FACTORS AFFECTING THE COMPOSITION OF LONG-CHAIN FATTY ACIDS IN THE AFRICAN BUFFALO (*SYNCERUS CAFFER*)

by

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ABSTRACT

The proportions of long-chain fatty acids in *M. Longissimus dorsi* (LD), subcutaneous (SCF), perirenal (PRF), pericardial (PCF) and omental (OMF) fat and the effects of age, gender and area on the proportions of long-chain fatty acids in these fat depots of the African buffalo were studied. Buffalo meat is an important commodity for tourists to the Kruger National Park (KNP) and the composition, colour and amount of carcass fat contributes significantly towards its quality. Previous research suggests significant breed, age, gender and anatomical differences in the composition of fatty acids in various domestic ruminant species. Little information is available on the composition of carcass fat in wild ruminants like the African buffalo. The LD, SCF, PRF, PCF and OMF depots were sampled from buffalo culled in three different areas in the KNP i.e. Mashatudrif at Houtboschrand (MH) (Mopane/Bushwillow woodlands on granite), Mthandanyathi at Lower Sabie (MLS) (thorn thickets on granite) and Mpanamana Dam at Crocodile Bridge (MD) (Knob thorn/Marula savannah on basalt). Samples were sterilised and stored at –20°C for subsequent lipid extraction with chloroform:methanol (2:1 v/v). Butylated hydroxy toluene (BHT) was included as an antioxidant. Fatty acids were measured by gas chromatography and expressed as a proportion of total long-chain fatty acids (w/w %).

Significant differences ($P<0.01$) were found in unsaturated (UFA) and saturated (SFA) fatty acids between the external (SCF and LD) and internal (PRF, PCF) fat depots. LD differed significantly ($P<0.01$) from OMF. Fatty acids from SCF and LD did not differ significantly ($P<0.01$). SCF and LD differed significantly ($P<0.01$) from PRF for the proportions of C13:0, C16:0, C16:1 and C18:1. The fatty acids present in PRF, PCF and OMF did not differ significantly, except for C16:1 being significantly ($P<0.01$) higher in PRF than OMF.

Age differences were noted for C13:0 (decreased in LD ($P<0.01$), C14:0 (increased in SCF ($P<0.01$)), C15:0 (decreased in PRF, OMF, PCF ($P<0.01$)), C16:0 (decreased in PRF ($P<0.01$) and PCF ($P<0.05$)), C18:0 (increased in SCF ($P<0.01$) and LD ($P<0.05$)), C18:1 (increased in SCF ($P<0.05$)), C18:3 (decreased in SCF ($P<0.05$)). The proportions of SFA and UFA did not change significantly with age. The proportions of UFA differed significantly between females and males and in particular C16:1 and C18:1 in SCF and LD. Significant differences ($P<0.05$) in the proportions UFA in SCF were found of buffalo's from different areas. Higher proportions of UFA were observed in animals from the MH than animals from MLS, while that of animals from MD was intermediate. Differences in the proportions of C13:0, C15:1, C16:0, C17:0 and C18:0 were noted between different buffalo herds sampled. Buffalo from MLS contained significantly higher proportions ($P<0.01$) of C13:0 compared to those from the other two areas. The internal fat depots appeared to be more stable, compared to the external depots and were not significantly influenced by area. The results suggest that area, age and gender significantly affected the composition of long-chain fatty acids in fat depots of the African buffalo and that the energy reserves of buffalo are progressively depleted during the dry winter season to meat the requirements for maintenance, growth and lactation.

OPSOMMING

Die verspreiding van langketting vetsure in *M. Longissimus dorsi* (LD), onderhuids (OHV), perirenaal (PRV), perikardaal (PKV) en omentum (OMV) vet en die effek van ouderdom, geslag en gebied op die verspreiding van vetsure in die vetreserwes van die Afrika buffel, is bestudeer. Buffelvleis is 'n belangrike kommoditeit vir toeriste wat die Nasionale Krugerwildtuin (NKW) besoek en die samestelling, kleur en hoeveelheid karkasvet dra betekenisvol by tot die kwaliteit van die eindproduk. Vorige navorsing dui op betekenisvolle verskille in ras, ouderdom, geslag en anatomiese lokalisasie ten opsigte van die samestelling van vetsure in verskillende plaasdierspesies. Min inligting is egter beskikbaar oor die samestelling van karkasvet in wilde herkouerspesies soos die Afrika buffel. Monsters is geneem van die LD, OHV, PRV, PKV en OMV vetreserwes van uitskot buffels van verskillende areas in NKW, naamlik Mashatudrif by Houtboschrand (MH) (Mopanie/Boswilger-bosveld op graniet), Mthandanyathi by Onder-Sabie (MLS) (doringveld op graniet) en Mpanamana-dam by Krokodilbrug (MD) (Knoppiesdoring/Maroela savanne op basalt). Monsters is gesteriliseer en by –20°C geberg vir daaropvolgende lipiedekstraksie met chloroform:methanol (2:1 v/v). Butielhidroksietoleen (BHT) is as anti-oksidant ingesluit. Vetsure is bepaal deur gaschromatografie en uitgedruk as 'n persentasie van die totale vetsuurinhoud (m/m %). Die resultate dui daarop dat gebied, ouderdom en geslag 'n betekenisvolle invloed op die samestelling van langketting vetsure in vetsreserwes van die Afrika buffel het. Betekenisvolle verskille is waargeneem ($P<0.01$) tussen die onversadigde (OVS) en versadigde (VVS) vetsure, tussen die eksterne (OHV en LD) en interne (PRV, PKV) vetreserwes. LD het betekenisvol verskil van OMV. Vetsure van OHV en LD het nie betekenisvol ($P<0.01$) verskil nie. OHV en LD het wel betekenisvol ($P<0.01$) verskil van PRV ten opsigte van C13:0, C16:0, C16:1 en C18:1. Die vetsure teenwoordig in PRV, PKV en OMV het nie betekenisvol verskil nie, behalwe in die geval van C16:1 wat wel betekenisvol ($P<0.01$) hoër was in PRV en OMV. Ouderdomsverskille is waargeneem ten opsigte van C13:0 (afname in LD ($P<0.01$)), C14:0 (toename in OHV ($P<0.01$)), C15:0 (afname in PRV, OMV en PKV ($P<0.01$)), C16:0 (afname in PRV ($P<0.01$) en PKV ($P<0.05$)), C18:0 (toename in OHV ($P<0.01$) en LD ($P<0.05$)), C18:1 (toename in OHV ($P<0.05$)) en C18:3 (afname in OHV ($P<0.05$)). VVS en OVS het nie betekenisvol verander met ouderdom nie. OVS het betekenisvol verskil tussen vroulike en manlike diere veral ten opsigte van C16:1 en C18:1 in OHV en LD. Betekenisvolle verskille ($P<0.05$) in OVS in die OHV is gevind in buffels van verskillende areas. Hoër verhoudings van OVS is waargeneem by diere van MH as by diere van MLS en MD. Verskille in C13:0, C15:1, C16:0, C17:0 en C18:0 is waargeneem by buffels in genoemde areas. Buffels van MLS het betekenisvol meer C13:0 ($P<0.01$) gehad as die buffels in die ander twee areas. Die interne vetreserwes kom meer stabiel voor as die onderhuidse vetreserwes en is nie betekenisvol deur die area beïnvloed nie. Die huidige resultate dui op 'n uitputting van energiereserwes om te voldoen aan die behoeftes vir onderhoud, groei en laktasie as gevolg van swak voedingstoestande gedurende die droë winter seisoen.

I declare that this thesis for the degree M.Sc. (Agirc) at the University of Pretoria has not been submitted by me for a degree at any other university.

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SUMMARY

The most important fatty acids in buffalo fat are C18:1, C18:0, C16:0 and C13:0. In the internal fat depots C18:1 was the most abundant fatty acid and was not influenced by age, gender or area. In subcutaneous fat (SCF) and muscle, C18:1 was the second most abundant fatty acid, since C13:0 was present in the highest proportions. Proportions of C18:1 increased with age and was higher in females than in males. The proportions of C13:0 was significantly higher in SCF and *M. Longissimus dorsi* (LD) than in the internal fat depots. It was highest in males, especially those from Mashatudrif at Lower Sabie (MLS) and Mpanamana dam at Crocodile Bridge (MD), with animals from MLS containing significantly higher proportions than those from Mtandanyathi at Houtboshrand (MH). Proportions of C18:0 increased with age and was highest in females and in buffalo sampled near MH. By contrast the proportion of C16:0 was higher in internal fat than SCF and LD, and highest in females. These results suggest that C16:0 is mobilised from the more labile energy stores, SCF and LD in all animals from MLS and MD during the depletion of adipose tissue due to poor nutritional status.

Keywords: long-chain fatty acids, African buffalo, age, gender, area, depot fat

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LIST OF ABBREVIATIONS

A	Buffalo calves and juveniles under 2 years of age
AMP	Adenosine monophosphate
AOAC	Association of Official Analytical Chemists
ATP	Adenosine triphosphate
B	Subadult buffalo between 2 and 6 years of age
BHT	Butylated hydroxy toluene
C	Adult buffalo older than 6 years of age
CO_2	Corbon dioxide
FAME	Fatty acid methyl ester
FFA	Free fatty acids
FMD	Foot and mouth disease
GC	Gas chromatograph
GLM	General linear Models
HDL	High-density-lipoprotein
KNP	Kruger National Park
LD	*M. Longissimus dorsi*
LDL	Low-density-lipoprotein
MD	Mpanamana dam at Crocodile Bridge
MH	Mtandanyathi at Houtboshrand
MLS	Mashatudrif at Lower Sabie
w/w %	Molar percentage
NADH	Nicotine adenine dinucleotide
NADPH	Nicotine adenine dinucleotide phosphate
NKW	Nasionale Krugerwildtuin
OHV	Onderhuidse vet
OIED	Onderstepoort Institute for Exotic Diseases
OMF	Omental fat
OMV	Omentum vet
OVI	Onderstepoort Veterinary Institute
OVS	Onversadigde vetsure
PCF	Pericardial fat
PKV	Perikardiale vet
PRF	Perirenal fat

PRV	Perirenale vet
PUFA	Polyunsaturated fatty acids
rpm	Revolutions per minute
SAS	Statistical Analysis System
SCF	Subcutaneous fat
SD	Standard deviation
SFA	Saturated fatty acids
TB	*Tuberculosis bovis*
TCA	Tricarboxylic acid cycle
UFA	Unsaturated fatty acids
VLDL	Very-low-density-lipoprotein
VVS	Versadigde vetsure

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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.

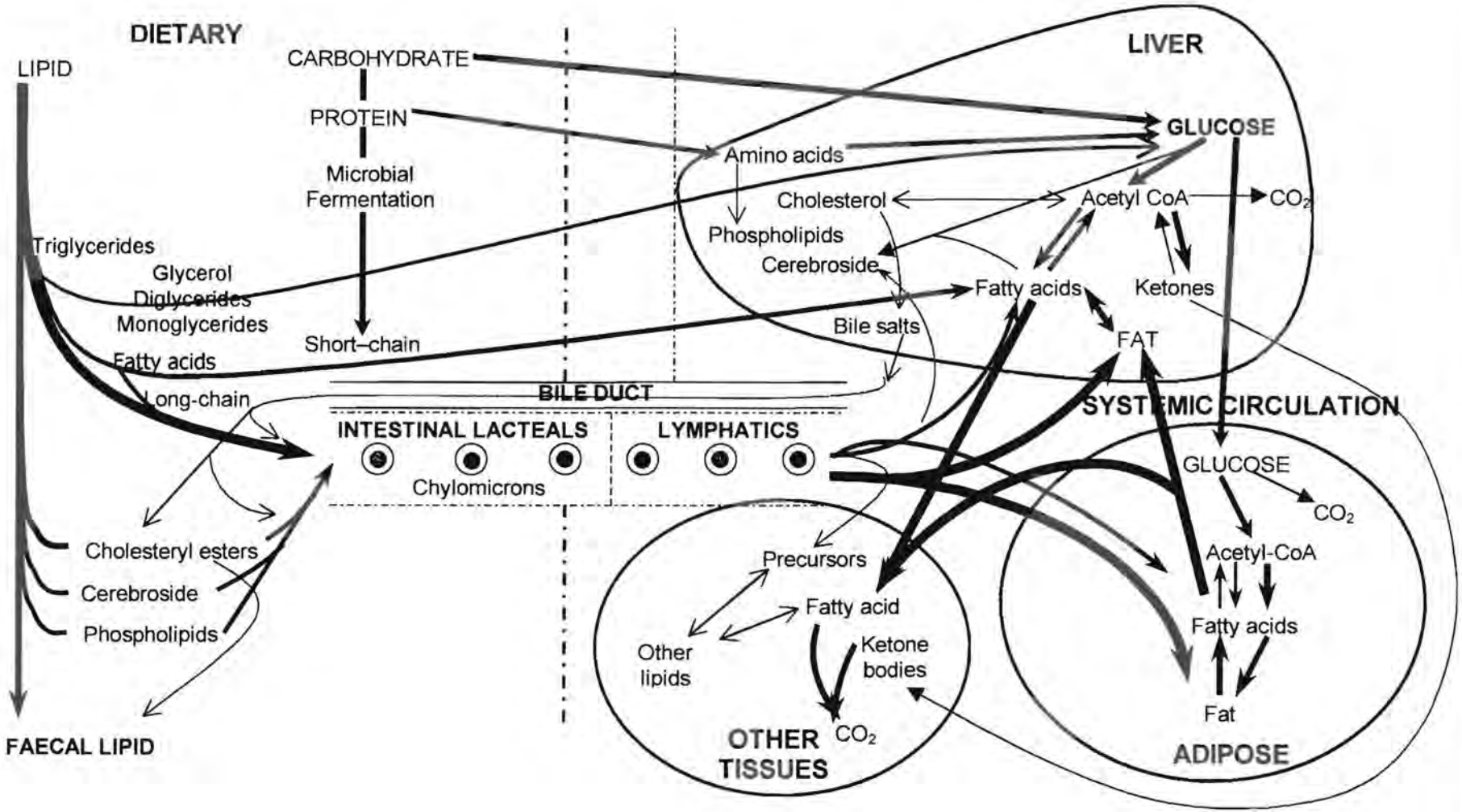
There is a close association between fat synthesis and active carbohydrate metabolism. Although changes in the level and composition of the diet influence the rate of fatty acid synthesis, it is directly, via the anabolic pathway, or indirectly by means of the throughput of substrates under hormonal 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.

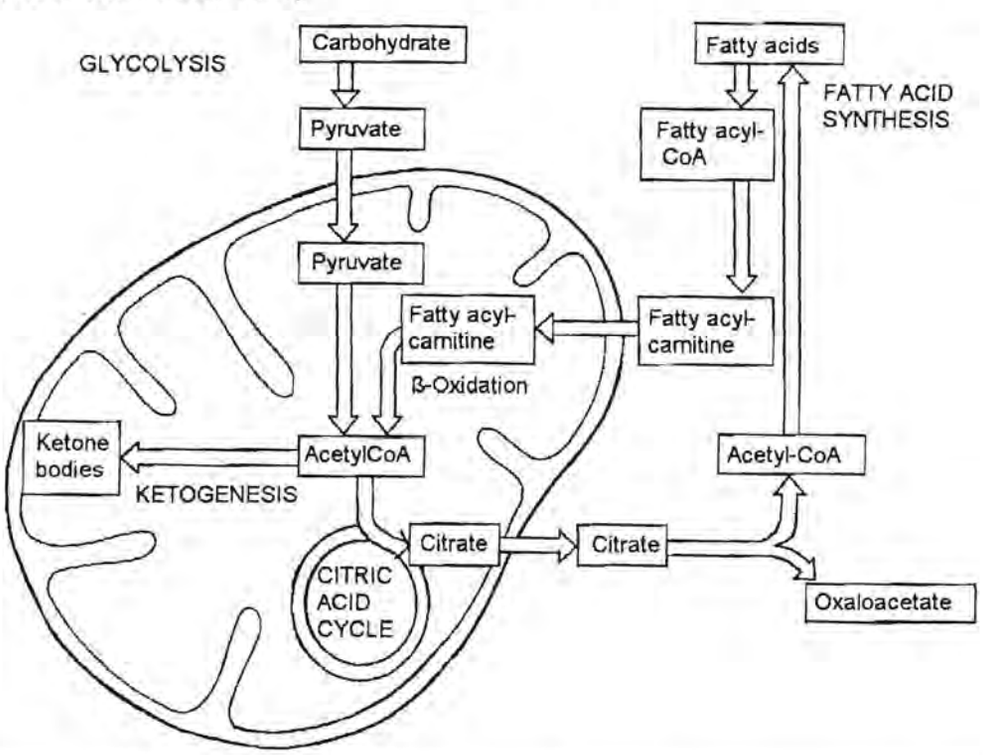
Fatty acids are normally synthesised by rumen microbes from glucose, but some fatty acids are synthesised *de novo* from short-chain fatty acids and are released and taken up by the animal, as the microorganisms themselves are digested (Noble, 1981). These fatty acids of both bacterial and protozoal 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*)

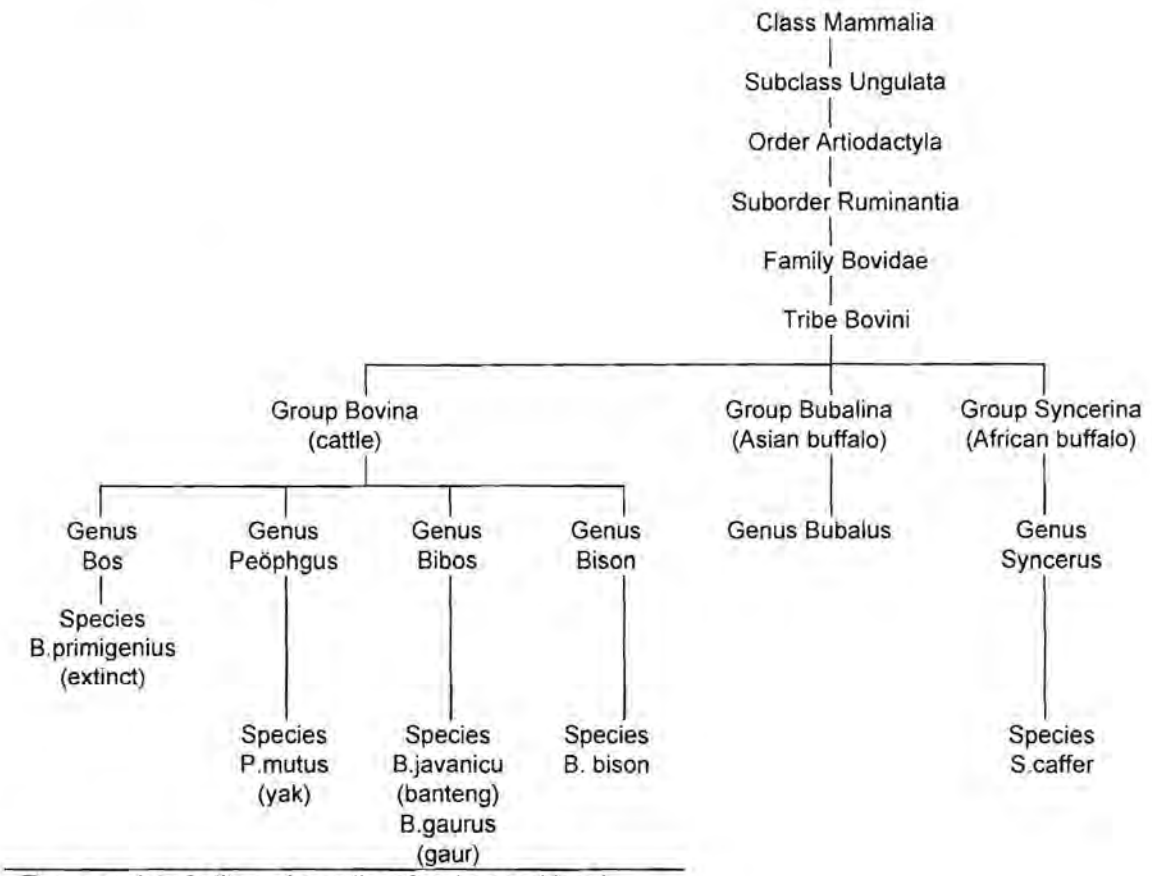
2.5.1 *General*

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.

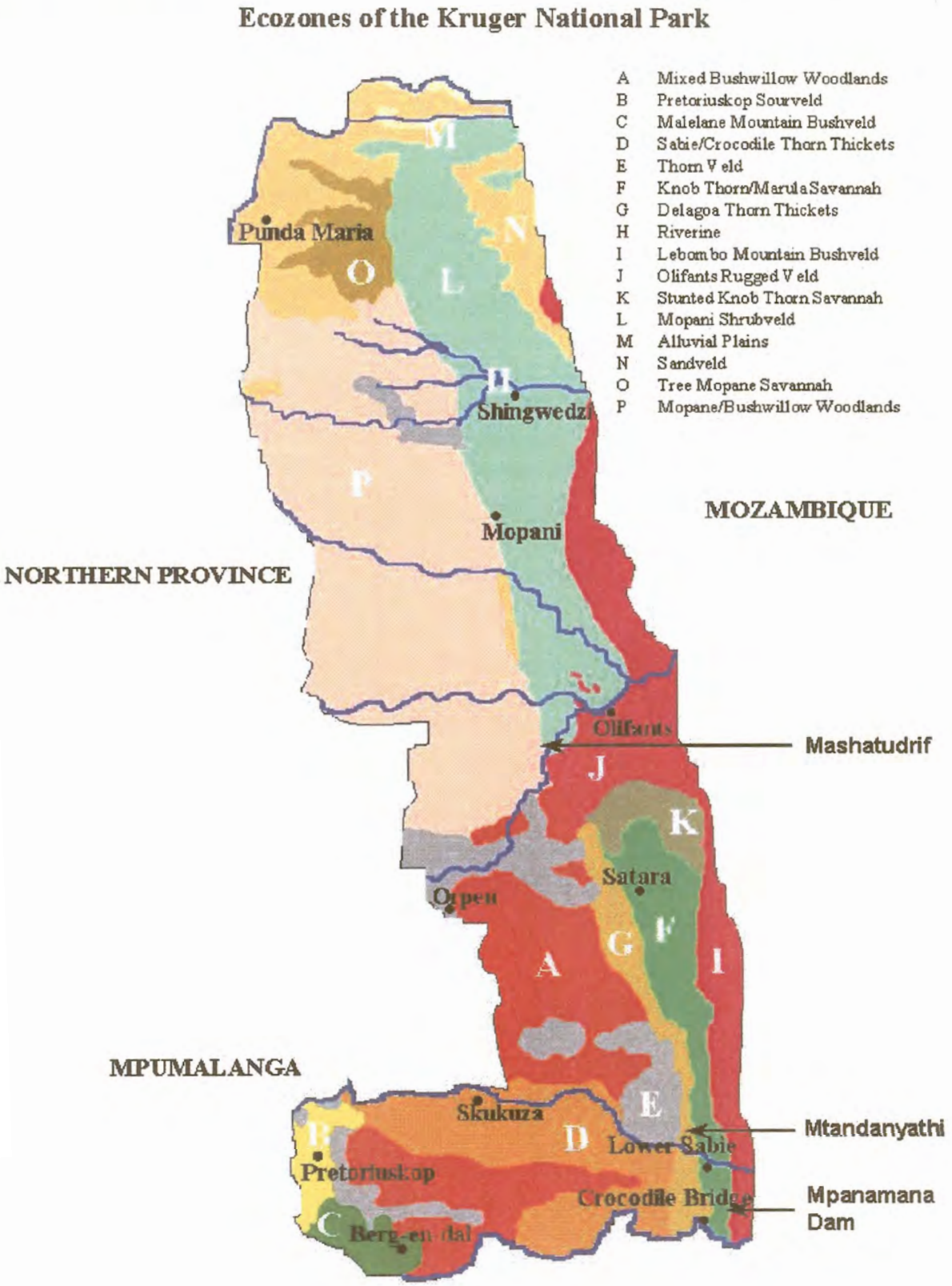
Table 2-2 Fatty acid content of depot lipids (% total fatty acids) of River buffalo and cattle carcasses (Adapted from de Francis and Moran, 1991).

	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).

