholesterol), which gives rise to cardiovascular disease.

Meat displays a polyunsaturated fatty acid to saturated fatty acid ratio (P:S) of 0.1, which differs from the recommended P:S ratio of 0.4 (Enser *et al*., 1996). The ratio of n-6: n-3 polyunsaturated fatty acids is another aspect necessary to consider in the development of cancer and coronary heart disease. Linoleic acid (18:2) produces n-6 polyunsaturated fatty acids, whereas n-3 polyunsaturated fatty acids are formed form α-linolenic acid (18:3). Ideally, the n-6: n-3 ratio should be less than 4. One can manipulate both the P:S and n-6: n-3 ratios to more favourable proportions (Wood *et al*., 2003). The content of meat n-3 fatty acids can be increased by rearing animals on grass-based systems. Cereals and grains are high in n-6 fatty acids and the n-6: n-3 fatty acid ratio is increased when animals are fed concentrate diets (Nuernberg *et al.*, 2005). The addition of formaldehyde treatments to protect dietary oils in feeds can increase polyunsaturated fatty acid concentration in meat (Wood *et al*., 2008). Cereal concentrate diets can also increase the amount of polyunsaturated fatty acids in meat.

Animals that are finished on pasture display lower levels of intramuscular fat and cholesterol, while increased levels of n-3 fatty acids are obtained from grass. Higher levels of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid are observed in the meat of lambs that were reared on grass-based systems. However, the concentration of these beneficial fatty acids in tissue is relatively small as high levels of their precursor, linolenic acid, is biohydrogenated in the rumen. Conjugated linoleic acid (CLA) contains anticarcinogenic properties and it exerts positive effects on the immune system. Conjugated linoleic acid also decreases the incidence of diabetes and atherosclerosis (Webb & Erasmus, 2013). Ruminant products contain the highest levels of CLA *cis*-9, *trans*-11.

Dietary lipids supply metabolic energy and are important for the production of phospholipids which form part of cell membranes. Linoleic acid plays a critical structural role in cellular membranes and contributes to the formation of tissue lipids. Long-chain fatty acids play an important structural role in the endothelium of arteries. Coronary artery disease involves the formation of plaques from the dysfunctional lining of artery endothelium (Webb & O'Neill, 2008). Endothelial relaxation, inhibition of arthrosclerosis and lower plasma levels of low-density lipoprotein (LDL) are some of the beneficial effects of n-3 fatty acids on cardiovascular health.

A balanced diet that contains adequate levels of unsaturated lipids (15 –20% of calories) and low amounts of saturated lipids (7 – 10%) could increase high-density lipoprotein concentrations. Research indicates that increased dietary carbohydrate intake and decreased total fat consumption is linked to the formation of triacylglycerols and lower levels of high-density lipoprotein (Roussell & Kris-Etherton, 2007). Undesirably high n-6: n-3 ratios can be alleviated by the inclusion of C20 and C22: n-3 polyunsaturated fatty acid in the diet (Enser *et al*., 1996)

2.5. Methods of traceability

Methods of traceability are employed to ascertain the production system from which a meat product originated. Certain meat compounds are associated with specific production systems. The techniques of gas chromatography and stable isotope ratio analysis are applied to establish the origin of production system.

2.5.1. Specific compounds

Dietary fatty acids influence tissue fatty acid composition. Linoleic acid (18:2n-6) is an important fatty acid in concentrate-based rations, whereas linolenic acid (18: 3n-3) is associated with grass-based systems. Linolenic acid serves as a precursor to the formation of docosahexaenoic acid (22: 6n-3), docosapentaenoic acid (22:5n-3) and eicosapentaenoic acid (20:5n-3) in meat. Linoleic acid serves as the precursor to arachidonic acid (20:4n-6) and conjugated linoleic acid (18: 2*cis*-9, *trans*-11) (Wood *et al*., 2008). Meat from animals reared on grass-based diets displays lower n-6 to n-3 fatty acid ratios compared to meat from animals fed grain-based diets (Nuernberg e*t al*., 2005). However, non-grass diets can give rise to meat fatty acid profiles that are similar to meat from animals kept on pasture-based systems and care must be taken not to assume dietary background solely on meat fatty acid analysis.

Carotenoids are present at higher levels in the tissue of animals that were reared on pasture-based diets. Carotenoid concentration can express information about dietary background and allows one to distinguish between grass-based diets and concentrates. An indirect method to establish carotenoid levels in tissue involves determining the reflectance spectra of adipose tissue. Carotenoids can also be measured directly in blood plasma (Prache & Theriez, 1999). Plasma concentrations of carotenoids decrease with a switch in diet from grass to grains. A period of time (13 days, according to Prache *et al.*, 2003) needs to pass in order for analysis of tissue carotenoid levels to reflect a switch in diet due to slow turnover rates. The rate at which new tissue replaces old tissue also affects the efficiency of tissue fatty acids, vitamin E and volatiles as indicators of dietary background.

Stereoisomers of α-tocopherol are unique to dietary background. Beef from cattle that were fed a grain-based diet along with vitamin supplementation displays all eight configurations of α- tocopherol stereoisomers. This phenomenon is associated with synthetic Vitamin E supplementation (Meglia *et al*., 2006). The RRR stereoisomer of vitamin E is prevalent in muscle from cattle that were reared on pasture diets.

Linolenic acid is an important fatty acid in grass. Linolenic acid is linked to the formation of 1-penten-3-ol and cis-2-penten-1-ol during cooking (Wood *et al*., 2003). Grass-based diets are associated with high concentrations of skatole (3-methylindole) in meat. Linolenic acid leads to the formation of skatole during oxidation (Webb & Erasmus, 2013). The compound known as 2,3-octanedione serves as an indicator of grass consumption.

Pentanal and hexanal are linked to concentrate diets since they are formed from the oxidation of linoleic acid. Linoleic acid is an important fatty acid in concentrate diets (Wood *et al.*, 2008). Grain-based diets lead to the production of large amounts of propionate in the rumen. Compounds such as 4-methyloctanoic acid and 4-methylnonanoic acid are branched-chain fatty acids formed from propionate (Priolo *et al*., 2002). The aforementioned compounds and their chemical processes are discussed in more detail in section 5.3.

2.5.2. Headspace analysis of volatile fatty acids

Techniques used to identify volatile flavour compounds in meat include simultaneous steam distillation-extraction (SDE), dynamic head-space entrainment on Tenax TA and solid-phase microextraction (SPME). Aromatic components that contribute to the development of flavour such as hydrocarbons, ketones, alcohols, carboxylic acids, ethers, furans, pyridines, pyrazines, alkylphenols, thiols, thiophenols, thiazoles, nitrogenous compounds, halogenated compounds and sulphur-containing compounds are isolated by these techniques (Madruga *et al*., 2009).

The method of gas chromatography is employed to measure meat volatile compounds. Volatile compounds include branched-chain fatty acids, lactones, aldehydes, indoles, 2,3-octanedione, terpenes, phenolic compounds and sulphur compounds. Compounds can be deposited directly into tissues or can be formed indirectly during the cooking of meat. Cooking involves chemical reactions between meat compounds that lead to the formation of lipid oxidation products and sulphur compounds (Monahan *et al*., 2018).

Liquid chromatography-mass spectrometry measures flavonoids and phenolic compounds. Gas chromatography-mass spectrometry and the human nose examine chemical compounds to establish differences in composition of meat samples between different grazing systems. Tissue and plant samples from lambs reared on Karoo vegetation display a headspace analysis that is concentrated. Meat, fat and browse samples are analysed by means of statistical analysis to diagnose chemical compounds that can be used as markers to authenticate diet or origin of production.

Dynamic headspace analysis can single out unique compounds present in meat that are associated with dietary background. Certain compounds are associated with meat from a grass-based or a grain-based diet. Skatole, cuminic alcohol, 2-methyl-1-butanol and 3-undecanone are chemicals that allow for the identification of dietary background in beef. Skatole (3-methylindole) is present at increased amounts in the fat of animals that are reared on pasture. Skatole is a by-product of the degradation of tryptophan by microbes. High levels of skatole are obtained in ruminant fat due to the low non-fibrous carbohydrate levels and high protein concentration of grass diets. This type of ratio causes increased deamination by ruminal microbes, which causes the increased production of skatole from tryptophan (Priolo *et al*., 2004).

Germacrene D is a terpenoid that is present in the meat of cattle that were fed a grass-based diet. Terpenes such as β-cubebene and β-gurjunene are used to ascertain whether or not animals were fed on pasture. Dietary terpene level is affected by seasonality and plant type, which will influence meat terpene signature. Terpene deposition in lipids may not be consistent between different lipid fractions in ruminants. Variability in terpene profile caused by lipid fraction, plant type and seasonality needs to be considered when terpenes are used to differentiate between production systems (Serano *et al*., 2007). The enzyme lipoxygenase is present at higher concentrations in herbage and is correlated with the chemical marker 2,3 – octanedione (Priolo *et al*., 2004). Mono- and sesquiterpenes are secondary plant metabolites that are present in tissues of animals that grazed pasture.

Madruga *et al*. (2009) discovered a range of C_2 to C_5 alkylformylcyclopentenes that contribute to the unique flavour expression associated with different livestock species. They also found that sheep fat contains the highest levels of phenols and alkylphenols, which is responsible for the characteristic aroma and taste of mutton and lamb. Sheep adipose tissue also contains higher concentrations of 4-methyloctanoic and 4-ethylloctanoic acids.

2.5.3. Stable isotope ratio analysis

Stable isotope ratio analysis (SIRA) determines the relative proportion of stable isotopes of organic material (Kelly *et al*., 2005). Isotopic signatures of carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), sulphur ($^{34}S/^{32}S$), oxygen ($^{18}O/^{16}O$) and hydrogen ($^{2}H/^{1}H$) are estimated by isotope ratio mass spectrometry (IRMS) and are expressed in units per mil (‰) as delta values. Isotope ratios of $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$, $^{18}O/^{16}O$ and $^{2}H/^{1}H$ are expressed by δ^{13} C, $\delta^{15}N$, $\delta^{34}S$, $\delta^{18}O$ and $\delta^{2}H$ values, respectively.

Diet influences the isotopic ratios of meat. Isotopes can be deposited into tissues when animals graze vegetation and may function as a method to identify the environment in which an animal was reared. Isotopic patterns of vegetation have the potential to be unique to a certain geographic area. The ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in meat can be linked to its geographic origin via diet consumed by the animal when a certain feed is associated with a specific area.

The type of plant material an animal consumes portrays a nitrogen isotope profile that is specific to that plant species. The ^{15}N value of a plant is affected by climate, plant species and soil type. $\delta^{15}N$ content is higher in plants that occur in arid environments, as plant ^{15}N values decrease with an increase in rainfall in an area. The application of organic fertilizers increases the $\delta^{15}N$ content of grazing. Legumes display lower levels of $\delta^{15}N$. Animal type (breed and species) and production system affect the degree to which ^{15}N is transferred to animal tissues from grazing. Separate locations may display the same $\delta^{15}N$ value, which demonstrates that $\delta^{15}N$ content is not expressive of a certain area (Erasmus *et al*., 2016).

The carbon isotope ratio of a plant depends on the type of photosynthetic pathway it utilizes. Plants that employ the C_4 route of metabolism utilise the four-carbon compound oxaloacetate, whereas the C_3 category of plants incorporates the three-carbon compound phosphoglycerate in photosynthesis (McDonald *et al*., 2011). Examples of C_3 plants are trees, bushes, shrubs, non-grassy herbs/forbs and temperate grasses. Tropical grasses are C_4 plants, whereas succulents fall into the Crassulacean Acid Metabolism (CAM) category. CAM plants have the ability to utilise both C_3 and C_4 pathways. The meat of livestock grazing C_4 plants displays higher levels of δ^{13} C compared to the meat from animals that are reared on vegetation consisting of browse. C_3 browse plants present with lower δ^{13} C levels. Grazed veldt that consists of both C_3 and C_4 gives rise to meat with δ^{13} C isotope values that are intermediate between that of animals that were reared exclusively on C_3 or C_4 vegetation (Erasmus *et al*., 2016).

Carbon and nitrogen isotopes act as guidelines of the type of diet an animal consumed but are not necessarily unique to a geographic location or production system. Further information can be obtained about the geographic region from which a meat product originated by establishing the isotope ratios of $^{34}S/^{32}S$, $^{18}O/^{16}O$ and $^{2}H/^{1}H$ along with carbon and nitrogen isotopes (Kelly et al., 2005; Zazzo *et al.,* 2011). Sulphur isotopic profiles are characteristic of a geographic area. $^{34}S/^{32}S$ ratios are influenced by geology, climate and soil fertilization. Latitude, altitude and precipitation are factors that influence sulphur isotope ratios. Oxygen and hydrogen isotopes are also associated with the climate and geographic attributes of an area. Livestock ingest oxygen and hydrogen isotopes by drinking water. Distance of production system from the sea affects sulphur, oxygen and hydrogen

isotopic values.

The approach to stable isotope ratio analysis of animal tissues has some limitations. Diet characteristics may change during the total period of feed consumption by an animal. Components of the ration may change due to changes in price or accessibility of feed ingredients. Changes in feed components will lead to a change in dietary isotopic values, which will affect meat isotope profiles.

Pasture isotope values are dependent on season. The addition of grains to pasture-based systems during winter or times of drought will lead to changes in meat isotope ratios. Organic production systems are linked to less seasonal variation in carbon isotopes, due to lower levels of concentrate incorporated into the diet. The period of time it takes for the meat isotopic profile to reflect changes in diet is also uncertain (Monahan *et al.,* 2018). Isotope values in meat may reflect feed isotope values of the diet prior to a change in diet due to slow metabolic turn-over rates in muscle. Grains and temperate grasses that both qualify as C_3 plant species display similar isotope ratios, which may not indicate a change in diet when muscle or adipose tissue samples are analysed.

Incremental tissue examination displays diet changes experienced by the animal. Hair, hoof and wool tissues are independent of metabolic processes and are not subjected to degradation activity. Incremental tissues are deposited continuously. Changes in diet are associated with corresponding changes in diet isotopes, which will reflect in incremental tissue analysis as isotopes will be stored within tissues. Samples of hair, wool or hoof tissues can be obtained from a live animal or from a carcass. Tail hair of cattle reflects nitrogen and carbon isotope profiles of dietary material near the time of slaughter at the root of the hair, whereas the hair tip will reflect isotope information of the diet consumed by the animal earlier in its life. Isotope ratio analysis of hair can discriminate between C_3 and C_4 plant species and can reflect the season in which the diet was consumed by livestock. Distinguishing between diets that display the same isotopic signature, for example, two C_3 plant species, proves to be more difficult. Analysis of sheep's wool can portray a sulphur isotope profile which is indicative of geographical origin. Stable isotope ratio analysis can be combined with the measurement of trace elements in tissue samples in order to obtain information about diet and to establish the origin of the production system. Carbon and nitrogen isotopes can be determined along with levels of Mg, K, Mn, Zn, Se and Zr (Zhao *et al*., 2013).

Erasmus *et al*. (2016) explored the application of stable isotope ratio analysis (SIRA) as a method to verify the origin of South African lamb meat. In the latter study, a total of seven farms were included in the study, each of which contained a distinctive topographic character and combination of plant species. The geographic origin of the farms included one farm from the Western Cape (Swellendam), one from the Free State (Boshof) and five from the Northern Cape (Carnarvon, Prieska, Loeriesfontein, Niewoudtville and Calvinia). Each farm supplied a total of ten Dorper lambs for the purpose of SIRA.

The farms were characterized by the type of on-farm vegetation and the diets they offered. The farm near Boshof in the Free State was representative of a grass diet, whereas the Swellendam farm in the Western Cape constituted a diet based on lucerne or alfalfa. The plant species from the Northern Cape farms consisted mainly of Karoo veldt and grasses. Carnarvon represented the central Karoo biome, Prieska represented the northern Karoo and Loeriesfontein was representative of the Hantam Karoo. The Knersvlakte area was represented by Niewoudtville and the Hantam Karoo biome was represented by Calvinia.

