CHAPTER 1

INTRODUCTION AND MOTIVATION

1.1 PROJECT THEME

Physiology of the African buffalo (*Syncerus caffer*).

1.2 PROJECT TITLE

Factors affecting the long-chain fatty acids in the African buffalo (*Syncerus caffer*).

1.3 AIM

To quantify the fatty acid composition of *Longissimus dorsi,* subcutaneous, perirenal, omental and pericardial adipose tissue depots in the African buffalo (*Syncerus caffer).*

To evaluate differences in the fatty acids composition as influenced by area, gender and age within these depots.

1.4 MOTIVATION

This project was a continuation of work reported by Webb (1992 and 1994) and Casey, Van Niekerk and Spreeth, (1988) on factors affecting fatty acid profiles in ruminants.

Research has been done on the fatty acid composition of a number of ruminant species – cattle, sheep, goats and even the Asian water buffalo. Since differences were found between species as well as within species and between different breeds (Malau-Aduli, *et al.*, 1997; Huerta-Leidenz *et al.*, 1993; Perry *et al.*, 1998; Zembayashi and Nishimura, 1996; de Francis and Moran, 1991), the composition and factors that may influence the composition of long-chain fatty acids in the African buffalo, were researched.

Fatty acid profiles are extremely important taking into account, not only the physiological importance, but also its role in the nutritional value of meat and the influence of long-chain fatty acids on the health of the consumer.

Previous research (Webb, 1992, 1994, 1995) suggest that the amount of fat and the concentration of fatty acids in adipocytes are directly dependent on the live weight and maturity of ruminants, while the profile (molar %) of fatty acids deposited is determined primarily by the diet. The concentrations of fatty acids increase with increasing live weight and differ between breeds. It is accepted that the composition of fatty acids differs at different anatomical locations. However, the composition of fatty acids at different locations in buffalo and many other wildlife species has not yet been quantified.

African buffalo, ranging the African planes, has been researched in many different aspects – behaviour, diseases and ecology, but little, if any data is available on carcass and meat quality. The latter aspect is becoming more important particularly since more buffalo are being bred outside of National Parks due to disease control and breeding of disease-free animals. Many tourists consume the meat of buffalo and other animals culled yearly, in the restaurants of the parks.

Buffalo meat is an important commodity and the quality thereof is important since many tourists to the Kruger National Park and African game ranches consume it daily. The composition, colour and amount of subcutaneous fat contribute significantly towards the quality of buffalo meat. Significant seasonal and environmental effects are expected in terms of the composition of fats due to fluctuations in the quality of the grazing and grazing patterns of buffalo. The effects of gender and age on the composition of fats have also been studied extensively (Banskalieva, 1996; Christie, 1981; Cramer and Marcello, 1964, as quoted by Webb, 1992; Huerta-Leidenz *et al.*, 1996; Kurbanov, 1978; Malau-

Aduli *et al.*, 1997; Nürnberg *et al.*, 1998; Perry *et al.*, 1998; Vernon, 1981; Webb and Casey, 1995; Westerling and Hedrick, 1979; Wu and Savell, 1992; Zembayashi and Nishimura, 1996; Zembayashi *et al.*, 1995), but again research in wildlife species and especially the African buffalo, is scarce (Moran, 1992).

This research will contribute significantly towards the current knowledge on the composition of animal fats and its contribution to the human diet. Meat fats have been labelled "saturated" and "unsafe", but recent research showed that the saturation levels of meat fats vary between 50 and 55%. Evidently this research is of vital importance in terms of improving the knowledge base of the composition of meat and the related effects on the quality of the products as well as the perceptions of meat in general.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The quality of fat and the composition of long-chain fatty acids of different fat depots are important aspects in the animal industry, especially due to the perception of 'unhealthy' highly saturated carcass fat. The quality of food produced by the red meat industry is affected by the carcass fat, which is influenced by the interactions between external and inherent factors.

Webb (1992, 1994 and 1995) suggested that the amount of fat and the concentration of fatty acids in adipocytes are dependent on the live weight and maturity of ruminants and that the profile (molar %) of fatty acids deposited is determined primarily by the diet. The concentrations of fatty acids increase with increasing live weight and differ between breeds (Perry *et al.*, 1998; Webb *et al.*, 1994). It is accepted that the composition of fatty acids differs at different anatomical locations.

2.2 LIPIDS

In the *Dictionary of Endocrinology and Related Biomedical Sciences* (Martin, 1995) lipids are defined as "fats, phospholipids, glycolipids, steroids, lipoproteins, waxes, terpenes and other organic compounds that are soluble in lipid solvents and insoluble in water". It is a heterogeneous class of natural organic compounds, composed of carbon, hydrogen and oxygen with some phospholipids, containing phosphorus and nitrogen. Living cells contain both simple fats and other fatlike materials. The latter, which are more complex substances, include lipids and sterols. In animal tissue, the most important lipids to be considered are fats, lipids found in biological membranes (phospholipids, glycolipids,

lipoproteins) and steroids. On a functional basis, lipids can be subdivided into two main groups (Mathews and van Holde, 1990; Egan, 1976):

1. Lipids primarily concerned in the structural organisation or specialised functional roles in body cells and tissue.
2. Lipids representing a source of energy deposited in largest quantities in specialised cells of adipose tissue.

In digestion, fats are hydrolysed or decomposed into their component glycerine and fatty acids. These are then synthesised to neutral fats, cholesterol compounds and phospholipids - fats, chemically united with phosphorus, that circulate in the blood. Fat may be synthesised into body structures or stored in the tissues for withdrawal when needed. Like glucose, it is then catabolised to carbon substances that are broken down into carbon dioxide and water.

2.2.1 *Structural and specialised lipids*

Included in this group of lipids, are the phospholipids, glycolipids, lipoptoteins and steroids. These lipids are not influenced to a great extend by energy availability and are not only found in cellular structures, but also in plasma, forming complexes with plasma proteins (lipoproteins).

1. ***Phospholipids***: Included in the phospholipids is lecithins, sphingomeyelins and cephalins. Phospholipids are important in the structure of all membranes. They are diglycerides that are derivatives of fatty acids, glycerol, phosphoric acid and nitrogen-containing bases, such as choline, serine and ethanolamine. The most common fatty acids are palmitic (C16:0), stearic (C18:0) and oleic acids (C18:1).
2. ***Glycolipids***: Glycolipids are lipids containing covalently linked carbohydrate groups. The glycolipids do not contain phosphorus but are derived from carbohydrates, fatty acids and nitrogen compounds.

3. ***Lipoproteins***: Lipoproteins can be designated to any compound composed of protein and lipid moieties. The best-known lipoproteins are those transporting triacylglycerols and cholesterol. Different kinds of lipoproteins are found namely:
   i. Chylomicrons: Transport digested fats (mainly triacylglycerols) into the circulation to be carried to the liver and other organs.
   ii. VLDL (very-low-density-lipoprotein): Carry fats, mostly triacylglycerols, throughout the body and carry only a small component of the cholesterol to the tissues.
   iii. LDL (low-density-lipoprotein): The primary molecular complexes that carry cholesterol in the blood to the organs and cells and contain the highest percentage of cholesterol.
   iv. HDL (high-density-lipoprotein): Pick up already used or unused cholesterol and cholesterol-esters, taking them back to the liver as part of the recycling process.
4. ***Sterols:*** Sterols are composed of complicated molecules, each containing 20 or more carbon atoms in an interlocking or fused cyclohexane ring structure with a hydroxyl group at one end of the molecule. Cholesterol, the precursor of bile acids, and steroid hormones are typical examples of natural steroids. Cholesterol assists in the health of the brain, nervous system, liver, blood and skin.

2.2.2 *Lipids in adipose tissue*

2.2.2.1 Fats

Fats, called triacylglycerols are the most abundant lipids and are composed of three fatty acid moieties linked by ester bonds to glycerol carbon atoms. Triacylglycerols are distributed throughout the body and provide a concentrated, efficient source of energy for the cells because of the hydrocarbon chains. They are predominantly aggregated in adipose tissue in the mesenteric fat around the intestine and kidney, in subcutaneous fat

layers and to some extend between fibres of skeletal muscle. Animals accumulate fat when in positive energy balance, and metabolises fat for energy, when in negative energy balance.

2.2.2.2 Fatty acids

Fatty acids, the simplest lipids, but also the most important lipid fraction, are constituents of more complex lipids. They consist of a hydrophilic carboxylate group attached to one end of a hydrocarbon chain. Fatty acids differ in chain length (short- (C1–C8); medium- (C9-C11); long-chain (C12-C26)), and degree of saturation. Table 2-1 summarises the classification of fatty acids (Christie, 1982a).

Saturated fatty acids are filled to capacity with hydrogen atoms, while unsaturated fatty acids contain one or more double bonds within their structure and are therefore subdivided into monounsaturated and polyunsaturated fatty acids. In most of the naturally occurring fatty acids, the orientation about double bonds is *cis* rather than *trans*, resulting in a bend in the hydrocarbon chain.

Due to the highly reduced methylene level of fatty acid carbons, a larger amount of metabolic energy is released on metabolic oxidation, than for carbohydrates and proteins. This is why fatty acid serves as the major source of energy for animal tissue. The brain is the only tissue that is unable to use fatty acids for energy, but can adjust during periods of starvation to use lipid-related compounds for energy.

Odd-numbered and branched-chain fatty acids found in animal tissue can either originate from the diet, or are synthesised *de novo* by rumen microorganisms. C_{13}, C_{14}, C_{15}, C_{16} and C_{17} branched chain fatty acids together with straight-chain fatty acids containing odd-number C-atoms, which are absent from the diet, are synthesised from volatile branched-

and straight chain fatty acids produced in the rumen and subsequently absorbed by the animal and incorporated into the tissue lipids (Noble, 1981).

Table 2-1 Classification of fatty acids after Christie (1982a).

Chain Length	Systematic Name	Trivial Name	Abbreviation
Short-chain	ethanoic	acetic	C2:0
Saturated fatty acids	propanoic	propionic	C3:0
$[CH_3(CH_2)_nCOOH]$	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	-	C11:0
Long chain	dodecanoic	lauric	C12:0
Saturated fatty acids	tridecanoic	-	C13:0
$[CH_3(CH_2)_nCOOH]$	tetradecanoic	myristic	C14:0
	pentadecanoic	-	C15:0
	hexadecanoic	palmitic	C16:0
	heptadecanoic	margaric	C17:0
	octadecanoic	stearic	C18:0
	nonadecanoic	-	C19:0
	eicosanoic	arachidic	C20:0
	heneicosanoic	-	C21:0
	docosanoic	behenic	C22:0
	tetracosanoic	lignoceric	C24:0
Monoenoic fatty acids	cis-9-dodeecenoic	lauroleic	C12:0 (n-3)
$[CH_3(CH_2)_mCH=CH(CH_2)_nCOOH]$	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[2]
	cis-11-octadecenoic	cis-vaccenic	C18:1 (n-7)
	Trans-11-octadecenoic	trans-vaccenic	C18:1[2]
	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)
Non-conjugated polyunsaturated fatty acids	9,12-octadecadienoic	linoleic	C18:2 (n-6)
$[(CH=CHCH_2)_m(CH_2)_sCH_2)_nCOOH]$	6,9,12-octadecatrienoic	δ-linolenic	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-docosapentaenoic	-	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)

[1] The (n-x) nomenclature is only used with fatty acids containing cis-double bonds

[2] The (n-x) nomenclature is only used with fatty acids containing cis-double bonds, and these fatty acids cannot be synthesised by the organism

Essential fatty acids are polyunsaturated fatty acids, linoleic, linolenic and arachidonic acids that cannot be synthesised in the body and need to be provided in the diet, either preformed or as suitable precursors.

2.3 LIPID METABOLISM

Lipid metabolism was described previously (Christie, 1981a,b; Webb, 1992; Webb, 1994) and will not be discussed in detail.