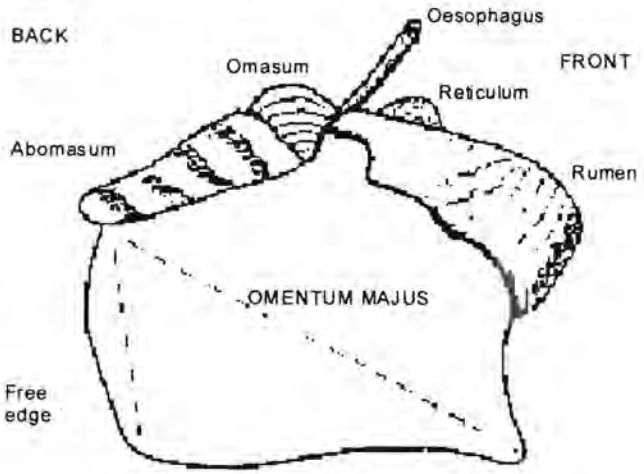
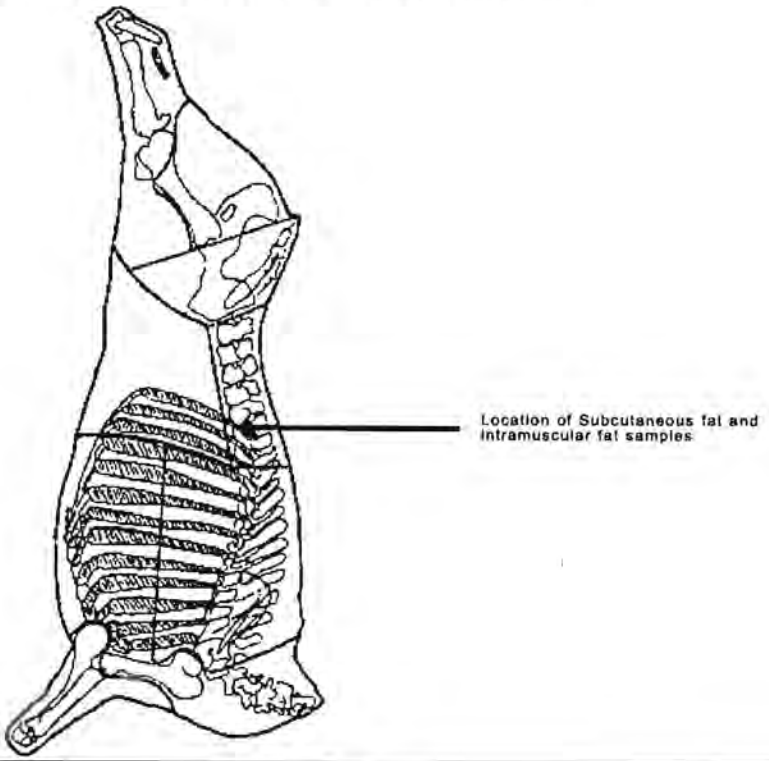


Figure 3-2 Sampling of subcutaneous fat on the carcass.



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.

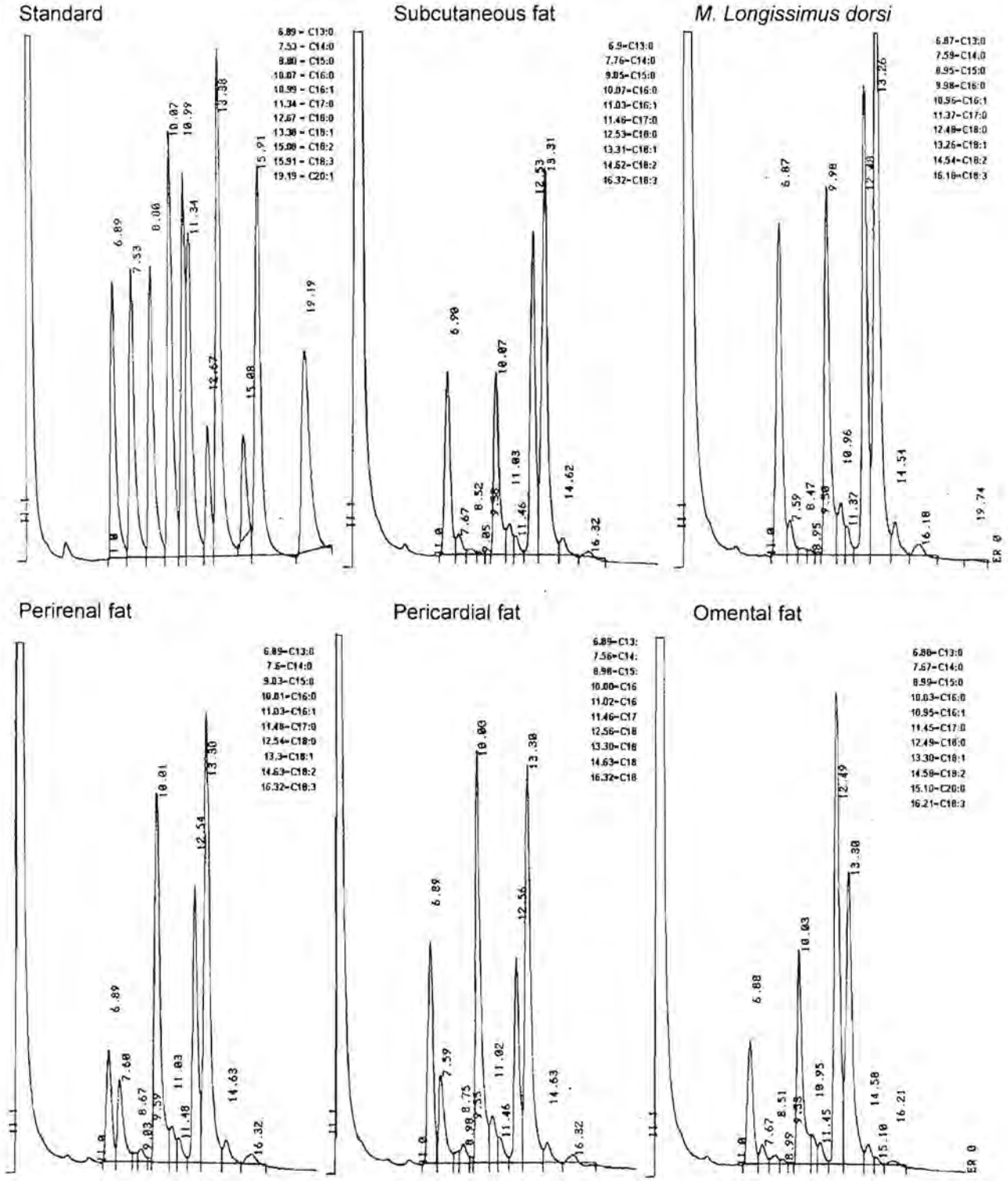
For the muscle samples, 4-5 g of the sample was used for long-chain fatty acid extraction as described for fat samples.

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.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 LONG-CHAIN FATTY ACIDS IN THE CARCASS FAT OF THE AFRICAN BUFFALO (*SYNCERUS CAFFER*)[1]

The most abundant fatty acids present in depot fat of African buffalo, in proportions higher than 10% of the total fatty acids present, were C18:1, C18:0, C16:0 and C13:0. The ranking order, differing between depots and sometimes also within a specific depot, depending on the age gender and habitat.

Table 4-1 Long-chain fatty acid composition (Mean±SD; w/w %) of the subcutaneous (SCF), M. Longissimus dorsi (LD), perirenal (PRF), pericardial (PCF), and omental (OMF) fat in the African buffalo.

(w/w %)	SCF (n = 36)	LD (n = 35)	PRF (n = 36)	PCF (n = 17)	OMF (n = 30)
C13:0	41.68^{A}±24.68	51.76^{A}±18.08	14.14^{B}±12.38	10.50^{B}±7.73	14.88^{B}±5.84
C14:0	3.13±1.20	2.10±1.48	28.03±26.12	3.29±1.64	15.68±22.86
C15:0	$0.61^{A,B}$±0.47	0.32^{B}±0.09	1.29^{A}±0.84	$0.52^{A,B}$±0.18	$1.10^{A,B}$±0.69
C16:0	11.91^{A}±5.14	$12.00^{A,C}$±4.06	19.54^{B}±7.84	$19.26^{B,C}$±7.36	19.27^{B}±5.92
C16:1	2.42^{A}±1.30	2.22^{A}±0.69	3.86^{B}±1.27	$3.10^{A,B}$±1.42	3.00^{A}±1.43
C17:0	1.22±0.80	1.03±0.47	2.11±0.16	2.11±0.50	2.20±0.04
C18:0	$14.30^{A,C}$±9.482	10.18^{A}±5.04	$17.86^{B,C}$±8.29	24.91^{B}±10.03	$19.65^{B,C}$±8.39
C18:1	$23.51^{A,C}$±10.33	19.28^{C}±7.47	28.69^{B}±8.42	$29.94^{A,B}$±3.85	$27.64^{A,B}$±6.00
C18:2	$1.94^{A,B}$±0.70	1.78^{B}±0.59	2.40^{A}±0.86	$2.17^{A,B}$±0.56	2.88^{A}±1.10
C18:3	$1.21^{A,B}$±0.68	0.83^{B}±0.35	1.74^{A}±0.37	1.76^{A}±0.90	$1.36^{A,B}$±0.62

$^{A, B, C}$ Means in the same row bearing different superscripts, differ (P<0.01)

C13:0 was present in the highest proportions in subcutaneous fat (SCF) and *M. Longissimus dorsi* (LD), followed by C18:1, C18:0 and C16:0 in SCF and C18:1, C16:0 and C18:0 in LD (Table 4-1). In all internal fat depots, the proportion of C18:1 was the highest in the pericardial (PCF) and omental fat (OMF), followed by C18:0, C16:0 and C13:0 and C16:0, C18:0 and C13:0 in the perirenal fat (PRF) (Table 4-1).

[1] Results presented at IX International Symposium on Ruminant Physiology, 1999 (Steenkamp *et al.*, 1999a).

Figure 4-1 Comparison of the long-chain fatty acid composition of different fat depots in the African buffalo.

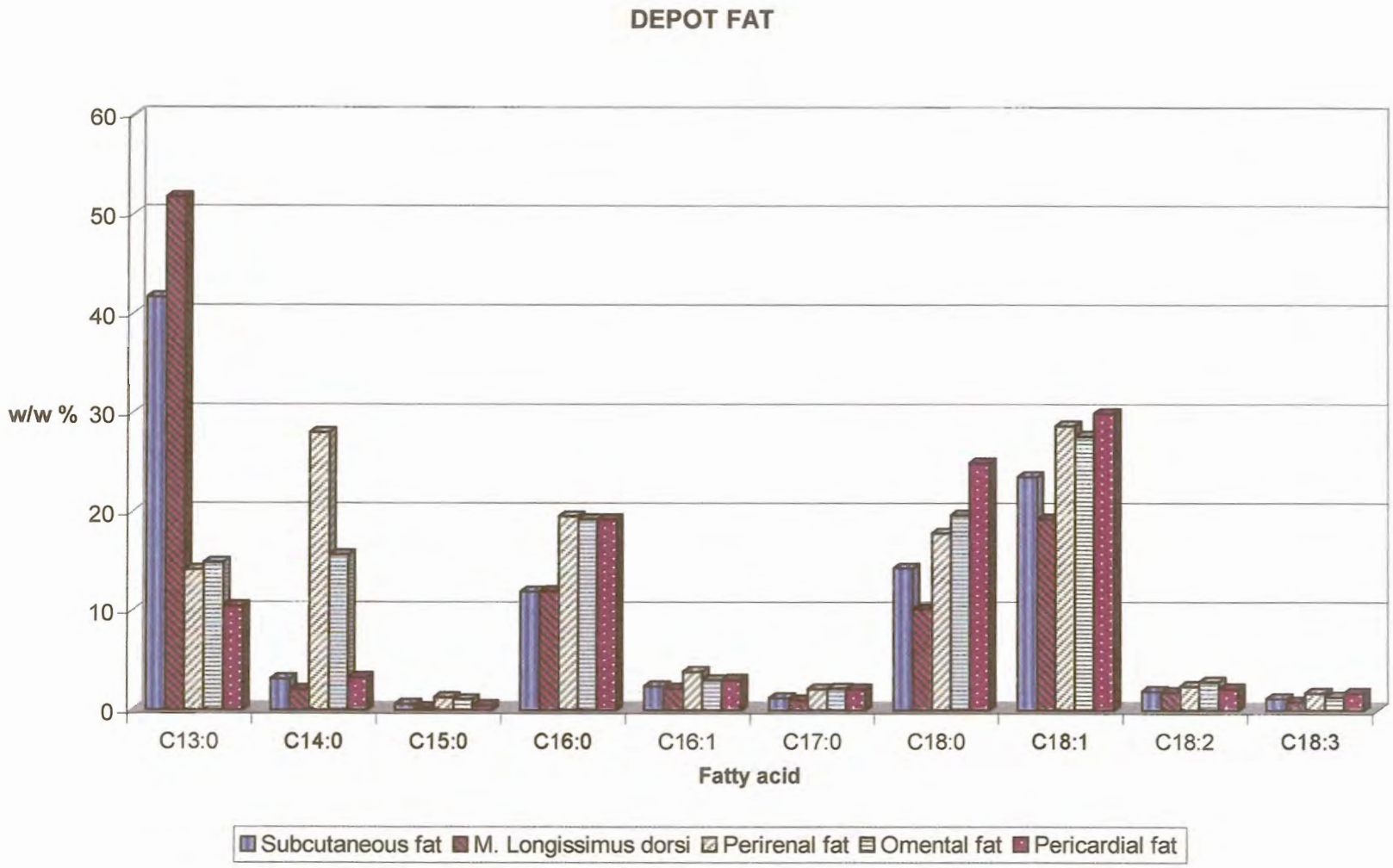
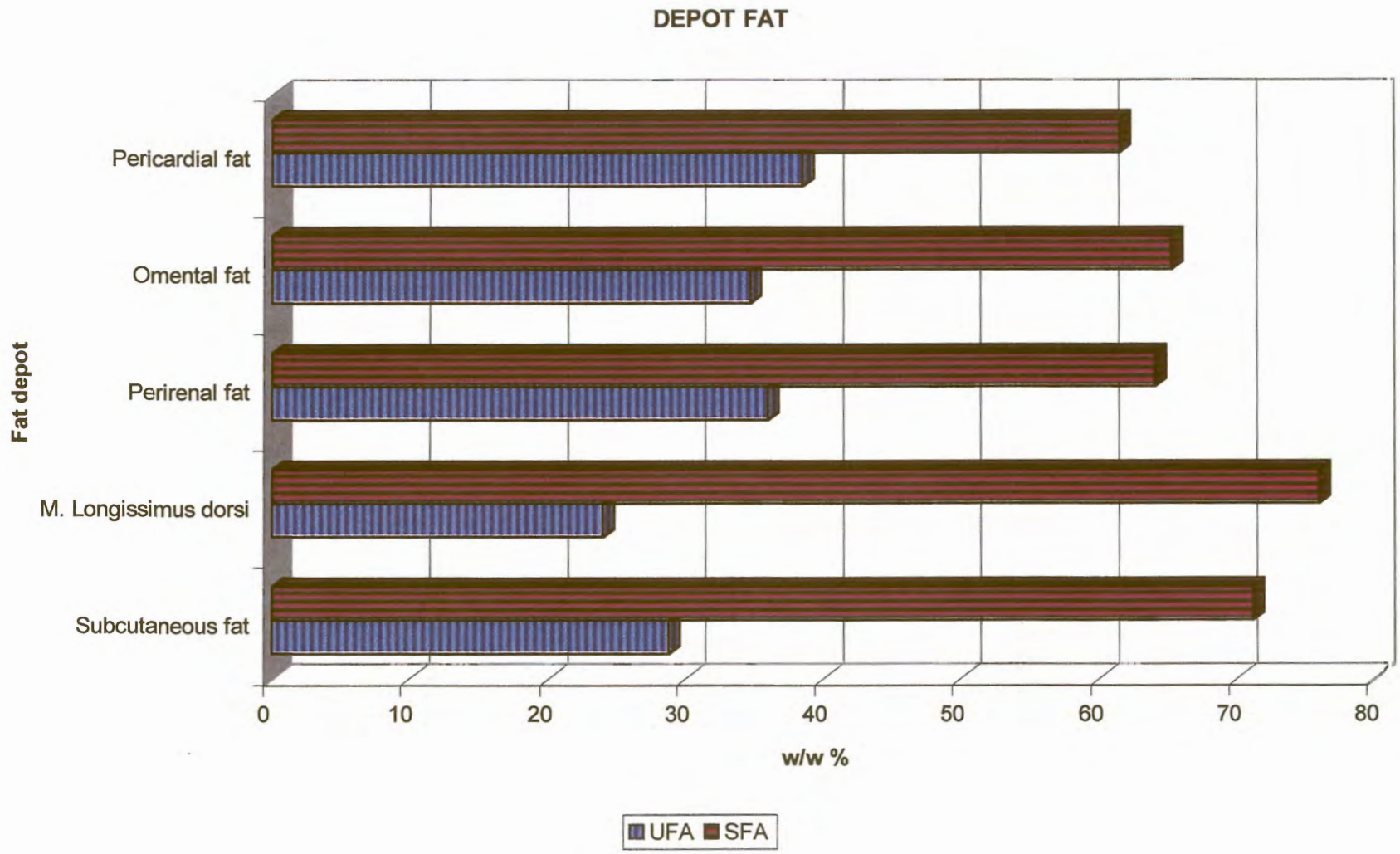


Figure 4-2 Differences between the proportions of total saturated and unsaturated long-chain fatty acids of different fat depots in the African buffalo.



C14:1 and C15:1 were detected in all fat depots, but in proportions lower than 1%. A small proportion of C20:1 (<1%) was detected only in LD, and C20:0 (<1%) only in the PRF and PCF. These are therefore not shown in Table 4-1.

In the literature, C13:0 is not usually listed in fatty acid profiles of ruminants and if listed (Link *et al.*, 1970; Kurbanov *et al.*, 1975) the proportion is generally lower than 1%. Possible reasons for the absence of C13:0 from previous reports may be that it was not present in the fatty acid standard as reference, due to very low proportions present, or the absence of C13:0 from the samples. Although it was difficult to separate C13:0 and C14:0, it was decided to include C13:0 in the report because of the prominent peak in all chromatograms (see results and Figure 2-3). The inclusion of C13:0 resulted in higher saturation levels of depot fat and relatively lower proportions of the other fatty acids, with the result that the proportions of fatty acids in fat depots from buffalo, can not accurately be compared to data from other ruminants (see Section 5.5 Critical Evaluation).

Highly significant differences ($P<0.01$) were found between the proportions of C13:0 in different depots. SCF and LD contained significantly higher proportions of C13:0 compared to PRF, PCF and OMF, indicated in Table 4-1. This corresponds with results of fat-tailed Karakul sheep reported by Kurbanov *et al.*, (1975) (internal fat: 0.27%; tail fat: 0.53%), although they found much lower proportions of C13:0.

PRF and OMF contained numerically higher proportions of C14:0 than the other fat depots (SCF, LD and PCF), although the differences were not statistically significant. This may be due to the problems encountered with the separation of C13:0 and C14:0, resulting in high standard deviations for C13:0 and C14:0 in PRF and OMF. Casey and van Niekerk (1985) also reported significantly lower proportions of C14:0 in SCF than in PRF in goats.

Table 4-2 Relative proportions (Mean±SD; w/w %) of the total saturated (SFA) and unsaturated (UFA) long-chain fatty acid content of the carcass fat in the African buffalo.

(w/w %)	UFA	SFA
Subcutaneous fat (SCF)	$28.77^{A,C}$±12.06	$71.15^{A,C}$±12.04
***M. Longissimus dorsi* (LD)**	23.97^{A}±8.63	75.94^{A}±8.63
Perirenal Fat (PRF)	35.96^{B}±11.00	64.04^{B}±11.01
Pericardial fat (PCF)	38.50^{B}±4.54	61.47^{B}±4.53
Omental fat (OMF)	$34.68^{B,C}$±7.29	$65.24^{B,C}$±7.22

$^{A, B, C}$ Means in the same column bearing different superscripts differ (P < 0.01)

The SFA and UFA content of the internal fat depots (PRF, OMF, PCF) did not differ significantly (P<0.01) (Table 4-2). The dominant fatty acids within the internal fat depots were C18:1, C18:0, C16:0 and C13:0 (Table 4-1). C16:1 differed significantly (P<0.01) between the PRF and OMF. The relative proportion of C16:1 in the PRF fat was higher (3.856±1.274%) than in omental fat (3.001±1.428%). The proportions of C16:1 in PCF (3.103±1.424%) did not differ significantly from either PRF or OMF. The proportions of C20:0 was less than 1% for all internal depots analysed.

SFA and UFA content of SCF and fat from the LD (Table 4-2) did not differ significantly (P<0.01) although the proportion of fatty acids in LD appeared to be more saturated compared to the SCF. The dominant fatty acids within SCF and LD were C13:0, C18:1, C18:0 and C16:0 respectively. No significant differences were found between the fatty acid composition of SCF and LD except for C18:1 being significantly lower in LD compared to SCF. This is in contrast to previous research where differences were found between individual fatty acids in SCF and LD (Zembayashi and Nishimura, 1996; Eichhorn *et al.*, 1985).

No significant difference was found between the proportions of SFA and UFA of SCF and OMF (P<0.01). OMF contained significantly higher proportions of C16:0 and lower proportions of C13:0 than SCF.

Table 4-2 Relative proportions (Mean±SD; w/w %) of the total saturated (SFA) and unsaturated (UFA) long-chain fatty acid content of the carcass fat in the African buffalo.

(w/w %)	UFA	SFA
Subcutaneous fat (SCF)	$28.77^{A,C}$±12.06	$71.15^{A,C}$±12.04
***M. Longissimus dorsi* (LD)**	23.97^{A}±8.63	75.94^{A}±8.63
Perirenal Fat (PRF)	35.96^{B}±11.00	64.04^{B}±11.01
Pericardial fat (PCF)	38.50^{B}±4.54	61.47^{B}±4.53
Omental fat (OMF)	$34.68^{B,C}$±7.29	$65.24^{B,C}$±7.22

$^{A, B, C}$ Means in the same column bearing different superscripts differ (P < 0.01)

The SFA and UFA content of the internal fat depots (PRF, OMF, PCF) did not differ significantly (P<0.01) (Table 4-2). The dominant fatty acids within the internal fat depots were C18:1, C18:0, C16:0 and C13:0 (Table 4-1). C16:1 differed significantly (P<0.01) between the PRF and OMF. The relative proportion of C16:1 in the PRF fat was higher (3.856±1.274%) than in omental fat (3.001±1.428%). The proportions of C16:1 in PCF (3.103±1.424%) did not differ significantly from either PRF or OMF. The proportions of C20:0 was less than 1% for all internal depots analysed.

SFA and UFA content of SCF and fat from the LD (Table 4-2) did not differ significantly (P<0.01) although the proportion of fatty acids in LD appeared to be more saturated compared to the SCF. The dominant fatty acids within SCF and LD were C13:0, C18:1, C18:0 and C16:0 respectively. No significant differences were found between the fatty acid composition of SCF and LD except for C18:1 being significantly lower in LD compared to SCF. This is in contrast to previous research where differences were found between individual fatty acids in SCF and LD (Zembayashi and Nishimura, 1996; Eichhorn *et al.*, 1985).

No significant difference was found between the proportions of SFA and UFA of SCF and OMF (P<0.01). OMF contained significantly higher proportions of C16:0 and lower proportions of C13:0 than SCF.

PCF contained significantly higher (P<0.01) proportions of C18:0 and C16:0 than SCF (Table 4-1). SCF was significantly (P<0.01) more saturated than PCF and PRF (Table 4-2). This is an unusual finding, as SCF are known to be more unsaturated than fat from internal fat depots of other ruminant species (Casey and van Niekerk, 1985; Webb *et al.*, 1998; Banskalieva, 1996). Animals were culled just after the dry season. The present results suggest that animals were in a poor body condition and that adipose reserves were mobilised to meet energy requirements for maintenance and other physiological conditions like lactation and growth during the dry season.

Significant differences (P<0.01) in the proportions of UFA and SFA were found between SCF and PRF depots (Table 4-1). This finding is in contrast with results obtained in a similar study on beef cattle (Webb *et al.*, 1998). SCF and PRF differed significantly (P<0.01) for the proportions of C13:0, C16:0, C16:1, C17:0 and C18:1 (Table 4-1). The PRF contained significantly higher proportions of C16:0, C16:1, C17:0 and C18:1 (P<0.01) than SCF (Table 4-1) and lower proportions of C13:0.

The fatty acid content of the LD differed significantly (P<0.01) from PRF (Table 4-2). LD was significantly more saturated than PRF. This is mainly due to the significantly higher proportions of C13:0 found in LD than in PRF. PRF, on the other hand, contained higher proportions of C15:0; C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3 than LD.

LD contained significantly more saturated fat than PCF (Table 4-2). Significant (P<0.01) differences were found in the proportions of C13:0, C18:0, C18:1 and C18:3. The proportions of C13:0 were generally higher (P<0.01) and C18:0, C18:1 and C18:3 lower in LD than in PCF (Table 4-1).

Highly significant differences (P<0.01) were found between the proportions of C13:0 in LD and OMF (Table 4-1). LD contained higher proportions of C13:0 than OMF resulting in

significantly higher proportions of total saturated fatty acids in LD than in OMF ($P<0.01$) (Table 4-2). The relative proportions of the other fatty acids did not differ significantly between the different depots.

4.2 INFLUENCE OF AGE[2]

4.2.1 *Subcutaneous fat*

The relative proportions of individual fatty acids in the subcutaneous fat of buffalo were influenced by age, but these differences did not significantly affect the overall proportion of saturated fatty acids. The proportion of C14:0 decreased significantly ($P<0.01$) from 6.896±6.622% in the A age (animals younger than 2 years of age) to 1.425±0.649% in the C age (animals older than 6 years of age).

A small, but significant ($P<0.05$) decrease with age was detected for the proportion of C18:3. The relative proportions of C18:0 ($P<0.001$) and C18:1 ($P<0.05$) were significantly lower in the A than the B or C ages.