$\delta^{13}C$ meat values depict the type of diet an animal consumes. $\delta^{13}C$ meat values from lambs grazing on grass from the Free State were the least negative. Lambs ingesting a diet consisting exclusively of C_4 plants displayed the highest $\delta^{13}C$ values (Erasmus *et al*., 2016). Carbon isotope ($\delta^{13}C$) meat values from lambs that grazed veldt from the central and northern Karoo biomes were intermediate when compared to isotope values from the rest of the farms. Erasmus *et al.* (2016) reported that the vegetation from the central and northern Karoo farms consisted of a mixture of both C_3 and C_4 plants. A diet existing of a mixture of C_3 and C_4 plant species will give rise to intermediate $\delta^{13}C$ meat values. $\delta^{13}C$ values from the Loeriesfontein farm (Hantam Karoo) were marginally higher than $\delta^{13}C$ values reported for the central and northern Karoo farms. Plant species from the Loeriesfontein (Hantam Karoo) farm did not contain C_4 grasses but consisted of CAM plant types. A diet composed of CAM plants will lead to intermediate $\delta^{13}C$ meat values.

The Swellendam and Calvinia (Hantam Karoo) farms displayed the second lowest $\delta^{13}C$ meat values. The plant composition of the Calvinia farm predominantly consisted of C_3-type browse species. A diet consisting of Karoo shrubs and bushes will cause $\delta^{13}C$ meat values to be more negative (lower $\delta^{13}C$ values). Erasmus *et al.* (2016) reported the most negative carbon isotope ($\delta^{13}C$) meat values from the Knersvlakte farm in Niewoudtville.

Erasmus *et al.* (2016) were able to differentiate effectively between farms based on $\delta^{15}N$ meat values. Large between-farm variation and low within-group variability allowed researchers to easily distinguish one farm from another based on $\delta^{15}N$ values. The Swellendam and Boshof farms, where diets based on lucerne or grass were consumed, respectively, portrayed the lowest $\delta^{15}N$ meat values. Higher $\delta^{15}N$ values were associated with farms from the northern Karoo, Calvinia (Hantam Karoo), Loeriesfontein (Hantam Karoo) and the Knersvlakte.

Higher $\delta^{15}N$ values from the Northern Cape environment can be explained by the prevalence of CAM plants and succulents, as well as the arid environment associated with these farms. Erasmus *et al.* (2016) reported that separate environments with different plant species may display comparable $\delta^{15}N$ meat values in that $\delta^{15}N$ values from the Free State farm resembled $\delta^{15}N$ values from the central Karoo farm.

Erasmus *et al.* (2016) confirmed the use of SIRA as a potential method to establish the origin of lamb meat production in South Africa. The ^{13}C isotope proved to be effective in designating differences in vegetation consumed by lambs. The isotopes of ^{2}H, ^{18}O and ^{34}S can further be utilized to take geographical origin into account. Future research needs to be conducted on establishing stable isotope ratios for the various regions so that these can be used as baseline profiles against which meat samples can be tested to authenticate the origin of the production systems.

2.5.4. Other techniques

Osorio *et al*. (2012) applied the technique of ^{1}H nuclear magnetic resonance (NMR) to analyse urine and muscle samples of cattle in order to discriminate between pasture-raised animals and animals that received a barley-based concentrate. Researchers were successfully able to distinguish between production systems based on urine and muscle samples from cattle that received different diets. Spectroscopic techniques such as near and mid-infrared spectroscopy (NIRS and MIRS, respectively) can be employed to pinpoint types of production systems and diet (Monahan *et al*., 2018). Assessment of perirenal fat between a spectrum of 400 and 2500 nm expressed a clear distinction for the longer wavelength between lambs that were raised on either pasture or concentrates (Dian *et al*., 2008).

Functional genomics enables researches to potentially distinguish between production systems. The application of functional genomics involves the use of proteome and transcriptome profiling (Hocquette *et al.*, 2009; Prache, 2009; Shibata *et al*., 2009). Cassar-Malek *et al*. (2009) identified Selenoprotein W as a potential marker in grass-fed animals in that it was under-expressed in Charolais cattle that were reared on pasture. Down-regulation of signal transducer and activator of transcription-5 (STAT5) and up-regulation of stearoyl-CoA desaturase, fatty acid synthase and Spot-14 occurred in the subcutaneous fat of grazing steers finished on a concentrate diet (Duckett *et al*., 2009).

3. MATERIALS AND METHODS

3.1. Ethical clearance

Techniques and research methods adhered to the University of Pretoria laboratory animal ethical code of research.

This research was accepted by the Animal Ethics Committee of the University of Pretoria (Reference: NAS018/2019. Please note the acceptance letter from the Ethics Committee from the Faculty of Natural and Agricultural Sciences in Appendix l.)

3.2. Specific objectives

The purpose of this study was to determine the efficacy of tissue fatty acid and headspace analyses of volatile components to distinguish between different meat samples of lambs that were reared on separate production systems. Meat samples from lambs reared on Karoo browse veldt and grass veldt were compared.

3.3. Hypothesis

H_0: The unique sensory characteristics of Karoo lamb can be distinguished by means of tissue fatty acid analysis and other volatile components.

H_A: The unique sensory characteristics of Karoo lamb cannot be distinguished by means of tissue fatty acid analysis and other volatile components.

3.4. Experimental design

A uniform group of typical Dorper lambs were obtained from a breeding farm in Upington in the Northern Cape province of the Republic of South Africa. The sheep were randomly allocated into two groups; one herd was raised on Karoo browse veldt, and the other group grazed Karoo grass veldt. The overall number of animals in the experiment amounted to twenty lambs, with ten per treatment group. Each group consisted of 5 ewes and 5 wethers. No supplements were provided and animals were allowed to graze on an *ad libitum* basis. Lambs were allowed to graze for a period of ninety days at an initial live mass of approximately 27 kg in order to reach an average target live mass of 40 kg. Lambs were subjected to standard animal husbandry procedures. Good quality drinking water was available on an *ad libitum* basis.

The following table depicts the experimental design as discussed;

Karoo browse veldt		Karoo grass veldt	
M	F	M	F
5	5	5	5

3.5. Slaughter procedures and carcass analysis

Animals were weighed and transported for slaughter at a registered local abattoir according to standard South African slaughter techniques (Cloete *et al.,* 2012). The average live mass at which animals were slaughtered was 40kg. Carcasses were classified according to the South African Carcass Classification System (Agricultural Product Standards Act, 1990). Carcass classification scores (A2 to A4) and carcass masses were obtained from abattoir records.

Carcasses were electrically stimulated (21 V, 60 Hz, 120 s) and stored in a cold room at ca. 4 °C for 24 hours. Each carcass was split in half along the vertebral column after the preceding period of 24 hours. The left half of each carcass was labelled and transported to the cold-room of the Animal Science research laboratory of the University of Pretoria. Carcass samples were stored in sealed polyethylene bags at −20 °C. Samples were taken from carcasses after they were allowed to thaw over a period of 24 hours. Techniques and research methods conformed to the laboratory animal ethical code of research of the University of Pretoria.

3.6. Collection of samples

Back fat depth was measured after the carcasses had thawed for 24 hours. Post-mortem fat and rib-cut samples were obtained from the *M. longissimus dorsi.* Samples for the analysis of fatty acids were acquired from 10 g of subcutaneous fat samples. These subcutaneous fat samples were acquired from the left side of each carcass at a location over the 13th rib, 25mm from the midline and samples were stored in sealed polyethylene bags at −20°C (Webb *et al*., 1994). Stored fat samples were subjected to medium and long-chain fatty acid analysis and volatile fatty acid analysis.

An estimate of total carcass composition was acquired by dissection and proximate analysis of a sample obtained from ribs 8-9-10 from the left section of the carcass. The ventral extremity of this three-rib sample formed part of a line drawn from the pubic symphysis to the middle of the first rib (Casey *et al*., 1988). Muscle samples of 10 g were subjected to ether-extract analysis to determine the total fat content of muscle.

Oesophageal samples were collected via an oesophageal cannula from all experimental animals. Samples were collected at the end of the feeding period from experimental animals on different veldt types. Oesophageal samples of plant material were stored in polyethylene bags and frozen at - 20°C until analyzed. Samples were dried in an oven and minced. Dry matter was determined according to the AOAC proximate analysis technique. The ether extract was done, and the lipid fraction was analyzed for amino acids after dry matter was determined.

3.7. Preparation of samples

Visible fat was removed from meat samples for the analysis of marbling content contained within the meat. Each meat sample was blended into a homogenous mixture after the removal of subcutaneous fat. Dry matter analysis was conducted by leaving 10 g of blended wet meat samples in an oven overnight at a temperature of 105 °C (Helrich, 1990). Samples that were not subjected to dry matter analysis were freeze dried and milled. Fatty acid analysis was conducted from dried milled meat samples.

3.8. Ether extraction

Ether extract was measured by application of the Soxtec method (Helrich, 1990). Roughly 2 g of milled samples were applied to filter paper. The filter paper along with the sample contents were placed in an extraction tube and plugged with fat-free cotton wool. The extraction process involved the placing of tubes into the extraction unit along with containers used to assemble the ether extract. The ether extract was weighed and calculated.

3.9. Medium and long chain fatty acids analysis

The removal of lipids was carried out based on the methods described by Webb and Casey (1995). Lipids were expelled from freeze-dried samples via an adaption (Ways & Hanahan, 1964) of the chloroform: methanol (2:1, v/v) method (Folch *et al*., 1957). Butylated hydroxytoluene (2.6 DI-tert-BUTYL-P-CRESOL) was incorporated as an antioxidant. Methyl esters of the fatty acid fraction of the neutral triglycerides were prepared according to the NaOH/ methanol procedure (Helrich, 1990). The methyl esters were segregated on a polar phase SP2330 column (2 m × 3 mm, packed with Silar 10C coated on Gas Chrom Q). The polar phase SP2330 column was fitted to a Shimadzu Tracera gas chromatograph with a barrier ionization discharge detector.

A standard solution consisting of methyl esters from common fatty acids existing in meat at known concentrations was applied to the gas chromatograph to compare retention times of sample fatty acids. Nu-Check-Prep, Inc. (Elysim, Minnesota, USA) provided standards for the fatty acids. Fatty acids were presented in terms of their molar proportions (normalised) and gravimetric concentrations (milligrams per gram fresh tissue) in order to establish differences in fatty acid profiles (Webb & Casey, 1995).

3.10. *Cis-trans* fatty acid analysis

The treatment of subcutaneous fat samples with *n*-hexane at 35°C for 24h, followed by the esterification of fatty acids according to the procedure of Van Wijngaarden (1967) produced profiles of the *cis-trans* fatty acids from subcutaneous adipose tissue. An SP2560 fused silica capillary column (100 m × 0.2 mm) fitted to a Varian 3700 gas chromatograph was incorporated to split *cis-trans* fatty acids isomers.

3.11. Long-chain fatty acids

Minced meat samples were freeze dried and combined with phosphate-buffered saline solution. This mixture was homogenised with an internal standard C15:0 and placed in a centrifuge. The supernatant was removed after centrifugation. A chloroform/NHCL mixture was added to the supernatant, which was again subjected to centrifugation. The chloroform was removed from the mixture and the supernatant was combined with aliquot chloroform. This mixture was centrifuged one last time. Chloroform was dissolved from the mixture after centrifugation by means of evaporation.

Methanolic KOH was added to the above mixture and heated. This mixture was allowed to cool down before BF3 in methanol was added and heated again. Following cooling down of the mixture, saturated NaCl in water and hexane was added. This mixture was subjected to centrifugation. Hexane was removed from the mixture and the remaining sample was administered into the Varian 3300 gas chromatograph. Fatty acid concentrations were established using Varian 3300 gas chromatograph fitted with an FID detector and WCOT fused silica coating CP-Sil 88, 100mm x 0.25mm DF 0.2µm column at a column temperature of 150°C and a run time of 40 minutes. The carrier gas was helium.

3.12. Comprehensive gas chromatography time of flight mass spectrometry (GC x GC-TOFMS)

Karoo browse lamb and grass-fed lamb tissue samples were analysed by comprehensive gas chromatography time of flight mass spectrometry (GC x GC-TOFMS). Samples consisted of cooked meat, cooked fat and raw meat. Five samples from each group were subjected to headspace sampling with solid-phase microextraction (SPME) and GC x GC-TOFMS. Refer to Table 3.1 for sample descriptions.

Table 3.1. Tissue samples subjected to GC x GC-TOFMS

Sample Description	Sample number
Karoo Cooked Meat	B1 402 – used for method development
Karoo Cooked Meat	B6 585
Karoo Cooked Meat	B8 347
Karoo Cooked Meat	B9 620
Karoo Cooked Meat	B10 804
Grass Cooked Meat	G1 139
Grass Cooked Meat	G2 537
Grass Cooked Meat	G4 910
Grass Cooked Meat	G8 620
Grass Cooked Meat	G9 600
Karoo Cooked Fat	B1 402
Karoo Cooked Fat	B6 585
Karoo Cooked Fat	B8 347
Karoo Cooked Fat	B9 817
Karoo Cooked Fat	B10 804
Grass Cooked Fat	G1 139
Grass Cooked Fat	G2 537
Grass Cooked Fat	G4 910
Grass Cooked Fat	G8 620
Grass Cooked Fat	G9 600
Karoo Raw Meat	B1 402
Karoo Raw Meat	B6 585
Karoo Raw Meat	B8 347
Karoo Raw Meat	B9 817
Karoo Raw Meat	B10 804
Grass Raw Meat	G1 139
Grass Raw Meat	G2 537
Grass Raw Meat	G4 910
Grass Raw Meat	G8 620
Grass Raw Meat	G9 600

3.13. Sample preparation: Headspace sampling with solid phase microextraction (SPME)

Lamb tissue samples were cut into small pieces using a sharp knife. Two grams of each sample were sealed in 24 ml glass vials. Vials were sealed by a screw cap, which consisted of a Teflon-lined septum with a hole in the centre. Samples contained within vials were submerged in a 40 °C water bath for a period ten minutes. Volatile compounds were extracted by a SUPELCO SPME column (Chromspec, Canada), which consisted of a 2-50/30µm DVB/Carboxen/PDMS StableFlex fibre. The fibre was inserted into the vial and held into the headspace above the sample for a duration of thirty minutes. The SPME device was subsequently withdrawn from the vial and placed into the GC x GC-TOFMS injection port for analysis.

3.14. GC x GC-TOFMS

Compound separation was done on a LECO Pegasus 4D GC x GC-TOFMS including an Agilent 7890 GC (LECO Africa (Pty) Ltd., Kempton Park, South Africa). The system consisted of a secondary oven and a dual-stage modulator. Synthetic air was used for the hot jets and liquid nitrogen was used to cool nitrogen gas for the cold jets. Helium was used as the carrier gas and was of ultra-high purity grade (Afrox, Gauteng, South Africa). Compounds sorbed onto the SPME fibre were desorbed in an SPME inlet liner of a GC inlet. Preliminary detection of compounds was based on comparison of the sample mass spectra to that of the National Institute of Standards and Technology (NIST08) library. LECO statistical compare software was used to establish similarities and differences between samples. Refer to Table 3.2. for GC x GC-TOFMS conditions.

Table 3.2. GC x GC-TOFMS conditions

GC:	Agilent 7890A
Detector:	LECO Pegasus 4D Time-of-Flight Mass Spectrometer
Acquisition Rate:	100 spectra/s
Stored Mass Range:	35 to 520 Da
Source Temperature:	230 °C, Ionisation mode EI+
Detector Voltage:	1815 Volts
GC Inlet Temperature:	250 °C
Splitless Time:	30 sec
SPME Desorption Time:	1.5 min
Carrier Gas:	Helium, 2 ml/min constant flow
Column 1:	Rxi-5Sil MS, 30m x 0.25mm ID x 0.25µm film thickness
Column 2:	Rxi-17Sil MS, 0.97m x 0.25mm ID x 0.25µm film thickness
Column 1 Oven:	35 °C for 1.5min, to 250 °C at 10 °C/min, hold 2 min
Column 2 Oven Offset:	5 °C (relative to primary oven)
Modulator Offset:	20 °C (relative to 2nd oven)
Modulation Period:	2 s (Hot pulse 0.4 s)
Transfer Line Temperature:	280 °C

3.15. Statistical Analyses

Animals were allocated to a completely randomised 2^2 factorial experiment (two sexes x two diets x five replicates). Differences in the subcutaneous fatty acid profile between and within sex, diets and their interactions (diet x sex) were analysed by the Bonferroni method of IBM SPSS Statistics (2017). Differences between means will be detected using the General Linear Model (GLM) procedure of SPSS (2017). Differences in the level of conditioning or fatness will be corrected for by the inclusion of crude fat (fat percentage) and subcutaneous fat thickness as covariates, respectively.