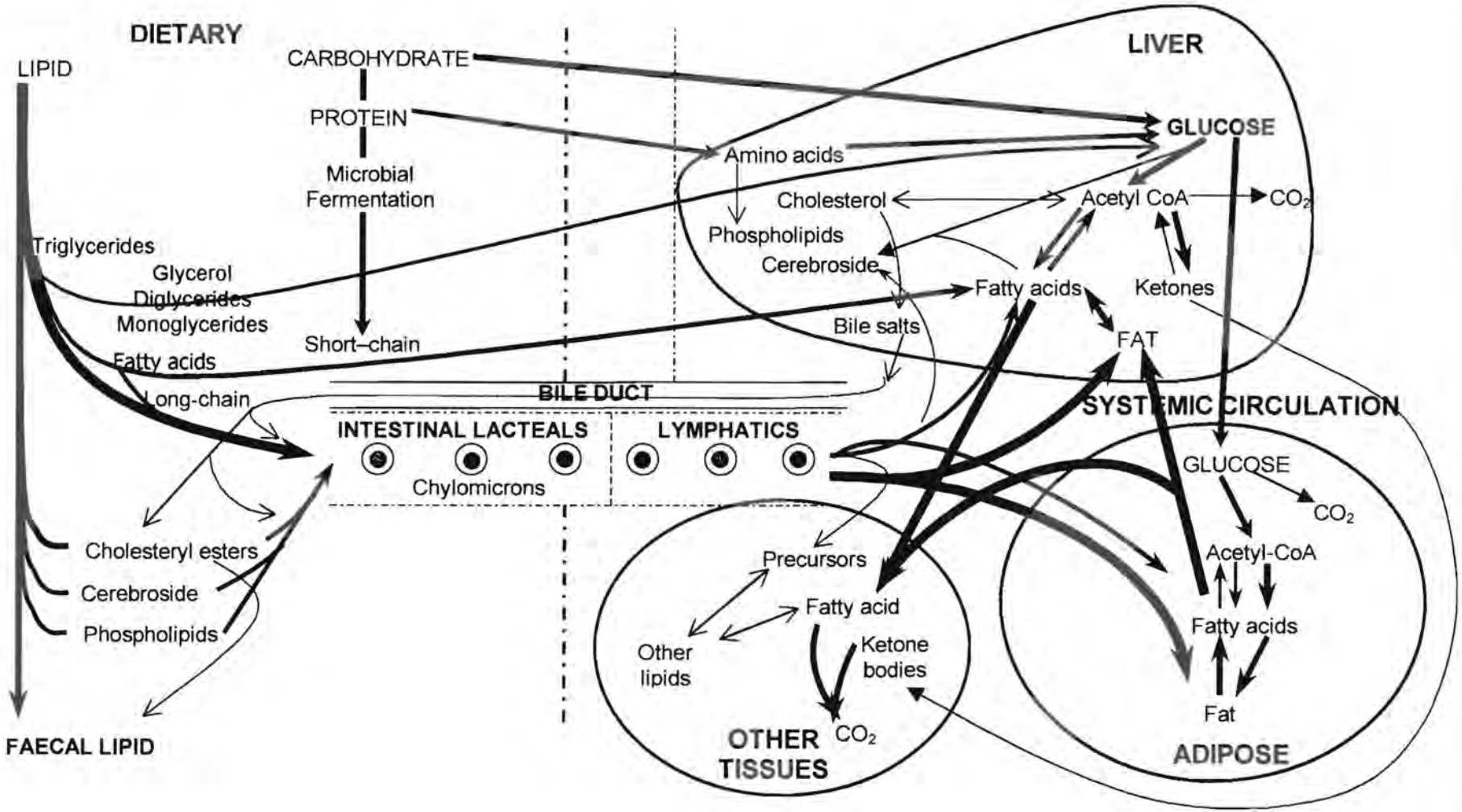
There is a close association between fat synthesis and active carbohydrate metabolism. Although changes in the level and composition of the diet influence the rate of fatty acid synthesis, it is directly, via the anabolic pathway, or indirectly by means of the throughput of substrates under hormonal control (Vernon, 1981, Mathews and van Holde, 1990; Egan, 1976, Nürnberg *et al.*, 1998). Free fatty acids can function as a readily available source of energy because of the extremely fast turnover rate combined with a rapid response to physiological metabolic and nutritional changes.

Fatty acids incorporated into the adipose tissue of ruminants are derived from two primary sources i.e. mobilisation of stored fat and the diet. The net increase in the quantity of triacylglycerols stored in adipose tissue is the result of *de novo* fatty acid synthesis, the uptake of exogenous fatty acids and lipolysis.

2.3.1 *Exogenous fatty acids*

Many of the dietary long-chain saturated fatty acids pass through the rumen unchanged and are subsequently absorbed and incorporated into animal tissues. Dietary short-chain fatty acids (C_{12}) are elongated before deposition into the tissue.

Figure 2-1 General pathways of lipid metabolism (Phyllis, 1971)



2.3.2 *De novo fatty acid synthesis*

2.3.2.1 Ruminal fatty acids

Ruminants differ from monogastric animals because of anaerobic and facultative anaerobic microbial fermentation occurring in the reticulo-rumen during digestion.

In the fed ruminant, metabolism is dominated by microbial fermentation of dietary carbohydrate and other organic constituents to short-chain fatty acids with acetic acid the most predominant of the three main volatile fatty acids (acetic, propionic and butyric acids) produced. It is absorbed and metabolised further in the ruminant body. The rate of acetate incorporation into adipose tissue differs between species (Ishida *et al.*, 1989), as well as within species and between breeds (Sinnett-Smith and Woolliams, 1988). Ishida *et al.* (1989) found that the incorporation of acetate into adipose tissue was lower in wild ruminants (deer) than in domesticated ruminants (sheep and goats).

Dietary unsaturated fatty acids, particularly linoleic and linolenic acids, are hydrogenated or partially hydrogenated by rumen microorganisms before absorption. According to Christie (1981a,b) the C_{18} polyunsaturated fatty acids in the diet are converted to stearic acid, together with smaller amounts of potential and geometrical isomers of other C_{18} components. Fat depots are especially rich in stearic acid due to microbial fermentation in the rumen.

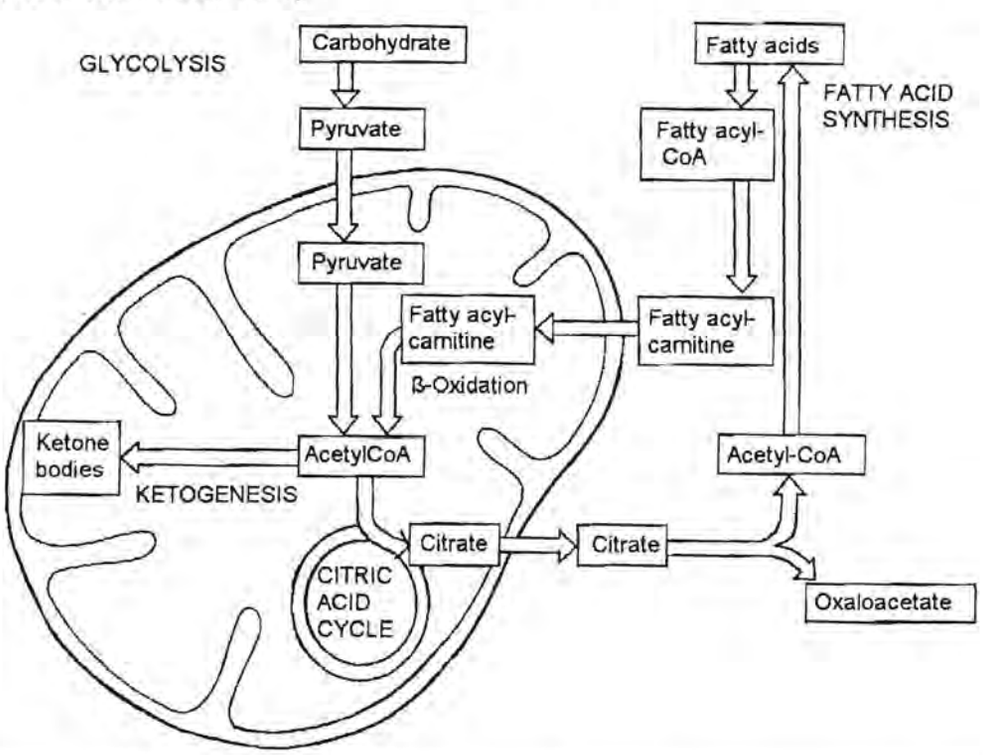
Fatty acids are normally synthesised by rumen microbes from glucose, but some fatty acids are synthesised *de novo* from short-chain fatty acids and are released and taken up by the animal, as the microorganisms themselves are digested (Noble, 1981). These fatty acids of both bacterial and protozoal lipids, contain high proportions of C_{13}, C_{14}, C_{15}, C_{16} and C_{17} branched chain fatty acids together with straight-chain fatty acids containing odd-

number C-atoms in addition to palmitic, stearic and C_{18} monoenoic fatty acids. These odd and branched-chain fatty acids, absent from the diet, are absorbed by the animal and are incorporated into the tissue lipids. The biosynthetic precursors are volatile branched- and straight chain fatty acids produced in the rumen (Noble, 1981).

2.3.2.2 In the tissue

Although additional fat is stored in periods of excess energy and metabolised whenever needed, the process of breakdown and resynthesis, or replacement, continues at all times in all body lipids, though at different turnover rates (dynamic state of fat). Triacylglycerols of adipose tissue and liver constantly release fatty acids from the glycerol esters for subsequent resynthesis of fats. Glycerol moieties released in the hydrolysis of fat, cannot be re-utilised for esterification of fatty acids, but are catabolised via the triose phosphates and glycolytic pathway in other tissues. Lipid metabolism in summarised is and Figure 2-2.

Figure 2-2 Fatty acid biosynthesis and breakdown in the cells of animal tissue (Mathews and van Holde, 1990).



In the animal, fatty acids are synthesised *de novo* from short-chain precursors. Most of the short-chain fatty acids produced by rumen fermentation, are oxidised in peripheral tissue and the surplus becomes the most important source of acetyl-CoA for synthesis *de novo* of long-chain fatty acids (Bell, 1981). In the liver, intestinal mucosa and adipose tissues, fatty acids are synthesised from acetyl-CoA. Propionate that escape hepatic metabolism is involved in the synthesis of long-chain fatty acids with an odd number of carbon atoms and abnormal saturated branched-chain fatty acids in adipose tissue triacylglycerols (Bell, 1981).

Most of the fatty acids synthesised *de novo* are esterified and incorporated into triacylglycerol, with the remainder incorporated into diacylglycerols. The rate of esterification is influenced by age, gender, breed, lactation and feeding of a low roughage diet. High-fat diets inhibit the contribution of fatty acids synthesised *de novo* to lipid deposition.

Absorbed fatty acids are modified by α- or β-oxidation, desaturation or chain elongation. All these fatty acids are susceptible to the same extent to dietary modification, resulting in the characteristic comparatively high concentrations of odd-chain and branched-chain fatty acids, of positional and configuration isomers of mono- and di-unsaturated fatty acids and at the same time comparatively low concentrations of polyunsaturated fatty acids. Other dietary components (phytol acid) are oxidised (Christie, 1981b).

Long-chain fatty acids are desaturated to their 9,10 cis-monounsaturated derivates in adipose tissue. Fatty acids synthesised *de novo* are readily desaturated and preferentially to fatty acids from exogenous origin. Some of the unchanged linoleic acid may be converted to arachidonic acid and other longer-chain fatty acids. Any of the long-chain fatty acids may be partially oxidised into C17:1 and C16:1 fatty acids.

Interconversion between saturated fatty acids occurs by means of the successive addition or removal of 2-C units. Fatty acids with one double bond are readily formed by hydrogenation of the saturated fatty acid of corresponding chain length. All fatty acids cannot be synthesised by means of interconversion between fatty acids because of a limit in the amount and positions of unsaturated double bonds to be created. These polyunsaturated fatty acids (linoleic, linolenic and arachidonic acids) are essential fatty acids and need to be supplied in the diet. The tissues utilise essential fatty acids highly efficient (Noble, 1981).

2.3.3 *Lipolysis*

For energy balance, there is a fine balance between energy intake and energy expenditure and for adipose deposition. The rate of fat metabolism and control of the balance of fat accumulation or breakdown of adipose tissue depend on the control of lipolysis. It was found that subcutaneous fat is preferentially used for lipolysis, compared to abdominal fat (Vernon, 1981).