Previous research (Banskalieva, 1996; Huerta-Leidenz *et al.*, 1996, Malau-Aduli *et al.*, 1997) on ruminants, suggest similar results for the proportions of C14:0, C18:1 and C18:3. For C18:0, contrasting results were reported. Banskalieva (1996) reported that C18:0 increase with age in sheep, while others (Huerta-Leidenz *et al.*, 1996; Malau-Aduli *et al.*, 1997) reported that C18:0 decrease with age in cattle. The reason for this may be that there are other, more important factors that influence the proportions of C18:0 e.g. energy balance of the animal (Vernon, 1981; Adrouni and Khachadurian, 1968) and diet (Casey and van Niekerk, 1985; Christie, 1981b; Banskalieva, 1996; Rumsey *et al.*, 1972; Wood *et al.*, 1991; Westerling and Hedrick, 1979). Saturated long-chain fatty acids are increasingly desaturated to unsaturated fatty acids with age (Banskalieva, 1996; Webb

[2] Results presented at IX International Symposium on Ruminant Physiology, 1999 (Steenkamp *et al.*, 1999b).

and Casey, 1995; Westerling and Hedrick, 1979; Zembayashi and Nishimura, 1996). Long-chain fatty acids are preferentially mobilised for physiological functioning compared to fatty acids of shorter chain length (Vernon, 1981). This may explain the decrease in the proportion of C13:0, although statistically insignificant, (Figure 4-3) and the relative increase of C18:0 and C18:1. Embleton and Leat, 1972 (as quoted by Christie, 1981b) reported that C18:0 and C16:0 increase linearly with age. Similar results were obtained in the present study, but C16:0 was not influenced to the same extend (Figure 4-3). This may be because the proportion of C16:0 is influenced by the energy balance of the animals and is a sensitive indicator of the mobilisation and synthesis of fatty acids (Vernon, 1981).

Table 4-3 The effect of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the long-chain fatty acid composition (Mean±SD; w/w %) of subcutaneous fat (SCF) in the African buffalo.

Age (w/w %)	A (< 2 years of age) (n = 12)	B (2 to 6 years of age) (n = 12)	C (> 6 years of age) (n = 12)
C13:0	39.97±24.87	43.97±24.09	27.41±20.70
C14:0	6.90^{A}±6.62	3.13±1.47	1.43^{B}±0.65
C14:1	6.87±0.18	0.73±0.09	0.67±0.27
C15:0	1.00±0.33	0.61±0.52	0.37±0.21
C15:1	0.22±0.04	0.20±0.05	0.17±0.12
C16:0	14.91±6.89	12.58±5.18	12.18±3.17
C16:1	3.35±1.64	2.59±1.66	2.30±0.64
C17:0	1.12±0.38	0.84±0.63	1.39±0.57
C18:0	9.76^{A}±4.48	12.06^{B}±7.04	21.14^{B}±8.72
C18:1	22.33^{a}±8.61	22.51^{b}±9.71	31.07^{b}±8.84
C18:2	1.96±0.87	2.09±0.67	2.27±0.31
C18:3	1.66^{a}±0.49	1.09^{b}±0.59	1.15±0.40
UFA	28.50±11.51	28.18±11.95	36.65±10.00
SFA	71.50±11.51	71.62±11.74	63.30±10.04

^{a,b} Means in the same row bearing different superscripts differ (P<0.05)
^{A,B} Means in the same row bearing different superscripts differ (P<0.01)

Figure 4-3 The effect of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the long-chain fatty acid composition of subcutaneous fat in the African buffalo.

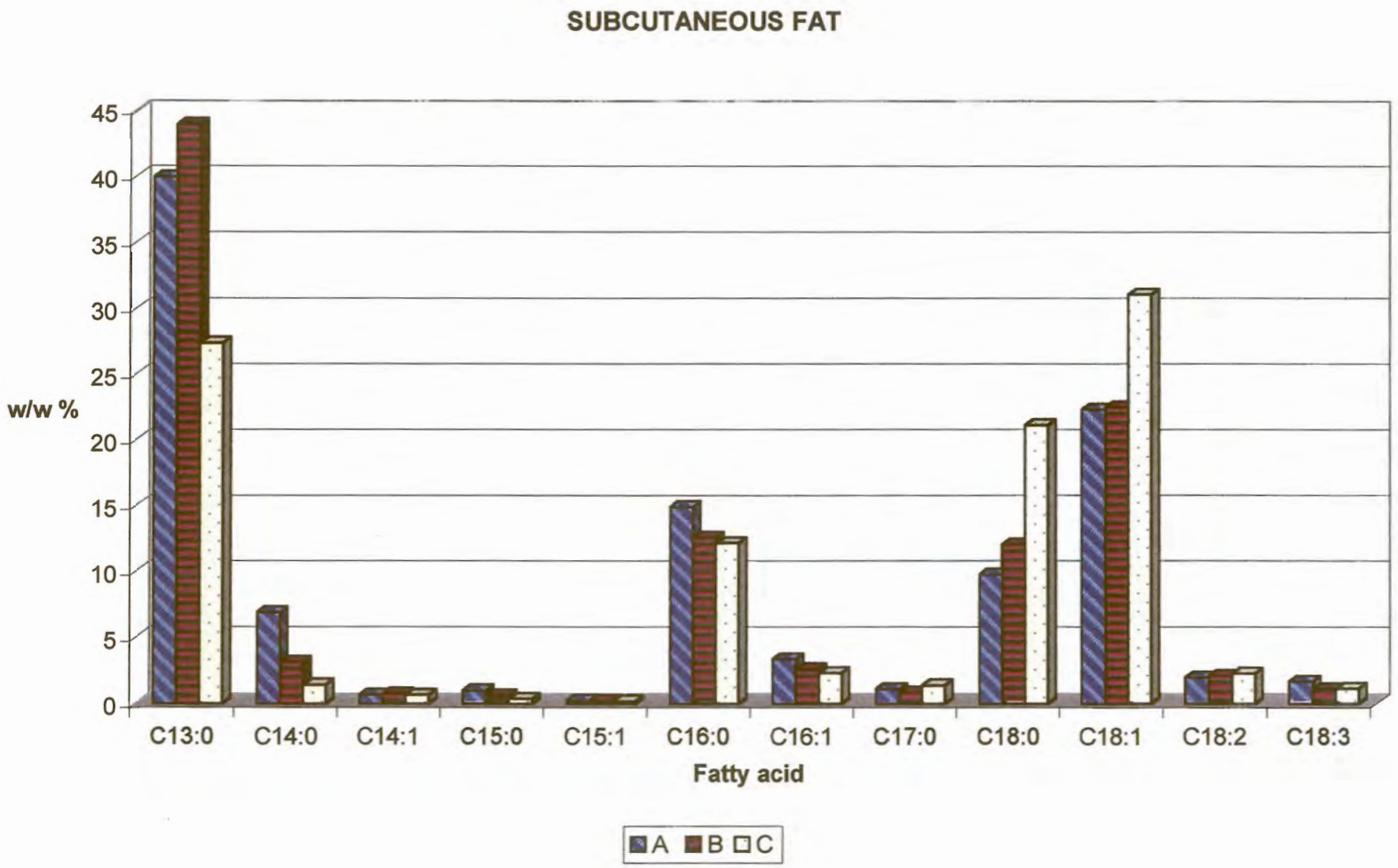
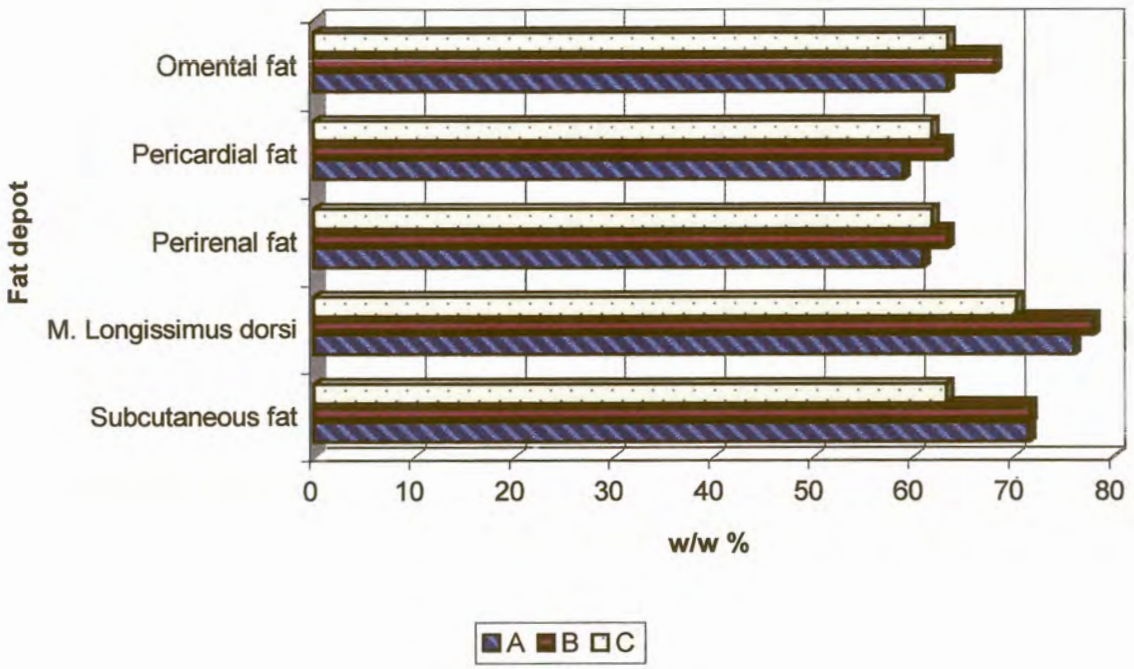
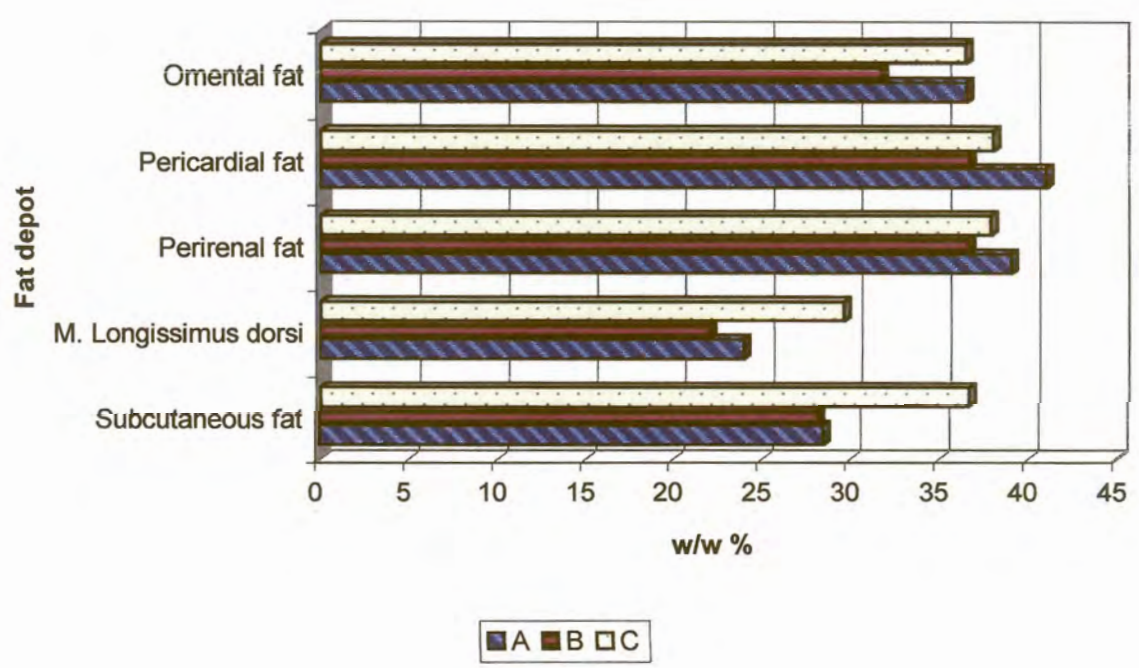


Figure 4-4 The effect of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the proportions of (a) total saturated and (b) total unsaturated long-chain fatty acids of different fat depots in the African buffalo.

a) SATURATED FATTY ACIDS



b) UNSATURATED FATTY ACIDS



4.2.2 *M. Longissimus dorsi*

The most abundant fatty acids in LD at all ages were C13:0, followed by C18:1, C16:0 and C18:0 (Figure 4-5). The proportions of total saturated fatty acids in LD changed with age (Table 4-4 and Figure 4-4). This is in agreement with previous results that reported the proportions of unsaturated fatty acids of carcass lipids (subcutaneous and intramuscular) to increase with age (or carcass fatness) (Zembayashi and Nishimura, 1996).

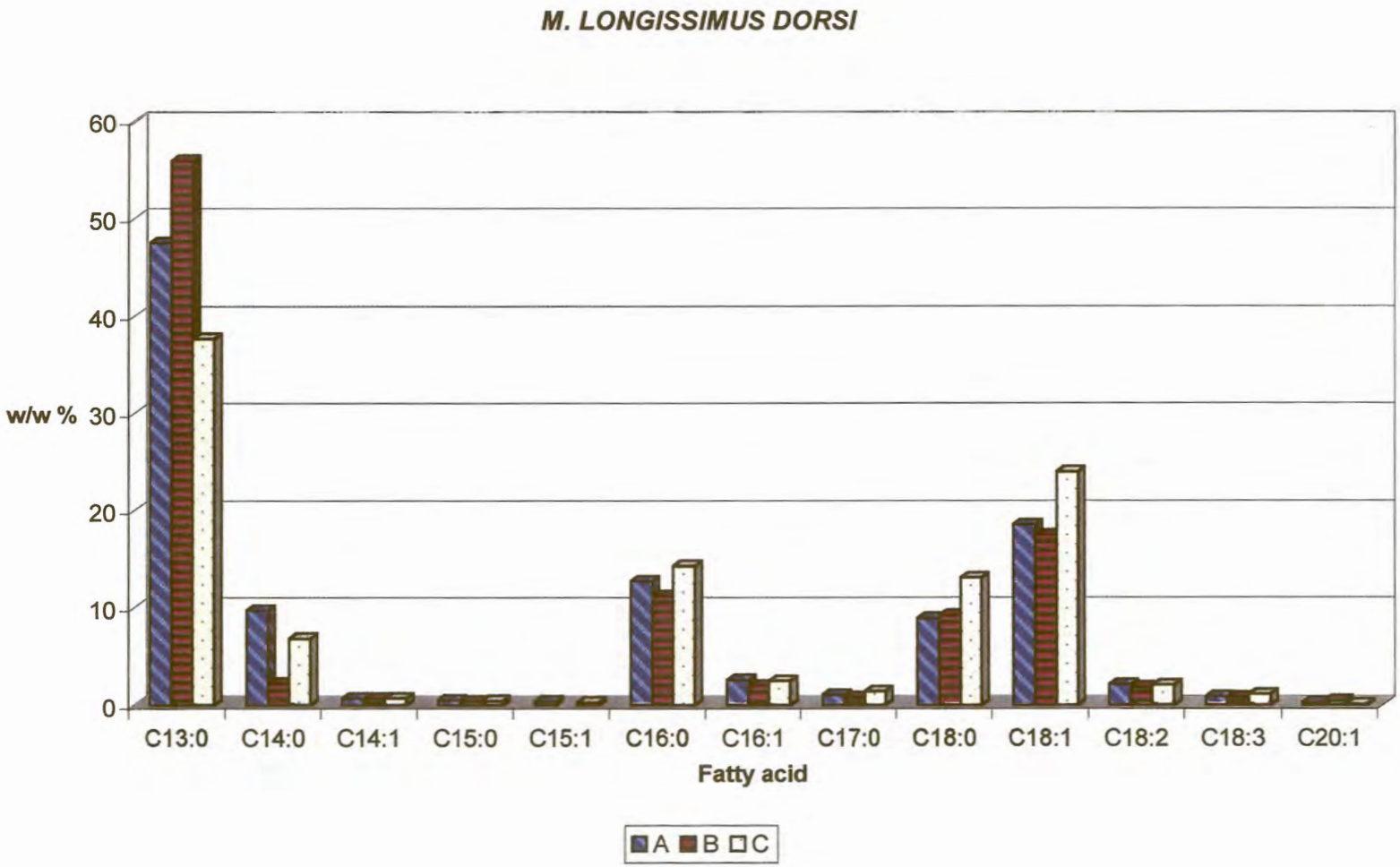
Table 4-4 The effect of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the long-chain fatty acid composition (Mean±SD; w/w %) of M. Longissimus dorsi (LD) in the African buffalo.

Age (w/w %)	A (< 2 years of age) (n = 12)	B (2 to 6 years of age) (n = 11)	C (> 6 years of age) (n = 12)
C13:0	47.50±15.83	55.89^{A}±13.00	37.68^{B}±21.98
C14:0	9.67±17.79	2.23±1.45	6.82±11.43
C14:1	0.70±00.32	0.71	0.72±0.18
C15:0	0.527±0.40	0.40±0.12	0.45±0.14
C15:1	0.37		0.28±0.20
C16:0	12.68±3.97	11.16±3.09	14.24±6.04
C16:1	2.56±0.46	2.00±1.00	2.47±1.11
C17:0	1.06±0.70	0.84±0.37	1.42±0.17
C18:0	8.88^{a}±5.16	9.18±3.11	13.04^{b}±6.15
C18:1	18.53±5.72	17.47^{a}±4.78	23.92^{b}±10.05
C18:2	2.13±1.23	1.85±0.59	2.07±0.81
C18:3	0.89±0.53	0.79±0.35	1.13±0.22
C20:1	0.31±0.21	0.43±0.26	0.11±0.02
UFA	23.90±7.19	21.98^{a}±6.25	29.62^{b}±11.99
SFA	75.99±7.23	77.90^{a}±6.22	70.35^{b}±12.02

^{a,b} Means in the same row bearing different superscripts differ (P<0.05)
^{A,B} Means in the same row bearing different superscripts differ (P<0.01)

More recent research (Webb *et al.*, 1998) indicate that the monounsaturated fatty acids increase, and that the polyunsaturated fatty acids, mainly part of the polar lipids, decrease with fatness. Animals older than 6 years (C age) had significantly more unsaturated fat (Figure 4-4b) than younger animals (P<0.05) as seen in Table 4-4.

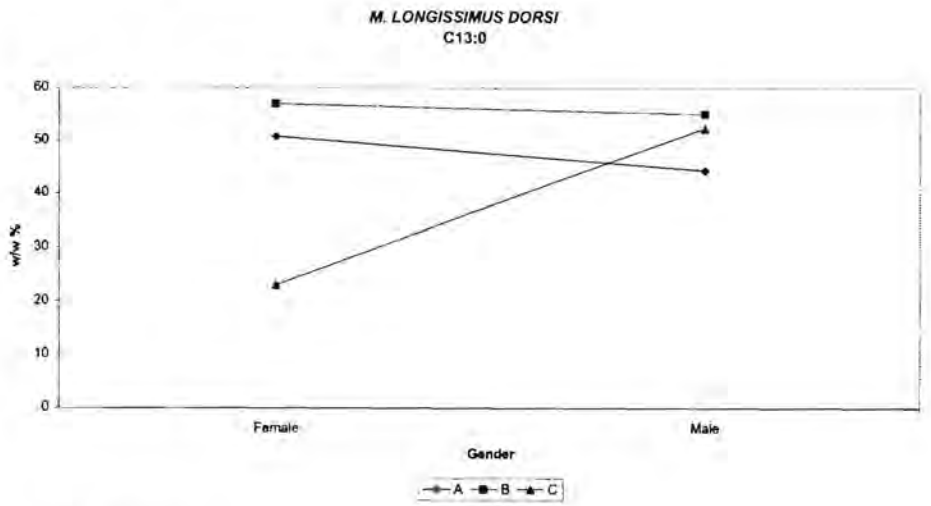
Figure 4-5 The effect of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the long-chain fatty acid composition of M. Longissimus dorsi in the African buffalo.



Significant interactions were found between age and gender for the proportions of C13:0, C18:0 and C18:1 and the total proportion of saturated fatty acids in LD (Addendum II). In male buffalo, the proportion of C13:0 increased with age (Figure 4-6), while C18:0 remained fairly constant (Figure 4-7) and C18:1 decreased slightly with age (Figure 4-8).

Figure 4-6 The effect of a) gender (male and femlae) and b) age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the proportion of C13:0 in M. Longissimus dorsi of the African buffalo (P<0.05).

a) Effect of gender



b) Effect of age

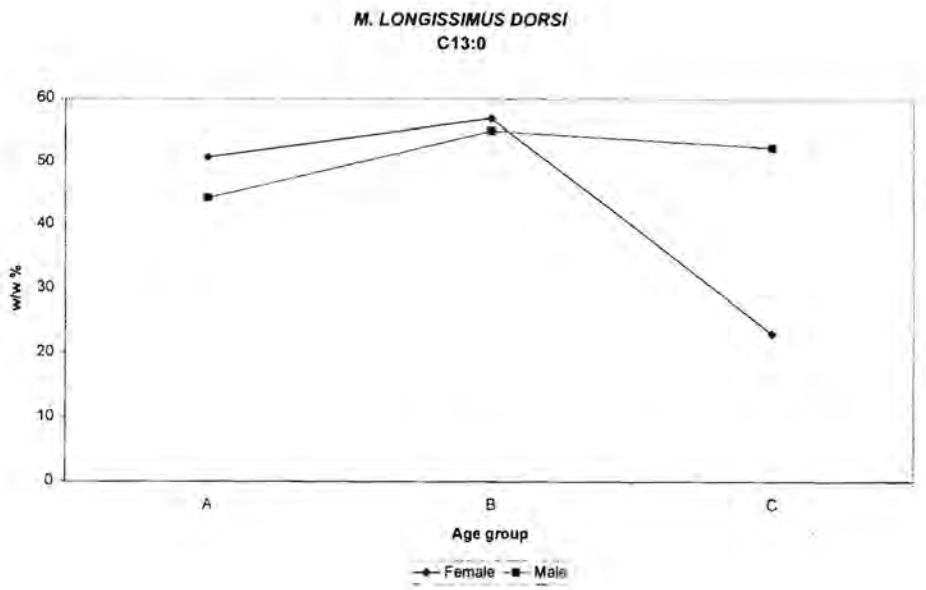
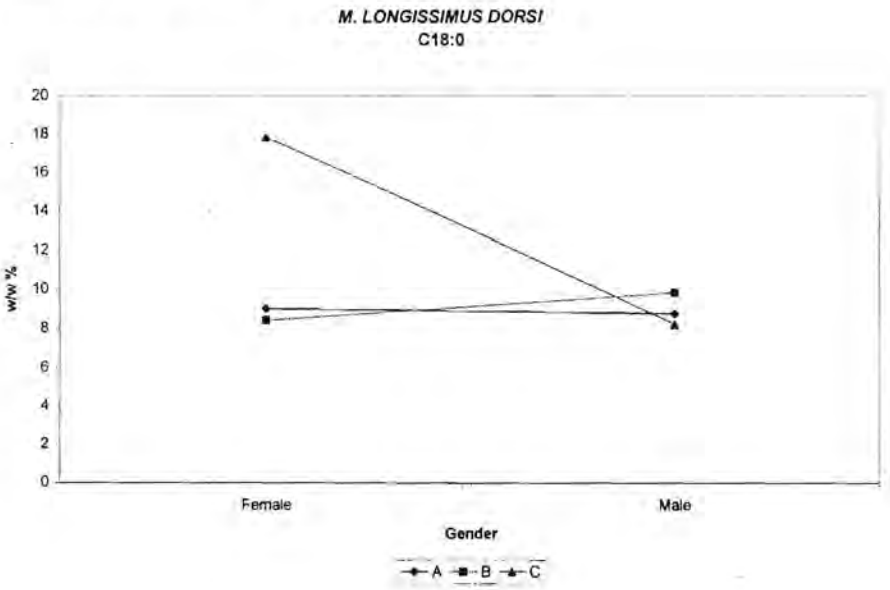
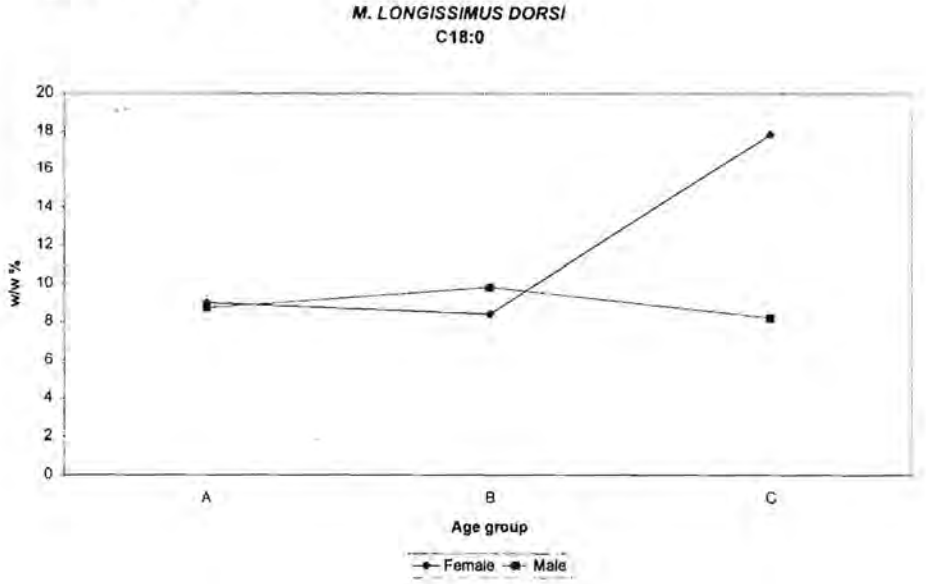


Figure 4-7 The effect of a) gender (male and female) and b) age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the proportion of C18:0 in M. Longissimus dorsi of the African buffalo (P<0.05).

a) Effect of gender



b) Effect of age

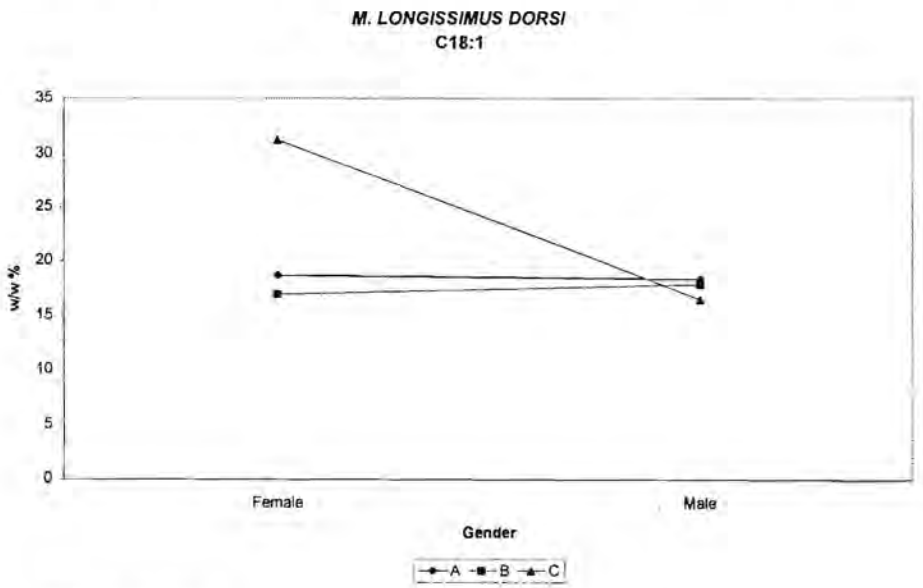


The relative proportions of fatty acids did not differ significantly between male and female buffalo within the A and B age groups. By contrast, female buffalo in the C age group, contained significantly higher proportions of C18:0 and C18:1 but lower proportions of C13:0, resulting in significantly higher proportions of UFA (P<0.05). The relative proportions of total saturated fatty acids in male buffalo increased slightly with age (Figure 4-9). Nürnberg *et al.*, 1996 (as quoted by Nürnberg *et al.*, 1998) found that saturated fatty

acids in lamb muscle increase with age. The current finding that adult (age C) females have more unsaturated fatty acids, may be due to physiological conditions, because most of the animals in the C age were either pregnant and/or lactating.