4: RESULTS

4.1. Gas chromatography analyses of subcutaneous adipose tissue and oesophageal samples of lambs from Karoo grass veldt and Karoo browse veldt biomes

4.1.1. Carcass characteristics of lambs from Karoo grass veldt and Karoo browse veldt

Table 4.1.1 Carcass characteristics of lambs from the Karoo browse veldt and Karoo grass veldt biomes ± standard deviation (SD) (in mm and %, respectively)

Carcass Characteristics	Biome			
	Karoo browse veldt		Karoo grass veldt	
	Female (n = 5)	Male (n =5)	Female (n = 5)	Male (n =5)
SCF thickness (mm)	$3.07_{A} \pm 0.201$	$3.25_{B} \pm 0.392$	$3.09_{A} \pm 0.454$	$3.35_{B} \pm 0.348$
Fat percentage (%)	14.62 ± 2.799	12.45 ± 2.993	11.75 ± 4.644	13.76 ± 1.981

A,B Means within the same row bearing different subscripts differ ($P < 0.05$).

SCF – subcutaneous fat.

4.1.2. Fatty acid molar proportions in subcutaneous adipose tissue samples

Table 4.1.2. Fatty acid molar proportions in subcutaneous adipose (fat) tissue samples of lambs from Karoo browse veldt and Karoo grass veldt biomes ± standard deviation (SD) (%)

Fatty acid	Biome			
	Karoo browse veldt		Karoo grass veldt	
	Female (n = 5)	Male (n = 5)	Female (n = 5)	Male (n = 5)
C12:0	0.41 ± 0.042	0.53 ± 0.242	0.26 ± 0.033	0.37 ± 0.157
C14:0	4.54 [a] ± 0. 538	5.65 [a] ± 1.800	3.67 [b] ± 0.490	3.89 [b] ± 1.070
C14:1	0.15 ± 0.017	0.19 ± 0.064	0.14 ± 0.031	0.12 ± 0.062
C16:0	28.37 ± 1.369	29.29 ± 1.525	27.58 ± 0.531	28.60 ± 4.000
C16:1	1.94 ± 0.140	2.20 ± 0.301	2.09 ± 0.317	2.05 ± 0.408
C17:0	1.93 ± 0.195	2.11 ± 0.156	1.67 ± 0.259	1.81 ± 0.228
C18:0	18.64 ± 1.147	18.65 ± 2.286	18.85 ± 3.599	14.72 ± 8.660
C18:1(n-9)t	2.14 ± 0.507	2.76 ± 0.735	2.03 ± 0.406	2.23 ± 0.438
C18:1(n-9)c	36.97 [a] ± 2.183	32.75 [a] ± 2.532	38.72 [b] ± 4.016	40.16 [b] ± 2.262
C18:2(n-6)c	2.20 [a] ± 1.320	2.12 [a] ± 0.419	1.66 [b] ± 0.454	1.94 [b] ± 0.867
C18:3(n-6)	0.03 ± 0.000	0.05 ± 0.010	0.04 ± 0.010	0.08 ± 0.041
C18:3(n-3)	0.59 ± 0.108	0.88 ± 0.124	0.58 ± 0.079	0.63 ± 0.222
C20:0	0.15 ± 0.019	0.17 ± 0.018	0.13 ± 0.016	0.15 ± 0.010
C20:1(n-9)	0.07 ± 0.006	0.05 ± 0.004	0.07 ± 0.022	0.09 ± 0.006
C20:2	0.05 ± 0.005	0.05 ± 0.006	0.06 ± 0.006	0.06 ± 0.034
C20:3(n-6)	0.05 ± 0.006	0.05 ± 0.020	0.06 ± 0.021	0.08 ± 0.042
C20:3(n-3)	0.02 ± 0.000	0.02 ± 0.005	0.02 ± 0.006	0.04 ± 0.036
C20:4(n-6)	0.57 ± 0.111	0.81 ± 0.416	0.84 ± 0.331	1.20 ± 0.583
C20:5(n-3)	0.15 ± 0.043	0.28 ± 0.131	0.23 ± 0.059	0.33 ± 0.208
C21:0	0.55 ± 0.051	0.80 ± 0.282	0.64 ± 0.173	0.56 ± 0.094
C22:0	0.14 [a] ± 0.013	0.16 [a] ± 0.089	0.29 [b] ± 0.134	0.38 [b] ± 0.128
C22:6(n-3)	0.07 ± 0.021	0.11 ± 0.040	0.15 ± 0.063	0.17 ± 0.125
C22:1(n-9)	0.01± 0.000	0.01 ± 0.000	0.03 ± 0.013	0.03 ± 0.283
C22:2(n-6)	0.00 ± 0.000	0.00 ± 0.006	0.01 ± 0.010	0.03 ± 0.015
C24:1	0.01 ± 0.000	0.01 ± 0.004	0.018 ± 0.005	0.06 ± 0.074
SFA: Total	54.72 [a] ± 2.904	57.36 [a] ± 1.540	52.97 [b] ± 4.517	50.48 [b] ± 4.441
MFA: Total	41.28 [a] ± 1.935	37.96 [a] ± 1.987	43.10 [b] ± 3.691	44.70 [b] ± 2.413
PUFA: Total	3.73 ± 1.454	4.38 ± 1.038	3.62 ± 0.903	4.54 ± 1.965
UFA: Total	45.01 ± 2.922	42.34 ± 1.590	46.72 ± 4.383	49.23 ± 4.352
SFA: UFA	1.22 ± 0.140	1.36 ± 0.088	1.15 ± 0.219	1.04 ± 0.186
***Cis*: Total**	39.17 [a] ± 3.125	34.87 [a] ± 2.511	40.38 [b] ± 4.304	42.10 [b] ± 3.003
***Trans:* Total**	2.17 ± 0.555	2.56 ± 0.580	2.03 ± 0.406	2.23 ± 0.438
Cis: Trans	19.17 [a] ± 5.188	13.55[a] ± 4.174	20.82 [b] ± 6.379	19.52 [b] ± 4.505

[a,b] Means within the same row bearing different superscripts differ ($P < 0.05$).

SFA – saturated fatty acids, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

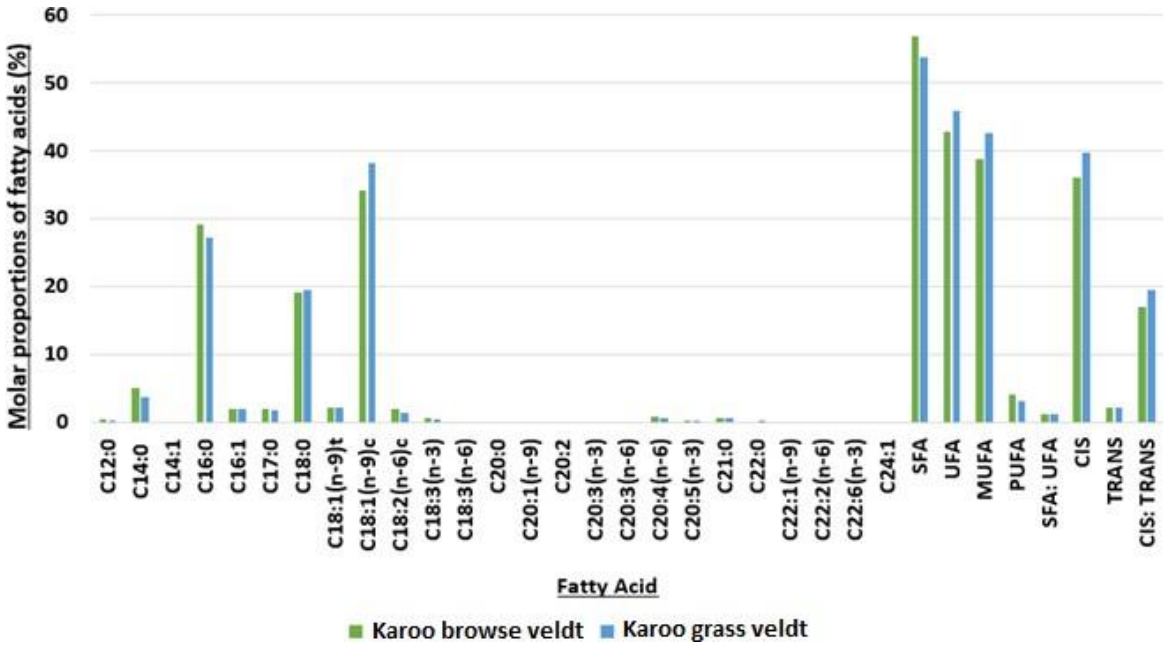

Figure 4.1.1. Fatty acid molar proportions in subcutaneous adipose tissue samples between Karoo browse veldt and Karoo grass veldt biomes.

4.1.3. Fatty acid molar proportions in oesophageal samples

Table 4.1.3. Fatty acid molar proportions from oesophageal samples of lambs from Karoo browse veldt and Karoo grass veldt biomes ± standard deviation (SD) (%)

Fatty acid	Biome			
	Karoo browse veldt		Karoo grass veldt	
	Female (n = 5)	Male (n = 5)	Female (n = 5)	Male (n = 5)
C12:0	2.78 ± 1.161	2.48 ± 0.818	ND	1.45 ± 0.533
C14:0	2.96 ± 1.026	3.47 ± 1.145	5.54 ± 1.485	5.36 ± 1.029
C16:0	30.70 [a] ± 10.678	30.78 [a] ± 5.08	40.60 [b] ± 6.866	38.46 [b] ± 2.477
C17:0	0.74 [a] ± 0.120	0.85 [a] ± 0.284	ND	1.28 [b]
C18:0	7.96 ± 3.248	9.49 ± 5.275	7.99 ± 2.531	10.07 ± 3.647
C18:1(n-9)c	6.89 ± 0.355	8.05 ± 2.903	7.27 ± 2.531	7.51 ± 1.731
C18:2(n-6)c	9.53 [a] ± 2.757	11.43 [a] ± 1.870	7.77 [b] ± 2.779	6.04 [b] ± 1.842
C18:3(n-3)	0.84 ± 0.615	0.64 ± 0.185	ND	1.13 ± 0.120
C20:0	2.22 ± 0.409	2.87 ± 0.938	3.35	4.19 ± 0.839
C20:1(n-9)	11.50 ± 7.188	11.07 ± 6.289	15.05 ± 0.940	11.00 ± 2.293
C20:4(n-6)	9.7175 ± 15.349	7.23 ± 4.466	ND	2.15 ± 1.139
C22:0	4.04 ± 1.067	3.09 ± 0.505	2.40	3.87 ± 0.447
C22:1(n-9)	3.24 ± 1.029	3.61 ± 1.479	6.40 ± 2.277	5.63 ± 3.096
C24:0	3.03 ± 1.232	3.31 ± 0.831	ND	3.20 ± 1.000
SFA: Total	53.35 [a] ± 11.695	56.14 [a] ± 2.598	57.00 [b] ± 2.956	64.94 [b] ± 3.017
MFA: Total	22.19 ± 6.773	22.89 ± 3.562	28.72 ± 0.686	24.13 ± 3.384
PUFA: Total	20.52 ± 15.834	19.14 ± 4.056	8.79 ± 1.336	9.22 ± 2.268
UFA: Total	42.76 [a] ± 11.542	42.03 [a] ± 2.526	37.50 [a] ± 0.651	33.35 [a] ± 2.796
SFA: UFA	1.37 ± 0.597	1.34 ± 0.143	1.52 ± 0.057	1.97 ± 0.267
***Cis*: Total**	16.42[a] ± 2.750	19.48 [a] ±1.457	15.04 [b] ± 5.310	13.54 [b] ± 3.498

[a,b] Means within the same row bearing different superscripts differ ($P < 0.05$).

SFA – saturated fatty acids, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

ND – not detected.

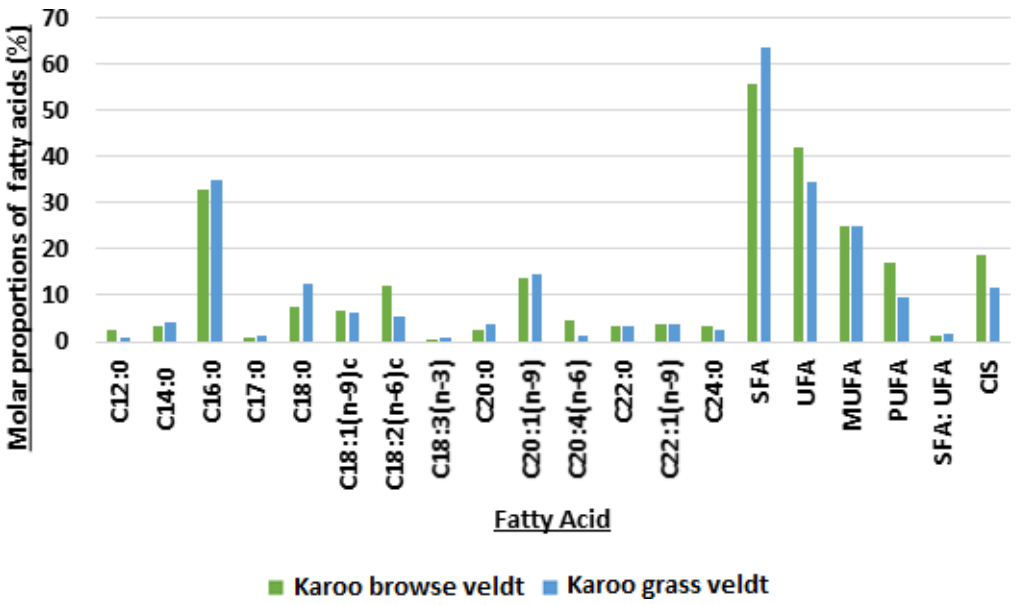

Figure 4.1.2. Fatty acid molar proportions from oesophageal samples of lambs from Karoo browse veldt and Karoo grass veldt biomes.

4.1.4. Fatty acid gravimetric proportions in subcutaneous tissue samples

Table 4.1.4. Fatty acid gravimetric proportions in subcutaneous adipose (fat) tissue of lambs from Karoo browse veldt and Karoo grass veldt biomes ± standard deviation (SD) (mg g^{-1} fresh tissue)

Fatty acid	Biome			
	Karoo browse veldt		Karoo grass veldt	
	Female (n = 5)	Male (n = 5)	Female (n = 5)	Male (n = 5)
C12:0	$0.21^{a} \pm 0.031$	$0.23^{a} \pm 0.099$	$0.13^{b} \pm 0.013$	$0.13^{b} \pm 0.048$
C14:0	2.30 ± 0.500	2.40 ± 0.729	1.79 ± 0.468	1.45 ± 0.597
C14:1	0.08 ± 0.018	0.08 ± 0.022	0.07 ± 0.013	0.04 ± 0.021
C16:0	14.32 ± 2.409	12.63 ± 1.938	13.41 ± 2.288	10.79 ± 3.752
C16:1	0.99 ± 0.208	0.94 ± 0.124	1.01 ± 0.149	0.75 ± 0.183
C17:0	0.97 ± 0.146	0.92 ± 0.219	0.82 ± 0.245	0.69 ± 0.290
C18:0	9.38 ± 1.416	8.17 ± 2.246	9.36 ± 3.342	6.53 ± 4.895
C18:1(n-9)t	1.07 ± 0.184	1.20 ± 0.367	1.00 ± 0.335	0.87 ± 0.445
C18:1(n-9)c	18.68 ± 3.295	14.33 ± 3.581	18.61 ± 1.666	15.29 ± 5.336
C18:2(n-6)c	1.08 ± 0.585	0.90 ± 0.155	0.78 ± 0.103	0.65 ± 0.041
C18:3(n-6)	0.02 ± 0.005	0.02 ± 0.004	0.02 ± 0.000	0.02 ± 0.019
C18:3(n-3)	0.29 ± 0.029	0.38 ± 0.061	0.28 ± 0.055	0.22 ± 0.022
C20:0	0.08 ± 0.013	0.07 ± 0.020	0.06 ± 0.019	0.06 ± 0.021
C20:1(n-9)	$0.04^{a}_{A} \pm 0.006$	$0.02^{a}_{B} \pm 0.004$	$0.04^{b}_{A} \pm 0.005$	$0.03^{b}_{B} \pm 0.010$
C20:2	0.02 ± 0.005	0.02 ± 0.004	0.03 ± 0.005	0.02 ± 0.021
C20:3(n-6)	0.02 ± 0.000	0.02 ± 0.005	0.03 ± 0.005	0.03 ± 0.006
C20:3(n-3)	0.01 ± 0.000	0.01 ± 0.000	0.01 ± 0.000	0.02 ± 0.015
C20:4(n-6)	0.29 ± 0.048	0.33 ± 0.098	0.39 ± 0.105	0.40 ± 0.103
C20:5(n-3)	0.08 ± 0.013	0.11 ± 0.034	0.11 ± 0.012	0.11 ± 0.042
C21:0	0.28 ± 0.037	0.34 ± 0.112	0.32 ± 0.136	0.21 ± 0.078
C22:0	0.07 ± 0.010	0.07 ± 0.022	0.13 ± 0.043	0.13 ± 0.033
C22:6(n-3)	0.04 ± 0.006	0.11 ± 0.141	0.07 ± 0.030	0.06 ± 0.031
C22:1(n-9)	ND	ND	0.01 ± 0.010	0.02 ± 0.014
C22:2(n-6)	ND	ND	0.01 ± 0.005	0.01 ± 0.008
C24:1	0.01 ± 0.006	0.001 ± 0.002	0.01 ± 0.005	0.03 ± 0.035

a,b Means within the same row bearing different superscripts differ ($P < 0.05$).