With mobilisation of adipose tissue, triacylglycerols are hydrolytically cleaved to free fatty acids (FFA) and glycerol. The fatty acids are re-esterified and metabolised within the tissue itself, or mobilised and transported to other tissues for metabolism, where the esterified fatty acids may undergo further anabolic reactions increasing chain length, desaturation reactions, or catabolic pathways. Glycerol passes into the blood to be metabolised in other tissues, particularly the liver. In the liver the state of the carbohydrates determines whether it is metabolised via α-glycerol phosphate or dihydroxy-acetone phosphate in either the glycolytic or the glucogenic directions. The relative rate of release of individual fatty acids is not necessarily related to their relative proportions in adipose tissue triacylglycerols. It was found though, that there is a

preferential release of palmitic acid as its entry rates, relative to those of stearic and oleic acids are greater (Vernon, 1981; Adrouni and Khachadurian, 1968).

Fatty acids are catabolised primarily by means of β-oxidation. The extent depends upon the nutritional and physiological state of the animal (Egan, 1976). With low energy intake, stored fatty acids are mobilised from the triacylglycerol depots to the liver and other tissues for oxidation. Fatty acid degradation results in acetyl-CoA production. These are channelled into the tricarboxylic acid cycle (TCA) for oxidation, with a net production of energy. Even chain fatty acids are completely broken down to acetyl-CoA without production of other TCA-cycle intermediates. Without adequate supply of TCA-cycle intermediates and if the influx of acetyl-CoA into the TCA-cycle is exceeded, aceto-acetate is formed, leading to the production of ketone bodies in a process known as ketogenesis (Figure 2-2).

2.4 FACTORS INFLUENCING LIPID COMPOSITION

2.4.1 *General*

The lipid composition of animal tissue is dependent on the fatty acid content. Adipose tissue is composed of triacylglycerols, the major component by far accompanied by small amounts of mono- and diglycerides, cholesterol, cholesteryl esters, unesterified fatty acids and phospholipids. Adipose tissue is the major site for fatty acid synthesis *de novo* and for desaturation of stearic acid to oleic acid.

Age, lactation and diet affect the rate of acetate oxidation, with the rate of acetate oxidation doubling during lactation (Vernon, 1981). Skeletal and muscular development as well as foetal growth and milk production usually precedents over fat accumulation. The proportions of fatty acids desaturated decrease with age. During the growth phase, fat accretion is related to both hyperplasia and hypertrophy in the adipocytes and to

hypertrophy only during the fattening phase. Lipogenesis in the tissue of the non-lactating growing ruminant is largely confined to adipose tissue.

2.4.2 *Anatomical Location*

Fat cells accumulate and grow in the extrafascicular spaces in the near proximity to the circulatory system. Adipose tissue of ruminants consists almost entirely of triacylglycerols and small amounts of unesterified fatty acids and other lipids. Skeletal muscle is infiltrated with adipocytes (intracellular free lipid droplets) largely triacylglycerols with appreciable amounts of phospholipids that are the constituents of the membranous structure. The amount of adipose tissue infiltration ("marbling") of the muscle tissue in domestic ruminants increases with age of the animal.

With marbling deposition, more triacylglycerols are deposited and begin to predominate over polar lipids, generally higher in poly-unsaturated fatty acids (Webb *et al.*, 1998; Xie *et al.*, 1996) resulting in lower proportions of C18:2 and C18:1 and higher proportions of C14:0, C14:1, C16:0 and C16:1 in *M. Longissimus dorsi* of cattle (Xie *et al.*, 1996).

In fat depots, an increase in fatness is associated with a decrease in saturation, related to a decrease in C16:0 and an increase in C17:1 (Perry *et al.*, 1998) and in C18:1 (Xie *et al.*, 1996).

The total saturated and unsaturated fatty acids present in subcutaneous and intramuscular fat may not differ significantly, but subcutaneous fat contained more palmitic and oleic and less linoleic, 11-eicosanoic and arachidonic acids than intramuscular fat (Westerling and Hedrick, 1979).

The intermuscular fat of animals that underwent compensatory growth is reportedly more saturated than either the subcutaneous or intramuscular fat and may be due to decreased intramuscular fat deposition and increased peripheral fat deposition, resulting in leaner meat (Hornick *et al.*, 1998).

In *M.Longissimus dorsi* (LD) of cattle, the proportions of C17:0, C17:1 and C18:3 were absent or too low for quantification. Changes in proportions of C18:0, C18:2 and C18:1 influenced the level of saturation of muscle (Rule *et al.*, 1997). Huang and Lin (1993) found that LD muscle contained the highest proportions of C18:1, followed by C16:0, C18:0 and C18:3. C18:0 of LD depot fat was higher than in muscle and C18:2 lower than in muscle.

In lambs, a maintenance diet reduced the numbers of subcutaneous and intermuscular adipocytes, but not those of perirenal fat, but the size of all three decreased due to reduced lipoprotein lipase activity of adipose tissue. The muscle tissue of animals fed on a maintenance diet contained more PUFA, especially C18:2 and C20:4 and less SFA, especially C14:0, C16:0 and C18:3 than animals on a diet containing more concentrates (Eichhorn *et al.*, 1986).

Desaturation occurs more in subcutaneous than in abdominal adipose tissue. This may be due to more fatty acids being synthesised *de novo* subcutaneously than abdominally, while unusual fatty acids from dietary origin tend to accumulate in abdominal (perirenal and omental) fat. Exogenously supplied polyunsaturated fatty acids are preferentially deposited in the intestinal tissue of sheep (Duncan and Garton, 1967 as quoted by Webb, 1992) and long-chain fatty acids absorbed from the intestine primarily influences the composition of the triacylglycerols of internal adipose tissues. Lipid deposition in omental

adipose tissue increased nearer to the rumen and abomasum, than further away (Bas *et al.*, 1992).

The internal fat depots of ruminants are more saturated than subcutaneous fat depots and have high levels of saturated components with appreciable quantities of odd-chain and particularly *trans*-unsaturated components (Banskalieva, 1996, Webb *et al.*, 1998).

Perirenal fat was reported to have, irrespective of diet, mass or gender, higher proportions of stearic acid, and lower concentrations of oleic acid and total fatty acids than subcutaneous adipose tissue (Kemp *et al.*, 1981 and Tichenor *et al.*, 1970 as quoted by Webb, 1992). The subcutaneous fat and internal adipose tissues of neonatal lambs were similar in composition (Christie, 1981b).

Casey and van Niekerk (1985) found that SCF was more unsaturated than kidney fat, with C14:0, C17:0 and C18:0 lower and C14:1, C16:1 and C18:1 higher than in perirenal fat. In SCF: C18:1>C16:0>C18:0 and PRF: C18:0>C16:0>C18:1. Kurbanov (1978) found palmitoleic acid to be lower in internal fat and high in tail fat.

2.4.3 *Age*

Development of adipose tissue involves both hyperplasia and hypertrophy of the adipocytes. For about the first month after birth, lipids are deposited into adipocytes. Thereafter, skeletal and muscle growth predominates and adipose tissue growth is retarded, and occurs due to hyperplasia, with subcutaneous and intermuscular fat developing faster than perirenal adipose tissue. During the fattening stage of development, adipose tissue depots develop in the order of abdominal, intermuscular, subcutaneous and finally intramuscular fat.

First, perirenal fat is deposited, followed by subcutaneous fat and lastly, inter and intramuscular fat. The development of perirenal adipose tissue is primarily due to hypertrophy of adipocytes. The greater rate of development of subcutaneous fat and intermuscular adipose tissue as compared to perirenal adipose tissue can be attributed to hyperplasia (Vernon, 1981).

Perirenal fat tissue matures before subcutaneous adipose tissue. During the growth phase, the amount of lipid deposition increase and at the end, the metabolic capacities of subcutaneous fat and perirenal fat are very similar. During the fattening and finishing phase, lipid deposition increases with greater increase in the deposition of perirenal than subcutaneous fat. Fattening is a switch of nutrient utilisation from muscle and skeletal growth to fat deposition. Stearic acid increase with age due to increased amounts of stearic acid absorbed with rumen development.

The effect of age on fatty acid composition is related to body fatness (Huerta-Leidenz *et al.*, 1996) and the percentage of saturated fat in lamb muscle increases with age (Nürnberg *et al.*, 1998; Huerta-Leidenz *et al.*, 1996). Age affected the saturation ratio, but not the fatty acid composition (Perry *et al.*, 1998). The degree of unsaturation of the subcutaneous fats increased with age of the animal mainly due to the increased concentration of C16:1. C18:0 decreased and C16:0 and C18:1 did not vary significantly (Christie, 1981b).

Malau-Aduli *et al.* (1997) found that more and more saturated fatty acids are converted into unsaturated fatty acids with age. Fatty acids deposited in adipose tissue during the first year of life are progressively being diluted by more unsaturated fatty acids. C16:0 (Palmitate) is converted into C16:1 (palmitoleate) and C18:0 (stearate) into C18:1

(oleate). This resulted in a decrease in saturation levels with increase in age. This is if weight loss, due to the mobilization of fat depots, is not taken into account.

Previous research in cattle indicated that mature female animals contained significantly lower proportions of C14:0 and C16:0 and higher proportions of C18:1 than yearlings (Malau-Aduli *et al.*, 1997).

Differences within a specific age group, especially sheep under 1 year of age, may occur as it was found that the composition of C14:0, C16:0 decreased and C18:0 and C18:1 increased post weaning (Banskalieva, 1996) and thereafter. With age, the total unsaturation of subcutaneous fat increases (Banskalieva, 1996, Webb and Casey, 1995, Westerling and Hedrick, 1979; Zembayashi and Nishimura, 1996). In very young animals, with underdeveloped rumen, the diet is reflected in the composition of the fat depots and the composition of adult mature animals are more influenced by diet than by physiological condition (Banskalieva, 1996).

Weaning usually causes a decrease in the rate of fat deposition and even a net loss of body fat, mainly due to the decreased energy intake and stress associated with weaning. The decreased plasma glucose levels, together with limited acetate supply form the incomplete developed ruminant digestion, limit fatty acid synthesis.

2.4.4 *Gender*

Gender influences the relative amount of adipose tissue distribution among different body sites and development patterns (Vernon, 1981). Differences in fatty acid composition are suspected to be mainly due to carcass fatness. At equal weights, males were leaner than females and contain more saturated fat (Nürnberg *et al.*, 1998). It was reported, though that females contained higher proportions of fatty acids with 16 or more carbons and

lower proportions of fatty acids with 16 or less carbons than males, regardless of the degree of saturation (Cramer and Marcello, 1964, as quoted by Webb, 1992).

Wu and Savell (1992) found higher proportions of C14:0, C16:0 and C18:2 and lower C18:0 in male than in female animals. Westerling and Hedrick (1979) found higher proportions of C18:2 and C20:4 in steers than in heifers, but no differences in the total saturated fatty acid content.

Zembayashi *et al.* (1995) found heifers to contain higher proportions of C18:1, C15:0 and C18:3 and total monounsaturated fatty acids and lower proportions of C14:0, C14:1, C16:0 and C16:1 in subcutaneous and intramuscular neutral lipids than steers. Differences were also found in the intramuscular phospholipid content, especially C16:0, C20:1 and C20:5.

The influence of gender on carcass tissue distribution in buffalo is reported to be similar to those found in cattle (Moran, 1992).

2.4.5 *Physiological State*

The reproductive state of females influences the adipose tissue composition and subsequent fatty acid composition of depot fat. During most of pregnancy, the animal is in a positive energy balance and accumulates lipid reserves. Late pregnancy may result in a negative energy balance and mobilisation of adipose tissue lipid occurs. During early lactation, the female is in a negative energy balance. Fatty acids are extensively mobilised from adipose tissue depots, comprising mainly of C18 components (Christie, 1981b) with the mammary gland taking up only plasma triacylglycerols, mainly C16:0, C18:0 and C18:1, and insignificant amounts of unesterified fatty acids (Moore and Christie, 1981).

During later lactation, the energy balance returns to a positive balance. The negative energy balance is not due to reduced food intake, but by exceptional demands of the growing foetus or milk production. Visceral fat also decreased during lactation (Vernon, 1981).

2.4.6 *Dietary Influences*

Dietary fats do not pass unchanged through the digestive system of ruminants. Depending on the dietary composition, polyunsaturated fats are usually biohydrogenated by rumen microorganisms into more saturated fatty acids (Nürnberg *et al.*, 1998).