Figure 4-8 The effect of a) gender (male and female) and b) age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the proportion of C18:1 in M. Longissimus dorsi of the African buffalo (P<0.05).

a) Effect of gender



b) Effect of age

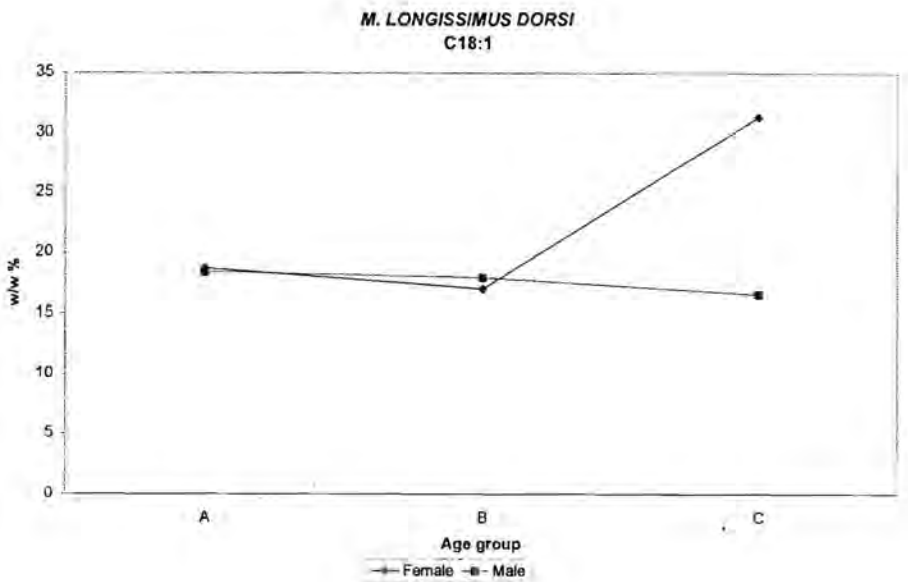
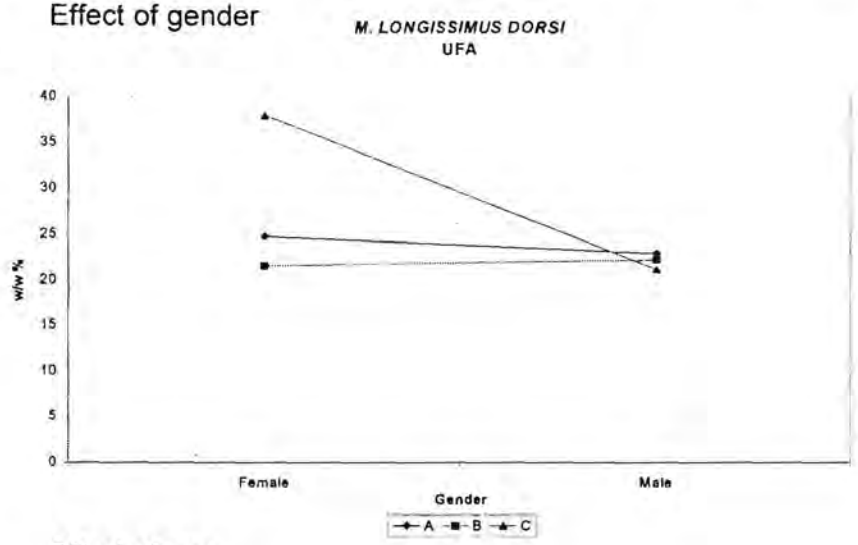
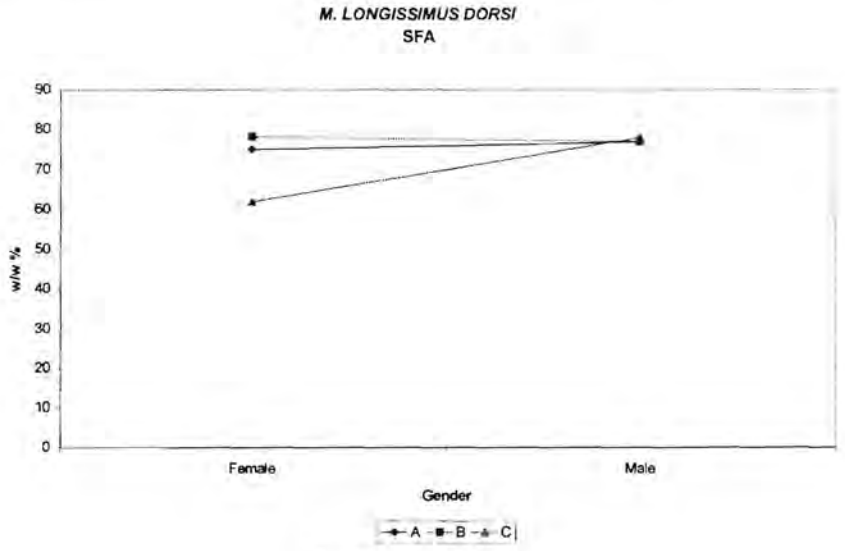


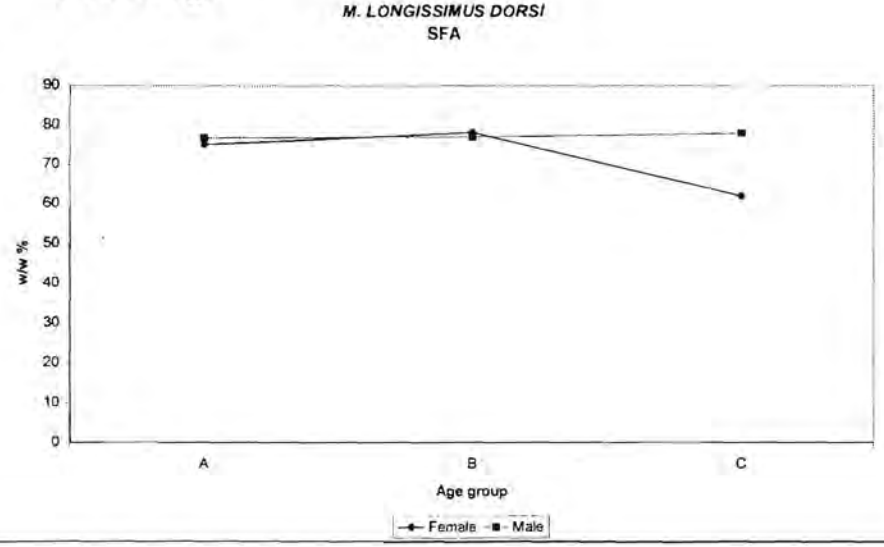
Figure 4-9 The effect of a) gender (male and female) and b) age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the saturation level of M. Longissimus dorsi in the African buffalo (P<0.05).

a) Effect of gender





b) Effect of age



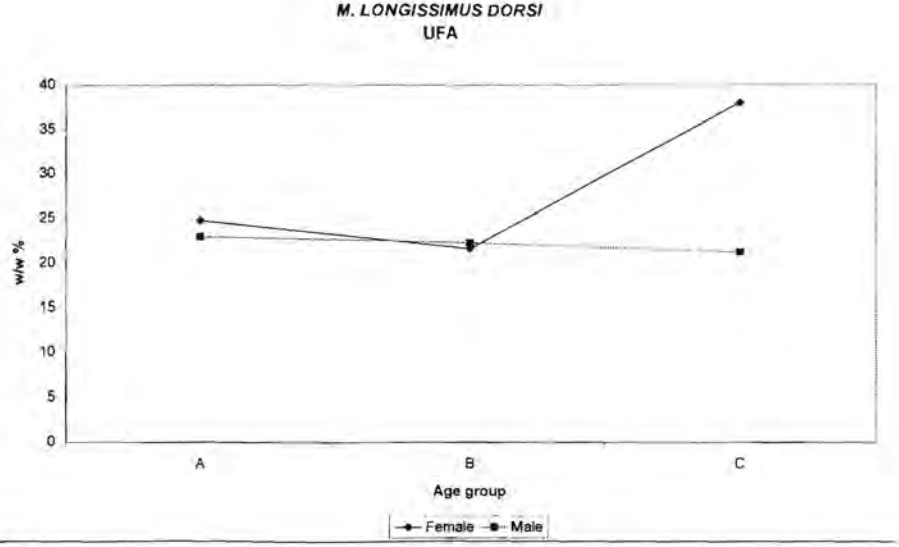
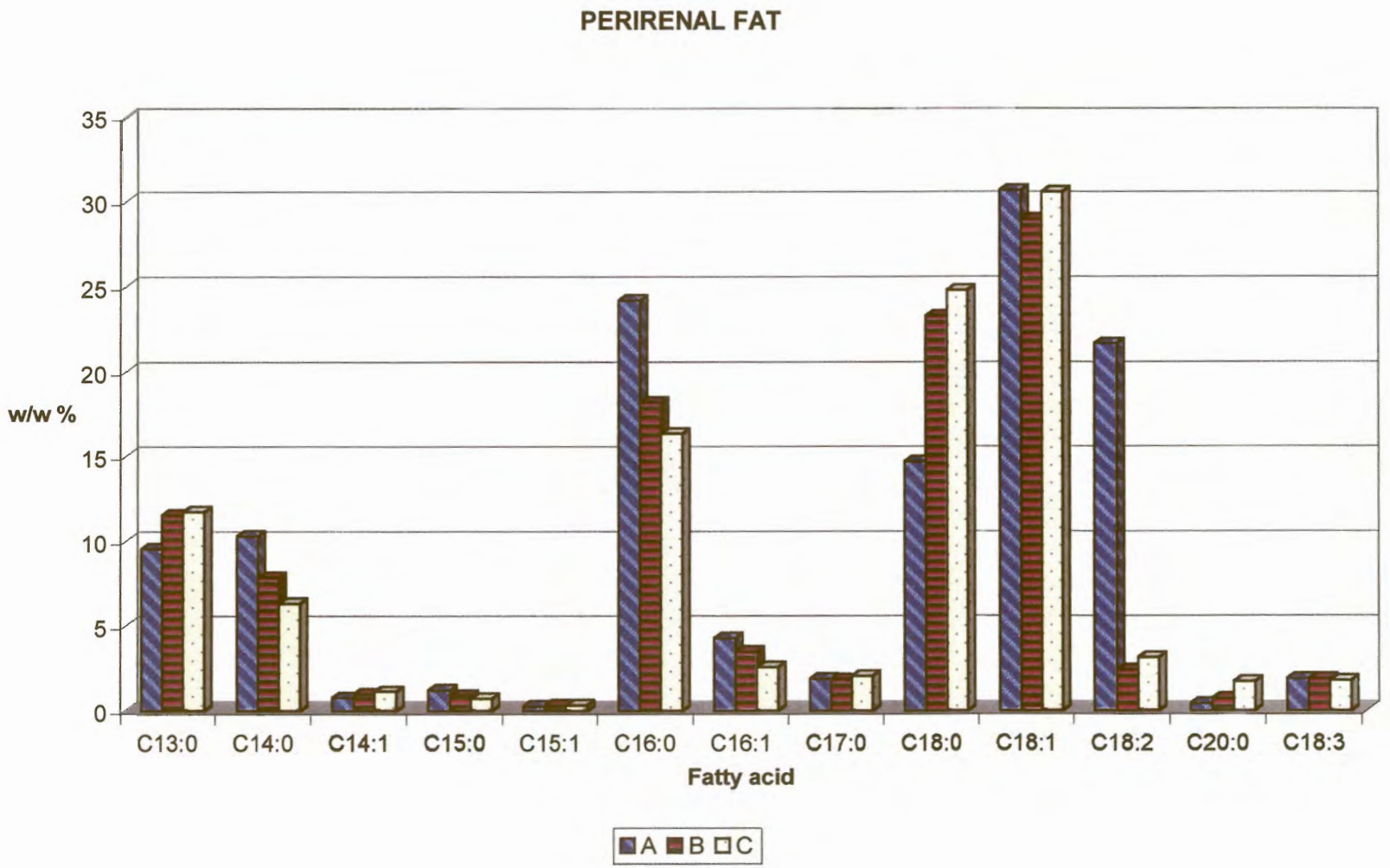


Figure 4-10 The effect of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the long-chain fatty acid composition of perirenal fat in the African buffalo.



4.2.3 *Internal fat*

In perirenal fat (PRF) of buffalo calves, C18:1 was the most abundant fatty acid, followed by C16:0, C18:0 and C13:0 (Figure 4-11). In subadult buffalo (B age group), C18:0 was the most abundant long-chain fatty acid, followed by C18:1 and in adult animals (C age group), C18:1 was the most abundant followed by C18:0 (Table 4-5). C16:0 and C13:0 were the other two important fatty acids (Table 4-5). The level of saturation of the perirenal fat did not change with age, but differences were noted between specific fatty acids (Table 4-5). The relative proportions of C15:0, C16:0 and C16:1 were significantly higher ($P<0.01$) in the A age group than the C group, while the proportions of C18:0 increased significantly ($P<0.01$) with age (Figure 4-10). Banskalieva (1996) also reported similar changes in C14:0, C16:0 and C18:0 with age. The proportions of C20:0 in PRF and PCF were generally lower than 1%.

Table 4-5 The effect of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the long-chain fatty acid composition (Mean±SD; w/w %) of perirenal fat (PRF) in the African buffalo.

Age (w/w %)	A (< 2 years of age) (n = 12)	B (2 to 6 years of age) (n = 12)	C (> 6 years of age) (n = 12)
C13:0	9.560±7.672	11.541±9.725	11.751±12.031
C14:0	10.313±14.139	7.842±12.707	6.321±8.909
C14:1	0.774±0.266	0.984±0.192	1.134±0.268
C15:0	1.204^{A}±0.513	0.884±0.256	0.734^{B}±0.185
C15:1	0.265±0.039	0.324±0.026	0.320±0.071
C16:0	24.191^{A}±7.002	$18.202^{A,B}$±6.646	16.345^{B}±4.333
C16:1	4.248^{a}±1.748	3.468±1.087	2.548^{b}±0.780
C17:0	1.862±0.279	1.850±0.257	2.019±0.545
C18:0	14.697^{A}±5.132	23.284^{A}±8.930	24.785^{B}±6.610
C18:1	30.686±7.659	28.976±7.542	30.578±4.703
C18:2	2.167±0.543	2.390±0.719	3.102±1.998
C20:0	0.421	0.737±0.246	1.705±1.099
C18:3	1.859±0.462	1.868±0.390	1.777±0.732
UFA	39.014±9.894	36.611±9.866	37.892±6.133
SFA	60.986±9.894	63.381±9.870	61.916±6.137

^{a,b} Means in the same row bearing different superscripts differ ($P<0.05$)
^{A,B} Means in the same row bearing different superscripts differ ($P<0.01$)

The three most abundant fatty acids within PCF and OMF were C18:1, C18:0 and C16:0 (Table 4-6 and Table 4-7). C13:0 also comprised a significant portion of OMF (Table 4-7).

This may be because dietary fatty acids are reflected in the omental fat (Bas *et al.*, 1992). The proportion of total saturated fatty acids in omental and pericardial fat did not change with age (Figure 4-4, Table 4-6 and Table 4-7), but differences were noted between the proportions of specific fatty acids (Table 4-6 and Figure 4-11; Table 4-7 and Figure 4-12).

Table 4-6 The effect of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the long-chain fatty acid composition (Mean±SD; w/w %) of pericardial fat (PCF) in the African buffalo.

Age (w/w %)	A (< 2 years of age) (n = 5)	B (2 to 6 years of age) (n = 5)	C (> 6 years of age) (n = 7)
C13:0	7.937±3.747	12.092±9.880	11.204±8.753
C14:0	4.798±2.714	3.578±2.063	4.217±5.895
C14:1	0.811±0.216	1.065±0.048	0.095±0.173
C15:0	1.403^{A}±0.753	1.186±0.670	0.643^{B}±0.331
C15:1	0.304±0.035	0.230±0.086	0.289±0.090
C16:0	23.921^{a}±8.558	20.701±8.386	14.904^{b}±2.596
C16:1	3.687^{A}±1.289	3.466±1.831	2.461^{B}±0.381
C17:0	2.021±0.313	1.921±0.508	2.013±0.263
C18:0	19.151±10.078	25.023±8.934	28.943±10.022
C18:1	32.091±4.380	28.740±2.961	29.271±3.911
C18:2	2.269±0.791	2.152±0.610	3.261±3.033
C20:0	0.869	0.766	0.808±0.240
C18:3	2.205±1.018	1.414±0.613	2.728±3.136
UFA	40.981±5.332	36.649±5.020	38.038±3.214
SFA	59.001±5.306	63.316±5.014	61.914±3.324

^{a,b} Means in the same row bearing different superscripts differ (P<0.05)
^{A,B} Means in the same row bearing different superscripts differ (P<0.01)

Table 4-7 The effect of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the long-chain fatty acid composition (Mean±SD; w/w %) of omental fat (OMF) in the African buffalo.

Age (w/w %)	A (< 2 years of age) (n = 10)	B (2 to 6 years of age) (n = 9)	C (> 6 years of age) (n = 11)
C13:0	15.598±15.923	17.346±7.597	15.257±6.880
C14:0	8.799±10.024	10.729±17.067	5.417±7.887
C14:1	0.815±0.171	0.955±0.186	0.870±0.324
C15:0	1.071^{A}±0.514	0.827±0.231	0.607^{B}±0.223
C15:1	0.316±0.032	0.312±0.034	0.345±0.081
C16:0	22.441^{a}±5.648	16.561^{b}±6.538	18.052±2.972
C16:1	3.144±0.978	2.370±1.440	2.653±1.081
C17:0	1.811±0.212	1.790±0.354	1.748±0.394
C18:0	15.221±4.556	21.873±7.829	23.265±7.281
C18:1	28.845±6.702	24.991±5.858	29.270±3.140
C18:2	2.364±0.594	3.564±1.611	3.387±1.100
C18:3	1.610^{a}±0.617	1.021^{b}±0.456	1.049±0.409
UFA	36.512±7.773	31.721±7.876	36.452±4.011
SFA	63.453±7.795	68.148±7.783	63.532±4.015

^{a,b} Means in the same row bearing different superscripts differ (P<0.05)
^{A,B} Means in the same row bearing different superscripts differ (P<0.01)

Figure 4-11 The influence of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the long-chain fatty acid composition of pericardial fat in the African buffalo.

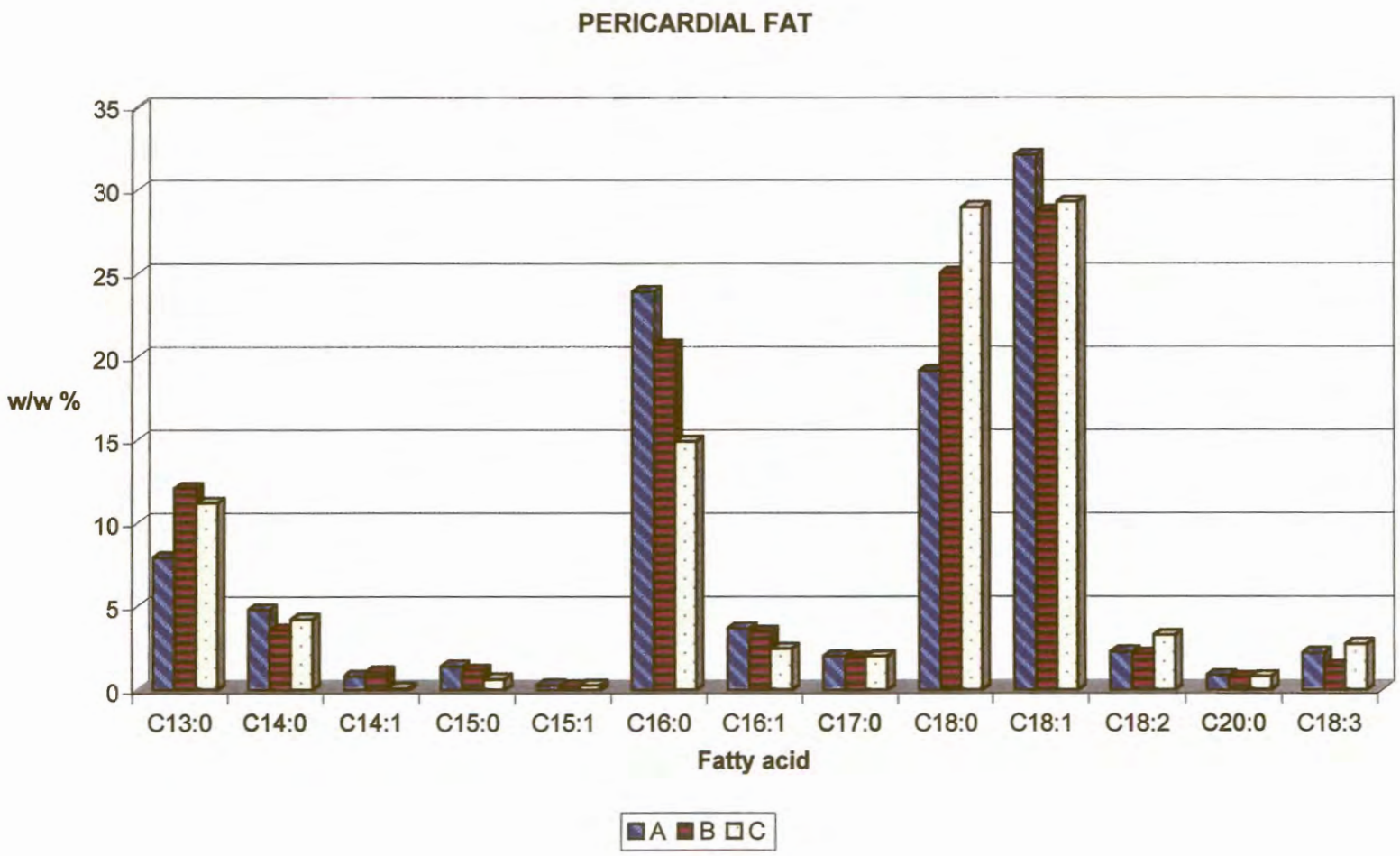
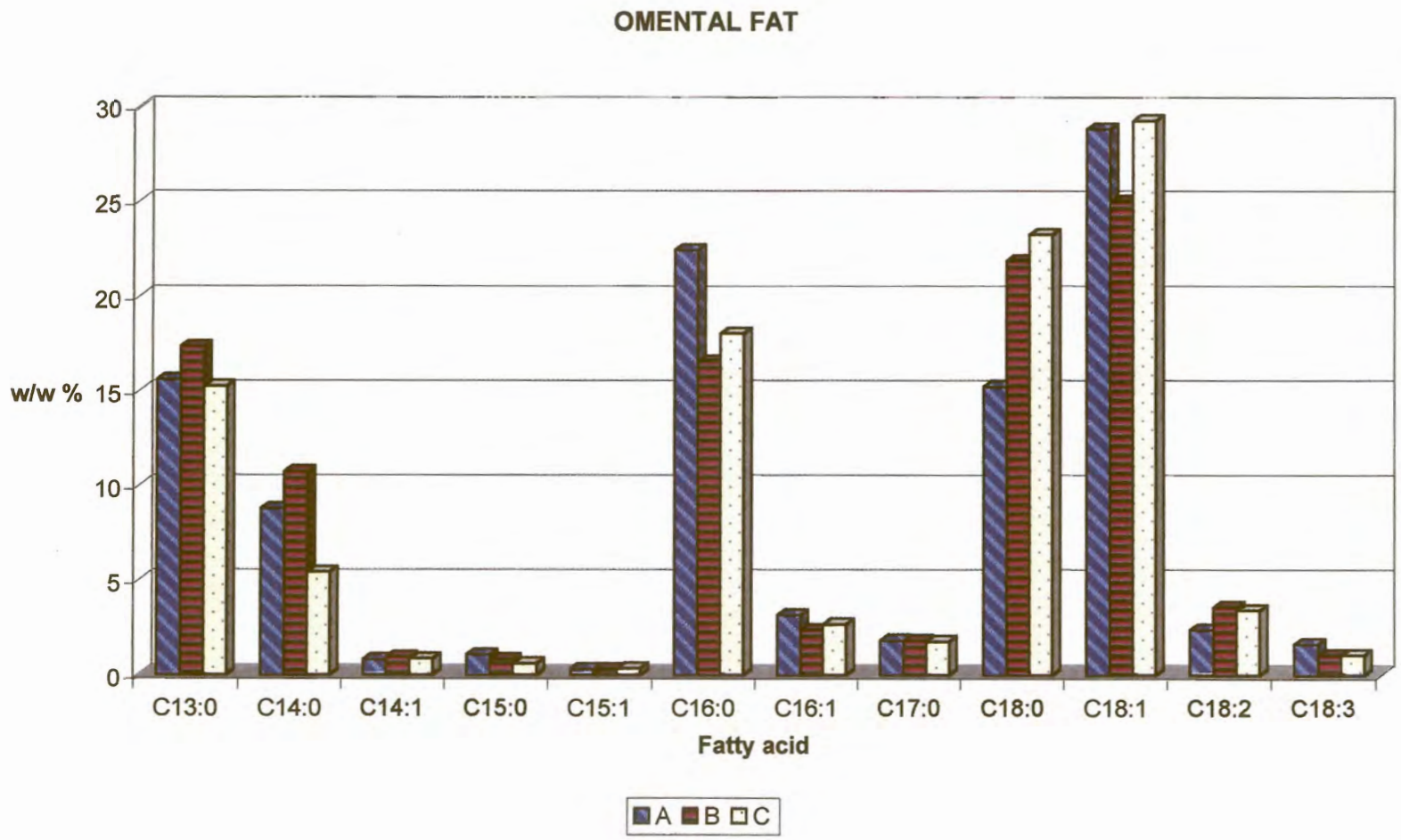


Figure 4-12 The influence of age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) on the long-chain fatty acid composition of omental fat in the African buffalo.