A,B Means within the same row bearing different subscripts differ ($P < 0.05$).

ND – not detected.

4.1.5. Fatty acid gravimetric proportions in oesophageal samples

Table 4.1.5. Fatty acid gravimetric proportions of oesophageal samples of lambs from Karoo browse veldt and Karoo grass veldt biomes ± standard deviation (SD) (mg g^{-1} fresh tissue)

Fatty acid	Biome			
	Karoo browse veldt		Karoo grass veldt	
	Female (n = 5)	Male (n = 5)	Female (n = 5)	Male (n = 5)
C12:0	0.09 ± 0.049	0.08 ± 0.010	ND	0.05 ± 0.012
C14:0	0.11 ± 0.056	0.11 ± 0.039	0.10 ± 0.028	0.16 ± 0.046
C16:0	0.95 ± 0.563	1.12 ± 0.322	0.71 ± 0.120	1.19 ± 0.420
C17:0	0.03 ± 0.007	0.41 ± 0.667	ND	0.07 ± 0.000
C18:0	0.26 ± 0.133	0.35 ± 0.241	0.14 ± 0.028	0.33 ± 0.217
C18:1(n-9)c	0.24 ± 0.111	0.30 ± 0.134	0.13 ± 0.042	0.24 ± 0.100
C18:2(n-6)c	0.37 ± 0.222	0.39 ± 0.042	0.14 ± 0.050	0.19 ± 0.087
C18:3(n-3)	0.04 ± 0.021	0.03 ± 0.000	ND	0.04 ± 0.014
C20:0	0.07 ± 0.030	0.09 ± 0.026	0.06 ± 0.000	0.13 ± 0.050
C20:1(n-9)	0.46 ± 0.394	0.45 ± 0.308	0.26 ± 0.014	0.37 ± 0.255
C20:4(n-6)	0.34 ± 0.560	0.24 ± 0.164	ND	0.07 ± 0.031
C22:0	0.13 ± 0.052	0.11 ± 0.014	0.04 ± 0.000	0.12 ± 0.043
C22:1(n-9)	0.10^{a} ± 0.034	0.12^{a} ± 0.024	0.11^{b} ± 0.042	0.17^{b} ± 0.062
C24:0	0.09 ± 0.028	0.11 ± 0.021	ND	0.11 ± 0.028

[a,b] Means within the same row bearing different superscripts differ ($P < 0.05$).

ND – not detected.

4.2. Relation between dietary and tissue fatty acids

4.2.1. Palmitic acid (C16:0)

Table 4.2.1. Fatty acid molar proportions divided by palmitic acid (C16:0) molar proportions ± standard deviation (SD)

Fatty acid ratio	Biomes			
	Karoo browse veldt		Karoo grass veldt	
	Female (n = 5)	Male (n = 5)	Female (n = 5)	Male (n = 5)
C12:0/C16:0	0.01 ± 0.002	0.02 ± 0.007	0.01 ± 0.001	0.01 ± 0.004
C14:0/C16:0	0.16 ± 0.017	0.19 ± 0.051	0.13 ± 0.018	0.14 ± 0.025
C14:1/C16:0	0.01 ± 0.001	0.01 ± 0.002	0.00 ± 0.001	0.00 ± 0.002
C16:0/C16:0	1.00 ± 0.000	1.00 ± 0.000	1.00 ± 0.000	1.00 ± 0.000
C16:1/C16:0	0.07 ± 0.006	0.07 ± 0.007	0.08 ± 0.013	0.07 ± 0.008
C17:0/C16:0	0.07 ± 0.004	0.07 ± 0.009	0.06 ± 0.009	0.06 ± 0.009
C18:0/C16:0	0.66 ± 0.021	0.64 ± 0.108	0.68 ± 0.124	0.55 ± 0.330
C18:1(n-9)t/C16:0	0.08 ± 0.016	0.09 ± 0.024	0.07 ± 0.014	0.08 ± 0.020
C18:1(n-9)c/C16:0	1.31 ± 0.134	1.12 ± 0.143	1.41 ± 0.160	1.42 ± 0.164
C18:2(n-6)c/C16:0	0.08 ± 0.053	0.07 ± 0.014	0.06 ± 0.017	0.07 ± 0.026
C18:3(n-3)/C16:0	0.02 ± 0.004	0.03 ± 0.004	0.02 ± 0.003	0.02 ± 0.007
C18:3(n-6)/C16:0	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001
C20:0/C16:0	0.01 ± 0.000	0.01 ± 0.001	0.00 ± 0.001	0.01 ± 0.000
C20:1/C16:0	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001	0.00 ± 0.002
C20:2/C16:0	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001
C20:3(n-3)/C16:0	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001
C20:3(n-6)/C16:0	0.00 ± 0.000	0.00 ± 0.001	0.00 ± 0.001	0.00 ± 0.001
C20:4(n-6)/C16:0	0.02 ± 0.005	0.03 ± 0.013	0.03 ± 0.012	0.04 ± 0.017
C20:5(n-3)/C16:0	0.01 ± 0.002	0.01 ± 0.004	0.00 ± 0.002	0.01 ± 0.007
C21:0/C16:0	0.02 ± 0.002	0.03 ± 0.008	0.02 ± 0.006	0.02 ± 0.005
C22:0/C16:0	0.00 ± 0.000	0.01 ± 0.003	0.01 ± 0.005	0.01 ± 0.004
C22:1(n-9)/C16:0	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001
C22:2/ C16:0	ND	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001
C22:6(n-3)/C16:0	0.00 ± 0.000	0.00 ± 0.001	0.01 ± 0.002	0.01 ± 0.005
C24:1/C16:0	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.003

ND – not detected

4.2.2. Stearic acid (C18:0)

Table 4.2.2. Fatty acid molar proportions divided by stearic acid (C18:0) molar proportions ± standard deviation (SD)

Fatty acid ratio	Biomes			
	Karoo browse veldt		Karoo grass veldt	
	Female (n = 5)	Male (n = 5)	Female (n = 5)	Male (n = 5)
C12:0/C18:0	0.02 ± 0.002	0.03 ± 0.018	0.01 ± 0.004	0.08 ± 0.137
C14:0/C18:0	0.24 ± 0.023	0.32 ± 0.149	0.20 ± 0.032	0.78 ± 1.189
C14:1/C18:0	0.01 ± 0.001	0.01 ± 0.005	0.01 ± 0.003	0.03 ± 0.044
C16:0/C18:0	1.52 ± 0.051	1.60 ± 0.297	1.50 ± 0.239	5.28 ± 7.731
C16:1/C18:0	0.10 ± 0.012	0.12 ± 0.033	0.11 ± 0.033	0.40 ± 0.593
C17:0/C18:0	0.10 ± 0.004	0.11 ± 0.008	0.09 ± 0.013	0.31 ± 0.437
C18:0/C18:0	1.00 ± 0.000	1.00 ± 0.000	1.00 ± 0.000	1.00 ± 0.000
C18:1(n-9)t/C18:0	0.11 ± 0.022	0.15 ± 0.052	0.11 ± 0.017	0.36 ± 0.488
C18:1(n-9)c/C18:0	1.99 ± 0.240	1.77 ± 0.155	2.13 ± 0.530	6.76 ± 9.304
C18:2(n-6)c/C18:0	0.12 ± 0.080	0.11 ± 0.025	0.09 ± 0.039	0.43 ± 0.685
C18:3(n-3)/C18:0	0.03 ± 0.005	0.05 ± 0.010	0.03 ± 0.008	0.13 ± 0.196
C18:3(n-6)/C18:0	0.00 ± 0.000	0.00 ± 0.001	0.00 ± 0.001	0.02 ± 0.025
C20:0/C18:0	0.01 ± 0.001	0.01 ± 0.000	0.01 ± 0.001	0.03 ± 0.035
C20:1/C18:0	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.002	0.02 ± 0.023
C20:2/C18:0	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001	0.01 ± 0.003
C20:3(n-3)/C18:0	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.01 ± 0.007
C20:3(n-6)/C18:0	0.00 ± 0.000	0.00 ± 0.001	0.00 ± 0.002	0.02 ± 0.030
C20:4(n-6)/C18:0	0.03 ± 0.007	0.04 ± 0.025	0.05 ± 0.024	0.27 ± 0.432
C20:5(n-3)/C18:0	0.01 ± 0.002	0.02 ± 0.007	0.01 ± 0.005	0.07 ± 0.113
C21:0/ C18:0	0.03 ± 0.004	0.04 ± 0.022	0.03 ± 0.006	0.09 ± 0.120
C22:0/ C18:0	0.01 ± 0.001	0.01 ± 0.005	0.02 ± 0.009	0.07 ± 0.111
C22:1(n-9)/ C18:0	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001	0.00 ± 0.002
C22:2/ C18:0	ND	0.00 ± 0.000	0.00 ± 0.001	0.00 ± 0.004
C22:6(n-3)/ C18:0	0.00 ± 0.001	0.01 ± 0.003	0.01 ± 0.004	0.03 ± 0.038
C24:1/ C18:0	ND	0.00 ± 0.000	0.00 ± 0.000	0.01 ± 0.009

ND – not detected.

4.2.3. Oleic acid (C18:1)

Table 4.2.3. Fatty acid molar proportions divided by oleic acid (C18:1) molar proportions ± standard deviation (SD)

Fatty acid ratio	Biomes			
	Karoo browse veldt		Karoo grass veldt	
	Female (n = 5)	Male (n = 5)	Female (n = 5)	Male (n = 5)
C12:0/C18:1(n-9)c	0.01 ± 0.002	0.02 ± 0.009	0.01 ± 0.001	0.01 ± 0.004
C14:0/C18:1(n-9)c	0.12 ± 0.019	0.18 ± 0.067	0.10 ± 0.023	0.10 ± 0.026
C14:1/C18:1(n-9)c	0.00 ± 0.000	0.01 ± 0.002	0.00 ± 0.001	0.00 ± 0.001
C16:0/C18:1(n-9)c	0.77 ± 0.076	0.90 ± 0.112	0.72 ± 0.088	0.71 ± 0.083
C16:1/C18:1(n-9)c	0.05 ± 0.003	0.07 ± 0.014	0.05 ± 0.010	0.05 ± 0.008
C17:0/C18:1(n-9)c	0.05 ± 0.008	0.06 ± 0.004	0.04 ± 0.012	0.05 ± 0.007
C18:0/C18:1(n-9)c	0.51 ± 0.061	0.57 ± 0.049	0.50 ± 0.156	0.37 ± 0.228
C18:1(n-9)t/C18:1(n-9)c	0.06 ± 0.017	0.09 ± 0.027	0.05 ± 0.016	0.06 ± 0.014
C18:1(n-9)c/C18:1(n-9)c	1.00 ± 0.000	1.00 ± 0.000	1.00 ± 0.000	1.00 ± 0.000
C18:2(n-6)c/C18:1(n-9)c	0.06 ± 0.033	0.07 ± 0.015	0.04 ± 0.010	0.05 ± 0.019
C18:3(n-3)/C18:1(n-9)c	0.02 ± 0.004	0.03 ± 0.005	0.02 ± 0.003	0.02 ± 0.005
C18:3(n-6)/C18:1(n-9)c	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001	0.00 ± 0.001
C20:0/C18:1(n-9)c	0.00 ± 0.000	0.01 ± 0.001	0.00 ± 0.001	0.00 ± 0.000
C20:1/C18:1(n-9)c	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001
C20:2/C18:1(n-9)c	ND	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001
C20:3(n-3)/C18:1(n-9)c	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001
C20:3(n-6)/C18:1(n-9)c	0.00 ± 0.000	0.00 ± 0.001	0.00 ± 0.000	0.00 ± 0.001
C20:4(n-6)/C18:1(n-9)c	0.02 ± 0.003	0.02 ± 0.014	0.02 ± 0.007	0.03 ± 0.013
C20:5(n-3)/C18:1(n-9)c	0.00 ± 0.001	0.01 ± 0.004	0.01 ± 0.001	0.01 ± 0.005
C21:0/ C18:1(n-9)c	0.01 ± 0.002	0.02 ± 0.010	0.02 ± 0.007	0.01 ± 0.002
C22:0/ C18:1(n-9)c	0.00 ± 0.000	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.003
C22:1(n-9)/ C18:1(n-9)c	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.001
C22:2/ C18:1(n-9)c	ND	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
C22:6(n-3)/ C18:1(n-9)c	0.00 ± 0.001	0.00 ± 0.001	0.00 ± 0.002	0.00 ± 0.003
C24:1/ C18:1(n-9)c	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.002

ND – not detected.

5. DISCUSSION

5.1. Gas chromatography analyses of subcutaneous adipose tissue and oesophageal samples of lambs from Karoo grass veldt and Karoo browse veldt biomes

5.1.1. Carcass characteristics of lambs from Karoo grass veldt and Karoo browse veldt

Carcass characteristics of lambs from Karoo grass veldt and Karoo browse veldt are presented in Table 4.1.1. Subcutaneous fat thickness (fat depth) and fat percentage (crude fat) did not differ significantly for sexes between treatments. Wethers from both biomes displayed higher subcutaneous fat thickness values compared to ewes ($P < 0.05$). Subcutaneous fat thickness was affected by the inclusion of fat percentage as a covariate ($P < 0.05$).

Webb and Casey (1995) conducted a study wherein they explored the genetic differences in fatty acid composition of subcutaneous adipose tissue in Dorper and SA Mutton Merino (SAMM) wethers at different live masses and breed × live mass interactions. They found that the subcutaneous fat thickness in Dorpers differed significantly from that of the SAMM breed ($P < 0.05$). The SAMM is a later maturing breed and contained less subcutaneous fat than the earlier maturing Dorper of the same live mass. In contrast, the earlier maturing female animals in this study displayed lower subcutaneous fat thickness values compared to the later maturing males with thicker subcutaneous fat depots.

Webb and Casey (1995) also found that subcutaneous fat thickness differed significantly between slaughter masses ($P < 0.05$), where an increase in the thickness of subcutaneous fat was associated with an increase in slaughter mass ($P < 0.05$). The effect of slaughter mass on carcass characteristics was not examined in this study, as lambs were reared to a single predetermined slaughter mass.

5.1.2. Fatty acid molar proportions in subcutaneous adipose tissue samples

Molar proportions of fatty acids from subcutaneous adipose tissue samples are presented in Table 4.1.2. The major fatty acid present in subcutaneous fat was oleic acid (C18:1(n-9) *cis*). Subcutaneous adipose tissue samples from Karoo grass-fed animals presented oleic acid concentrations of 38.72 ± 4.016 % and 40.16 ± 2.262 % for ewes and wethers respectively. Oleic acid concentrations for ewes and wethers from Karoo browse veldt were 36.97 ± 2.183 % and 32.75 ± 2.532 %, respectively. Adipose tissues from Karoo browse veldt contained significantly lower oleic acid concentrations compared to tissue samples from Karoo grass veldt ($P < 0.05$). Palmitic acid (C16:0) and stearic acid (C18:0) were also present at higher concentrations in subcutaneous adipose tissue samples from both groups.

Webb *et al*. (1994) studied fatty acids in the subcutaneous adipose tissue of intensively fed South African Mutton Merino and Dorper wethers at different live masses. A diet with a medium level of energy (10.2 MJ ME/kg DM (M)) and another with a high plane of energy (11.8 MJ ME/kg DM (H)) were fed to wethers of both breeds. The diets consisted of maize meal, *Eragrostis* tef hay, cottonseed oil cake, urea, feed lime, sodium bicarbonate, dicalcium phosphate and salt.