Dietary long-chain saturated fatty acids pass through the rumen unchanged and is absorbed and incorporated into animal tissues. Fatty acids synthesised *de novo* by rumen microorganisms are absorbed by the animal after digestion of the microorganisms (Christie, 1981a). Under some circumstances, appreciable amounts of a whole range of branched-chain fatty acids and greater amounts of normal odd-chain fatty acids than usual, can be accumulated in the adipose tissue of ruminants (Christie, 1981a).

A typical ruminant diet contains small amounts of lipid (< 5%) with forages containing largely phospholipids and glycolipids. The fatty acid composition is dominated by high proportions of unsaturated fatty acids especially C18:2 (linoleic acid) and C18:3 (linolenic acid). Small proportions of C18:1 (oleic acid) are also present (Noble, 1981).

Concentrate diets are characterised by the presence of triacylglycerols, with high proportions of linoleic acid. The addition of concentrates to the diet of ruminants therefore increases the intake of unesterified fatty acids, particularly triacylglycerols, resulting in softer, more unsaturated fat in the carcass, mainly as a result of higher concentrations of C18:1 and lower concentrations of C16:0 and C18:0 (Banskalieva, 1996; Rumsey *et al.*,

1972; Wood *et al.*, 1991). Casey and van Niekerk (1985) also found that an increase in energy level of diet increased the levels of C18:1 in perirenal and subcutaneous fat, while C18:0 decreased in subcutaneous fat. Westerling and Hedrick (1979) found both intramuscular and subcutaneous fat of grass-fed animals, to contain more saturated fatty acids (palmitic and stearic) and less unsaturated fatty acids (primarily oleic) than did fat from grain-fed animals.

Most changes due to different diets, appear to be primarily due to altered rumen fermentation as under certain circumstances, different populations of bacteria and protozoa arise within the rumen with different capacities for biohydrogenation of dietary fatty acids (Christie, 1981a,b). Differences in C18:2 and C18:3 can be expected due to diet since both are essential fatty acids and cannot be synthesised by the animal (Malau-Aduli *et al.*, 1997).

The increase in unsaturation levels of reserve fat is due to exogenous fatty acid changes occurring in the rumen (Banskalieva, 1996). C16:0 is synthesised and elongated to C18:0. C18:0 is then desaturated to C18:1, the major end point of *de novo* fatty acid synthesis (Rule *et al.*, 1997).

Large proportions of concentrates in the diet increase fat deposition in adipose tissue, while restricted feed intake results in a reduced growth rate, reducing the rate of fatty acid synthesis and more so in subcutaneous fat than abdominal fat (Adrouni and Khachadurian, 1968).

The proportions of C16:0, C18:1, C18:2 and C18:3 are significantly influenced by environment (Perry *et al.*, 1998).

2.4.7 *Breed*

Previous research indicated significant differences within species, between breeds (Malau-Aduli, *et al.*, 1997) as well as between species. Breed differences seem to become more distinct with age, since less difference can be detected in younger animals than in mature animals (Malau-Aduli, *et al.*, 1997). Differences between breeds depend upon carcass fat (Nürnberg, 1998). Differences are mainly due to maturity types with later developing breeds having more internal fat, and less subcutaneous at the same body weight. Later maturing breeds have more saturated adipose tissue than earlier maturing breeds (Malau-Aduli, *et al.*, 1997; Huerta-Leidenz *et al.*, 1993; Perry *et al.*, 1998; Zembayashi and Nishimura, 1996).

Perry *et al.* (1998) suggested that among-breed differences in fatty acid composition at the same age, are associated with variation in stage of maturity at slaughter. This is reflected by differences in fat percentage, because a decrease in saturation is either due to a decrease in C16:0 and an increase in C17:1 or a decrease in C18:0 and an increase in C16:1. Different fatty acids (C14:0, C16:0 and C17:1) are regarded as distinguishing between the different breeds (Perry *et al.*, 1998; Webb *et al.*, 1994).

Wu and Savell (1992) found odd-numbered carbons (C15:0, C17:0 and C17:1) to be more abundant in Karakul sheep when compared to goat (Angora and Spanish) and other sheep breeds (Rambouillet, Barbados Blackbelly).

The fat of African ruminants is reported to contain higher proportions of polyunsaturated fatty acids than reported in domestic and wild animals from more temperate regions. This may be due to diet or differences in rumen microflora, particularly with regard to biohydrogenating efficiency (Crawford *et al.*, 1970 as quoted by Christie, 1981a). The

nature and biological composition of the rumen micro flora may also vary between herds or even animals in the same herd (Christie, 1981a).

River buffalo deposited less fat intramuscularly than cattle and the buffalo meat contained less fat and more protein than cattle of similar total carcass fat content (de Francis and Moran, 1991).

2.5 THE AFRICAN BUFFALO (*SYNCERUS CAFFER*)

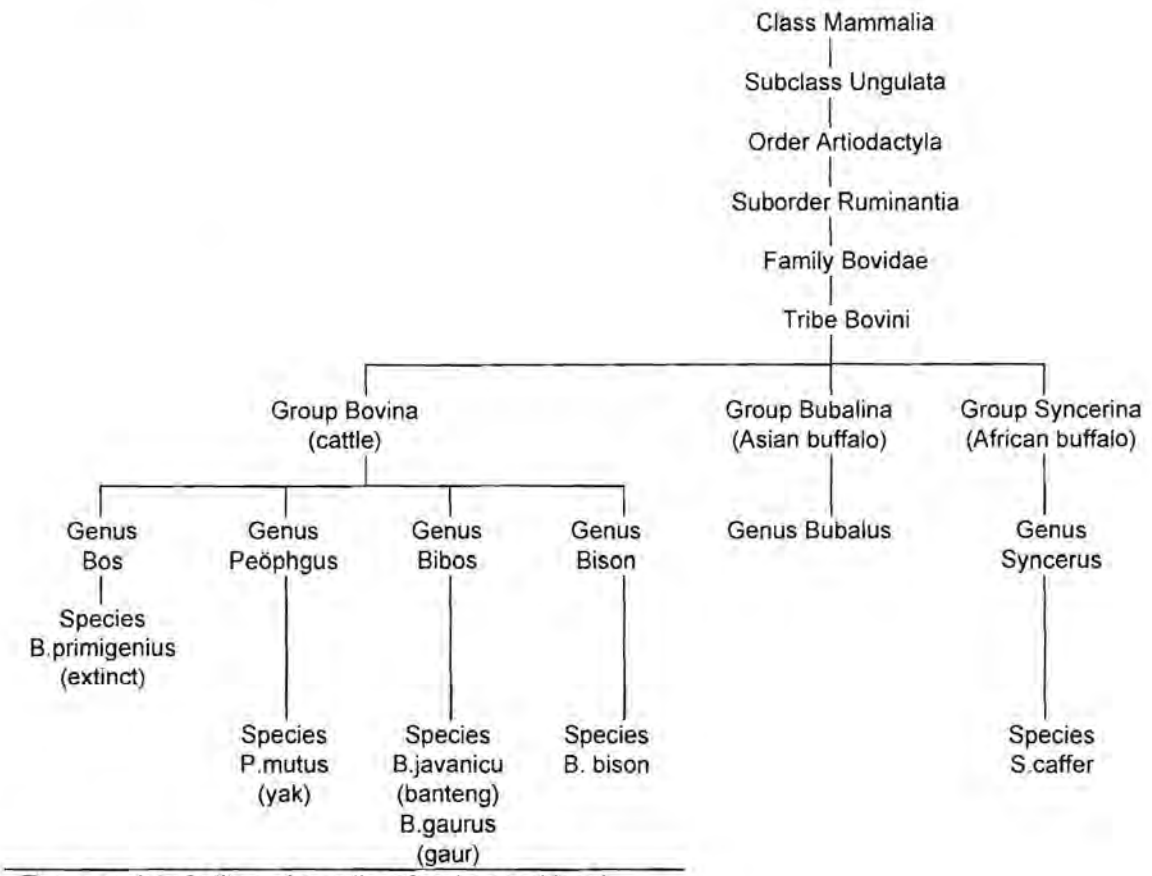
2.5.1 *General*

The African buffalo belong to the family Bovidae, sub-family Bovini of the sub-order Ruminantia. There are two groups of buffalo, the African buffalo, *Syncerus caffer* (Sparrman), and *Bubalis*, the Asian buffalo (Figure 2-3). The water buffalo, *Bos bubalus bubalis*, was studied to a great extend throughout the years as, especially in Asian countries, it was not only a source of animal traction, but also a source of milk, butter fat and meat (Hill, 1988; Mahadevan, 1992; Tulloh and Holmes, 1992). Behaviour and ecology of the African buffalo was studied, but little is known about meat quality (Prins, 1996, Grobler, 1996).

2.5.2 *Diet of the African Buffalo*

African buffalo are gregarious, large herbivores and are classified as bulk and roughage grazers (Prins, 1996). Studies on buffalo have demonstrated selective feeding (grass and other plant species) with a definite dependence on surface water (Sinclair, 1977; Prins, 1996). Little is known about the diet composition of the African buffalo found in the Kruger National Park (KNP).

Figure 2-3 The position of the African buffalo in the classification of the Bovini (Mahadevan, 1992).



The energy and nutrients required by buffalo to live, grow or reproduce need to be met by the ingested food. The most critical requirements are energy and protein. Food intake of buffalo in terms of digestible protein and metabolisable energy depend on the crude protein concentration of the food. Grazing buffalo need to satisfy their needs for energy and protein simultaneously by an optimal balance between the requirements for energy and for protein. Seasonal variation exists in the ability of buffalo to satisfy their protein and energy needs (Prins, 1996). It was found that buffalo ingest a balanced diet and not a diet maximal in protein or energy content. This may result in the use of body reserves of lactating animals during periods of declining food quality, in order to continue production (Prins, 1996).

Grasses comprise the greatest part of the diet. Buffalo were found to switch to browse species more during the dry seasons and especially in drought years (Prins, 1996; Stark, 1986). During the rainy seasons, the quality of the food consumed is high, but during the post-rainy season the quality decline and even falls below maintenance requirements for some animals. On average, though, the food quality is still high enough for milk production or for growth. During the late dry season, when buffalo were not able to select for very high quality food any more, and the food quality is still above maintenance requirements, milk production or growth was hardly possible and lactating females had to use body reserves to continue production.

The quality of the buffalo diet varies with season. Buffalo consume a combination of species parts to ingest a balanced diet with regard to their requirements for protein and energy during all seasons (Prins and Beekman, 1989).

Buffalo do not use available habitat types in proportions of occurrence and show seasonal variation in habitat selection. The presence of acceptable forage, the cover available to predators, the proximity of water and the mobility of the herd are involved in the selection of habitat. During the summer, when there are young calves in the herd, buffalo prefer areas with a lack of cover for predators e.g. mixed tree savannah are selected above sandveld. Grass communities are generally not selected (Funston *et al.*, 1994). During the winter, the herds ranges more widely, as well as in the riverine habitat types, because of the die back of the vegetation, providing less cover for predators. During the pre-summer, buffalo prefer the sandveld woodlands and mixed tree savannah where they intensely utilise *Panicum maximum*. Knob thorn habitats are usually ignored, because of the cover provided for predators.

The home range of buffalo varies with season and may extend from 40 km^2 during the summer to 120 km^2 during the dry winter months (Funston *et al.*, 1994). In the pre-summer, herds select intensively for the area surrounding a particular watering hole, whereas herds move great distances in search for grazing during the winter months. The mean distance travelled per day varies between 7 and 10 km per day, depending on the season and availability of food and water (Funston *et al.*, 1994; Stark, 1986).

Research on the comparative utilisation of feeds by buffalo (river and swamp buffalo) has indicated that buffalo utilise fibre better than cattle (Devendra, 1992; Ranjhan, 1992) and it may be related to the larger rumen volume and/or slower rumen movements of buffalo compared to other ruminants.

2.5.3 *Age and Gender Distribution*

Buffalo can be subdivided into calves and juveniles (younger than 3 years of age), subadults (3 to 5 years of age) and adults (6 years and older) (Prins, 1996). Mixed herds normally consist of adult cows, subadult cows, juveniles and calves. Bulls are adults by the age of 7 years. Males and females have the same weight development up to the age of 6 years. Thereafter cows do not appear to gain much more weight while bulls keep growing (Prins, 1996).