Within both depots, OMF and PCF, C15:0 ($P<0.01$) and C16:0 ($P<0.05$) decreased significantly (Table 4-6 and Table 4-7) with age. The proportions of C18:0 increased as the animal grew older (Table 4-7), although it was statistically insignificant ($P>0.05$). In PCF, the proportions of C16:1 decreased significantly after 6 years of age ($P<0.01$) (Figure 4-11). The proportions of the other fatty acids detected in the PCF and OMF remained relatively constant. Changes in the fatty acid composition of PCF and OMF with age were similar compared to those detected in PRF.

4.3 INFLUENCE OF GENDER[3]

The most abundant fatty acids in the subcutaneous fat, present in proportions higher than 10%, include C18:1, C13:0, C18:0 and C16:0 in females[4] and C13:0, C18:1, C16:0 and C18:0 in males[5] (Figure 4-14).

Significant differences ($P<0.05$) were found in the proportions of C13:0, C16:0, C17:0, C18:0, C18:1 and SFA, between males and females. The proportions of C13:0 were significantly higher ($P<0.01$) in male buffalo (46.10±22.97%) than in female buffalo (28.13±21.45%) (Table 4-8 and Figure 4-14), while the proportions of C16:0, C17:0, C18:0 and C18:1 were significantly ($P<0.05$) higher in females than in males. The proportion of C16:1 was numerically higher in females than in males, but these differences were not statistically significant (Table 4-8). Zembayashi *et al.* (1995) and Westerling and Hedrick, (1979) also reported higher proportions of C16:0 and C18:0 in heifers compared to steers. Although Nürnberg *et al.* (1998) reported higher proportions of C16:0, C17:0 and C18:0 and more saturated fat in females (in sheep, cattle and pigs), subcutaneous fat of male buffalo was significantly ($P<0.05$) more saturated (SFA =

[3] Results presented at IX International Symposium on Ruminant Physiology, 1999 (Steenkamp *et al.*, 1999b).
[4] Female animals or females will be used whenever no differentiation is made between calves and juveniles, subadult and adult cows. Calves and juveniles, subadult cows and adult cows will be referred to in specific cases.
[5] Male animals or males will be used whenever no differentiation is made between young and old bulls. Bull calves or juveniles, subadult bulls and adult bulls will be referred to in specific cases.

72.95±11.37%) compared to female buffalo (SFA = 64.66±10.33%) (Figure 4-15). The current results are also more pronounced due to the high proportions of C13:0 observed in males in this study (Table 4-8).

Table 4-8 The influenced of gender (male and female) on the long-chain fatty acid composition (Mean±SD; w/w %) of subcutaneous fat (SCF) in the African buffalo.

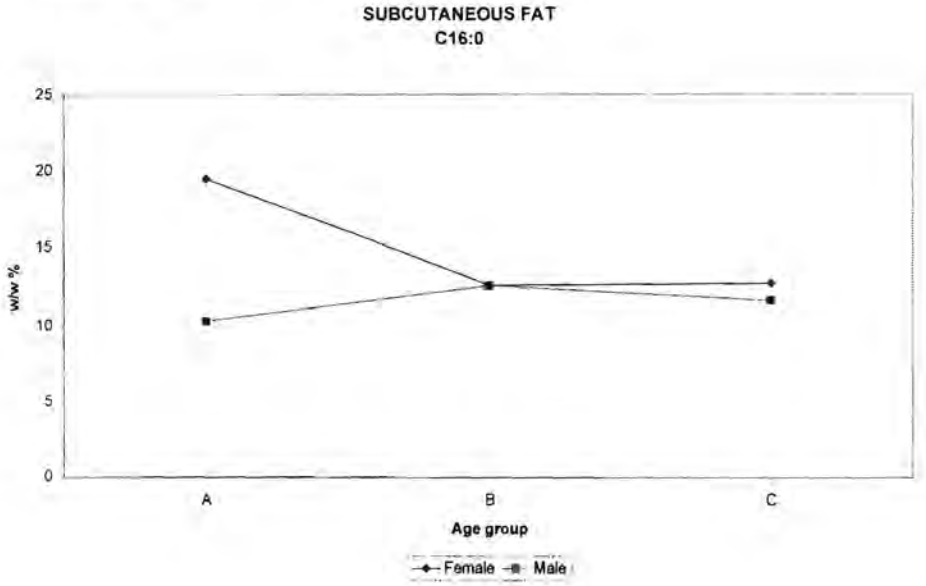
(w/w %)	Female (n = 18)	Male (n = 18)
C13:0	28.128^{A}±21.446	46.100^{B}±22.966
C14:0	3.233±3.031	5.560±6.614
C14:1	0.693±0.193	0.665±0.236
C15:0	0.667±0.477	0.620±0.428
C15:1	0.209±0.073	0.167±0.041
C16:0	14.931^{a}±5.482	11.516^{b}±4.630
C16:1	3.054±1.537	2.331±1.167
C17:0	1.382^{a}±0.551	0.873^{b}±0.530
C18:0	17.234^{A}±8.840	11.412^{B}±7.019
C18:1	28.345^{a}±9.010	22.264^{b}±9.697
C18:2	2.199±0.398	2.020±0.828
C18:3	1.464±0.479	1.032±0.572
UFA	35.221^{a}±10.448	27.003^{b}±11.426
SFA	64.661^{a}±10.328	72.950^{b}±11.369

^{a,b} Means in the same row bearing different superscripts differ (P<0.05)
^{A,B} Means in the same row bearing different superscripts differ (P<0.01)

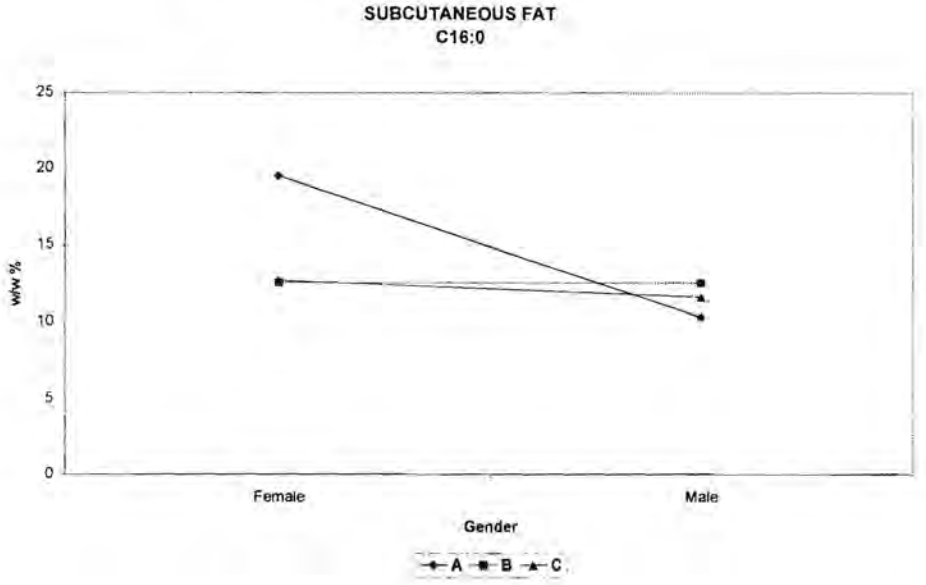
In the subcutaneous fat of buffalo, the relative proportion of C16:0 was significantly influenced by the interaction of age and gender (Figure 4-13). Within the A age group (juveniles and calves), female animals contained higher levels of C16:0 compared to young male buffalo (Figure 4-13). By contrast, there was little difference in the proportions of C16:0 (Figure 4-13) between gender in older animals (P<0.05). In males, the proportions of C16:0 appeared to be fairly constant between the different age groups. Female buffalo of the B and C age groups appeared not to be different from males of the same age (Addendum I) but female calves of the A age contained significantly higher proportions of C16:0 (Figure 4-13).

Figure 4-13 The effect of a) age (A = animals younger than 2 years of age; B = animals from 2 to 6 years of age; C = animals older than 6 years of age) and b) gender (male and female) on the proportion of C16:0 in subcutaneous fat of the African buffalo (P<0.05).

a) Effect of age



b) Effect of gender



In the LD of females, C14:1 and C15:1 were present in proportions lower than 1%, but these two fatty acids were not detected in males (Figure 4-14). High proportions of C13:0 were present in both genders, followed by C18:1, C16:0 and C18:0 (Figure 4-16).

Figure 4-14 The effect of gender (male and female) on the long-chaon fatty acid composition of subcutaneous fat in the African buffalo

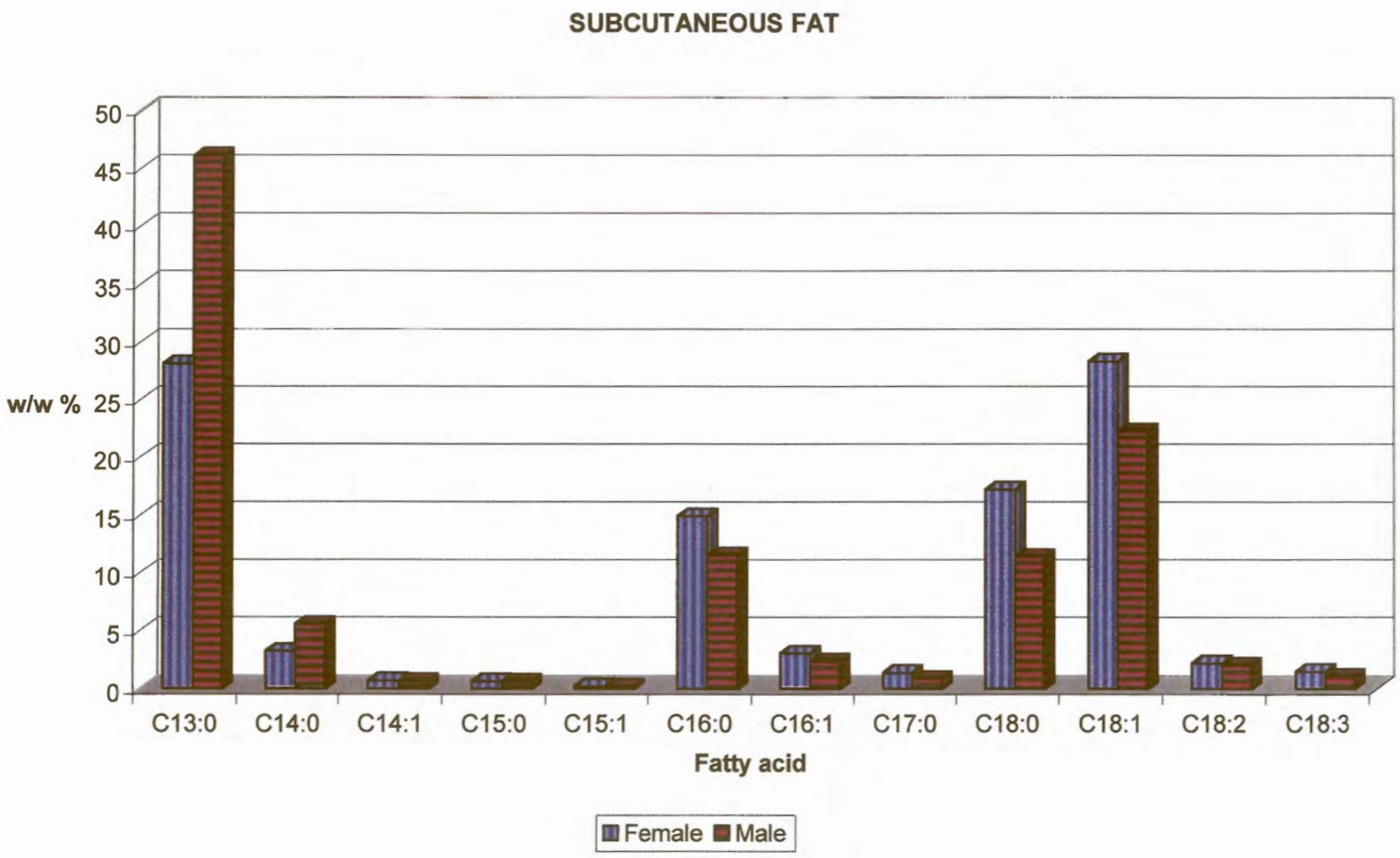


Figure 4-15 Illustration of the effect of gender (male and female) on the proportions of (a) total saturated and (b) total unsaturated long-chain fatty acids in fat depots of the African buffalo.

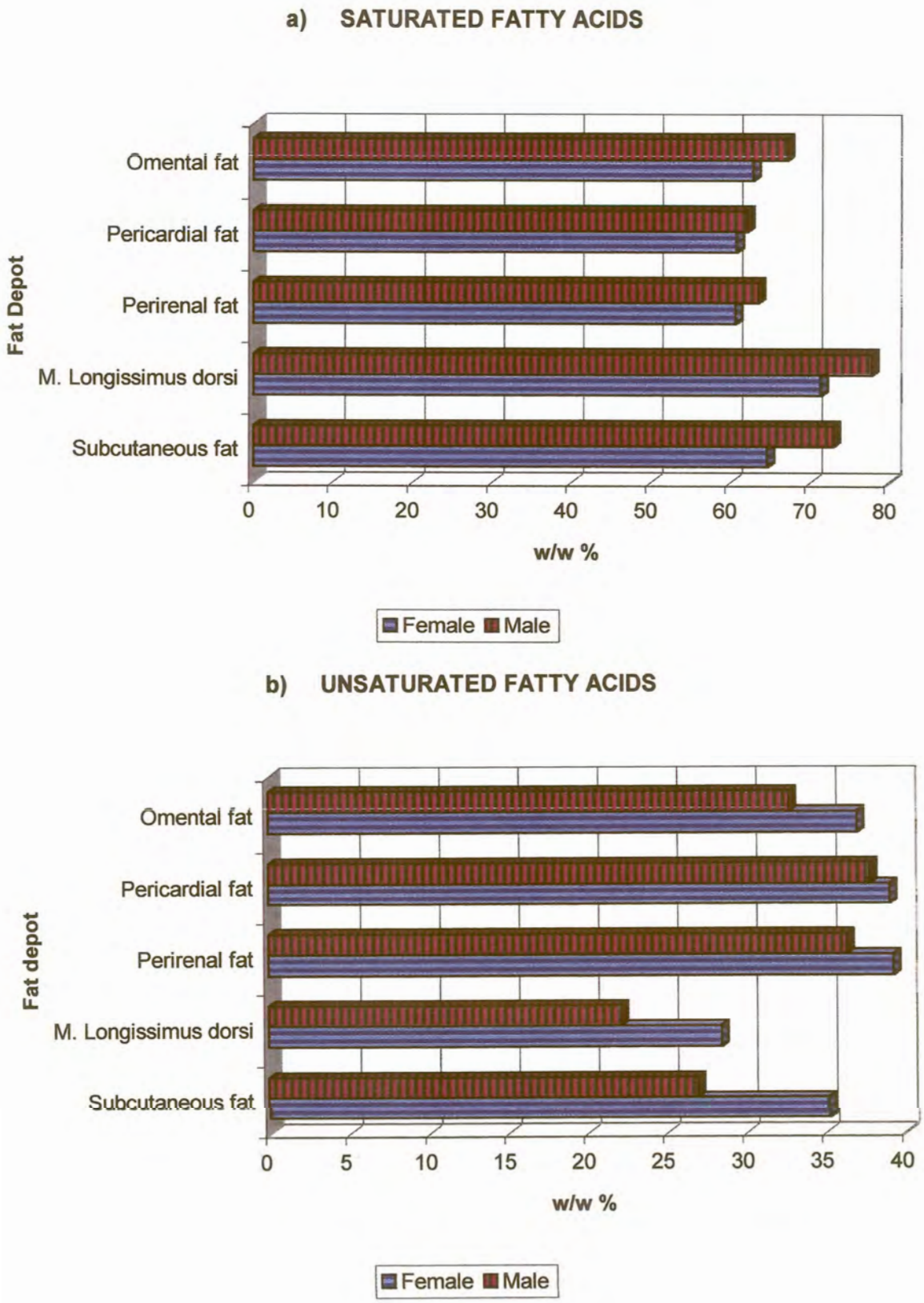


Figure 4-16 The effect of gender (male and female) on the long-chain fatty acid composition of M. Longissimus dorsi in the African buffalo.

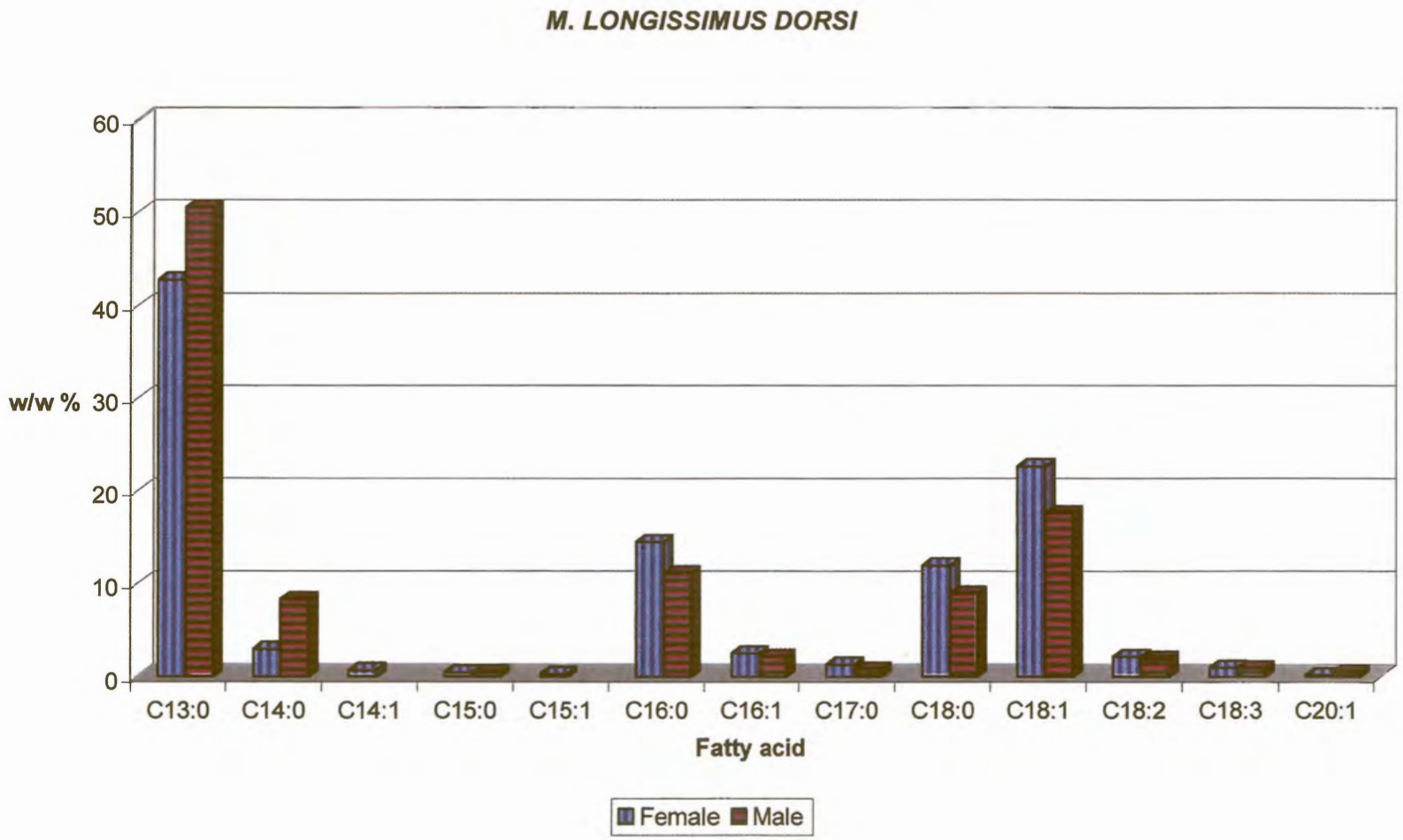


Table 4-9 The effect of gender (male and female) on the long-chain fatty acid composition (Mean±SD; w/w %) of M. Longissimus dorsi (LD) in the African buffalo.

(w/w %)	Female (n = 17)	Male (n = 18)
C13:0	42.791±21.163	50.527±15.309
C14:0	2.953±1.136	8.304±15.0.842
C15:0	0.462±0.256	0.446±0.093
C16:0	14.478[a]±4.739	11.088[b]±3.949
C16:1	2.516±0.703	2.155±1.076
C17:0	1.307±0.451	0.790±0.194
C18:0	11.956±6.305	8.927±3.567
C18:1	22.637[a]±7.840	17.593[b]±6.747
C18:2	2.135±1.100	1.921±0.686
C18:3	0.991±0.485	0.903±0.268
UFA	28.513[a]±9.210	22.181[b]±8.407
SFA	71.387[a]±9.218	77.736[b]±8.395

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

The proportion of saturated fatty acids in muscle was significantly influenced by gender (Figure 4-15a). The muscle of females contained a higher proportion (P<0.05) of unsaturated fatty acids (28.51±9.21%) than males (22.18±8.41%) (Table 4-9 and Figure 4-15b). This is mainly due to significantly higher (P<0.05) proportions of C18:1 and numerically lower proportions of C13:0 (Figure 4-16). The proportions of C16:0 were significantly higher in female than in males (P<0.05; Table 4-9).

Table 4-10 The effect of gender (male and female) on the long-chain fatty acid composition (Mean±SD; w/w %) of perirenal fat (PRF) in the African buffalo.

(w/w %)	Female (n = 18)	Male (n = 18)
C13:0	9.734±9.595	12.167±9.990
C14:0	6.286±7.014	10.031±15.307
C14:1	0.931±0.259	1.037±0.281
C15:0	1.069±0.505	0.849±0.225
C15:1	0.307±0.053	0.294±0.055
C16:0	20.867±6.988	18.292±6.622
C16:1	3.685±1.566	3.043±1.180
C17:0	1.897±0.442	1.927±0.323
C18:0	21.037±6.438	20.807±9.862
C18:1	31.028±4.088	29.132±8.470
C18:2	2.225±0.343	2.784±1.637
C20:0	1.323±1.092	0.842±0.292
C18:3	1.907±0.575	1.749±0.461
UFA	39.298±5.650	36.380±10.783
SFA	60.699±10.805	63.490±10.805

Gender did not have any significant influences on the fatty acid composition of the perirenal fat of buffalo (Table 4-10 and Figure 4-17) although some numerical differences were found. In the pericardial fat, only the proportions of C15:0 and C16:0 were significantly ($P<0.05$) influenced by gender (Table 4-11). Both fatty acids were significantly higher in males than in females. Numerical differences were noted for the proportions of C18:0 (Figure 4-18), but these differences were not significant ($P<0.05$) and did not significantly influence the proportions of saturated fatty acids in PCF.

Table 4-11 The effect of gender (male and female) on the long-chain fatty acid composition (Mean±SD; w/w %) of pericardial fat (PCF) in the African buffalo.