The major fatty acid in the high energy concentrate diet was linoleic acid (C18:2), while oleic acid (C18:1) was the fatty acid present at highest concentrations in the medium energy concentrate diet. Higher concentrations of palmitic acid (C16:0; $P < 0.01$) and stearic acid (C18:0; $P < 0.01$) were present the subcutaneous adipose tissue of wethers fed the medium energy concentrate diet. The main subcutaneous fatty acids from wethers fed the high energy concentrate diet. were pentadecanoic acid(C15:0), heptadecanoic acid(C17:1) and margaric acid (C17:0) ($P < 0.01$).

The result from the present study agree with those of Webb and Casey (1995), who reported that stearic acid (C18:0) and palmitic acid (C16:0) accounted for 85% of long-chain saturated fatty acids in subcutaneous adipose tissue, with stearic acid making up most of the saturated fatty acids. The major monounsaturated fatty acid was oleic acid (C18:1), while linoleic acid (C18:2) was the most prevalent polyunsaturated fatty acid.

Molar fatty acid proportions differed significantly between dietary treatments for myristic acid (C14:0), behenic acid (C22:0) and linoleic acid (C18:2(n-6)) in this study. The concentrations of myristic acid and linoleic acid were significantly higher in the subcutaneous adipose tissue of lambs from Karoo browse veldt, whereas the concentration of behenic acid was significantly higher in the subcutaneous adipose tissue of lambs from Karoo grass veldt ($P < 0.05$). Diet and sex interactions displayed a tendency towards significance for α-Linoleic acid (18:3(n-3); $P = 0.086$). Wethers from Karoo browse veldt contained more α-Linoleic acid within their subcutaneous adipose tissue compared to the

subcutaneous fat of wethers from grass veldt. Female subcutaneous adipose tissue contained more or less the same amount of α-Linoleic acid between diets.

Arachidic acid (C20:0; $P < 0.05$) and eicosatrienoic acid (C20:3(n-3); $P = 0.053$) differed significantly between dietary treatments after fat percentage was included as a covariate in the current study. Arachidic acid was lower in subcutaneous fat samples from Karoo browse-fed animals, whereas C20:3(n-3) was higher in the subcutaneous fat from animals that were raised on Karoo grass veldt.

Eicosatrienoic acid (C20:3(n-3)) also differed significantly between diets after the inclusion of subcutaneous fat thickness (fat depth) as a covariate ($P < 0.05$). Diet and sex interactions were significant for C20:2 when subcutaneous fat thickness was included as a covariate. Both wethers and ewes from Karoo grass veldt contained higher concentrations of C20:2 in fat than animals from Karoo browse veldt. The inclusion of subcutaneous fat thickness lead to a significant difference in crude fat between treatments ($P < 0.05$).

Animals that grazed Karoo browse veldt displayed unsaturated fatty acid concentrations of 45.01 ± 2.922% and 42.34 ± 1.590% for ewes and wethers, respectively. The concentrations of unsaturated fatty acids recorded for animals from Karoo grass veldt were 46.72 ± 4.383% and 49.23 ± 4.352% for ewes and wethers respectively. Saturated fatty acid concentrations were present in the subcutaneous fat of Karoo grass-fed ewes and wethers at 52.97 ± 4.517 % and 50.48 ± 4.441 % respectively. Karoo browse veldt animals displayed saturated fatty acid concentrations of 54.72 ± 2.904 % and 57.36 ± 1.540 % for ewes and wethers respectively. Saturated fatty acids were therefore present at significantly higher concentrations in the subcutaneous fat of lambs reared on Karoo browse veldt ($P < 0.05$). Monounsaturated fatty acid concentrations were significantly lower in the subcutaneous fat of lambs from Karoo browse veldt compared to the subcutaneous fat of lambs from Karoo grass veldt ($P < 0.05$).

Webb *et al.* (1994) described a higher concentration of unsaturated fatty acids in the subcutaneous fat of early maturing Dorpers compared to SAMM wethers ($P < 0.01$). The high energy concentrate diet displayed a decrease in the saturated to unsaturated fatty acid ratio ($P < 0.01$) compared to the medium energy concentrate diet, because it contained a higher concentration of unsaturated fatty acids ($P < 0.01$) and a lower concentration of saturated fatty acids ($P < 0.01$).

Webb and Casey (1995) reported that around 53% of fatty acids present in subcutaneous adipose tissue were in the form of saturated fatty acids for an *ad libitum* maize-based diet, with a total of 42% of fatty acids consisting of monounsaturated fatty acids. The remaining fatty acids present in subcutaneous adipose tissue consisted of polyunsaturated fatty acids (5%). The Karoo grass veldt

diet presented fatty acid values for subcutaneous adipose tissue samples that fell within approximately the same range as those in the study of Webb and Casey (1995). However, slightly higher concentrations of saturated fatty acids are reported for subcutaneous fat samples from animals reared on Karoo browse veldt.

Saturated fatty acid to unsaturated fatty acid ratios (SFA:UFA) for the respective subcutaneous adipose tissue samples are displayed in Table 4.1.2. The SFA:UFA ratios for animals from Karoo grass veldt were 1.15 ± 0.219 and 1.04 ± 0.186 for ewes and wethers respectively. Karoo browse veldt displayed SFA:UFA ratios of 1.22 ± 0.140 and 1.36 ± 0.088 for ewes and wethers respectively. Subcutaneous adipose tissue samples from Karoo browse veldt therefore exhibit marginally higher SFA:UFA ratios compared to subcutaneous fat samples from Karoo grass veldt. Webb and Casey (1995) reported a SFA:UFA ratio of 1.141 ± 0. 0.143 for the subcutaneous adipose tissue from animals that ingested an *ad-libitum* maize-based diet.

The concentrations of *cis* fatty acids recorded from subcutaneous fat samples for Karoo grass-fed ewes and wethers were 40.38 ± 4.304 % and 42.10 ± 3.003 % respectively. Total *cis* fatty acids for Karoo browse veldt ewes and wethers amounted to 39.17 ± 3.125 % and 34.87 ± 2.511 % respectively. Higher proportions of *trans* fatty acids were present in the subcutaneous fat of animals reared on Karoo browse veldt. The ratio of c*is: trans* fatty acids was higher in the subcutaneous fat of animals from Karoo grass veldt. The inclusion of fat percentage (crude fat) as a covariate significantly affected the ratio of *cis: trans* fatty acids ($P < 0.05$), which appeared to be higher in the samples from animals that were reared on Karoo grass veldt.

Previous research by Webb *et al.* (1994) showed that high inclusion levels of maize meal in diets lead to a lower *cis: trans* fatty acid ratio in subcutaneous adipose tissue. Only *trans*-11-octadecenoic acid (C18:1(*trans*)) was present in subcutaneous adipose tissue (Webb *et al.,* 1994). Tissue samples from wethers fed the high energy concentrate diet consisted of higher concentrations of *trans*-11-octadecenoic acid ($P < 0.01$). Branched-chain subcutaneous fatty acids were present at elevated concentrations in wethers that were fed the high energy concentrate diet ($P < 0.01$). Webb and Casey (1995) reported higher percentages of C18:1 (*cis*) in the subcutaneous fat samples of Dorpers.

It was also shown that the changes in pH as described by Webb *et al.* (1994) affected the microbial population of the rumen. A change in ruminal pH influenced important bacterial species as certain groups of bacteria thrive within a specific pH range (Mackie *et al.,*1978, as cited in Webb *et al.,*1994). The ingestion of the high energy concentrate diet caused an increase in the lactate-utilizing bacterial population of the rumen. An increase in lactate-utilizing bacteria affected the end-products of rumen fermentation by decreasing the acetate to propionate ratio (Mackie & Gilchrist,1979, as cited in

Webb *et al.,* 1994). There was consequently an increase in the amount of propionate formed via lactate as intermediate. The liver did not metabolize high levels of propionate and methylmalonate effectively. Therefore, propionate and methylmalonate were synthesised to odd-numbered and branched and/or unsaturated fatty acids in subcutaneous tissue.

An *ad libitum* maize diet fed to animals in the study of Webb & Casey (1995) was significantly higher in energy than a diet based on Karoo browse veldt, which may, in turn, explain the difference in subcutaneous adipose tissue fatty acid profiles obtained between the two studies. However, the fatty acid profile obtained from Karoo grass veldt resembles the fatty acid profile described by Webb & Casey (1995). Webb *et al.* (1994) found that diets consisting of high levels of maize may significantly influence the subcutaneous fatty acid profiles. A diet that is high in energy may significantly elevate the amount of unsaturated fatty acids in subcutaneous fat. This phenomenon may explain the lower concentrations of subcutaneous adipose tissue unsaturated fatty acids obtained in lambs from Karoo browse veldt compared to the results obtained by Webb and Casey (1995).

Ewes reach physiological maturity at an earlier chronological stage than wethers. Earlier-maturing ewes may therefore potentially produce a meat fatty acid profile that is different to the fatty acid composition contained within wethers' meat. The fatty acid profiles of subcutaneous adipose tissue did not display significant differences between ewes and wethers in this study. Differences in fatty acid composition between ewes and wethers remained insignificant after the inclusion of fat percentage and subcutaneous fat thickness as covariates.

Webb and Casey (1995) described higher concentrations of margaric acid (C17:0), oleic acid (C18:1) and heptadecenoic acid (C17:1) in the subcutaneous fat of Dorpers compared to SAMM. The difference in molar proportions of fatty acids between Dorper and SAMM breeds reinforce the notion that genetic differences may influence molar proportions of subcutaneous fatty acids. SAMM breeds mature later, whereas Dorper breeds are earlier maturing. The molar proportions of subcutaneous unsaturated – and monounsaturated fatty acids were reported to be higher in Dorpers compared to SAMM wethers ($P < 0.01$) (Webb and Casey, 1995).

The difference in the subcutaneous fatty acid profiles of Dorpers grazing Karoo browse veldt and Karoo grass veldt biomes is compared in Figure 4.1.1. Oleic acid (C18:1(n-9) *cis*) was slightly higher in the subcutaneous fat samples of animals reared on Karoo grass veldt, whereas palmitic acid (C16:0) was present at higher concentrations in the subcutaneous adipose tissue of animals from Karoo browse veldt. Higher concentrations of subcutaneous saturated fatty acids and polyunsaturated fatty acids were available in lamb tissue from Karoo browse veldt. Unsaturated fatty acids, monounsaturated fatty acids and *cis* fatty acids were higher in the subcutaneous adipose tissue from lambs reared on

Karoo grass veldt. The *cis* fatty acid to *trans* fatty acid ratio obtained from subcutaneous fat samples was higher in lambs from Karoo grass veldt.

The fatty acid composition of subcutaneous adipose tissue and muscle of loin samples in sheep is presented in Table 5.1.1 (Adapted from Webb & O'Neil, 2008). The molar proportions of palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2(n-6)) in the subcutaneous adipose tissue samples of this study were higher than the concentrations displayed in Table 5.1.1. The subcutaneous fat samples in this study displayed lower concentrations for palmitoleic acid (C16:1), stearic acid (C18:0) and α-Linolenic (C18:3(n-3)) compared to the molar proportions described by Webb and O'Neil (2008). Subcutaneous adipose tissue samples from Karoo browse veldt displayed higher molar proportions for myristic acid (C14:0) compared to Table 5.1.1., while samples from Karoo grass veldt were lower in C14:0 concentration than the C14:0 concentrations displayed by Webb and O'Neil (2008). The fatty acid composition of muscle and meat (Tables 5.1.1. and 5.1.2. respectively) are also presented. Meat and muscle samples were not subjected to gas chromatography in the study by Webb & O'Neil, 2008.

Table 5.1.1. Fatty acid composition (w/w%) of subcutaneous adipose tissue and muscle of loin samples in sheep (adapted from Webb & O'Neil, 2008)

	Adipose tissue	Muscle
C14:0	4.1	3.3
C16:0	21.9	22.2
C16:1*cis*	2.4	2.2
C18:0	22.6	18.1
C18:1cis-9	28.7	32.5
C18:2n-6	1.3	2.7
C18:3n-3	1.0	1.37
C20:4n-6	ND	0.64
C20:5n-3	ND	0.45
n-6:n-3	1.4	1.3
P:S	0.09	0.15
Total	70.6	4.9

Table 5.1.2. Comparison between fatty acid composition (w/w%) of freeze-dried meat (muscle and fat) of sheep (adapted from Webb & O'Neil, 2008)

Fatty acid	Breed	
	Dorper sheep	Damara sheep
C14:0	8.4	7.4
C16:0	24.3	22.5
C16:1	4.0	3.4
C17:0	2.1	2.1
C18:0	14.4	16.4
C18:1	37.6	38.9
C18:3	3.2	3.9
C20:1	2.2	2.0
SFA	52.8	51.8
UFA	47.2	48.2
MUFA	43.9	44.3
PUFA	3.3	3.9
P:S	0.06	0.07

5.1.3. Fatty acid molar proportions in oesophageal samples

Oesophageal samples were collected to analyse diet fatty acid composition. Molar proportions of fatty acids from oesophageal samples are presented in Table 4.1.3. Palmitic acid (C16:0) was the most abundant fatty acid in both diets. Palmitic acid occurs regularly in almost all animal and plant oils (Webb, 1994). Samples from ewes and wethers that consumed Karoo grass veldt consisted of 40.60 ± 6.866 % and 38.46 ± 2.477% palmitic acid (C16:0) respectively. Oesophageal samples from Karoo browse veldt contained 30.70 ± 10.678 % and 30.78 ± 5.08 % palmitic acid (C16:0) for ewes and wethers respectively. Palmitic acid was therefore significantly higher in the oesophageal samples from Karoo grass veldt.

The percentage of saturated fatty acids in oesophageal samples of lambs from Karoo browse veldt was 53.35 ± 11.695 % and 56.14 ± 2.598 % for ewes and wethers respectively. The Karoo grass veldt diet contained a saturated fatty acid percentage of 57.00 ± 2.956 % and 64.94 ± 3.017 % for ewes and wethers respectively. The percentage of unsaturated fatty acids in the Karoo grass veldt diet for ewes and wethers was 37.50 ± 0.651% and 33.35 ± 2.796 % respectively, whereas the Karoo browse veldt samples displayed unsaturated fatty acid concentrations of 42.76 ± 11.542 % and 42.03 ± 2.526 % for ewes and wethers respectively. The saturated fatty acid to unsaturated fatty acid ratio was higher in oesophageal samples from ewes and wethers fed Karoo grass veldt (1.52 ± 0.057 and 1.97 ± 0.267 respectively) compared to Karoo browse veldt (1.37 ± 0.597, 1.34 ± 0.143 for ewes and wethers respectively).

Margaric acid (C17:0) differed significantly between dietary treatments ($P < 0.05$). Margaric acid was detected at significantly elevated molar proportions in the oesophageal samples of wethers raised on Karoo grass veldt compared to other fatty acids present in samples. Linoleic acid (C18:2(n-6); $P = 0.086$) displayed a tendency toward significance and was present at higher concentrations in samples from Karoo browse veldt. Rumen biohydrogenation processes limit the effect of differences in the dietary linoleic acid (C18:2) concentrations on fatty acid tissue profiles (Webb, 1994).

Molar fatty acid concentrations from oesophageal samples of lambs from Karoo browse veldt and Karoo grass veldt biomes are depicted in Figure 4.1.2. Palmitic acid (C16:0) and stearic acid (C18:0) were higher in the oesophageal samples of lambs that consumed Karoo grass veldt. Oesophageal samples of lambs from Karoo browse veldt were higher in linoleic acid (C18:2(n-6)) concentrations. The molar proportions of saturated fatty acids were higher in the oesophageal samples of lambs raised on Karoo grass veldt, whereas the oesophageal samples of lambs from Karoo browse veldt contained higher concentrations of unsaturated fatty acids, polyunsaturated fatty acids and *cis* fatty acids.

Webb *et al.* (1994) reported that linoleic acid (C18:2) was the predominant fatty acid associated with the high energy concentrate diet, while oleic acid (C18:1) was present at highest concentrations in the medium energy concentrate diet. The medium energy concentrate diet also contained considerable amounts of palmitic (C16:0) and stearic acids (C18:0).

5.1.4. Gravimetric proportions of fatty acids in subcutaneous tissue samples

The gravimetric proportions of fatty acids from subcutaneous adipose tissue samples are presented by Table 4.1.4. Oleic acid (C18:1(n-9) *cis*) was the most abundant fatty acid present in adipose fat, followed by palmitic acid (C16:0) and stearic acid (C18:0), respectively. Webb and Casey (1995) reported that oleic acid (C18:1(n-9) *cis*)) was present at the highest gravimetric concentrations in the subcutaneous adipose tissue of lambs raised on an ad-libitum maize-based diet. High concentrations of stearic acid (C18:0) and palmitic acid (C16:0) were also recorded by Webb and Casey (1995).