Bulls stay in the mixed herd in which they were born until adulthood. After leaving the mixed herd, adult bulls are encountered in bachelor groups of about 4 animals. Matings in mixed herds are by adult bulls only. After a period the bulls leave the herd and associate in bachelor groups for the periods outside mixed herds and rarely return to the same social environment more than twice (Prins, 1996).

2.5.4 *Reproduction*

Little information has been published on reproduction in the African buffalo (Bertschinger, 1996). Females reach puberty at about 3 or 4 years of age and calves at an age of about 5 years for the first time. Body mass is the determining factor for puberty. The mating season, starting mid-December (the rainy season), lasts for about four months (Krüger, 1996) with conception occurring especially towards the end of the season (March to May) resulting in the peak calving season during the summer. The mean duration of pregnancy of buffalo is about 11,5 months (340 days) (Bertschinger, 1996; Whyte, 1996; Prins, 1996). Calves are normally weaned at 4-5 months of age. Longer periods were observed and may be tolerated for up to 15 months, but then lactation ceased by the time the cow reached 7 months of gestation (Bertschinger, 1996). Lactating animals loose condition.

Calving interval depends primarily on environmental factors, e.g. condition, weaning date, and may be up to 2 years. The calving interval in the Kruger National Park is approximately 15 months (Sinclair, 1977 as quoted by Prins 1996).

2.5.5 *The African buffalo as meat animal*

The fatty tissue of Swamp buffalo have a lower carcass ether extract and also lower carcass energy contents than cattle (Moran, 1992). Carcass tissues of River and Swamp buffalo grew at similar rates than those of cattle, but Swamp buffalo contained lower subcutaneous to intermuscular fat ratios and a higher proportion of kidney and channel fat compared to cattle (De Francis and Moran, 1991). The contrary though is true for River buffalo when compared to Friesians. The lack of comparative data makes it difficult to ascertain the differences between buffalo and cattle in terms of the distribution of carcass bone, muscle and fat (Moran, 1992).

Earlier this century, meat from the African buffalo was used extensively as a protein source to supply African mine and sugar cane workers. Today, no more commercial exploitation of the buffalo is found and with every trophy bagged, a carcass of roughly 400 kg is available for consumption. However, due to regulations and disease restrictions, the utilisation of buffalo meat to its fullest potential is limited. Diseases of buffalo impose severe limitations on the use and disposal of meat and meat by-products of buffalo unless treated according to the requirements of the Directorate of Animal Health. The abattoir in the KNP was designed to accommodate these requirements and meat (canned and biltong - not uncooked) and other products are processed accordingly and can be sold outside of the "Red-line" (Whyte, 1996).

Buffalo are described to be big boned, rather massive animals compared to domestic cattle breeds, with bodies set low on strong legs with large hooves (Grobler, 1996). Grobler seems to believe that it is virtually impossible to foresee a time when buffalo will become commercially exploited for their meat production potential alone, because "disease-free" buffalo fetches grossly inflated prices at game sales and the income from trophy hunting also exceeds the potential income from meat by far. Buffalo though, produces a fine meat carcass, with the quality depending directly on the health status, condition and age of the animal. Buffalo meat, together with most other game meat, is regarded as a delicatessen, and is available only to tourists to the KNP and game ranches (Grobler, 1996).

Little research has been done on the carcass characteristics of African buffalo, while River and Swamp buffalo have been extensively studied and compared to cattle (de Francis and Moran, 1991). Buffalo are reported to contain less intramuscular fat than beef. Buffalo depot fat contains higher proportions of stearic and oleic fatty acids than cattle and lower proportions of palmitic acid (Table 2-2). The proportion of stearic acid in

entire male buffalo was lower than for castrates. Entire males also had significantly lower proportions of saturated to unsaturated fatty acids.

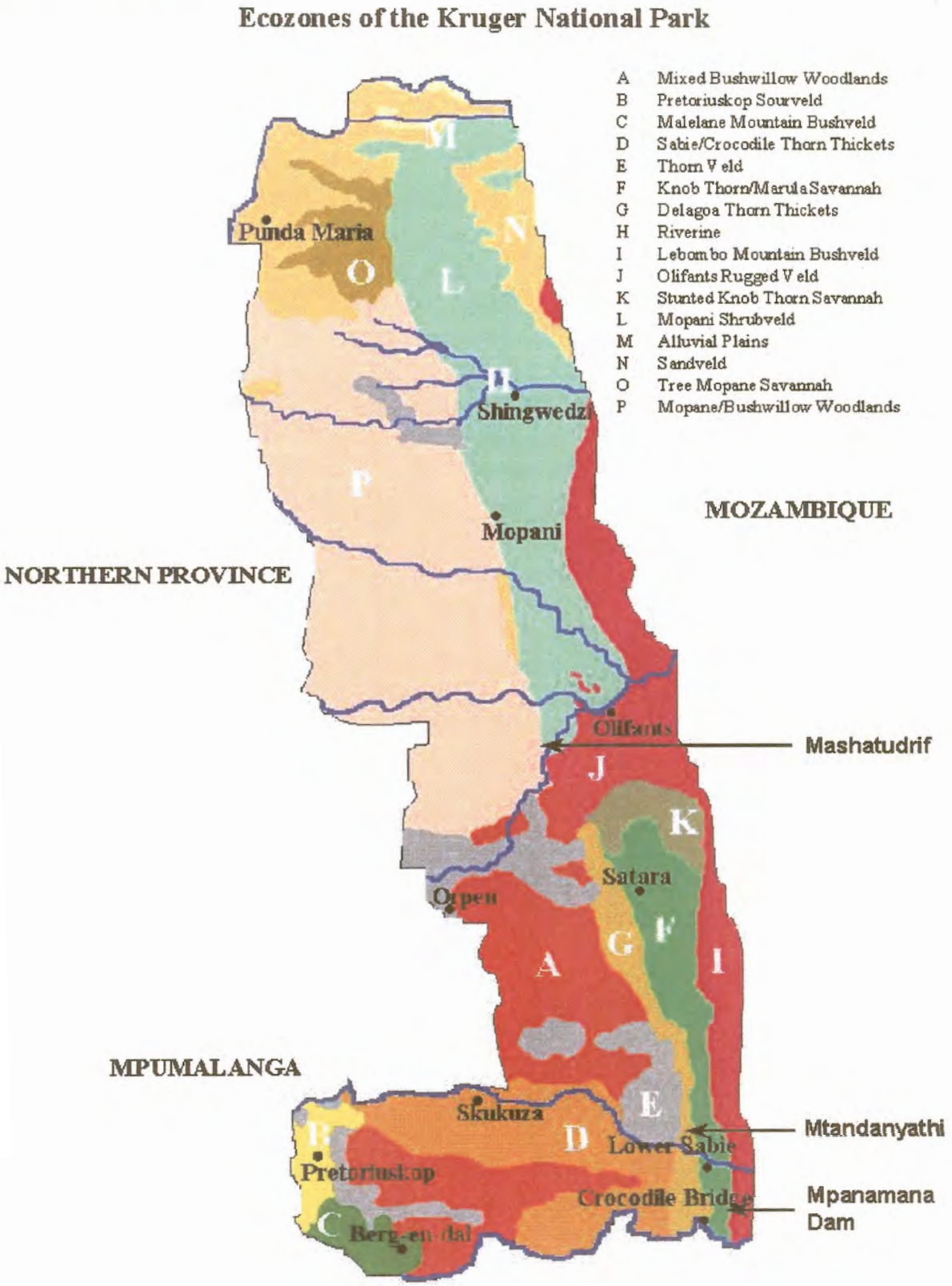
Table 2-2 Fatty acid content of depot lipids (% total fatty acids) of River buffalo and cattle carcasses (Adapted from de Francis and Moran, 1991).

	Buffalo	Cattle
Palmitic (C16:0)	18.3	29.2
Stearic (C18:0)	24.4	21.0
Palmitoleic (C16:1)	3.0	4.4
Oleic (C18:1)	44.1	31.5
Linoleic (C18:2)	2.9	1.6
Linolenic (C18:3)	0.9	1.2
Arachidonic (C20:4)	0.2	1.0

2.5.6 *Vegetation of the Kruger National Park*

The Kruger National Park is subdivided into different ecological zones according to geomorphological and vegetational information (Figure 2-4). Only the relevant areas where buffalo were sampled will be discussed i.e. Mpanamana Dam (near Crocodile Bridge), Lower Sabie and Houtboschrand. The plant species utilised by grazers and browsers are summarised in Table 2-3. The vegetation of Mashatudrif, near Houtboschrand (MH), is different from those of Mpanamana Dam (MD), and Mtandanyathi, near Lower Sabie (MLS) (Figure 2-4 and Table 2-3). Riverine vegetation was observed to be more prevalent in especially MLS than in MH.

Figure 2-4 Ecozone map of the Kruger National Park with indication of the three areas of importance to the study.



2.5.6.1 Mpanamana Dam

The area is in the southeastern corner of the Park near Crocodile Bridge and is mainly Knob thorn / Marula savannah veld on basalt. Mpanamana dam itself is situated in Ecozone F (Knob thorn / Marula savannah veld on basalt) of the Park, but the herd could also have grazed in the bordering Ecozone I (Lebombo Mountain bushveld on rhyolite).

2.5.6.1.1 *Ecozone F: Knob thorn/Marula savannah on Basalt*

Trees and shrubs favoured by browsers:

- *Acacia nigrescens* (Knob thorn)
- *Acacia tortilis* (Umbrella thorn)
- *Acacia xanthoploea* (Fever tree)
- *Combretum imberbe* (leadwood)
- *Lonchocarpus capassa* (Rain tree)
- *Sclerocarya birrea* (Marula)
- *Dichrostahys cinerea* (Sickle bush)
- *Grewia species* (Raisin bush)
- *Pterocarpus rotundifolius* (Round-leafed teak)
- *Ziziphus muscronata* (Buffalo thorn)

The main "sweet" grass species found that are generally palatable and nutritious are:

- *Digitaria eriantha* (Finger grass) grows in sandy areas of most soils, especially on damp soils along rivers and vleis in tall grassland. The grass is a highly digestible and palatable pasture grass with a grazing value that is mostly very high.
- *Panicum maximum* (Buffalo grass) grows in damp places with fertile soil (rivers and shade) of all soil types. It is a valuable pasture grass, very palatable and with a very high grazing value.

- *Themeda triandra* (Rooigras) grows in grassland areas on basalt, gabbro and dolerite. It is utilised by buffalo. The palatability is high with a grazing value of high to very high. The nutritional value is low in winter.
- *Setaria incrassata* (Vlei bristle grass) grows in wet areas as vleis, marshes and riverbanks Basalt, Gabbro, black clay soils. It is a palatable species with a grazing value of average to high.

The grasses that are generally not palatable or nutritious and only grazed when young and tender are:

- *Heteropogon contortus* (Spear grass) grows in stony soil (along roadside). It is a relatively good, hardy and fast-growing pasture grass. The grazing value is average to high and declines as the season progresses.
- *Enneapogon cenchroides* (Nine-awned grass) grows in sandy soils, in disturbed areas (roadside) and in natural veld after drought. The grazing value is variable but usually low. The grass is able to withstand long droughts and heavy grazing.
- *Bothriochloa radicans* (Stinking grass) grows in drier basalt areas and clay soil, near vleis and other low-lying areas. It is an unpalatable grass with a grazing value of low to very low.
- *Phragmites australis* grows near water and serves as dry season grazing for buffalo.

2.5.6.1.2 *Ecozone I: Lebombo mountain bushveld on Rhyolite*

Trees and shrubs favoured by browsers:

- *Acacia nigrescens* (Knob thorn)
- *Combretum apiculatum* (Red bushwillow)
- *Combretum zeyheri* (Large-fruited bushwillow)
- *Kirkia acuminata* (White seringa)
- *Sclerocarya birrea* (Marula)

- *Dichrostahys cinerea* (Sickle bush)
- *Grewia* species (Raisin bush)
- *Pterocarpus rotundifolius* (Round-leafed teak)
- *Ziziphus muscronata* (Buffalo thorn)

The main "sweet" grass species that are generally palatable and nutritious:

- *Digitaria eriantha* (Finger grass) grows in sandy areas of most soils, especially on damp soils along rivers and vleis in tall grassland. The grass is a highly digestible and palatable pasture grass with a grazing value that is mostly very high.
- *Panicum maximum* (Buffalo grass) grows in damp places with fertile soil (rivers and shade) of all soil types. It is a valuable pasture grass, very palatable and with a very high grazing value.
- *Themeda triandra* (Rooigras) grows in grassland areas on basalt, gabbro and dolerite. It is utilised by buffalo. The palatability is high with a grazing value of high to very high. The nutritional value is low in winter.