(w/w %)	Female (n = 9)	Male (n = 8)
C13:0	12.868±8.373	7.845±6.410
C14:0	2.572±2.233	5.906±4.996
C14:1	0.877±0.173	1.094±0.049
C15:0	0.722^{a}±0.376	1.417^{b}±0.712
C15:1	0.274±0.065	0.321±0.067
C16:0	15.882^{a}±6.470	23.063^{b}±6.691
C16:1	2.950±1.081	3.306±1.525
C17:0	1.961±0.406	2.016±0.271
C18:0	27.336±11.352	22.181±8.146
C18:1	29.843±4.835	30.058±2.668
C18:2	2.948±2.775	2.300±0.233
C20:0	0.903±0.122	0.628±0.180
C18:3	2.787±2.708	1.521±0.509
UFA	39.082±5.264	37.835±3.808
SFA	60.860±5.232	62.155±3.822

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

No statistical differences were found between the proportions of saturated and unsaturated fatty acids in the omental fat of the African buffalo (Figure 15). Individual fatty acids were affected by the gender (Table 4-12). C15:0, C15:1, C17:0 and C18:0 were significantly higher ($P<0.05$) in females than in males (Table 4-12 and Figure 4-19) and the proportions of C18:3 were significantly ($P<0.01$) higher in females than in males. The omental fat of female buffalo contained significantly lower proportions of C15:0 ($P<0.01$) and C16:0 ($P<0.05$) than males (Table 4-12 and Figure 4-18).

Table 4-12 The effect of gender (male and female) on the long-chain fatty acid composition (Mean±SD; w/w %) of omental fat (OMF) in the African buffalo.

(w/w %)	Female (n = 16)	Male (n = 14)
C13:0	13.130±8.258	19.275±12.201
C14:0	4.989±6.603	11.963±15.543
C14:1	0.922±0.214	0.741±0.188
C15:0	1.043^{a}±0.449	0.650^{b}±0.294
C15:1	0.330^{a}±0.032	0.267^{b}±0.018
C16:0	20.048±5.226	17.948±5.925
C16:1	2.903±1.120	2.591±1.195
C17:0	1.918^{a}±0.299	1.558^{b}±0.202
C18:0	22.374^{a}±7.525	17.643^{b}±6.605
C18:1	29.633±3.815	25.800±6.534
C18:2	2.618±1.080	3.650±1.204
C18:3	1.471^{A}±0.591	0.943^{B}±0.360
UFA	37.042±4.741	32.779±8.184
SFA	62.919±4.759	67.143±8.115

^{a,b} Means in the same row bearing different superscripts differ (P<0.05)
^{A,B} Means in the same row bearing different superscripts differ (P<0.01)

The proportion of C15:0 present in the omental fat of the African buffalo was significantly (P<0.01) influenced by area and gender (Addendum VIII). In MD and MLS, with similar vegetation types (Figure 2-4), the proportion for C15:0 was lower in males than in females. A higher proportion of C15:0 was observed in males than in females in MH where the vegetation appeared to be different from MLS and MD (Figure 2-4). The shift observed in the proportions of C15:0 between gender may be the consequence of the shift in C13:0 due to the ingestion of riverine vegetation (e.g. the common reed) which is more prevalent in MD and MLS or the mobilisation of C16:0 to yield energy (Table 4-12).

Figure 4-17 The effect of gender (male and female) on the long-chain fatty acid composition of perirenal fat in the African buffalo.

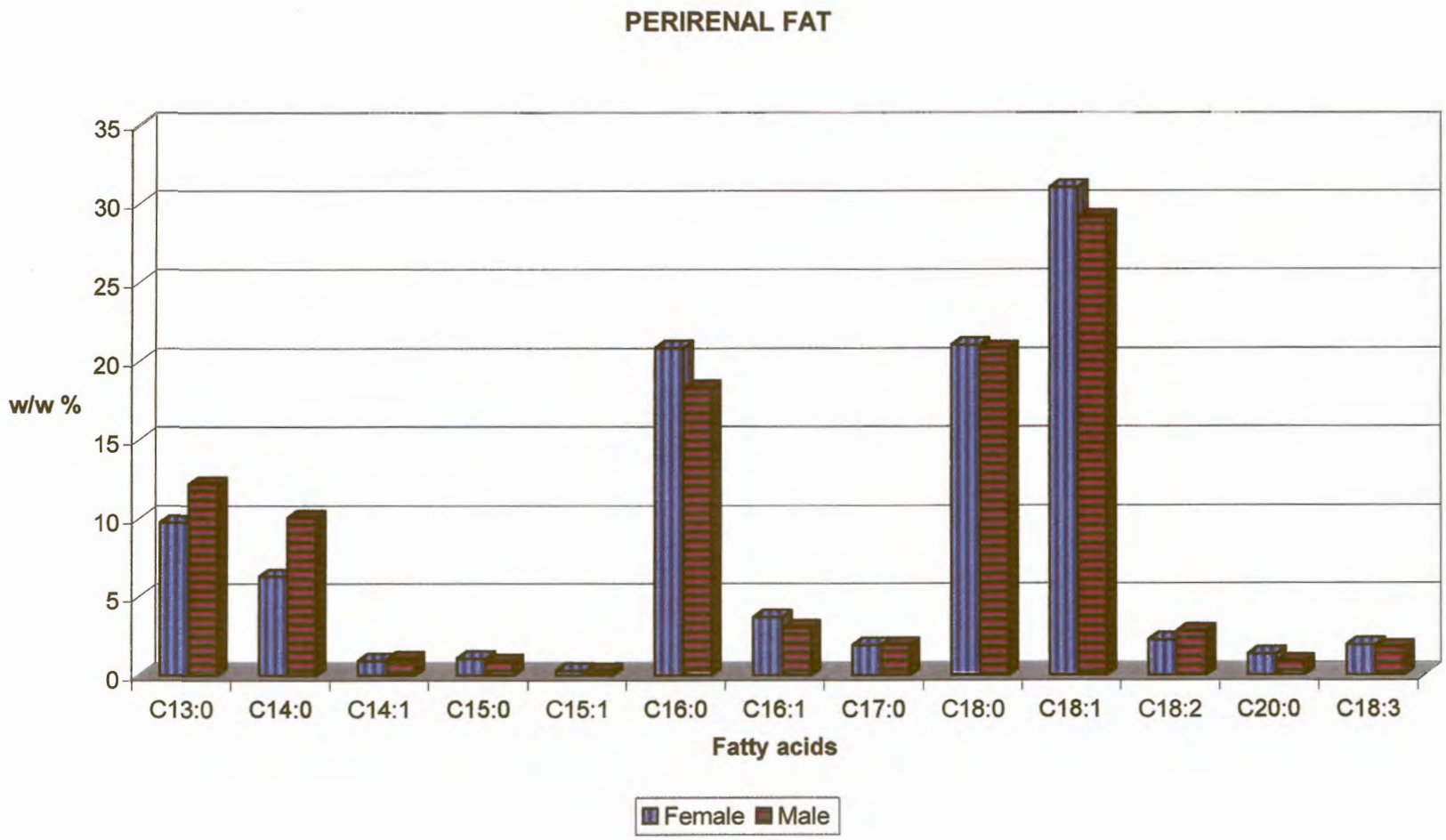


Figure 4-18 The effect of gender (male and female) on the long-chain fatty acid composition of pericardial fat in the African buffalo.

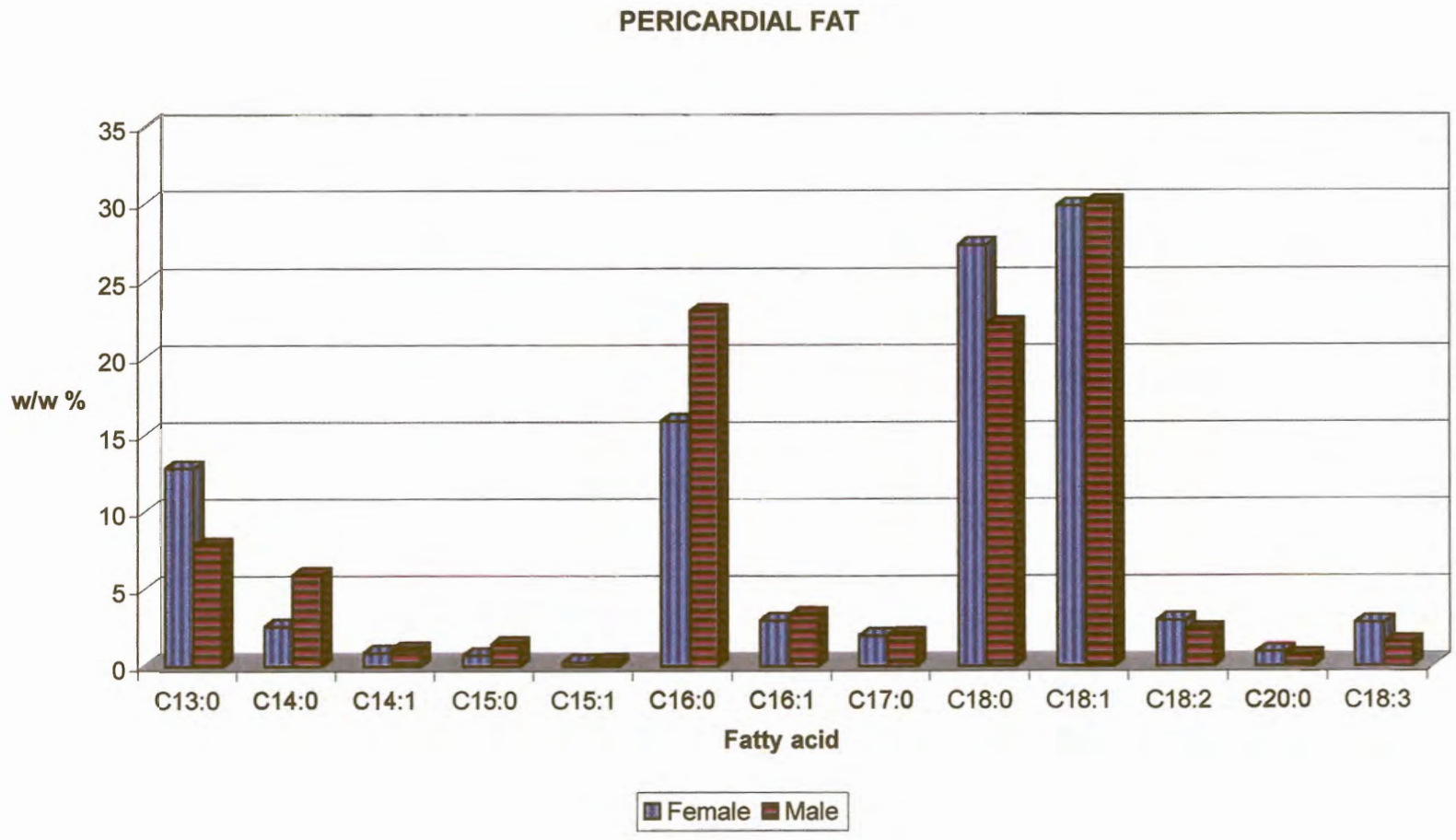
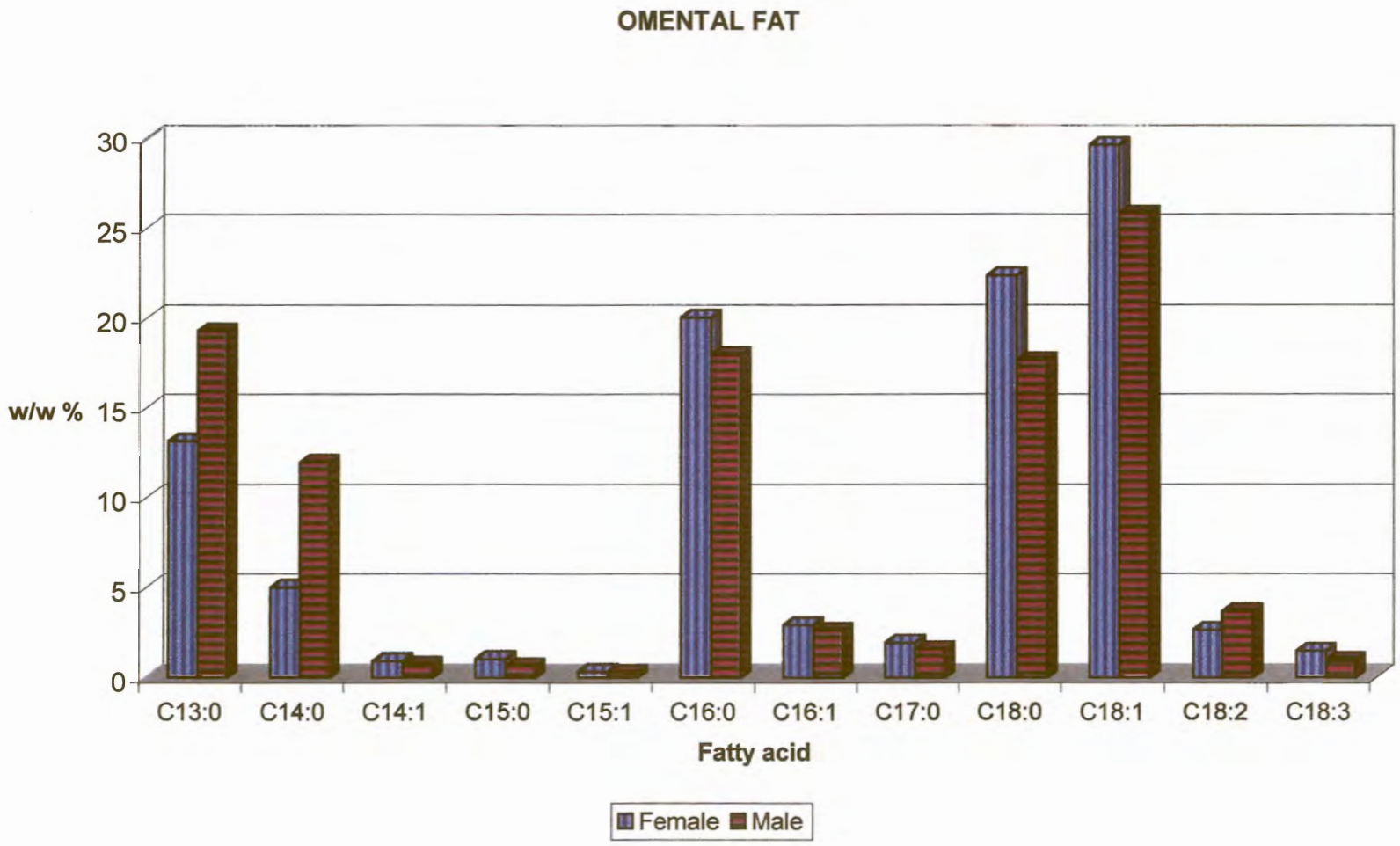


Figure 4-19 The effect of gender (male and female) on the long-chain fatty acid composition of omental fat in the African buffalo.



4.4 INFLUENCE OF AREA[6]

The home range areas of the buffalo differed in ecology as discussed in Chapter 2. In summary, Mpanamana dam (MD) is mainly Marula savannah, Mtandanyathi (MH), Mopane/Bushwillow woodlands and Mashatudrif (MLS), thorn thickets. There is more overlapping in the vegetation types of MD and MLS than with MH. Some plant species were encountered in al three areas but to a different extend depending on the geology of the area. More overlapping were found in the southern region (MD and MLS) than with the central region (MH) of the KNP. Although some species occurs in all of the areas, it does not necessarily mean that it was utilised to the same extend by the different herds, as buffalo tend to balance their diet by utilisation of different species available (Prins, 1991).

4.4.1 *Subcutaneous fat*

The most abundant fatty acids in the subcutaneous fat of buffalo from the southern region (MLS and MD) were C13:0, followed by C18:1 in both regions (Figure 4-22). C16:0 was third most abundant in MLS, followed by C18:0, whereas the proportion of C18:0 was higher than C16:0 in MD (Figure 4-22). Buffalo from MH contained the highest proportion of C18:1 subcutaneously, followed by C13:0, C18:0 and C16:0.

A higher proportion of unsaturated fatty acids was observed in animals from the Mopane/Bushwillow woodlands (MH) than animals from the thorn thickets (MLS), while that of animals from the Marula savannah (MD) was intermediate (Figure 4-23b).

Statistically significant differences ($P<0.05$) were observed between the different areas for the proportions of C13:0, C14:0, C15:1, C16:0, C16:1, C17:0 and C18:0 (Table 4-13). Animals from MLS contained significantly higher proportions of C13:0 compared to those

[6] Results presented at IX International Symposium on Ruminant Physiology, 1999 (Steenkamp *et al.*, 1999b).

from the other two areas (P<0.01) (Table 4-13), explaining the significantly higher proportion of saturated fatty acids in SCF of these animals (Figure 4-23a).

Table 4-13 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the long-chain fatty acid composition (Mean±SD; w/w %) in the subcutaneous fat (SCF) of the African buffalo.

(w/w %)	MH (n = 10)	MLS (n = 13)	MD (n = 13)
C13:0	20.686^{A}±11.545	51.329^{B}±23.027	35.537±23.755
C14:0	5.425^{a}±7.210	5.094±3.734	2.385^{b}±1.186
C14:1	0.647±0.229	0.587±0.201	0.765±0.161
C15:0	0.763±0.463	0.551±0.428	0.616±0.504
C15:1	0.179^{a}±0.074	0.220±0.086	0.221^{b}±0.066
C16:0	16.70^{a}±5.369	10.637^{b}±4.642	13.13^{b}±4.604
C16:1	3.683^{a}±1.523	1.976^{b}±0.747	2.592±1.384
C17:0	1.614^{a}±0.352	$0.782^{a,b}$±0.516	1.246^{b}±0.575
C18:0	18.412^{a}±9.063	10.063^{b}±6.700	15.438±8.030
C18:1	30.134±6.504	20.210±10.259	26.684±9.452
C18:2	2.231±0.423	1.852±0.725	2.312±0.689
C18:3	1.328±0.426	1.171±0.741	1.380±0.493
UFA	37.806^{a}±6.362	24.669^{b}±11.848	32.407±11.635
SFA	62.152^{a}±6.414	75.175^{b}±11.750	67.554±11.587

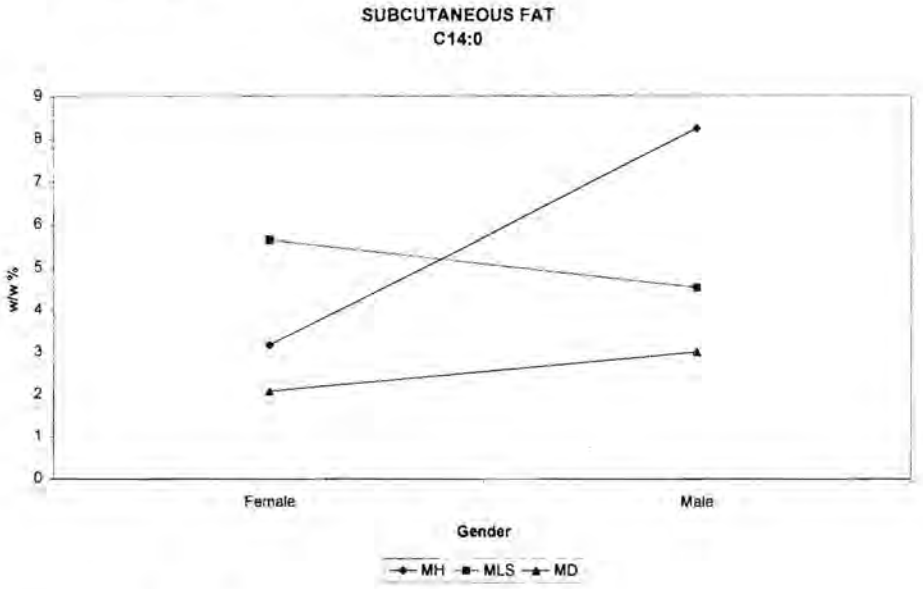
^{a,b} Means in the same row bearing different superscripts differ (P<0.05)
^{A,B} Means in the same row bearing different superscripts differ (P<0.01)

MH contained significantly higher (P<0.05) proportions of C14:0 and C17:0 and lower proportions of C15:1 than MD, but neither differed from MLS (Table 4-13). MH contained significantly higher (P<0.05) proportions of C16:0, C16:1 and C18:0 than MLS with the proportions found in MD intermediate and not significantly different form either MH or MLS (Table 4-13).

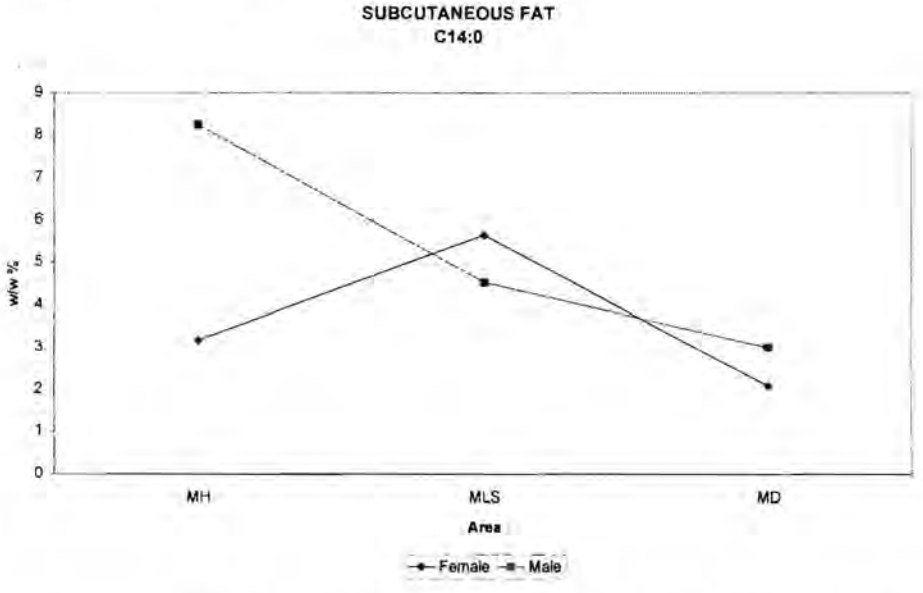
The significant higher proportion of C14:0 found in MH is due to higher proportions noted in male buffalo from MH (Figure 4-20) than in females from the same area. The proportions noted in males and females in the other two areas appeared to be fairly constant with males from MLS having slightly less C14:0 than females and males from MD had slightly more C14:0 than females from the same area.

Figure 4-20 The effect of a) gender (male and female) and b) area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the proportion of C14:0 in subcutaneous fat of the African buffalo (P<0.05).

a) Effect of gender



b) Effect of area

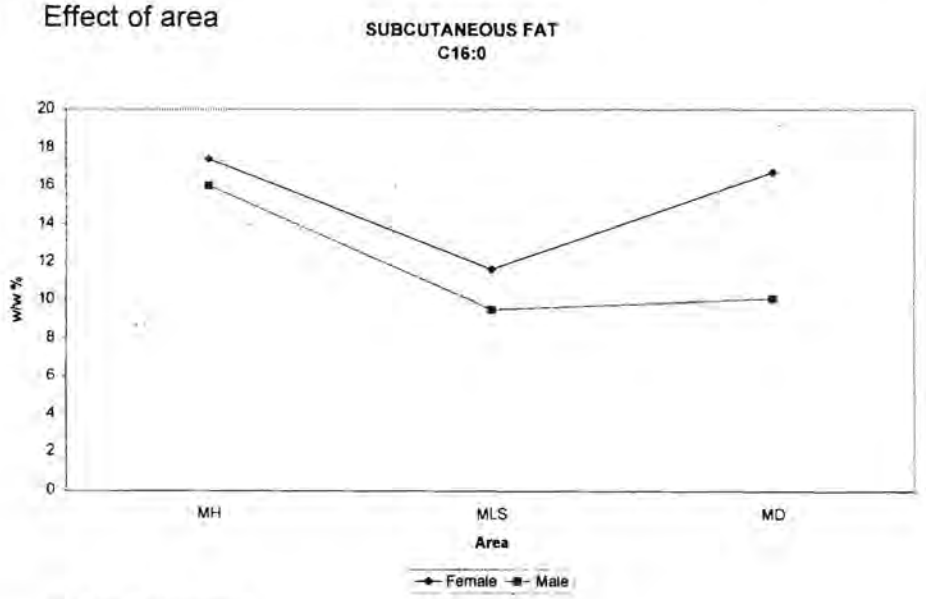


The proportion of C16:0 in SCF was significantly different between the male and female buffalo in the different areas and in particular for buffalo from MD (Figure 4-21). In all three areas, the proportion of C16:0 in SCF of females was higher than for males (as reported earlier in this chapter). Differences were not as significant in MH and MLS as in MD where the proportion of C16:0 was much higher for females than for males (Figure 4-

21). The relatively high proportion of C16:0 in SCF of buffalo sampled at MH and females sampled at MD, may reflect the better condition of these animals, while the low proportions of C16:0 of animals from MLS and males from MD indicate that the energy reserves of these animals were mobilised for energy. C16:0 is reportedly the first fatty acid to be mobilised from adipose tissue stores during periods of restricted energy intake (Vernon, 1981; Adrouni and Khachadurian, 1968).