The difference in the gravimetric proportions of lauric acid (C12:0) was highly significant between dietary treatments ($P < 0.01$) in this study. The subcutaneous adipose tissue of animals from Karoo browse veldt contained significantly higher concentrations of lauric acid compared to the subcutaneous fat of animals reared on Karoo grass veldt. Gondoic acid (C20:1) differed significantly between diets and sexes ($P < 0.05$). Gondoic acid was significantly higher in the subcutaneous fat of animals reared on Karoo grass veldt and was also present at higher proportions in the fat of ewes from both browse and grass veldt compared to wethers. Significant interactions for diet and sex occurred

for lauric acid (C12:0; $P = 0.002$) and gondoic acid (C20:1, $P = 0.015$). Ewes and wethers from Karoo browse veldt both contained higher concentrations of lauric acid compared to animals from Karoo grass veldt.

Webb and Casey (1995) found differences in the gravimetric concentrations of subcutaneous fatty acids between the Dorper and the SA Mutton Merino ($P < 0.05$). The Dorper contained higher concentrations of subcutaneous myristic acid ([C14:0]; $P < 0.05$), pentadecanoic acid ([C15:0]; $P < 0.05$), palmitic acid ([C16:0]; $P < 0.05$), palmitoleic acid ([C16:1]; $P < 0.05$), margaric acid ([C17:0]; $P < 0.01$), heptadecenoic acid ([C17:1]; $P < 0.01$), stearic acid ([C18:0]; $P < 0.05$) and oleic acid ([C18:1]; $P < 0.05$). The difference in fatty acid profile between breeds described by Webb and Casey (1995) illustrates the effect of earlier vs. later-maturing breeds on subcutaneous adipose tissue fatty acid composition. As such, Gondoic acid (C20:1) was detected at significantly increased gravimetric concentrations in the subcutaneous fat of earlier-maturing ewes compared to that of later-maturing wethers in this study.

Differences between dietary treatments were significant for lauric acid (C12:0, $P < 0.05$) and myristoleic acid (C14:1, $P = 0.004$) after the inclusion of fat percentage as a covariate in this study. Myristoleic acid was higher in the subcutaneous fat of ewes and wethers reared from Karoo browse veldt. Myristoleic acid (C14:1) and gondoic acid (C20:1) were significantly higher in the fat samples from ewes of both veldt types compared to wethers ($P < 0.05$). Highly significant diet and sex interactions occurred for lauric acid (C12:0; $P = 0.001$) and myristoleic acid (C14:1; $P = 0.002$) after fat percentage was included as a covariate. Subcutaneous fat thickness differed significantly between lambs from different veldt types after fat percentage was included as a covariate ($P < 0.05$).

The inclusion of subcutaneous fat thickness as a covariate in this study highlighted the significant interactions between diet and sex for lauric acid (C12:0; $P < 0.05$) and myristoleic acid (C14:1, $P < 0.05$). Fat percentage also differed significantly between treatments with the inclusion of subcutaneous fat thickness as a covariate ($P < 0.05$).

The inclusion of carcass fatness (subcutaneous fat thickness) as a covariate caused some differences in the fatty acid concentrations between breeds to be negligible in the study by Webb and Casey (1995). Important breed differences in gravimetric fatty acid concentrations present after the inclusion of carcass fatness as a covariate were limited to heptadecenoic acid ([C17:1]; $P < 0.05$) and margaric acid ([C17:0]; $P < 0.05$). Webb and Casey (1995) found that important differences in the concentrations of unsaturated fatty acids between breeds persisted after carcass fat was included as a covariate in the ANOVA model. Breed differences in fatty acids concentrations can be explained by

the earlier maturing Dorper breed storing moderately greater concentrations of subcutaneous unsaturated fatty acids than the SAMM.

Webb and Casey (1995) discovered an association between heavier slaughter mass and certain fatty acids. Slaughter mass was an important factor that influenced gravimetric fatty acid concentration, even though it played a negligible role regarding molar proportions of fatty acids. The concentrations of myristic acid ([C14:0]; $P < 0.05$), palmitic acid ([C16:0]; $P < 0.05$), palmitoleic acid ([C16:1]; $P < 0.05$), stearic acid ([C18:0]; $P < 0.05$) and oleic acid ([C18:1]; $P < 0.05$) increased in both Dorpers and SAMM with an increase in slaughter masss. The effect of slaughter mass on subcutaneous fatty acid composition was not examined in this study, as lambs were reared to a single predetermined slaughter mass.

5.1.5. Fatty acid gravimetric proportions in oesophageal tissue samples

The gravimetric proportions of fatty acids from oesophageal samples are summarized in Table 4.1.5. Oesophageal samples represented feed composition. The highest gravimetric proportions of fatty acids from feed samples were recorded for palmitic acid (C16:0). Erucic acid (C22:1(n-9); $P < 0.05$) differed significantly between samples from Karoo browse veldt and Karoo grass veldt. Erucic acid was higher in the oesophageal samples obtained from Karoo grass veldt compared to samples from Karoo browse veldt. Lignoceric acid (C24:0) was present in oesophageal samples, but not present in the samples from subcutaneous adipose tissue.

5.2. Relation between dietary and tissue fatty acids

The relative proportion in which one fatty acid is deposited from plant origin into the subcutaneous adipose tissue compared to which a fatty acid is synthesized *de novo* may highlight the differences of fatty acid metabolism between different diets. Fatty acid molar proportions were divided by the molar proportions of palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1), respectively, to investigate the possible difference in fatty acid metabolism between diets.

5.2.1. Palmitic acid (C16:0)

The tissue fatty acid ratios of C18:3(n-3)/C16:0 (P = 0.068) and C12:0/C16:0 (P = 0.069) showed tendencies toward significance – both ratios were higher in the subcutaneous tissue from animals raised on Karoo browse veldt compared to Karoo grass veldt. The interaction between diet and sex was significant for the fatty acid ratio of C18:3(n-3)/C16:0 ($P < 0.05$). It was significantly higher for tissue samples from Karoo browse veldt compared to Karoo grass veldt.

5.2.2. Stearic acid (C18:0)

No significant ratios were found for C18:0 as a denominator.

5.2.3. Oleic acid (C18:1)

The subcutaneous fatty acid ratios of C12:0/C18:1 (P = 0.059) and C20:0/C18:1 (P = 0.090) displayed tendencies toward significance for diet. The aforementioned fatty acid ratios were present at higher levels in the subcutaneous tissue from animals raised on Karoo browse veldt. The ratio of C18:3(n-3)/C18:0 reported a tendency toward significance between diet and sex interactions (P = 0.086).

5.3. Comprehensive gas chromatography time of flight mass spectrometry (GC x GC-TOFMS)

Compounds detected in samples of lambs from the Karoo (Karoo browse veldt) and grass-biome (Karoo grass veldt) treatments are reported in Appendices II and III. The sulphur-containing compound 3-methylthio-2-butanone was preliminarily identified as a potential marker for Karoo lamb. It was detected in four of the five Karoo lamb raw meat samples (B1, B6, B8, B9 Karoo raw meat), while it was present in only one of the four Karoo cooked meat samples (B8). The compound 3-methylthio-2-butanone was not found in any of the five Karoo cooked fat samples.

The GC elution temperature of 3-methylthio-2-butanone was 74 °C. The possible loss or destruction of 3-methylthio-2-butanone at high cooking temperatures may explain the absence of this compound in cooked samples. Karoo raw meat samples from Karoo browse veldt would consequently be better suited for examination as samples are not subjected to higher temperatures, leading to the destruction of 3-methylthio-2-butanone. This compound was not isolated in any of the fifteen grass-fed lamb tissue samples (grass cooked meat, cooked fat and raw meat). An authentic reference standard should be used to verify the presence of 3-methylthio-2-butanone in Karoo raw meat.

Caryophyllene was present in three of the five Karoo grass-fed lamb samples (G2, G8, G9 raw meat, cooked meat and cooked fat). β-Caryophyllene is a sesquiterpene and can serve as a biomarker in

meat to designate a grass diet (Priolo *et al*., 2004). Caryophyllene was absent in all of the fourteen examined Karoo lamb samples (Karoo cooked meat, cooked fat and raw meat). β-caryophyllene detection in tissue samples should be compared to a certified reference standard.

Average peak areas for compounds detected in Karoo browse and Karoo grass-fed samples are presented by bar charts in Appendix III. Figure 1 (Appendix III) displays a significantly higher peak area for 3-methylthio-2-butanone in raw meat Karoo samples. Caryophyllene was present at higher levels in raw meat, cooked meat and cooked fat samples from Karoo grass-fed lamb (Figures 1, 2 and 3 of Appendix III).

Multidimensional scaling was applied to determine compound variance between Karoo browse and Karoo grass-fed lamb for each treatment (Appendix III). Forty compounds for cooked fat, thirty-eight compounds for cooked meat, and thirty-three compounds for raw meat were selected for analysis. Multidimensional scaling demonstrated discrimination between Karoo browse and Karoo grass-fed lamb for raw meat (Figure 4, Appendix III). Significant overlap existed between treatments for cooked meat and fat samples (Figures 5 & 6, Appendix III).

A biomarker needs to be identified that is unique to the whole Karoo geographical region. Failing that, the Karoo district can be subdivided into smaller regions, each of which is associated with a distinctive marker compound. However, the identification of a biomarker for Karoo lamb for each of the sub-regions will be challenging and costly when using GC x GC-TOFMS. The untargeted analysis of samples by GC x GC-TOFMS requires the examination of more than a thousand compounds to identify a potential marker for each sub-region. Extensive funding is therefore required for the analysis of each sample when a compound associated with a treatment is unknown. Ascertaining the trace elements within lamb bones by ICP-MS may be an easier, affordable approach to establish the origin of production system. However, the identification of 3-methylthio-2-butanone in raw Karoo meat samples allows for targeted analysis of samples. Targeted analysis of samples and the automation of analysis by the use of an SPME autosampler will significantly reduce costs.

Gas chromatography-combustion-carbon isotope mass spectrometry (GC-C-IRMS) can also be applied to pinpoint trace biomarkers to establish geographical origin. Cymene may serve as a potential marker since it was present in each of the Karoo browse and Karoo grass-fed lamb samples. Cymene isotope ratios may then be applied to establish region of production. The effect of grain finishing on potential biomarkers should be taken into account as Karoo lamb flocks are finished on grain prior to slaughter. The Karoo meat analysed in this study was not finished on grain.

6. CONCLUSION

The majority of small stock production systems in South Africa follow extensive grazing practices. Sheep farming in the Karoo is a sensible alternative to cattle farming as sheep are adapted to the semi-arid environment and deliver animal products of exceptional quality under harsh circumstances (Zervas & Tsiplakou, 2011). The Karoo landscape proves to be too challenging to incorporate crop production. Lamb that originates from Karoo farms has distinctive taste and sensory characteristics. The unique flavour associated with Karoo lamb is linked to free-range grazing by flocks on indigenous Karoo vegetation. Problems arise when some businesses falsely claim that their meat product originates from the Karoo. The Karoo Meat of Origin certification ensures that sheep have been reared exclusively on Karoo veldt ('Karoo Meat of Origin', 2019).

Grazing activity affects meat quality and characteristics. The Dorper breed selects woody-type vegetation. They tend to favour Karoo browse species, whereas Merino-type breeds prefer the soft, herbaceous parts of plants (Brand, 2000). Nutrition and environment influence meat quality and characteristics. Meat fatty acid composition is affected by diet (Wood *et al*., 2008). The degree of saturation of meat fatty acids affects shelf-life and fat firmness. Studies have shown that less tender meat in cattle is associated with grass-based systems (Nuernberg *et al*., 2005). Meat flavour is influenced by nutrition directly or indirectly (Resconi *et al*., 2009). Biohydrogenation processes in the rumen will affect meat characteristics and quality since they change the composition of vegetation consumed by the animal. Fermentation end-products are absorbed by the gastrointestinal tract, metabolised and deposited into muscle (Leek, 1993).

Various methods exist to pinpoint the diet and environment linked to a meat product. Specific compounds associated with diet include carotenoids, skatole and 2,3-octanedione. Meat volatile compounds are measured by dynamic headspace analysis. Stable isotope ratio analysis can be used to determine diet and geographic origin (Monahan *et al*., 2018). The use of traceability techniques to verify the origin of production system will be beneficial to Karoo farmers and associated business partners. Meat samples from lambs reared on Karoo browse veldt and grass veldt were compared to identify compounds responsible for the unique flavour of Karoo lamb via the application of gas chromatography.

Higher subcutaneous fat thickness values were displayed by wethers from both biomes compared to ewes. Fat percentage and subcutaneous fat thickness did not differ significantly between treatments. Biohydrogenation processes within the rumen limited the effect of differences in diet on the fatty acid profiles of subcutaneous adipose tissue.

Oleic acid (C18:1(n-9) *cis*) was the most abundant fatty acid present in the subcutaneous adipose tissue of lambs from both treatments. Subcutaneous adipose tissue samples from Karoo grass veldt contained significantly higher molar concentrations of oleic acid compared to tissue samples from Karoo browse veldt ($P < 0.05$). Subcutaneous fat samples from Karoo browse veldt displayed fatty acid profiles that were significantly higher in myristic acid (C14:0) and linoleic acid (C18:2(n-6)) ($P < 0.05$).

Interesting differences in longer-chain fatty acids (> C20) were reported between treatments. The molar proportions of behenic acid (C22:0) were significantly higher in the subcutaneous adipose tissue of lambs from Karoo grass veldt ($P < 0.05$). Eicosatrienoic acid (C20:3(n-3); $P = 0.053$) was higher in the subcutaneous adipose tissue of animals that were raised on Karoo grass veldt, while arachidic acid (C20:0; $P < 0.05$) was lower in subcutaneous fat samples from Karoo browse-fed animals after fat percentage was included as a covariate.

The subcutaneous fatty acid profiles of lambs from Karoo browse veldt contained significantly higher concentrations of saturated fatty acids and significantly lower concentrations of monounsaturated fatty acids compared to the subcutaneous fat of lambs from Karoo grass veldt ($P < 0.05$). The subcutaneous adipose tissue from Karoo browse veldt displayed marginally higher saturated fatty acid to unsaturated fatty acid ratios compared to subcutaneous adipose tissue samples from Karoo grass veldt. Subcutaneous adipose tissue of lambs from Karoo grass veldt displayed higher *cis* fatty acid to *trans* fatty acid ratios. The subcutaneous fatty acid profiles of animals raised on Karoo browse veldt displayed higher *trans* fatty acid molar concentrations.

The subcutaneous fat of lambs from Karoo browse veldt contained significantly elevated lauric acid (C12:0) gravimetric concentrations compared to the subcutaneous fat of animals reared on Karoo grass veldt. Gondoic acid (C20:1) was present at higher gravimetric proportions in the fat of ewes from both Karoo browse veldt and Karoo grass veldt compared to wethers and was significantly increased in the subcutaneous adipose tissue of animals reared on Karoo grass veldt ($P < 0.05$). Differences in the gravimetric proportions of lauric acid (C12:0, $P < 0.05$) and myristoleic acid (C14:1, $P = 0.004$) were significant between dietary treatments and sexes after fat percentage and subcutaneous fat thickness were included as covariates in this study.

Diet fatty acid composition was obtained by the analysis of oesophageal samples. The most abundant fatty acid in both Karoo browse veldt and Karoo grass veldt was palmitic acid (C16:0). The oesophageal samples of lambs from Karoo grass veldt displayed higher saturated fatty acid to unsaturated fatty acid ratios compared to oesophageal samples from Karoo browse veldt.

Margaric acid (C17:0) and erucic acid (C22:1(n-9)) was present at significantly higher molar and gravimetric proportions, respectively, in the oesophageal samples of lambs raised on Karoo grass veldt compared to the oesophageal samples from lambs that consumed Karoo browse veldt ($P < 0.05$). Lignoceric acid (C24:0) was absent in the samples obtained from subcutaneous adipose tissue, while it was detected in oesophageal samples.

Longer chain fatty acids (> C20) displayed interesting differences between dietary treatments. Erucic acid (C22:1(n-9)) was significantly lower in the oesophageal samples obtained from Karoo browse veldt compared to oesophageal samples from lambs that grazed Karoo grass veldt ($P < 0.05$). Lignoceric acid (C24:0) was detected in oesophageal samples, while it was absent in the samples from subcutaneous adipose tissue.