The grasses generally not palatable or nutritious and only grazed when young and tender are:

- *Heteropogon contortus* (Spear grass) grows in stony soil (along roadside). It is a relatively good, hardy and fast-growing pasture grass. The grazing value is average to high and declines as the season progresses.
- *Enneapogon cenchroides* (Nine-awned grass) grows in sandy soils, in disturbed areas (roadside) and in natural veld after drought. The grazing value is variable but usually low. The grass is able to withstand long droughts and heavy grazing.
- *Bothriochloa radicans* (Stinking grass) grows in drier basalt areas and clay soil, near vleis and other low-lying areas. It is an unpalatable grass with a grazing value of low to very low.

- *Phragmites australis* grows near water and serves as dry season grazing for buffalo.

2.5.6.2 Mtandanyati at Lower Sabie

Lower Sabie is situated in the southern region of the Park. Within a radius of 20 kilometres, 4 different ecological zones are found. The buffalo herd from this area could have grazed in all of these regions i.e. Ecozone D (Sabie/Crocodile thorn thickets on Granite), Ecozone E (Thorn veld on gabbro), Ecozone F (Knob thorn/Marula savannah on Basalt) and Ecozone G (Delagoa Thorn thickets on ecca shales).

2.5.6.2.1 *Ecozone D: Sabie/Crocodile Thorn Thickets on Granite*

Trees and shrubs favoured by browsers

- *Acacia grandicornuta* (Horned thorn)
- *Acacia nigrescens* (Knob thorn)
- *Acacia nilotica* (Scented thorn)
- *Acacia tortilis* (Umbrella thorn)
- *Albizia forbesii* (Broad-pod false thorn)
- *Balanites maughamii* (Green thorn)
- *Bolusanthus speciosus* (Tree wistaria)
- *Combretum apiculatum* (Red bushwillow)
- *Combretum hereroense* (Russet bushwillow)
- *Combretum zeyheri* (Large-fruited bushwillow)
- *Lonchocarpus capassa* (Rain tree)
- *Sclerocarya birrea* (Marula)
- *Dichrostahys cinerea* (Sickle bush)
- *Grewia species* (Raisin bush)
- *Pterocarpus rotundifolius* (Round-leafed teak)
- *Ziziphus muscronata* (Buffalo thorn)

The main "sweet" grass species found that are generally palatable and nutritious are:

- *Digitaria eriantha* (Finger grass) grows in sandy areas of most soils, especially on damp soils along rivers and vleis in tall grassland. The grass is a highly digestible and palatable pasture grass with a grazing value that is mostly very high.
- *Panicum maximum* (Buffalo grass) grows in damp places with fertile soil (rivers and shade) of all soil types. It is a valuable pasture grass, very palatable and with a very high grazing value.
- *Themeda triandra* (Rooigras) grows in grassland areas on basalt, gabbro and dolerite. It is utilised by buffalo. The palatability is high with a grazing value of high to very high. The nutritional value is low in winter.

The grasses that are generally not palatable or nutritious and only grazed when young and tender are:

- *Heteropogon contortus* (Spear grass) grows in stony soil (along roadside). It is a relatively good, hardy and fast-growing pasture grass. The grazing value is average to high and declines as the season progresses.
- *Phragmites australis* (Reeds) grows near water and serves as dry season grazing for buffalo.

2.5.6.2.2 *Ecozone E: Thorn veld on Gabbro*

Trees and shrubs favoured by browsers

- *Acacia nigrescens* (Knob thorn)
- *Acacia tortilis* (Umbrella thorn)
- *Bolusanthus speciosus* (Tree wistaria)
- *Sclerocarya birrea* (Marula)
- *Colophospermum mopane* (Mopane)
- *Dichrostahys cinerea* (Sickle bush)
- *Grewia species* (Raisin bush)

- *Ziziphus muscronata* (Buffalo thorn)

The main "sweet" grass species found that are generally palatable and nutritious are:

- *Cenchrus ciliaris* (Blue Buffalo grass) grows in most soils and on termitaria. It is very palatable when young.
- *Panicum maximum* (Buffalo grass) grows in damp places with fertile soil (rivers and shade) of all soil types. It is a valuable pasture grass, very palatable and with a very high grazing value.
- *Themeda triandra* (Rooigras) grows in grassland areas on basalt, gabbro and dolerite. It is utilised by buffalo. The palatability is high with a grazing value of high to very high. The nutritional value is low in winter.
- *Setaria incrassata* (Vlei bristle grass) grows in wet areas as vleis, marshes and riverbanks Basalt, Gabbro, black clay soils. It is a palatable species with a grazing value of average to high.

The grasses that are generally not palatable or nutritious and only grazed when young and tender are:

- *Bothriochloa radicans* (Stinking grass) grows in Drier basalt areas and clay soil, near vleis and other low-lying areas. It is an unpalatable grass with a grazing value of low to very low.
- *Phragmites australis* grows near water and serves as dry season grazing for buffalo.

2.5.6.2.3 *Ecozone F: Knob thorn/Marula savannah on Basalt*

Trees and shrubs favoured by browsers

- *Acacia nigrescens* (Knob thorn)
- *Acacia tortilis* (Umbrella thorn)
- *Acacia xanthoploea* (Fever tree)

- *Combretum imberbe* (leadwood)
- *Lonchocarpus capassa* (Rain tree)
- *Sclerocarya birrea* (Marula)
- *Dichrostahys cinerea* (Sickle bush)
- *Grewia species* (Raisin bush)
- *Pterocarpus rotundifolius* (Round-leafed teak)
- *Ziziphus muscronata* (Buffalo thorn)

The main "sweet" grass species found that are generally palatable and nutritious are:

- *Digitaria eriantha* (Finger grass) grows in sandy areas of most soils, especially on damp soils along rivers and vleis in tall grassland. The grass is a highly digestible and palatable pasture grass with a grazing value that is mostly very high.
- *Panicum maximum* (Buffalo grass) grows in damp places with fertile soil (rivers and shade) of all soil types. It is a valuable pasture grass, very palatable and with a very high grazing value.
- *Themeda triandra* (Rooigras) grows in grassland areas on basalt, gabbro and dolerite. It is utilised by buffalo. The palatability is high with a grazing value of high to very high. The nutritional value is low in winter.
- *Setaria incrassata* (Vlei bristle grass) grows in wet areas as vleis, marshes and riverbanks Basalt, Gabbro, black clay soils. It is a palatable species with a grazing value of average to high.

The grasses that are generally not palatable or nutritious and only grazed when young and tender are:

- *Heteropogon contortus* (Spear grass) grows in stony soil (along roadside). It is a relatively good, hardy and fast-growing pasture grass. The grazing value is average to high and declines as the season progresses.

- *Enneapogon cenchroides* (Nine-awned grass) grows in sandy soils, in disturbed areas (roadside) and in natural veld after drought. The grazing value is variable but usually low. The grass is able to withstand long droughts and heavy grazing.
- *Bothriochloa radicans* (stinking grass) grows in Drier basalt areas and clay soil, near vleis and other low-lying areas. It is an unpalatable grass with a grazing value of low to very low.
- *Phragmites australis* Grows near water and serves as dry season grazing for buffalo.

2.5.6.2.4 *Ecozone G: Delagoa Thorn thickets on Ecca Shales*

Trees and shrubs favoured by browsers

- *Acacia welwitschii* (Delagoa thorn)
- *Albizia petersiana* (Many-stemmed false thorn)
- *Bolusanthus speciosus* (Tree wistaria)
- *Combretum hereroense* (Russet bushwillow)
- *Combretum imberbe* (leadwood)
- *Sclerocarya birrea* (Marula)
- *Dichrostahys cinerea* (Sickle bush)
- *Grewia species* (Raisin bush)
- *Ziziphus muscronata* (Buffalo thorn)

The main "sweet" grass species found that are generally palatable and nutritious are:

- *Panicum maximum* (Buffalo grass) grows in damp places with fertile soil (rivers and shade) of all soil types. It is a valuable pasture grass, very palatable and with a very high grazing value.

The grasses that are generally not palatable or nutritious and only grazed when young and tender are:

- *Bothriochloa radicans* (stinking grass) grows in drier basalt areas and clay soil, near vleis and other low-lying areas. It is an unpalatable grass with a grazing value of low to very low.
- *Phragmites australis* grows near water and serves as dry season grazing for buffalo.

2.5.6.3 Mashatudrif at Houtboschrand

Mashatudrif at Houtboschrand are situated in the central region of the Park. The buffalo herd from this area, could have grazed in two different ecozones i.e. Ecozone L (Mopane shrubveld on Basalt) and Ecozone P (Mopane/Bushwillow Woodlands on Granite).

2.5.6.3.1 *Ecozone L: Mopane shrubveld on Basalt*

Trees and shrubs favoured by browsers

- *Acacia nigrescens* (Knob thorn)
- *Acacia tortilis* (Umbrella thorn)
- *Combretum imberbe* (leadwood)
- *Lonchocarpus capassa* (Rain tree)
- *Sclerocarya birrea* (Marula)
- *Colophospermum mopane* (Mopane)
- *Dichrostahys cinerea* (Sickle bush)
- *Grewia species* (Raisin bush)
- *Ziziphus muscronata* (Buffalo thorn)

The main "sweet" grass species found that are generally palatable and nutritious are:

- *Digitaria eriantha* (Finger grass) grows in sandy areas of most soils, especially on damp soils along rivers and vleis in tall grassland. The grass is a highly digestible and palatable pasture grass with a grazing value that is mostly very high.

- *Panicum maximum* (Buffalo grass) grows in damp places with fertile soil (rivers and shade) of all soil types. It is a valuable pasture grass, very palatable and with a very high grazing value.
- *Themeda triandra* (Rooigras) grows in grassland areas on basalt, gabbro and dolerite. It is utilised by buffalo. The palatability is high with a grazing value of high to very high. The nutritional value is low in winter.

The grasses that are generally not palatable or nutritious and only grazed when young and tender are:

- *Heteropogon contortus* (Spear grass) grows in stony soil (along roadside). It is a relatively good, hardy and fast-growing pasture grass. The grazing value is average to high and declines as the season progresses.
- *Enneapogon cenchroides* (Nine-awned grass) grows in sandy soils, in disturbed areas (roadside) and in natural veld after drought. The grazing value is variable but usually low. The grass is able to withstand long droughts and heavy grazing.
- *Bothriochloa radicans* (Stinking grass) grows in drier basalt areas and clay soil, near vleis and other low-lying areas. It is an unpalatable grass with a grazing value of low to very low.
- *Phragmites australis* grows near water and serves as dry season grazing for buffalo.

2.5.6.3.2 *Ecozone P: Mopane/Bushwillow Woodlands on Granite*

Trees and shrubs favoured by browsers

- *Acacia nigrescens* (Knob thorn)
- *Combretum apiculatum* (Red bushwillow)
- *Combretum hereroense* (Russet bushwillow)
- *Combretum zeyheri* (large-fruited Bushwillow)

- *Lonchocarpus capassa* (Rain tree)
- *Sclerocarya birrea* (Marula)
- *Colophospermum mopane* (Mopane)
- *Dichrostahys cinerea* (Sickle bush)
- *Grewia species* (Raisin bush)
- *Pterocarpus rotundifolius* (Round-leafed teak)
- *Ziziphus muscronata* (Buffalo thorn)

The main "sweet" grass species found that are generally palatable and nutritious are:

- *Digitaria eriantha* (Finger grass) grows in sandy areas of most soils, especially on damp soils along rivers and vleis in tall grassland. The grass is a highly digestible and palatable pasture grass with a grazing value that is mostly very high.
- *Panicum maximum* (Buffalo grass) grows in damp places with fertile soil (rivers and shade) of all soil types. It is a valuable pasture grass, very palatable and with a very high grazing value.