Figure 4-21 The effect of a) area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) and b) gender (male and female) on the proportion of C16:0 present in subcutaneous fat of the African buffalo (P<0.05).

a) Effect of area



b) Effect of gender

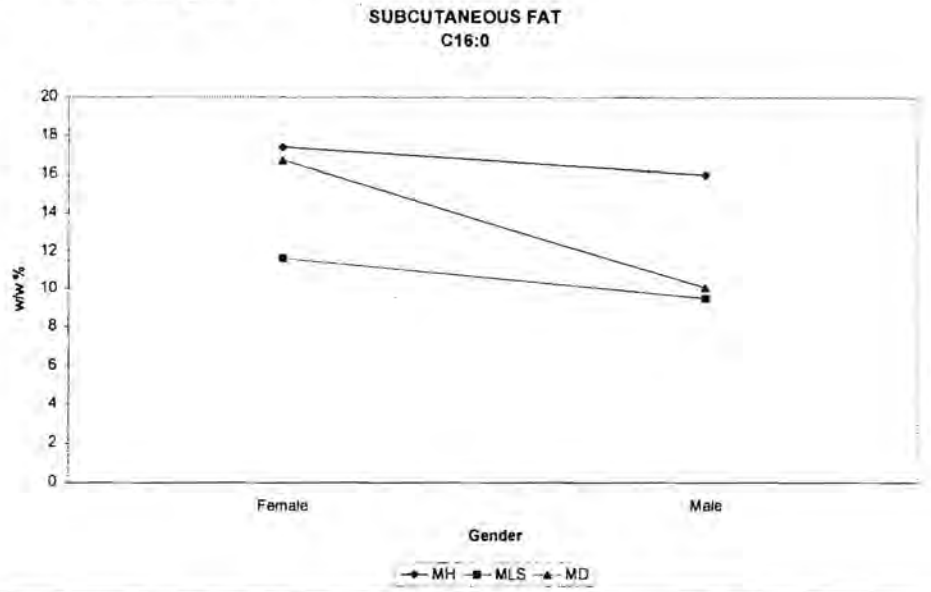


Figure 4-22 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the long-chain fatty acid composition of subcutaneous fat in the African buffalo.

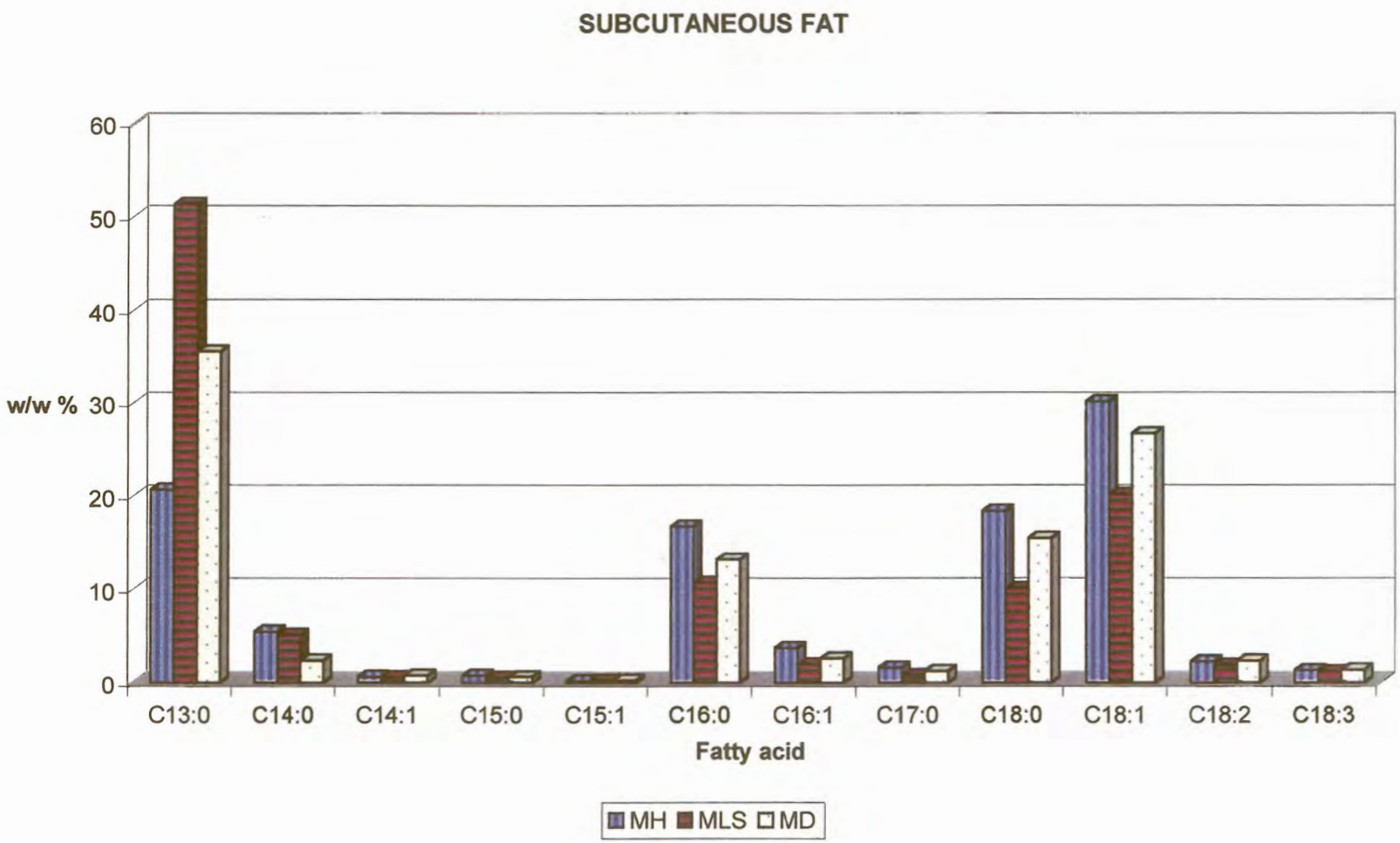
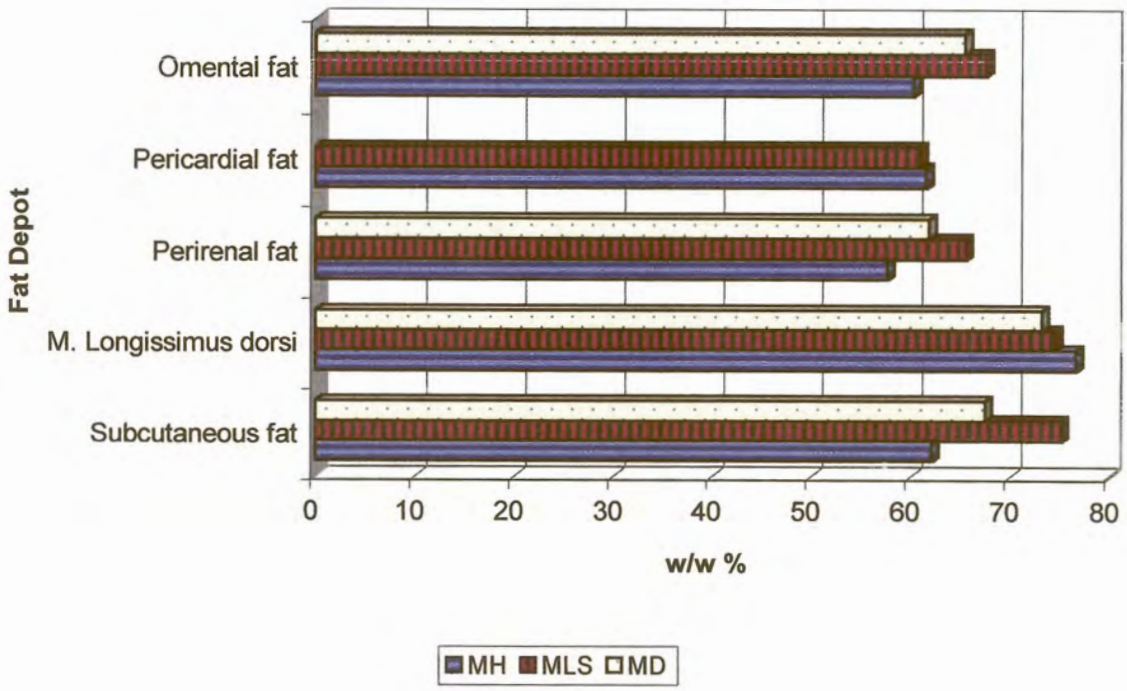


Figure 4-23 Illustration of the effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the proportions of (a) total saturated and (b) total unsaturated long-chain fatty acids of depot fat in the African buffalo.

a) SATURATED FATTY ACIDS



b) UNSATURATED FATTY ACIDS

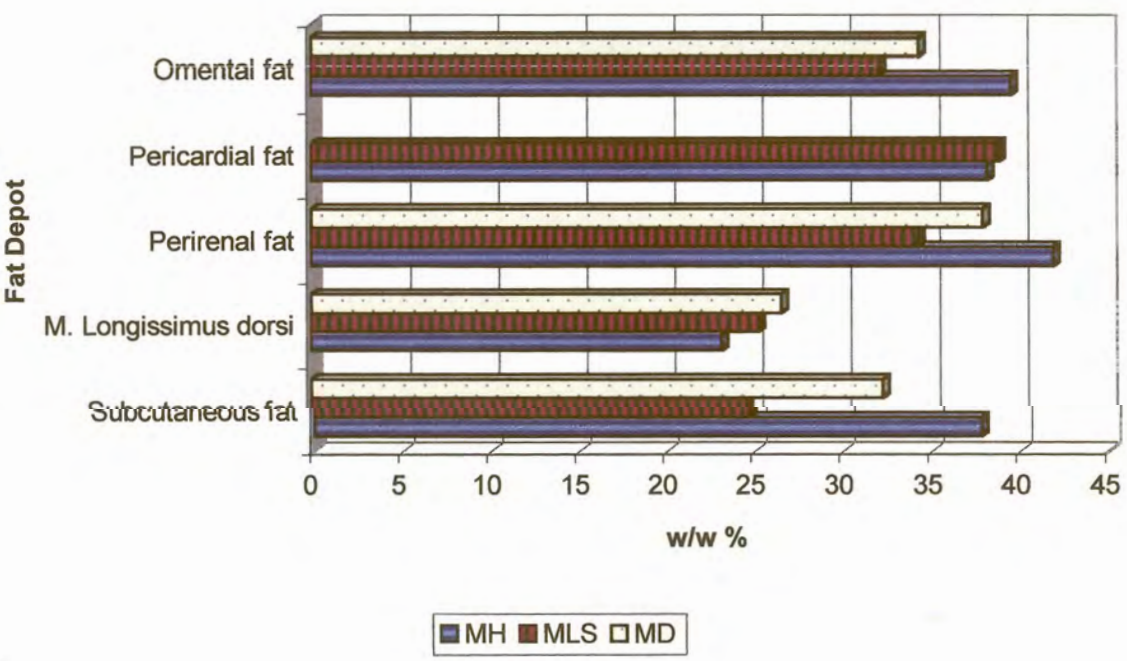
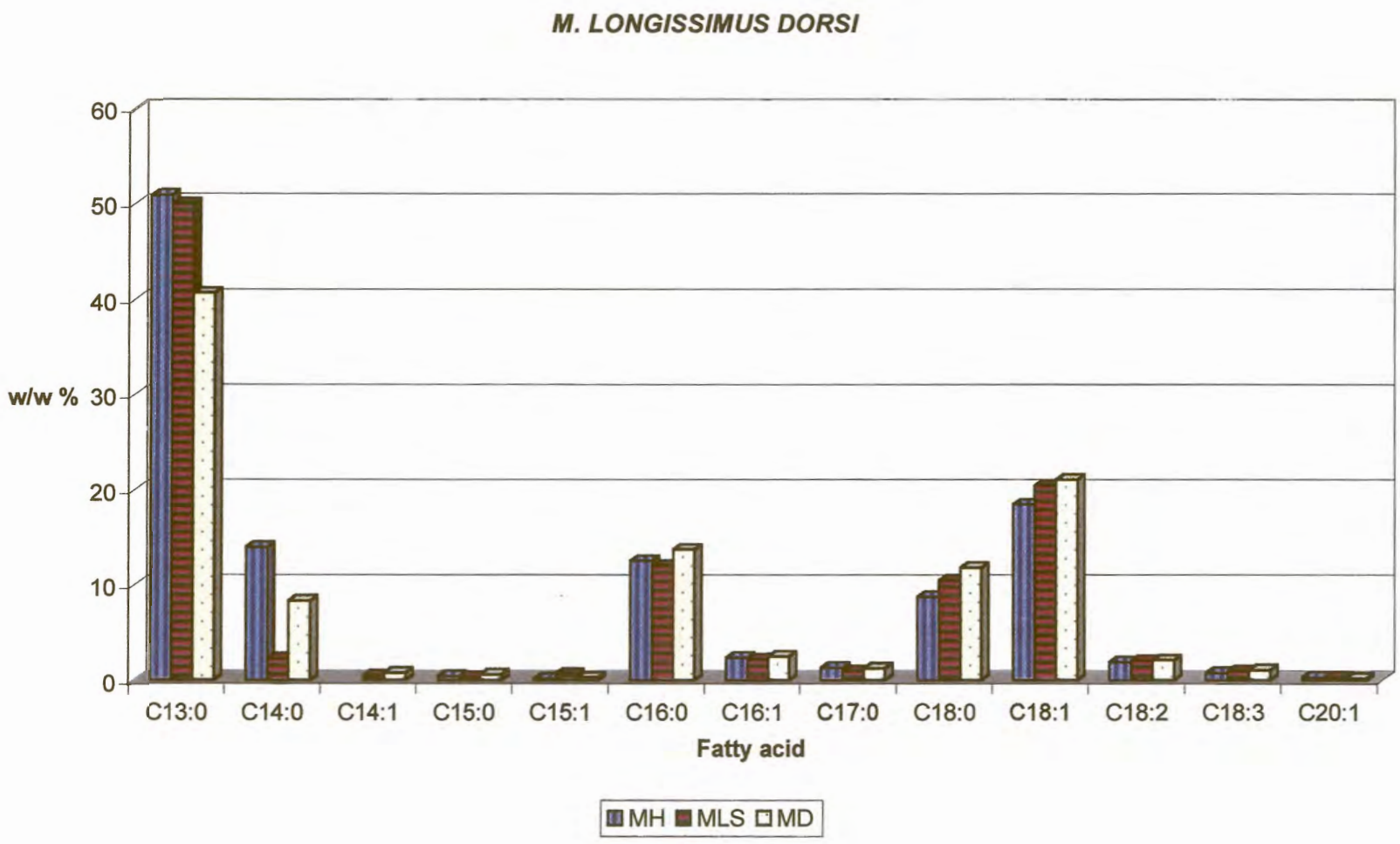


Figure 4-24 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the long-chain fatty acid composition of M. Longissimus dorsi in the African buffalo.



4.4.2 *M. Longissimus dorsi*

The only statistically significant difference in LD between areas was in the proportion of C15:1 (Table 4-14). The area in which the herds grazed had other effects on the composition of the *M. Longissimus dorsi,* although some minor differences (Figure 4-24). The proportions of C13:0 and C14:0 were numerically different between different areas, but these could be due to problems in the separation of the peaks as explained earlier in this chapter as well as Chapter 3.

Table 4-14 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the long-chain fatty acid composition (Mean±SD; w/w %) of M. Longissimus dorsi (LD) in the African buffalo.

(w/w %)	MH (n = 10)	MLS (n = 12)	MD (n = 13)
C13:0	50.893±18.189	49.962±16.424	40.651±20.310
C14:0	13.925±18.204	2.263±1.181	8.309±15.973
C14:1		0.476	0.769±0.145
C15:0	0.423±061	0.324±0.082	0.610±0.281
C15:1	0.161^{A}±0.001	0.637^{B}	0.275±0.085
C16:0	12.482±4.967	11.949±3.078	13.653±5.624
C16:1	2.360±0.748	2.194±0.954	2.474±1.017
C17:0	1.288±0.146	0.956±0.493	1.252±0.494
C18:0	8.678±5.108	10.379±4.458	11.739±5.896
C18:1	18.410±8.205	20.359±7.053	21.007±8.098
C18:2	1.913±1.431	2.042±0.499	2.096±0.730
C18:3	0.790±0.289	0.957±0.378	1.077±0.461
C20:1	0.306±0.302	0.328±0.250	0.182±0.157
UFA	23.270±9.438	25.371±8.550	26.680±10.154
SFA	76.614±9.402	74.547±8.570	73.240±10.175

^{A,B} Means in the same row bearing different superscripts differ (P<0.01)

4.4.3 *Internal fat depots*

The internal fat depots were relatively stable and with no significant differences between areas. In the perirenal fat (PRF), C18:1 was numerically the most abundant fatty acid present in samples from all areas, followed by C16:0, C18:0 and C13:0 in samples from MH and MLS and C18:0 C16:0 and C13:0 in samples from MD (Figure 4-26). Only C13:0 was significantly influenced (P<0.05) by area namely higher proportions in buffalo from MLS, compared to buffalo from MH (Table 4-15).

Table 4-15 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the long-chain fatty acid composition (Mean±SD; w/w %) of perirenal fat (PRF) in the African buffalo.

(w/w %)	MH (n = 10)	MLS (n = 13)	MD (n = 13)
C13:0	6.435[a]±2.732	16.857[b]±12.973	8.518±6.415
C14:0	5.640±4.629	11.066±17.645	7.190±8.325
C14:1	1.022±0.283	0.931±0.193	0.966±0.317
C15:0	0.830±0.195	1.164±0.636	0.913±0.218
C15:1	0.289±0.053	0.302±0.046	0.314±0.063
C16:0	22.722±5.359	17.904±8.254	18.838±5.893
C16:1	3.895±2.031	3.546±1.151	2.836±0.835
C17:0	2.195±0.153	1.953±0.335	1.743±0.411
C18:0	20.974±7.668	17.244±8.925	24.968±6.532
C18:1	32.797±3.389	27.862±9.675	30.208±3.781
C18:2	2.639±0.817	2.140±0.567	2.752±1.787
C20:0	1.156	2.181±1.200	0.705±0.300
C18:3	1.752±0.371	1.622±0.456	2.114±0.627
UFA	42.000±3.924	34.412±12.241	38.065±5.266
SFA	57.770±3.654	65.581±12.246	61.935±5.266

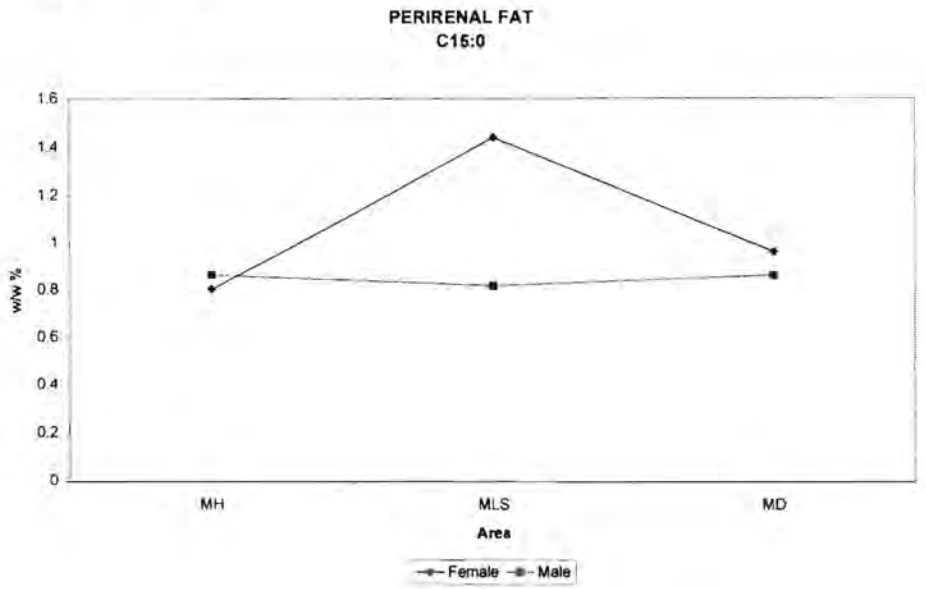
[a,b] Means in the same row bearing different superscripts differ (P<0.05)

In PRF, the proportion of C15:0 was reasonably constant in males sampled in different areas (Figure 4-25a), while it varied in females between the different areas. The most significant difference between gender was found in MLS where females had higher proportions of C15:0. In MH and MD, the differences between males and females were small and the relative proportion of C15:0 appeared to be about the same. This may be due to differences in diet because the proportion of C15:0 in females from MD was higher than males from the same areas, while the proportion of C15:0 was lower in females than in males from MH (Figure 4-25b).

The pericardial fat from buffalo at Mpanamana dam was not collected. The only significant difference (P<0.01) in the fatty acid composition of the pericardial fat between the areas collected, was for the proportion of C16:1 (Table 4-16). Area differences did not significantly influence the rest of the fatty acids present in PRF. Animals from MH had higher proportions (3.685±1.976%) of C16:1 than animals from MLS (2.825±0.633%) (P<0.01) (Table 4-16).

Figure 4-25 The effect a) area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) and b) gender on the proportion of C15:0 in perirenal fat of the African buffalo (P<0.01).

a) Effect of area



b) Effect of gender

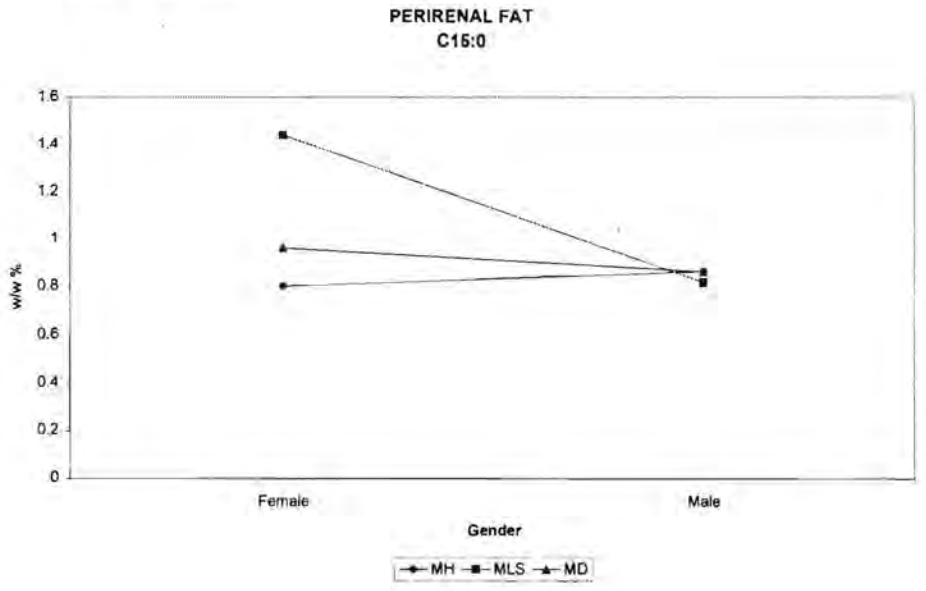


Figure 4-26 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the long-chain fatty acid composition of perirenal fat in the African buffalo.

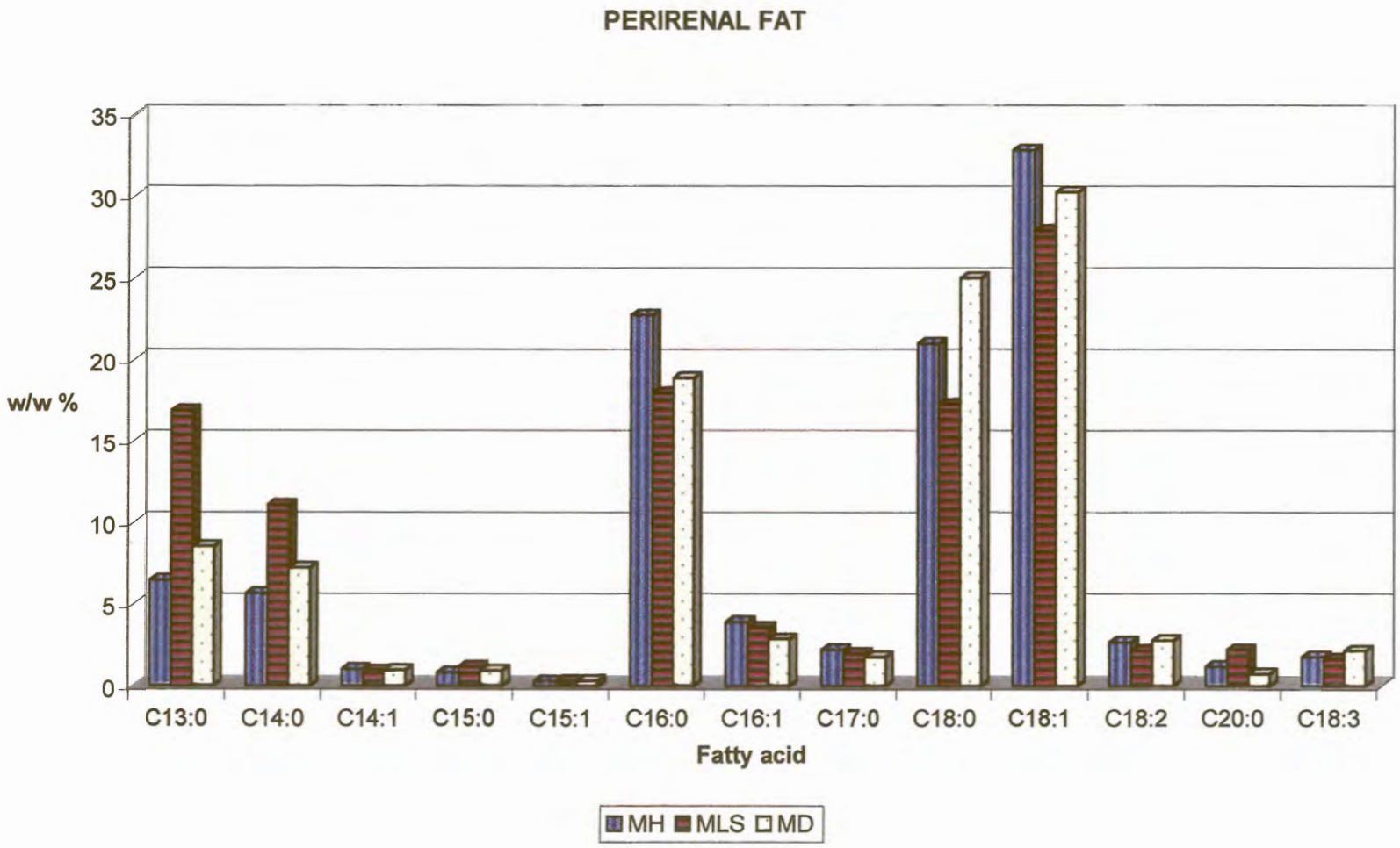


Table 4-16 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the long-chain fatty acid composition (Mean±SD; w/w %) of pericardial fat (PCF) in the African buffalo.