The subcutaneous adipose tissue of lambs from Karoo grass veldt contained significantly higher molar proportions of behenic acid (C22:0) compared to the subcutaneous fat of lambs from Karoo browse veldt ($P < 0.05$). Gondoic acid (C20:1) was significantly higher in the subcutaneous fat of animals reared on Karoo grass veldt and was present at higher gravimetric proportions in the fat of ewes from both Karoo browse veldt and Karoo grass veldt compared to wethers ($P < 0.05$). Longer chained fatty acids are subjected to extensive biohydrogenation processes within the rumen and *de novo* fatty acid metabolism within the subcutaneous adipose tissue of animals.

The difference in fatty acid metabolism between diets can be represented by the rate at which one fatty acid is deposited from the diet into subcutaneous adipose tissue compared to the rate at which a fatty acid is synthesized *de novo.* The fatty acid molar proportions were divided by the molar proportions of palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1), respectively, to investigate the possible difference in fatty acid metabolism between diets. The relation between dietary and tissue fatty acids displayed tendencies toward significance between different biomes, with interactions between diet and sex displaying significance for the ratio of C18:3(n-3)/ C16:0 ($P < 0.05$). No differences in fatty acid metabolism were reported between Karoo browse veldt and Karoo grass veldt in this study ($P < 0.05$).

Extensive biohydrogenation processes within the rumen produce saturated fatty acids. Biohydrogenation therefore changes the fatty acid profile of the ingested plant material in such a way that it proves to be challenging to isolate a specific fatty acid that is associated with a specific diet. Therefore, conjugated fatty acid chains are prone to originate from the diet as fatty acids are saturated in the rumen. *Trans*-type bonds in fatty acids are produced by microbial enzymes within the rumen. Generally, uneven numbered fatty acid chains originate from plants, as mammalian enzyme systems add two carbon units at a time to a fatty acid chain (Webb, 1994).

Although statistical analyses indicate a significant difference between treatments, actual differences in fatty acid profiles between treatments were still marginal. The practical application of isolating specific fatty acids associated with a treatment proves to be difficult as fatty acids are present in small amounts in tissue samples. Similarity in the fatty acid profiles between veldt types can also be attributed to little variation between veldt types. Both veldt types contain vegetation associated with the Karoo biome, the difference being that the Karoo grass veldt type consists of more grasses and the Karoo browse veldt type contains more species of browse. Tissue fatty acid profiles will vary more between treatments if the diets vary more, for example, a feed that is high in concentrates vs. natural veldt types. Longer growth periods allow for a longer period of metabolism of fatty acids that originate from plants, while the tissue from a short growth period (associated with a diet that is high in energy) will more closely resemble a fatty acid profile where fatty acids are deposited from the diet because less time is allowed for plant fatty acids to be metabolised *de novo*.

The difference in tissue fatty acid profiles between sexes can be explained by the age at which sexual maturity is obtained. Females reach sexual maturity at an earlier stage compared to males. Females therefore have a longer period wherein they can metabolise fatty acids from plant origin. Males will resemble a tissue fatty acid profile with higher levels of plant fatty acids, while females will display a fatty acid profile that contains more fatty acids from *de novo* fatty acid synthesis. Selective grazing behaviour between sexes may also explain the difference in tissue fatty acid profiles. Variation in oesophageal samples may also be an indication of selective behaviour between sexes.

The compound 3-methylthio-2-butanone was identified as a potential marker for Karoo lamb by the application of comprehensive gas chromatography time of flight mass spectrometry. The GC elution temperature of 3-methylthio-2-butanone was 74°C. Karoo raw meat samples from Karoo browse veldt would consequently be better suited for examination as samples are not subjected to higher temperatures, leading to the destruction of 3-methylthio-2-butanone. Caryophyllene was present in

three of the five Karoo grass-fed lamb samples. Cymene may serve as a potential marker since it was present in each of the Karoo browse and Karoo grass-fed lamb samples.

A biomarker needs to be identified that is specific to the whole Karoo geographical district. Alternatively, unique marker compounds can be identified that are associated with smaller regions within the larger Karoo biome. The untargeted analysis of samples by GC x GC-TOFMS is a costly procedure which involves the examination of more than a thousand compounds to identify a potential marker for each sub-region. Considerable funding is necessary for the analysis of each sample in order to identify an unknown compound associated with a treatment. The identification of 3-methylthio-2-butanone in raw Karoo meat samples allows for targeted sample analysis.

Ascertaining the trace elements within lamb bones by ICP-MS may be an easier and more affordable approach to establish the origin of production system. Gas chromatography-combustion-carbon isotope mass spectrometry (GC-C-IRMS) can also be applied to pinpoint trace biomarkers to establish geographical origin. Future research needs to be conducted on establishing stable isotope ratios for certain Karoo regions to compare meat samples in order to authenticate the origin of the production system.

Meat provenance may involve the application of different techniques to pinpoint the geographic environment from which a product originated and may not be dependent on solely one method to establish origin of production. The differences in fatty acid profiles between subcutaneous adipose tissue samples of lambs from Karoo browse veldt and Karoo grass veldt were small due to relatively minor differences in diet (both diets were based on Karoo veldt) and extensive biohydrogenation processes within the rumen. H_0 is accepted because differences were detected between the subcutaneous adipose tissue samples of lambs raised on Karoo browse veldt and Karoo grass veldt, respectively. Unique compounds were detected by GC x GC-TOFMS that were associated specifically with each respective diet treatment.

This study was representative of actual Karoo farming conditions in that lambs were allowed to graze freely on indigenous veldt of the Karoo. Ewes and wethers were included in the study and the Dorper breed is very popular in extensive lamb production systems. Future research can lengthen the grazing period and widen the sample group of animals to include more lambs. More biopsies or samples can be obtained in future research to study seasonal variation. However, the inclusion of more lambs into a sample group or the acquisition of biopsies from live animals may be compromising from an ethical perspective.

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APPENDIX I

UNIVERSITEIT VAN PRETORIA
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Faculty of Natural and Agricultural Sciences
Ethics Committee

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ETHICS SUBMISSION: LETTER OF APPROVAL

Ms E Mostert
Department of Animal and Wildlife Sciences
Faculty of Natural and Agricultural Science
University of Pretoria

Reference number: NAS018/2019
Project title: The effect of Karoo – and veldt feeding on long-chain fatty acids and volatile fatty acid components in lamb.

Dear Ms E Mostert,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Natural and Agricultural Sciences Research Ethics committee.

Please note the following about your ethics approval:

- Please use your reference number (NAS018/2019) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
- Please note that ethical approval is granted for the duration of the research (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
- The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Post approval submissions including application for ethics extension and amendments to the approved application should be submitted online via the Ethics work centre.

We wish you the best with your research.

Yours sincerely,

Chairperson: NAS Ethics Committee

APPENDIX II

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15 September 2014
Department of Animal and Wildlife Sciences

Dear Prof Eddie Webb,

Karoo Meat of Origin: Analysis of headspace profiles of lamb meat and fat from animals grazing on grass and Karoo veldt by comprehensive gas chromatography time of flight mass spectrometry (GC x GC-TOFMS) – a preliminary investigation

Terms of reference

1. The contents of this report are intended for use as an Appendix to Oliver Mwale's PhD thesis. Please reference the contributors below.
2. Use of the contents of this report is restricted. Contents may not be reproduced or distributed, in part or in full, without the prior written consent of Dr Yvette Naudé and Prof Egmont Rohwer.

Contributors

1. Method development, sample preparation, analyses by comprehensive gas chromatography time of flight mass spectrometry (GC x GC-TOFMS), data processing, results evaluation and interpretation were performed by Madelien Wooding (BSc Chemistry (Hons) student) and Yvette Naudé.
2. Funding and facilities were provided by Prof Egmont Rohwer.

Samples of Karoo lamb and Grass fed lamb were analysed by GC x GC-TOFMS to identify a potential marker for Karoo Meat of Origin.

Experimental

Table 1: Samples

Sample description	Sample number
Karoo Cooked Meat	B1 402 – used for method development
Karoo Cooked Meat	B6 585
Karoo Cooked Meat	B8 347
Karoo Cooked Meat	B9 620
Karoo Cooked Meat	B10 804
Grass Cooked Meat	G1 139
Grass Cooked Meat	G2 537
Grass Cooked Meat	G4 910
Grass Cooked Meat	G8 620
Grass Cooked Meat	G9 600
Karoo Cooked Fat	B1 402
Karoo Cooked Fat	B6 585
Karoo Cooked Fat	B8 347
Karoo Cooked Fat	B9 817
Karoo Cooked Fat	B10 804
Grass Cooked Fat	G1 139
Grass Cooked Fat	G2 537
Grass Cooked Fat	G4 910
Grass Cooked Fat	G8 620
Grass Cooked Fat	G9 600
Karoo Raw Meat	B1 402
Karoo Raw Meat	B6 585
Karoo Raw Meat	B8 347
Karoo Raw Meat	B9 817
Karoo Raw Meat	B10 804
Grass Raw Meat	G1 139
Grass Raw Meat	G2 537
Grass Raw Meat	G4 910
Grass Raw Meat	G8 620
Grass Raw Meat	G9 600

Headspace sampling with solid phase microextraction (SPME)

Two grams of lamb meat or fat, cut into small pieces, were placed in a 24 ml glass vial. The vial was sealed with a screw cap with centre hole and a Teflon-lined septum. The sample was immersed in a water bath at 40°C for 10 min to equilibrate. The extractions were done with a SUPELCO SPME device which was fitted with a 2-50/30µm DVB/Carboxen/PDMS StableFlex fibre. The fibre was exposed to the headspace above the sample for 30 min. The SPME device was then removed from the vial and inserted into the GC x GC-TOFMS injection port (Table 2).

GC x GC-TOFMS

Compound separation was done on a LECO Pegasus 4D GC x GC-TOFMS including an Agilent 7890 GC (LECO Africa (Pty) Ltd., Kempton Park, South Africa). The system included a secondary oven and a dual stage modulator. Liquid nitrogen was used to cool nitrogen gas for the cold jets and synthetic air was used for the hot jets. The carrier gas, helium, was of ultra-high purity grade (Afrox, Gauteng, South Africa). Compounds sorbed onto the SPME fibre were desorbed in a SPME inlet liner of a GC inlet.

Table 2: GC x GC-TOFMS conditions

GC:	Agilent 7890A
Detector:	LECO Pegasus 4D Time-of-Flight Mass Spectrometer
Acquisition Rate:	100 spectra/s
Stored Mass Range:	35 to 520 Da
Source Temperature:	230°C, Ionisation mode EI+
Detector Voltage:	1815 Volts
GC Inlet Temperature:	250°C
Splitless Time:	30 sec
SPME Desorption time:	1.5 min
Carrier Gas:	Helium, 2 ml/min constant flow
Column 1:	Rxi-5Sil MS, 30m x 0.25mm ID x 0.25µm film thickness
Column 2:	Rxi-17Sil MS, 0.97m x 0.25mm ID x 0.25µm film thickness
Column 1 Oven:	35°C for 1.5min, to 250°C at 10°C/min, hold 2min
Column 2 Oven Offset:	5°C (relative to primary oven)
Modulator Offset:	20°C (relative to 2nd oven)
Modulation Period:	2 s (Hot pulse 0.4 s)
Transfer Line Temperature:	280°C

Tentative identification of compounds was based on comparison of the sample mass spectra to that of the National Institute of Standards and Technology (NIST08) library. LECO statistical compare software was used to determine similarities and differences between samples.

Results and discussion

Marker for Karoo Meat Raw

A sulphur containing compound, 3-(Methylthio)-2-butanone, was tentatively identified as a possible marker for Karoo lamb meat since it was detected in four (B1, B6, B8, B9 Karoo Raw Meat) of the five Karoo lamb raw meat samples analysed. 3-(Methylthio)-2-butanone was detected in only one (B8) of the four cooked Karoo lamb meat samples analysed, while it was not detected in any of the five Karoo cooked fat samples analysed. 3-(Methylthio)-2-butanone was not detected in any of the fifteen Grass Fed lamb samples (Grass Fed Cooked and Raw Meat, and Cooked Fat) analysed.

The GC elution temperature of 3-(Methylthio)-2-butanone was 74°C which may explain its absence in cooked samples due to the possible loss/destruction thereof during the cooking process. Therefore, raw meat samples should be used for analysis to eliminate effects of the cooking process. Confirmation of the presence of 3-(Methylthio)-2-butanone in Karoo Raw Meat should be sought using a certified reference standard. Reasons as to the absence of 3-(Methylthio)-2-butanone in one (B10 Karoo Raw) of the five Karoo Raw meat samples analysed should be investigated, e.g. possible incorrect sample labelling.

Marker for Grass Fed Lamb

The sesquiterpene, β-caryophyllene, was reported by Priolo et al. (2004) to be present in the fat of grass fed lamb and not in the fat of grain fed lamb [1]. β-Caryophyllene can thus be used as a biomarker in meat products to indicate grass feeding. Caryophyllene was detected in three (G2, G8, G9) of the five grass fed lamb cooked and raw meat samples and cooked fat samples analysed. Samples G1 and G2 should be traced to determine reasons for the absence of caryophyllene in these grass fed lamb samples. Confirmation of the presence of β-caryophyllene should be sought using a certified reference standard. Caryophyllene was not detected in any of the Karoo lamb samples analysed (n=14, cooked and raw meat, cooked fat).

Establishment of a routine forensic technique for off-the-shelf produce requires a marker that is unique to all of the Karoo geographical regions. Alternatively, the region should be divided into smaller areas and markers should be identified for each sub-region. This is a major analytical challenge requiring extensive funding due to the fact that for each sample 100s to 1000s of compounds were detected by GC x GC-TOFMS. A simpler and cheaper alternative may be the determination of trace elements within lamb bones by ICP-MS to verify terroir. Gas Chromatography-Combustion-Carbon Isotope Mass Spectrometry (GC-C-IRMS) may also be considered to identify trace biomarkers to determine geographical origin. Here, compound-specific isotope ratios (^{13}C) are determined to trace produce to its origin [2]. Cymene shows promise as a marker as it was detected in all of the lamb samples (Karoo and grass fed) analysed: isotope ratios of cymene may then be compared to establish terroir specificity.

According to a Karoo lamb meat farmer (personal communication Madelien Wooding) all Karoo lamb are finished on grain prior to slaughter. The effect of grain finishing on potential markers should be considered.

Conclusion

3-(Methylthio)-2-butanone in raw meat may be further investigated as a potential marker of Karoo meat of origin.

Recommendations

1. The presence of the compound tentatively identified as 3-(Methylthio)-2-butanone must be confirmed with an authentic reference standard.
2. Analysis of raw meat by GC-C-IRMS, or the determination of trace elements within lamb bones by ICP-MS, to trace the geographical origin of lamb.
3. Investigation of the effect of grain finishing on potential markers.

References

[1] Priolo A, Cornu A, Prache S, Krogmann M, Kondjoyan N, Micol D, et al. (2004) Fat volatiles tracers of grass feeding in sheep. Meat Sci **66** 475-481.

[2] Van Leeuwen KA, Prenzler PD, Ryan D, Camin F (2014) Gas chromatography–combustion- isotope ratio mass spectrometry for traceability and authenticity in foods and beverages. Compr Rev Food Sci F **13** 814-837.

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Dr Tim Laurens, Department of Chemistry, for suggesting the use of ICP-MS.
Dr Peter Gorst-Allman, LECO Africa, for loan of the LECO statistical compare software.
Marc Bouwer for performing Multidimensional Scaling.

Yours sincerely,

Dr Yvette Naudé

APPENDIX III

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25 September 2014
Department of Animal and Wildlife Sciences

Dear Prof Eddie Webb,

Appendix to Karoo Meat of Origin: Analysis of headspace profiles of lamb meat and fat from animals grazing on grass and Karoo veldt by comprehensive gas chromatography time of flight mass spectrometry (GC x GC-TOFMS) – a preliminary investigation

Terms of reference

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Contributors

1. Method development, sample preparation, analyses by comprehensive gas chromatography time of flight mass spectrometry (GC x GC-TOFMS), data processing, results evaluation and interpretation were performed by Madelien Wooding (BSc Chemistry (Hons) student) and Yvette Naudé.
2. Funding and facilities were provided by Prof Egmont Rohwer.