The grasses that are generally not palatable or nutritious and only grazed when young and tender are:

- *Heteropogon contortus* (spear grass) grows in stony soil (along roadside). It is a relatively good, hardy and fast-growing pasture grass. The grazing value is average to high and declines as the season progresses.
- *Phragmites australis* grows near water and serves as dry season grazing for buffalo.

(Trollop, Potgieter and Zambatis, 1989; Van Oudtshoorn, 1992; Kruger National Park. 1993)

Table 2-3 Summary of the different plant species found in the homerange areas of the three herds.

	Mpanamana Dam Crocodile Bridge			Mtandanyati Lower Sabie		Mashatudrif Houtboschrand	
Ecozone	I	F	D	E	G	P	L
Veld type	Lebombo mountain bushveld	Knob thorn/Marula savannah	Sabie/Crocodile thorn thickets	Thorn veld	Delagoa Thorn thickets	Mopane/Bushwillow Woodlands	Mopane shrubveld
Soil type	Rhyolite	Basalt	Granite	Gabbro	Ecca Shales	Granite	Basalt
Trees	*Acacia nigrescens* *Combretum apiculatum* *Combretum zeyheri* *Kirkia acuminata* *Sclerocarya birrea*	*Acacia nigrescens* *Acacia tortilis* *Acacia xanthoploea* *Combretum imberbe* *Lonchocarpus capassa* *Sclerocarya birrea*	*Acacia grandicomuta* *Acacia nigrescens* *Acacia nilotica* *Acacia tortilis* *Albizia forbesii* *Balanites maughamii* *Bolusanthus speciosus* *Combretum apiculatum* *Combretum hereroense* *Combretum zeyheri* *Lonchocarpus capassa* *Sclerocarya birrea*	*Acacia nigrescens* *Acacia tortilis* *Bolusanthus speciosus* *Sclerocarya birrea*	*Acacia welwitschii* *Albizia petersiana* *Bolusanthus speciosus* *Combretum hereroense* *Combretum imberbe* *Sclerocarya birrea*	*Acacia nigrescens* *Combretum apiculatum* *Combretum hereroense* *Combretum zeyheri* *Lonchocarpus capassa* *Sclerocarya birrea*	*Acacia nigrescens* *Acacia tortilis* *Combretum imberbe* *Lonchocarpus capassa* *Sclerocarya birrea*
Shrubs (shrub-like trees)	*Dichrostahys cinerea* *Grewia species* *Pterocarpus rotundifolius* *Ziziphus muscronata*	*Dichrostahys cinerea* *Grewia species* *Pterocarpus rotundifolius* *Ziziphus muscronata*	*Dichrostahys cinerea* *Grewia species* *Pterocarpus rotundifolius* *Ziziphus muscronata*	*Colophospermum mopane* *Dichrostahys cinerea* *Grewia species* *Ziziphus muscronata*	*Dichrostahys cinerea* *Grewia species* *Ziziphus muscronata*	*Colophospermum mopane* *Dichrostahys cinerea* *Grewia species* *Pterocarpus rotundifolius* *Ziziphus muscronata*	*Colophospermum mopane* *Dichrostahys cinerea* *Grewia species* *Ziziphus muscronata*
Grasses (A: sweet)	*Digitaria eriantha* *Panicum maximum* *Themeda triandra*	*Digitaria eriantha* *Panicum maximum* *Setaria incrassata* *Themeda triandra*	*Digitaria eriantha* *Panicum maximum* *Themeda triandra*	*Cenchrus ciliaris* *Panicum maximum* *Setaria incrassata* *Themeda triandra*	*Panicum maximum*	*Digitaria eriantha* *Panicum maximum*	*Digitaria eriantha* *Panicum maximum* *Themeda triandra*
Reeds	*Phragmites australis*	*Phragmites australis*	*Phragmites australis*	*Phragmites australis*	*Phragmites australis*	*Phragmites australis*	*Phragmites australis*
Grasses (C: not nutritious)	*Bothriochloa radicans* *Enneapogon cenchroides* *Heteropogon contortus*	*Bothriochloa radicans* *Enneapogon cenchroides* *Heteropogon contortus*	*Heteropogon contortus*	*Bothriochloa radicans*	*Bothriochloa radicans*	*Heteropogon contortus*	*Bothriochloa radican* *Enneapogon cenchroides* *Heteropogon contortus*

CHAPTER 3

MATERIALS AND METHODS

3.1 SAMPLING PROCEDURE

This project formed part of a greater bovine tuberculosis (TB) monitoring programme in the Kruger National Park (KNP) launched during the spring of 1996. Three groups of African buffalo (*Syncerus caffer*) were culled in the KNP. "Culling" implies the removal of selected animals from a herd or population (Whyte, 1996). In this case a predetermined proportion of animals was removed randomly from three populations. The groups were from three different areas in the KNP (Figure 2-4):

1. Mpanamana Dam (MD) in the south east of the Park near Crocodile Bridge (47 animals).
2. Mtandanyathi, near Lower Sabie (MLS) (61 animals).
3. Mashatudrif at Houtboschrand (MH) south of Olifants Camp (46 animals).

According to KNP policy, a predetermined amount of animals need to be culled, in order to maintain a natural population structure. To achieve this, a method was used which is as close to random as possible by splitting off any group of buffalo from the herd by helicopter. Therefore the cull for one day will not be strictly "random" as it will likely be animals of a similar sex and status (Whyte, 1996).

Culling was done by means of a helicopter and using scoline as drug. Scoline is an immobilising agent, which blocks the passage of signals from nerve to muscle, resulting in paralysis of the diaphragm and intercostal muscles, almost simultaneously with general skeletal paralysis. Hypoxia sets in quickly, brain activity decreases simultaneously in proportion to the degree of hypoxaemia and the animals are insensible within a short time

after going down (De Vos *et al.*, 1983). The advantage of the use of scoline as drug is that it obviates wounding, which is a great safety factor as a wounded buffalo pose a great danger to ground personnel. The scoline dart placed anywhere in the muscle of the buffalo's body will allow absorption of the scoline, paralysis and death of the animal. Another advantage is that its use does not affect the meat and can be used for human consumption (Whyte, 1996).

After all the animals were recumbent, the ground crew moved in and any animal still alive was brain shot. Throats were immediately cut to allow proper bleeding and to minimise deterioration of meat quality. All animals were inspected for foot-and-mouth disease. The age of each animal was obtained from the tooth eruption sequence in younger animals and molar wear in older ones. Each animal was eviscerated and the reproductive tracts (uterus) of all females removed for examination and assessment of reproductive status. Carcasses were recovered by a seven-ton truck fitted with a hydraulic crane, loaded onto a 14-ton transport vehicle and transported to the By-products Depot at Skukuza where the meat and hides were processed.

The Department of Nature Conservation kindly allowed us to sample all buffalo culled. The carcasses were sampled the following day at the abattoir. Approximately 5 g samples of subcutaneous, perirenal, intramuscular (*M. Longissimus dorsi* (L1-L6)) and omental fat were collected (Webb *et al.*, 1994; Figure 3-1 and Figure 3-2). As some of the carcasses were found to have little perirenal fat and more fat around the heart, some pericardial fat samples were also collected (Table 3-1). In general it was difficult to obtain samples from pericardial and omental fat. The samples were clearly marked (age group, gender, anatomical location, area) and stored in polyethylene bags at -20°C.

The diseases of buffalo (especially TB and foot-and-mouth disease (FMD)) impose severe limitations on the use and disposal of meat and meat by-products and therefore any muscle or fat samples of buffalo, unless treated according to the requirements of the Directorate of Animal Health. This significantly limited sampling procedures and subsequent analyses. The abattoir in the KNP has been designed to meet these requirements, so that meat (canned and biltong - not uncooked) and other products can be processed and sold outside of the "Red-line" (Whyte, 1996).

Figure 3-1 Sampling of omental fat (Bas et al., 1992).

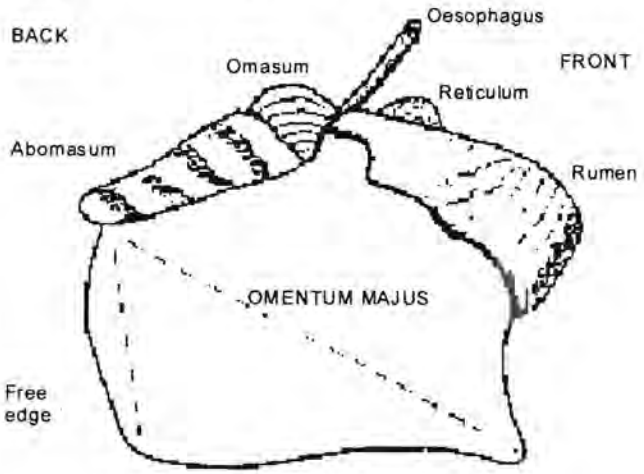
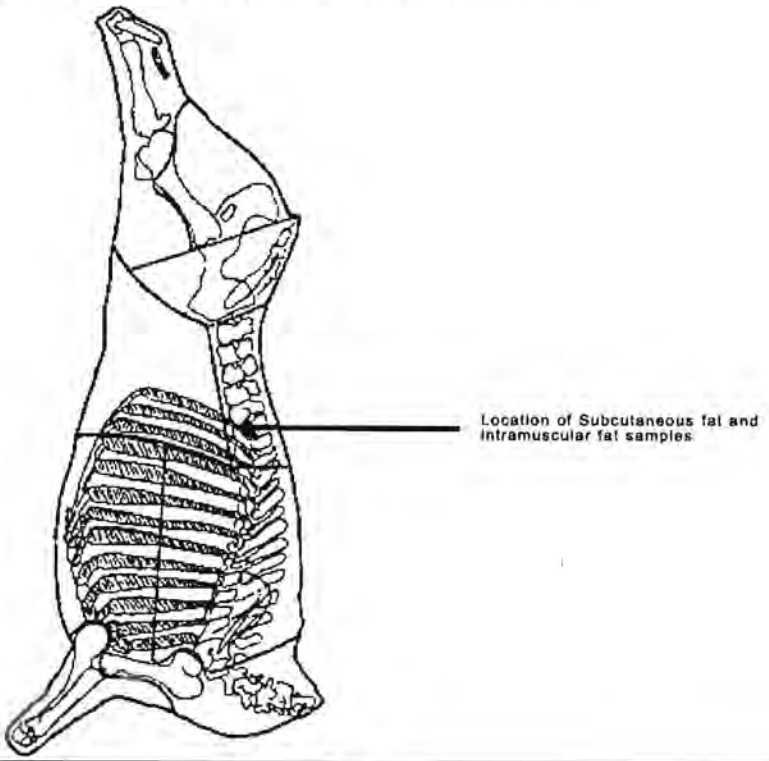


Figure 3-2 Sampling of subcutaneous fat on the carcass.



The KNP do not have the facilities to conduct basic proximate analyses. The KNP is behind the FMD red-line, and precautions had to be taken to prevent the foot-and-mouth disease virus from spreading to other parts of the country. In addition TB posed a potential health threat and therefore samples could not be analysed at the Department of Animal and Wildlife Sciences at the University of Pretoria, unless sterilised at 70°C for at least 30 minutes. This could only be done under quarantine conditions at the Onderstepoort Institute for Exotic Diseases (OIED). The facilities available limited the analyses of the samples to the extraction of lipid from the samples. A permit was obtained to transport the sealed containers with the fat and muscle samples from Skukuza to Pretoria. At the OIED, samples were kept at -20°C until sterilised.

3.1.1 *Disease Security Regulations*

On entering the OIED, a form was signed which signified the acceptance of the conditions imposed as stated: All visitors to the high security areas are subject to the following:

i) May for five days not visit farms, shows and markets where cloven-hoofed animals are held or usually held and/or exhibited; abattoirs, zoos, the Onderstepoort Veterinary Institute (OVI) and the Faculty of Veterinary Science, artificial insemination centres or premises where food or any other product intended for consumption by cloven-hoofed animals are produced or stored.

ii) Must for three days avoid contact with persons known to come into contact with cloven-hooded animals or products which are intended for cloven-hooded animals.

iii) Must for three days after exposure not visit any game reserves or game parks, which are outside the FMD red-line.

vi) No equipment or personal possessions may be taken into the quarantine area unless permission has been obtained from the Disease Security Officer.

On entering and leaving the laboratories of the high security area, certain safety regulations also had to be obeyed.

Table 3-1 Experimental design: number of animals sampled.

	Age group	Amount of samples collected				
		Subcutaneous fat	Intramuscular fat	Perirenal fat	Pericardial fat	Omental fat
Male	<2yrs (A)	6	6	6	2*	4*
	2-6yrs (B)	6	6	6	3*	5*
	>6yrs (C)	6	6	6	3*	5*
Female	<2yrs (A)	6	6	6	3*	6
	2-6yrs (B)	6	5*	6	2*	5*
	>6yrs (C)	6	6	6	4*	6
Area	MD**	13	13	13	0	8
	MH**	10	10	10	7	9
	MLS**	13	12	13	10	13

* although the original proposal was to sample 6 animals per treatment combination, not enough animals were available in this group. Samples were collected at the same anatomical location of each animal (as proposed for sheep by Webb, 1994 and cattle Webb and Casey, 1995).

** MD = Mpanamana Dam; MH = Mashatudrif, Houtboschrand; MLS = Mtandanyathi, Lower Sabie

3.1.2 *Sample preparation*

Sterilisation and extraction were done at the OIED. Approximately 0.5 g of fat and 5 g of the muscle samples were weighed into heat resistant containers (e.g. centrifuge tube or test tube). Lipids were extracted with chloroform: methanol (2:1; v/v) (Folch *et al.*, 1957; Ways and Hanahan, 1964). Butylated hydroxy toluene was included as antioxidant. 3 ml chloroform (Chloroform + 0.1% butylated hydroxy toluene (BHT)) was added to the sample. Using a glass rod, the sample was crushed and thoroughly blended with the chloroform. It was then cooked in a waterbath at 70°C for 30 minutes. About 10 ml Chloroform was added during this period of time (the boiling point of Chloroform, $CHCl_3$ is 61°C and evaporated very fast at 70°C (Kotz and Purcell, 1987) whereafter the samples were removed from the waterbath. The extracted fat was then transferred into a small plastic bottle and kept in a freezer until all samples were extracted. Everything, including the samples were sterilised upon leaving the laboratory to ensure that there was no risk of spreading the foot-and-mouth disease virus by means of any of the materials leaving the laboratory.

The extracted fat samples were then transferred to the Department of Animal and Wildlife Sciences, University of Pretoria and stored in a freezer (-20°C) until analysed for fatty acid composition. The residues of the muscle and fat samples were destroyed soon after extraction of the fat was completed without leaving the OIED.

3.2 FATTY ACID DETERMINATION

3.2.1 *Modification of lipid extraction*

The preferred method of lipid extraction at the Department of Animal and Wildlife Sciences, University of Pretoria, is by means of chloroform:methanol (2:1 v/v) as described by Folch *et al.* (1957) with the modifications of Ways and Hanahan (1964). Butylated hydroxy toluene (BHT) is included as antioxidant to prevent lipid oxidation. Usually lipid is extracted by means of 3 ml chloroform (chloroform + 0.1% BHT) at 2-4°C and the samples are shaken every hour for 4-6 hours. However, the directorate of Animal Health requires all samples collected in the KNP to be sterilised before further analyses. In the BF_3/Methanol method (AOAC, 1975) lipid is extracted from samples subsequent to heating. Since the apparatus required for the BF_3/Methanol method was not available at the OIED, it was decided to modify the chloroform:methanol method by increasing the temperature during the extraction procedure. This also allowed for the sterilisation of samples as required by the directorate of Animal Health.

One gram of the fat sample was weighed into a heat resistant container (e.g. a centrifuge tube or test tube) together with 1 ml chloroform (Chloroform + 0.1 % BHT) and heated at 60°C. Using a glass rod, the sample was crushed and thoroughly blended with the chloroform. After 10 minutes, more chloroform (1 ml) was added and heated for another five minutes. After heating, another 5ml chloroform was added. The fluid was then extracted and esterified as described previously (Webb *et al.*, 1994). The volumes of lipid extracted were higher compared to samples extracted with the "cold extraction" method.

For a smaller peak, 0.5 g of the sample was used together with 3 ml chloroform (chloroform + 0.1 % BHT). This was heated at 60°C for 10 minutes and an additional 3 ml chloroform was added after removal from the waterbath. This mixture could either be stored at 2-4°C until required or immediately used for esterification. Of the extracted sample, 0.5 ml was used for esterification.

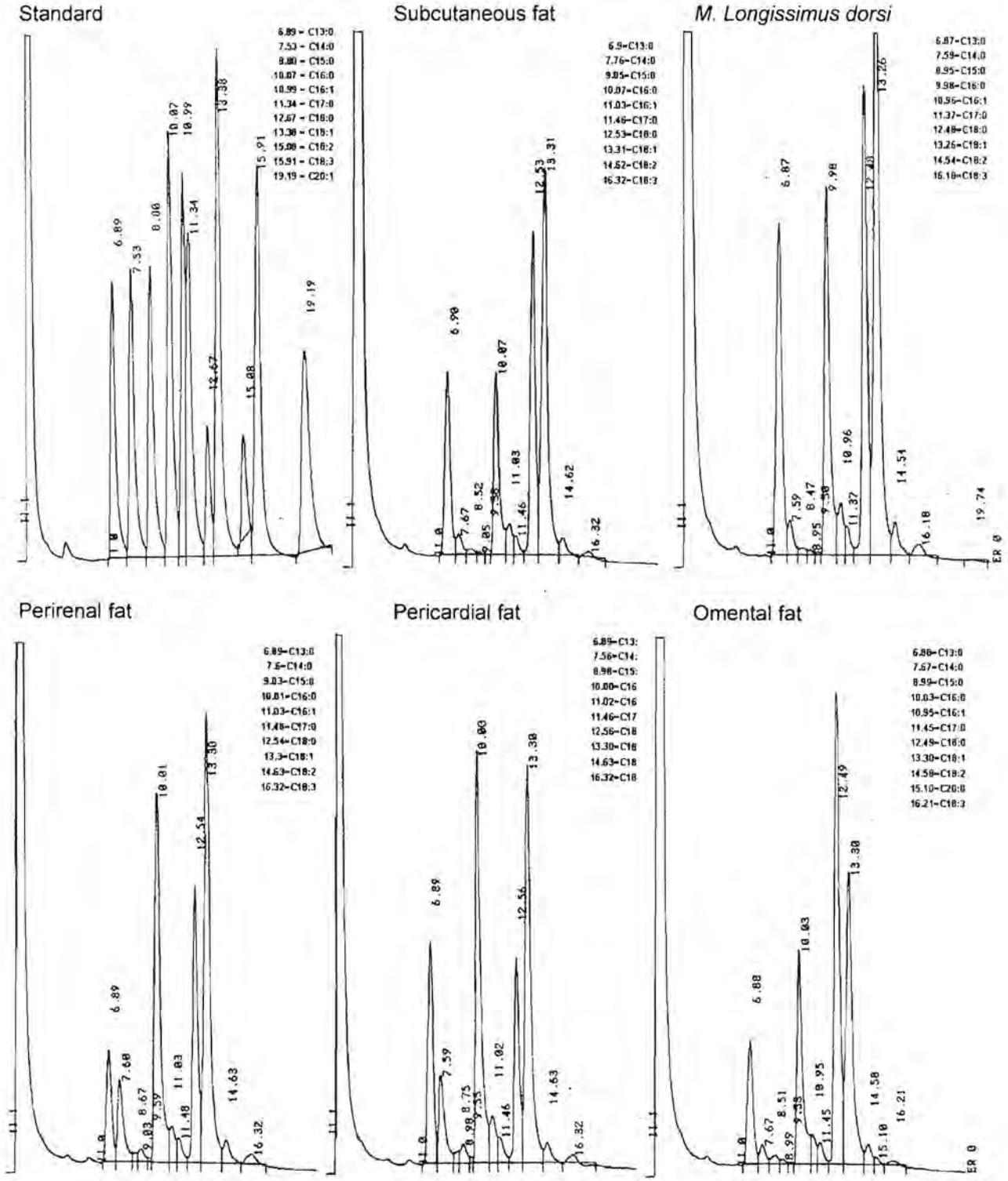
For the muscle samples, 4-5 g of the sample was used for long-chain fatty acid extraction as described for fat samples.

3.2.2 *Preparation of fatty acid methyl esters*

The separation of C13:0 and C14:0 were a problem. In the original method used, esterification occurred at a temperature of about 50°C for 20 minutes (Marcello, Cook, Slanger, Johnson, Fischer and Dinusson, 1983; Luddy, Bradford and Riemenschneider, 1960). At first the temperature was elevated from 50°C to 60°C. No differences were found between esterifications for 10 minutes at 60°C and 20 minutes at 50°C. A lengthening of time to 30 minutes at 60°C resulted in better separation of peaks for fatty acids. The method used was modified and all samples were esterified at 60°C for 30 minutes.

1 ml of 2M sodium hydroxide in methanol solution (8 g NaOH in 100ml methanol) was mixed with 5 ml chloroform. To this mixture, 0.5 ml of the sample extract (1 ml for muscle samples) was added, mixed thoroughly and placed in a waterbath at 60°C for 30 minutes. After 30 minutes the samples were removed from the waterbath, allowed to cool and centrifuged at 5000 rpm for 15 minutes. A portion of the clear supernatant was then pipetted into a clean plastic container and stored in a freezer until required. Fatty acids were measured by gas chromatography (Webb *et al.*, 1994; Webb and Casey, 1995) (Figure 3-3) and expressed as proportions of long-chain fatty acids (w/w %) present in the sample.

Figure 3-3 GC chromatograms for description of the results obtained after modification of the method for long-chain fatty acid determination (the examples of chromatograms presented were reduced in size to fit on one page. Retention times of specific fatty acids are indicated above each peak for identification).



3.2.3 *Settings of the GC column*

A 2 meter glass column (ID: 3 mm, packed with 10% SP 2330 on Chromosorb W/HP 100/120) was used. The GC and integrator settings and programmes were as follows:

Flame ionisation detector gas: H_2 - 300 KPa; O_2 - 300 KPa

Carrier gas: N_2 - 300 KPa (25 ml/min) 15 psi (cold)

GC Programme:
- GC Attenuation: 64
- Two minutes to stabilise
- starting temperature: 150°C
- hold for 2 minutes
- temperature rise: 5°C/min
- final temperature: 210°C
- hold for 8 min

Integrator setting:
- Attenuation: 64
- chart speed: 0.5 cm/min
- dialogue: 21 min - end run
- Injector temperature: 220°C
- Detector temperature: 240°C
- Initial attenuation: INF
- Initial range: 11
- Method complete: 22 minutes

1 µl of the samples and the standard were injected. For muscle samples, 2 µl was injected. The column had to be conditioned before and between runs by setting the oven temperature at 225°C overnight. The carrier gas flow was maintained at all times (15 ml/min). Identification of the sample fatty acids was then made by comparison of the relative retention times of the fatty acid methyl ester (FAME) peaks from the samples with those of the standard.

3.2.4 *Standard*

A standard solution containing methyl esters of the fatty acids (C13:0 – C20:0) to be determined in approximately the same concentrations as that expected for the samples was prepared and injected in order to determine and check the retention times of the different fatty acids.

3.3 DATA ANALYSIS

Data were initially recorded as a listing of the proportions of long-chain fatty acids (w/w %) in the sample. In previous studies, C13:0 was omitted from the reports.

Fatty acids were classified into saturated (SFA, no double bonds) and unsaturated (UFA, one or more double bonds). Unidentified peaks were not included in calculations. Differences between depots were determined over ages, gender and areas. This resulted in large standard deviations for specific fatty acids. Interactions have been analysed and will be discussed in the relevant chapter. All data were statistically analysed by means of multifactor analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of SAS (1992). In most cases the data was unbalanced and therefore the Bonferroni multiple range test was used. Significant differences are quoted at the $P<0.01$ and $P<0.05$ levels.

3.4 RECOMMENDATION

Always use fresh NaOH. With time crystallisation occurs resulting an inaccurate molality. The esterification process is influenced and the peaks of C13:0 and C14:0 do not separate properly and cannot be identified.

3.5 TERMS OF REFERENCE

Gender — Gender was used because animals were either female or male.

Area — The area defined from where the different herds were located, consists of a number of habitats.