Appendix

Average Peak Areas Karoo vs. Grass fed

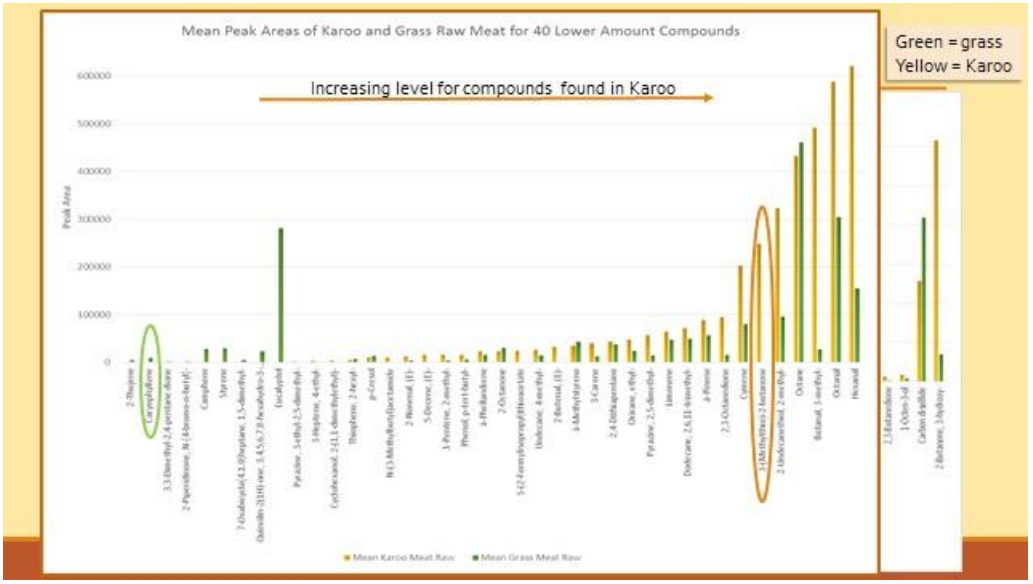

Figure 1. Average Peak Areas (arranged in increasing values for Karoo) for compounds detected in Karoo (n=5) and Grass (n=5) fed lamb: raw meat.

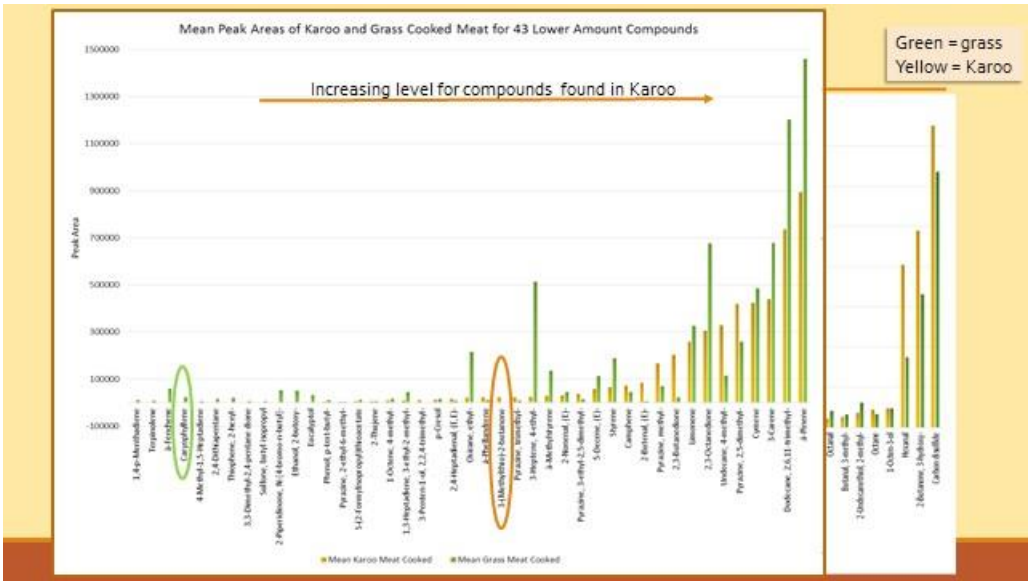

Figure 2. Average Peak Areas (arranged in increasing values for Karoo) for compounds detected in Karoo (n=4) and Grass (n=5) fed lamb: cooked meat.

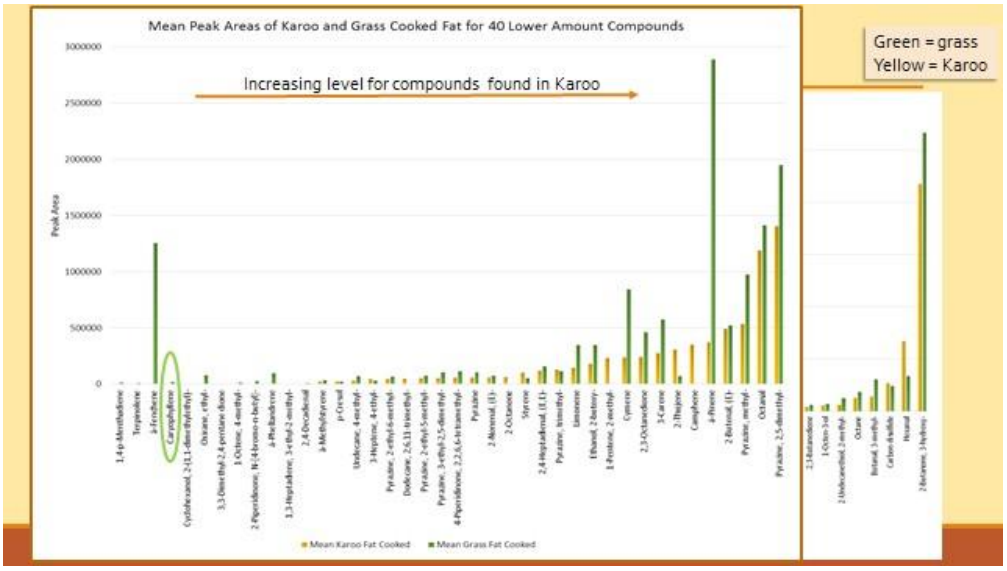

Figure 3. Average Peak Areas (arranged in increasing values for Karoo) for compounds detected in Karoo (n=5) and Grass (n=5) fed lamb: cooked fat.

Caution: The bar charts depict average values for compound peak areas. Values reported do not imply that the compounds were present in all samples within a feed type. For example, Eucalyptol was present in only one (G9) of the five grass raw meat samples analysed. Therefore, it cannot be interpreted that Eucalyptol is a marker for grass fed lamb, or that the absence of Eucalyptol is indicative of Karoo fed lamb (Fig. 1).

Multidimensional Scaling

Leco Statistical Compare software was first used to determine compound variance between the two feed types for each treatment. From this, thirty three compounds for raw meat, thirty eight compounds for cooked meat, and forty compounds for cooked fat were selected for multidimensional scaling. Multidimensional Scaling (Vegan package software (version R-3.1.0) employing non-metric multidimensional scaling techniques was used), based on the Bray-Curtis dissimilarity index, was then performed on the relative peak area ratios of the selected compounds:

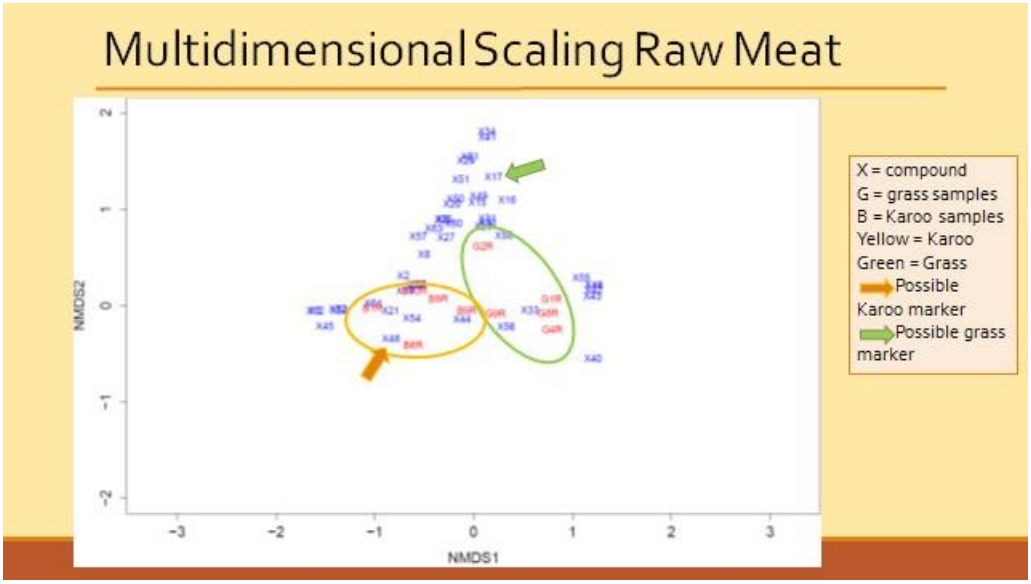

Figure 4. X17 Caryophyllene, X48 3-(Methylthio)-2-butanone.

X	
2	2-Butenal, (E)-
6	á-Pinene
8	Cymene
16	á-Phellandrene
17	Caryophyllene
19	Pyrazine, 2,5-dimethyl-
20	Limonene
21	3-Carene
23	à-Methylstyrene
25	1-Octen-3-ol
27	Undecane, 4-methyl-
29	2,4-Dithiapentane
30	Thiophene, 2-hexyl-
31	Oxirane, ethyl-
33	Carbon disulfide
39	2-Undecanethiol, 2-methyl-
41	Camphene
42	Styrene
45	2,3-Butanedione
48	3-(Methylthio)-2-butanone
49	Octane
50	Dodecane, 2,6,11-trimethyl-
51	Phenol, p-tert-butyl-
52	Butanal, 3-methyl-
53	5-Decene, (E)-
54	2-Butanone, 3-hydroxy-
55	Ethanol, 2-butoxy-
57	Hexanal
58	p-Cresol
60	Octanal
61	2-Octanone
63	2-Nonenal, (E)-
64	2,3-Octanedione

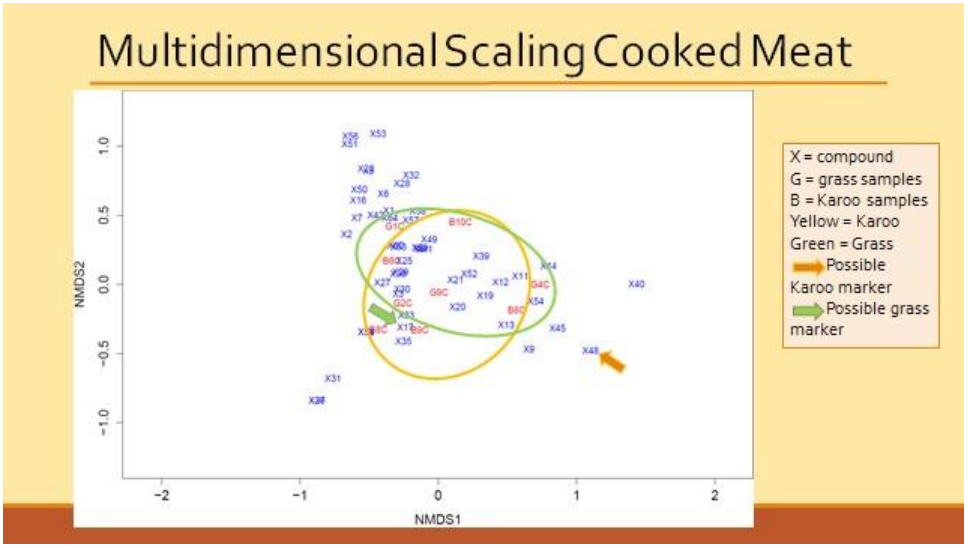

Figure 5

X	
1	1,4-p-Menthadiene
2	2-Butenal, (E)-
3	3-Heptene, 4-ethyl-
5	Terpinolene
6	á-Pinene
7	à-Fenchene
8	Cymene
9	Pyrazine, trimethyl-
11	Pyrazine, methyl-
12	Pyrazine, 3-ethyl-2,5-dimethyl-
16	á-Phellandrene
17	Caryophyllene
19	Pyrazine, 2,5-dimethyl-
20	Limonene
21	3-Carene
23	à-Methylstyrene
25	1-Octen-3-ol
27	Undecane, 4-methyl-
28	1,3-Heptadiene, 3-ethyl-2-methyl-
29	2,4-Dithiapentane
30	Thiophene, 2-hexyl-
31	Oxirane, ethyl-
33	Carbon disulfide
35	S-(2-Formylisopropyl)thioacetate
39	2-Undecanethiol, 2-methyl-
41	Camphene
42	Styrene
45	2,3-Butanedione
49	Octane
50	Dodecane, 2,6,11-trimethyl-
52	Butanal, 3-methyl-
54	2-Butanone, 3-hydroxy-
57	Hexanal
58	p-Cresol
59	2,4-Heptadienal, (E,E)-
60	Octanal
63	2-Nonenal, (E)-
64	2,3-Octanedione

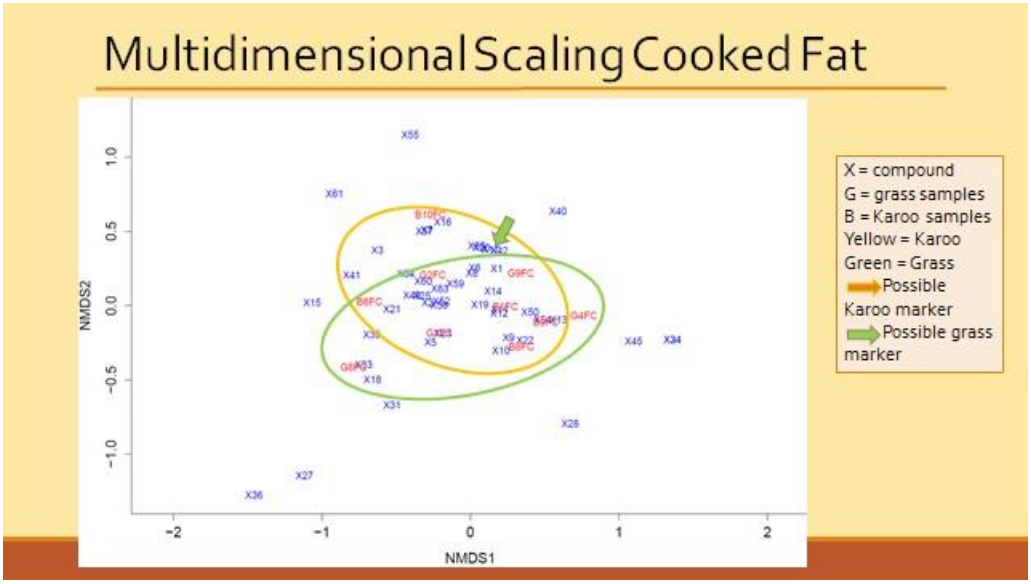

Figure 6

X	
1	1,4-p-Menthadiene
2	2-Butenal, (E)-
3	3-Heptene, 4-ethyl-
5	Terpinolene
6	á-Pinene
7	à-Fenchene
8	Cymene
9	Pyrazine, trimethyl-
10	Pyrazine
11	Pyrazine, methyl-
12	Pyrazine, 3-ethyl-2,5-dimethyl-
13	2-Thujene
14	Pyrazine, 2-ethyl-6-methyl-
16	á-Phellandrene
17	Caryophyllene
18	4-Piperidinone, 2,2,6,6-tetramethyl-
19	Pyrazine, 2,5-dimethyl-
20	Limonene
21	3-Carene
22	Pyrazine, 2-ethyl-5-methyl-
23	à-Methylstyrene
25	1-Octen-3-ol
27	Undecane, 4-methyl-
31	Oxirane, ethyl-
33	Carbon disulfide
39	2-Undecanethiol, 2-methyl-
41	Camphene
42	Styrene
45	2,3-Butanedione
49	Octane
52	Butanal, 3-methyl-
54	2-Butanone, 3-hydroxy-
55	Ethanol, 2-butoxy-
57	Hexanal
58	p-Cresol
59	2,4-Heptadienal, (E,E)-
60	Octanal
61	2-Octanone
63	2-Nonenal, (E)-
64	2,3-Octanedione

Multidimensional scaling showed discrimination between Karoo and Grass fed lamb for raw meat, while for cooked meat and cooked fat the two feed types exhibited considerable overlap.

Acknowledgement

Marc Bouwer for Multidimensional Scaling.

Yours sincerely,

Dr Yvette Naudé