(w/w %)	MH (n = 7)	MLS (n = 10)
C13:0	7.107±4.074	13.133±8.814
C14:0	3.562±2.888	3.240±1.947
C14:1	0.894±0.192	0.990±0.168
C15:0	0.884±0.647	1.213±0.721
C15:1	0.250±0.299	0.318±0.062
C16:0	20.668±8.843	18.704±7.090
C16:1	3.685^{A}±1.976	2.825^{B}±0.633
C17:0	2.124±0.216	1.932±0.387
C18:0	27.451±12.727	23.529±9.057
C18:1	30.038±1.875	30.090±4.890
C18:2	2.009±0.205	3.047±2.570
C18:3	1.620±0.449	2.556±2.627
UFA	38.304±2.699	38.947±5.576
SFA	61.626±2.733	61.035±5.551

^{A,B} Means in the same row bearing different superscripts differ (P<0.01)

Table 4-17 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the long-chain fatty acid composition (Mean±SD; w/w %) of omental fat (OMF) in the African buffalo.

(w/w %)	MH (n = 9)	MLS (n = 13)	MD (n = 8)
C13:0	10.760±6.340	20.753±13.238	14.162±5.950
C14:0	4.836±3.119	12.039±16.213	5.917±9.354
C14:1	0.843±0.208	0.922±0.256	0.872±0.252
C15:0	0.846±0.340	1.052±0.643	0.796±0.222
C15:1	0.308±0.038	0.319±0.018	0.341±0.062
C16:0	22.034±5.211	17.414±6.031	18.419±4.265
C16:1	3.208±1.296	2.747±1.183	2.197±0.621
C17:0	1.860±0.358	1.795±0.314	1.687±0.299
C18:0	20.593±7.933	16.869^{a}±7.340	25.043^{b}±3.790
C18:1	31.564±2.772	25.671±6.989	27.193±2.563
C18:2	2.978±1.304	2.840±1.132	3.657±1.299
C18:3	1.474±0.636	1.056±0.493	1.350±0.574
UFA	39.630±3.033	32.250±8.487	34.458±4.003
SFA	60.342±3.048	67.655±8.429	65.514±4.026

^{a,b} Means in the same row bearing different superscripts differ (P<0.05)

C18:1 was the most abundant fatty acid present in OMF in all three areas, followed by C16:0, C18:0 and C13:0 in MH; C13:0, C16:0 and C18:0 in MLS; C18:0, C16:0 and C13:0 in MD (Table 4-17). The area did not affect the total proportions of SFA and UFA in the OMF. Animals sampled in MLS appeared to have a higher proportion of saturated fatty acids (67.655±8.429%) than those from MD (65.514±4.026%) and MH (60.342±3.048%), but these differences were only numerical and not significant (Table 4-17). C18:0 differed

between the areas with MD containing significantly (P<0.05) higher proportions of C18:0 than MLS. MH was intermediate, but not significantly different from the other areas. The proportion of C18:1 was numerically, but not statistically higher in MH than in MLS with MD intermediate. Although numerical differences were found for the proportions of C13:0 and C16:0 these were not significant (Figure 4-29).

Although the mean proportions of C16:0 in OMF of buffalo did not differ significantly between either area or gender, the interaction between area and gender for C16:0 was significant (P<0.05). The proportions of C16:0 in OMF of females remained fairly constant between the different areas (Figure 4-27). In both MH and MD, slightly lower proportions of C16:0 were noted for females than for males, while in MLS significantly higher proportions of C16:0 were noted for females than for males (Figure 4-27).

Figure 4-27 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) and gender (male and female) on the proportion of C16:0 in omental fat of the African buffalo (P<0.05).

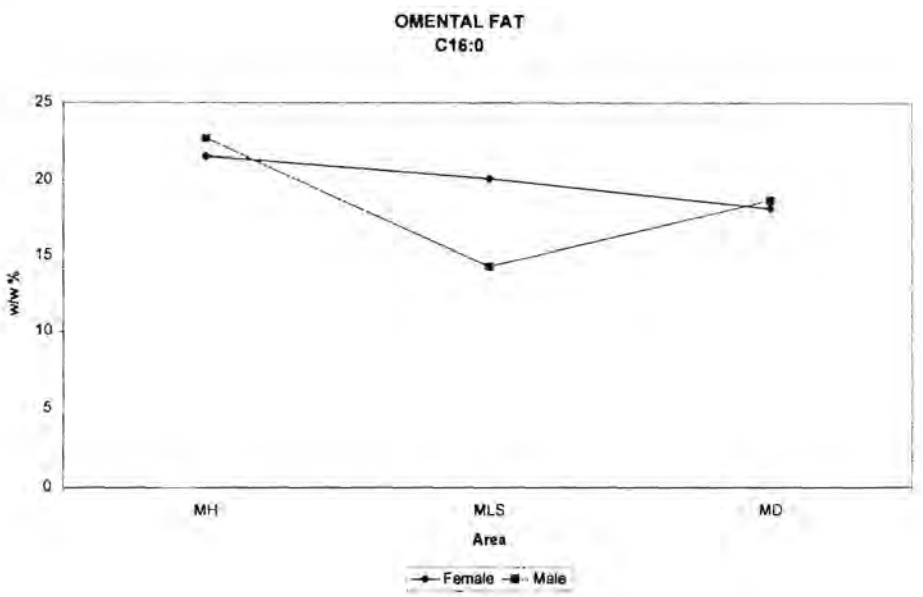


Figure 4-28 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the long-chain fatty acid composition of pericardial fat in the African buffalo.

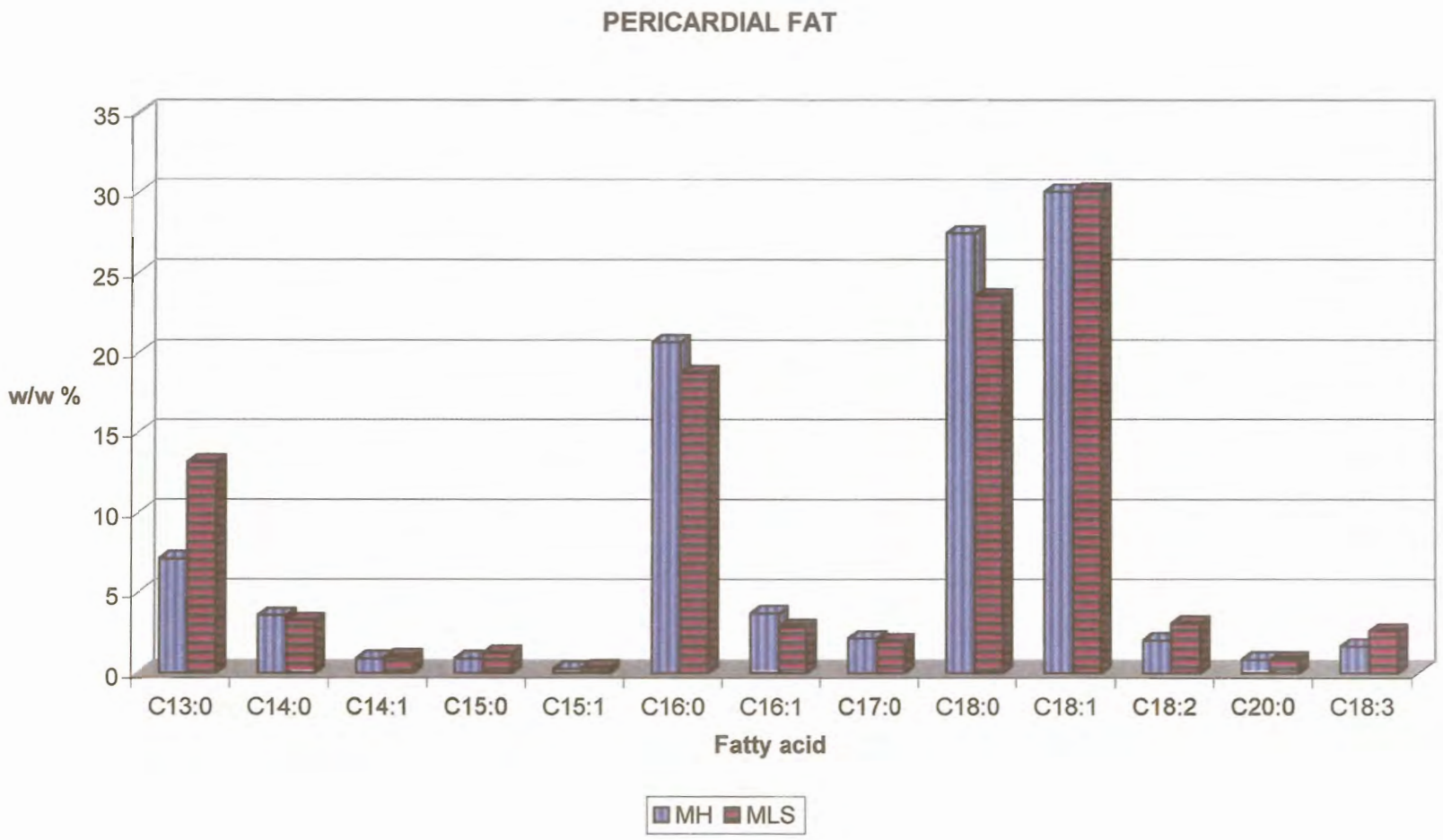
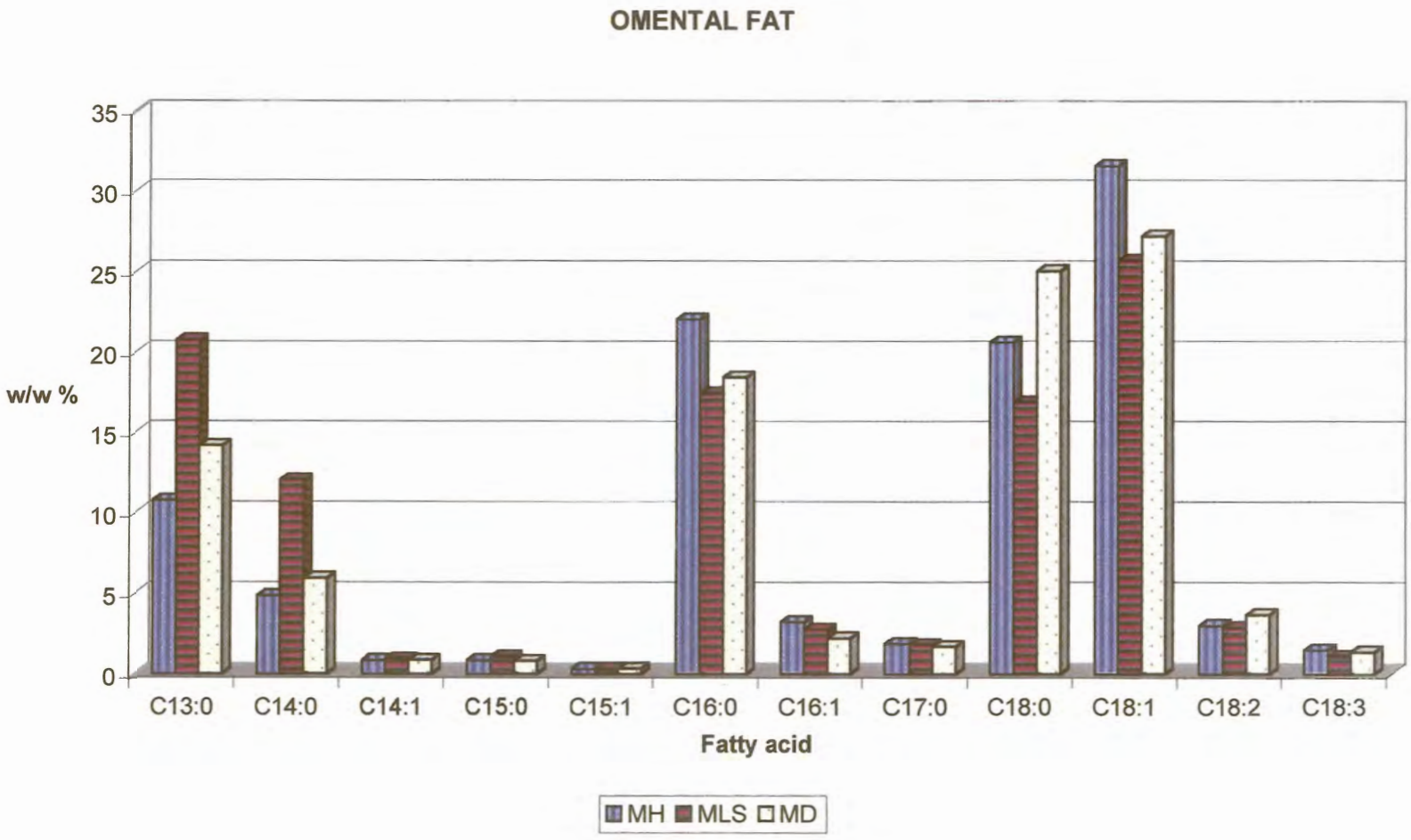


Figure 4-29 The effect of area (MH = Mtandanyathi at Houtboschrand; MLS = Mashatudrif at Lower Sabie; MD = Mpanamana dam at Crocodile Bridge) on the long-chain fatty acid composition of omental fat in the African buffalo.



Addendum I *Interaction effects of age and gender on the long-chain fatty acid composition (Mean±SD; w/w %) of subcutaneous fat in the African buffalo.*

	A		B		C		P
	Female (n = 6)	Male (n = 6)	Female (n = 6)	Male (n = 6)	Female (n = 6)	Male (n = 6)	
C13:0	24.172±6.047	55.772±26.916	42.876±27.614	45.054±22.618	17.336±18.578	37.473±18.823	0.155
C14:0	5.249±3.701	9.368±9.754	2.548±1.271	3.569±1.630	1.225±0.663	1.925±0.264	0.051
C14:1	0.757±0.054	0.415	0.742±0.117	0.698	0.620±0.283	0.883	0.039
C15:0	1.001±0.366	0.977	0.628±0.598	0.595±0.490	0.372±0.235	0.363	0.410
C15:1	0.228±0.046	0.196	0.225±0.029	0.138	0.172±0.115		
C16:0	19.490±3.410	10.336±6.520	12.585±6.168	12.576±4.588	12.719±3.886	11.636±2.508	0.037
C16:1	4.033±1.444	2.333±1.506	2.561±1.970	2.623±1.464	2.568±0.586	2.036±0.612	0.377
C17:0	1.374±0.191	0.728±0.125	0.939±0.602	0.666±0.768	1.830±0.182	1.025±0.510	0.243
C18:0	12.994±3.167	6.532±3.002	12.783±7.381	11.343±7.308	25.926±8.072	16.361±6.863	0.195
C18:1	28.054±3.921	16.611±8.322	22.757±10.844	22.267±9.478	34.224±7.999	27.916±9.163	0.327
C18:2	2.163±0.461	1.713±1.223	1.988±0.359	2.177±0.886	2.453±0.246	2.118±0.281	0.695
C18:3	1.756±0.380	1.469±0.730	1.249±0.542	0.857±0.664	1.370±0.402	0.879±0.172	0.882
UFA	36.575±4.781	20.430±10.596	28.584±13.280	27.777±11.731	40.505±9.130	32.803±10.052	0.236
SFA	63.426±4.780	79.570±10.597	71.149±13.034	72.082±11.529	59.409±9.190	67.199±10.052	0.241

Addendum II *Interaction effects of age and gender on the long-chain fatty acid composition (Mean±SD; w/w %) of M. Longissimus dorsi in the African buffalo.*

	A		B		C		P
	Female (n = 6)	**Male (n = 6)**	**Female (n = 5)**	**Male (n = 6)**	**Female (n = 6)**	**Male (n = 6)**	
C13:0	50.778±21.150	44.215±8.825	56.952±11.785	55.013±14.985	23.003±11.304	52.354±20.455	0.026
C14:0	3.766±0.317	11.632±20.597	4.474	1.782±1.050	2.167±0.717	11.464±15.713	0.816
C14:1	0.700±0.316		0.712		0.720±0.177		
C15:0	0.551±0.491	0.456	0.357±0.098	0.545	0.472±0.156	0.392±0.102	0.764
C15:1	0.369				0.283±0.201		
C16:0	13.399±4.610	11.961±3.486	11.444±3.352	10.914±3.157	18.085±3.926	10.388±5.418	0.109
C16:1	2.618±0.532	2.483±0.406	2.240±0.930	1.795±1.090	2.645±0.706	2.258±1.533	0.978
C17:0	1.851	0.664±0.170	0.763±0.603	0.916±0.140	1.416±0.166		0.076
C18:0	9.016±6.880	8.742±3.328	8.415±1.833	9.814±3.941	17.845±3.556	8.225±3.882	0.011
C18:1	18.692±5.885	18.376±6.105	16.956±3.510	17.889±5.943	31.317±3.295	16.513±8.942	0.017
C18:2	2.320±1.732	1.948±0.480	1.670±0.295	2.005±0.758	2.338±0.703	1.810±0.880	0.716
C18:3	0.884±0.689	0.903±0.229	0.867±0.436	0.718±0.274	1.202±0.207	1.056±0.216	0.998
C20:10	0.139±0.144	0.472±0.066	0.429±0.256		0.107±0.024		
UFA	24.808±7.735	22.996±7.212	21.603±5.081	22.287±7.568	37.978±4.382	21.261±11.364	0.046
SFA	75.125±7.788	76.847±7.254	78.225±5.172	77.062±7.464	61.951±4.417	78.515±11.364	0.046

Addendum III *Interaction effects of age and gender on the long-chain fatty acid composition (Mean±SD; w/w %) of perirenal fat in the African buffalo.*

	A		B		C		P
	Female (n = 6)	**Male (n = 6)**	**Female (n = 6)**	**Male (n = 6)**	**Female (n = 6)**	**Male (n = 6)**	
C13:0	5.669±1.025	13.452±9.596	7.255±2.947	15.827±12.462	16.278±14.989	7.223±6.674	0.056
C14:0	5.086±1.984	15.541±19.243	5.109±3.384	10.575±18.051	8.664±11.904	3.978±4.443	0.434
C14:1	0.846±0.311	0.629±0.021	0.902±0.185	1.148±0.028	1.103±0.332	1.157±0.261	0.217
C15:0	1.359±0.665	1.018±0.165	0.921±0.266	0.809±0.271	0.784±0.253	0.705±0.158	0.097
C15:1	0.276±0.045	0.247±0.024	0.329±0.028	0.310±0.013	0.317±0.093	0.322±0.066	0.484
C16:0	27.505±4.372	20.876±7.899	19.660±4.814	16.745±8.301	15.437±5.806	17.254±2.369	0.576
C16:1	5.028±1.904	3.311±1.049	3.487±0.544	3.440±1.746	2.542±0.794	2.555±0.841	0.423
C17:0	1.950±0.172	1.797±0.351	1.935±0.245	1.723±0.250	1.800±0.801	2.194±0.161	0.225
C18:0	16.301±5.141	13.092±5.032	24.813±5.422	21.755±11.850	21.995±6.321	27.575±6.124	0.188
C18:1	33.006±2.719	28.366±10.429	31.417±1.644	26.535±10.399	28.661±5.928	32.496±2.165	0.226
C18:2	2.257±0.262	2.077±0.750	2.057±0.315	2.723±0.880	2.428±0.442	3.552±2.542	0.553
C20:0	0.421		0.674±0.266	0.831±0.267	2.273±1.055	0.854±0.428	0.124
C18:3	1.950±0.309	1.750±6.225	1.770±0.425	2.064±0.262	2.050±1.061	1.559±0.308	0.213
UFA	43.035±2.803	34.996±12.988	39.962±2.478	33.260±13.456	34.898±7.350	40.887±2.684	0.138
SFA	56.965±2.803	65.007±12.988	60.031±2.481	66.733±13.462	65.103±7.350	58.730±2.115	0.126

Addendum IV *Interaction effects of age and gender on the long-chain fatty acid composition (Mean±SD; w/w %) of omental fat in the African buffalo.*

	A		B		C		P
	Female (n = 6)	**Male (n = 4)**	**Female (n = 4)**	**Male (n = 5)**	**Female (n = 6)**	**Male (n = 5)**	
C13:0	9.552±3.408	24.669±23.626	19.429±11.296	15.680±3.452	12.508±8.297	18.555±2.710	0.417
C14:0	4.818±1.213	16.762±15.988	1.916±0.845	17.780±21.028	7.208±10.742	3.267±0.836	0.226
C14:1	0.832±0.185	0.726	1.066±0.082	0.844±0.218	0.977±0.297	0.548	0.895
C15:0	1.288±0.418	0.637±0.443	0.990±0.117	0.664±0.199	0.589±0.269	0.661	0.351
C15:1	0.326±0.018	0.254	0.328±0.025	0.279	0.345±0.081		
C16:0	25.128±2.644	18.411±6.925	16.382±4.115	16.705±8.528	17.412±3.838	18.821±1.523	0.441
C16:1	3.639±0.895	2.402±0.554	2.288±0.686	2.432±1.956	2.474±1.194	2.869±1.015	0.192
C17:0	1.879±0.191	1.640±0.206	1.846±0.400	1.679±0.328	2.040±0.345	1.456±0.130	0.468
C18:0	16.739±3.677	12.943±5.311	25.664±5.884	18.840±8.412	25.816±8.637	20.205±4.219	0.156
C18:1	32.068±3.976	24.011±7.515	27.525±0.729	22.963±7.529	28.605±3.951	30.068±1.917	0.404
C18:2	2.130±0.183	2.715±0.854	3.220±2.026	3.839±1.377	2.703±0.646	4.208±0.978	0.462
C18:3	1.925±0.482	0.980±0.224	1.219±0.539	0.873±0.393	1.078±0.385	1.000±0.533	0.527
UFA	40.781±3.697	30.108±8.206	34.073±2.539	29.839±10.453	35.283±4.729	37.855±2.786	0.317
SFA	59.160±3.701	69.892±8.206	65.905±2.550	69.942±10.354	64.688±4.744	62.145±2.786	0.310

Addendum V *Interaction effects of area and gender on the long-chain fatty acid composition (Mean±SD; w/w %) of subcutaneous fat in the African buffalo.*

	MH		MLS		MD		P
	Female (n = 5)	Male (n = 5)	Female (n = 7)	Male (n = 6)	Female (n = 6)	Male (n = 7)	
C13:0	16.203±7.965	25.169±13.646	46.264±23.535	57.238±23.025	16.908±9.730	51.504±20.131	0.074
C14:0	3.170±2.458	8.245±10.559	5.656±5.674	4.532±1.307	2.075±1.020	3.007±1.467	0.018
C14:1	0.645±0.223	0.649±0.331	0.587±0.202		0.781±0.181	0.698	
C15:0	0.696±0.499	0.876±0.470	0.617±0.442	0.218	0.687±0.589	0.437±0.220	0.175
C15:1	0.175±0.082	0.196	0.220±0.086		0.238±0.058	0.138	
C16:0	17.408±6.672	15.992±4.369	11.620±4.962	9.491±4.387	16.731±3.414	10.055±2.947	0.045
C16:1	4.199±1.55	3.168±1.467	1.875±0.825	2.151±0.634	3.476±1.345	1.835±0.936	0.105
C17:0	1.899±0.088	1.424±0.330	0.972±0.521	0.400±0.239	1.667±0.284	0.825±0.470	0.322
C18:0	19.997±11.280	16.826±7.162	12.047±7.908	7.748±4.557	20.983±5.168	10.686±7.043	0.289
C18:1	32.866±6.899	27.402±5.377	20.949±8.744	19.348±12.617	33.206±4.559	21.095±9.077	0.162
C18:2	2.357±0.479	2.104±0.365	2.063±0.414	1.606±0.960	2.237±0.236	2.362±0.903	0.717
C18:3	1.591±0.354	1.064±0.331	1.195±0.645	1.122±1.073	1.626±0.290	0.888±0.466	0.309
UFA	41.575±5.037	34.037±5.493	26.143±10.433	22.949±14.132	40.518±5.550	25.455±11.086	0.170
SFA	58.341±5.108	65.963±5.493	73.629±10.235	76.978±14.085	59.466±5.580	74.487±11.004	0.176

Addendum VI *Interaction effects of area and gender on the long-chain fatty acid composition (Mean±SD; w/w %) of M. Longissimus dorsi in the African buffalo.*

	MH		MLS		MD		P
	Female (n = 5)	Male (n = 5)	Female (n = 6)	Male (n = 6)	Female (n = 6)	Male (n = 7)	
C13:0	44.344±17.592	57.442±18.102	50.292±18.841	49.632±15.432	33.996±25.843	46.356±13.644	0.536
C14:0		13.925±18.204	2.537±1.286	2.126±1.226	3.266±1.085	11.671±20.607	0.436
C14:1			0.476		0.769±0.145		
C15:0	0.407±0.076	0.456	0.325±0.091	0.320	0.662±0.345	0.505±0.057	0.594
C15:1	0.161±0.001		0.637		0.275±0.085		
C16:0	15.236±3.742	9.728±4.749	11.585±2.693	12.313±3.643	16.739±6.054	11.008±3.877	0.113
C16:1	2.453±0.445	2.244±1.094	2.168±0.666	2.220±1.248	2.918±0.798	2.030±1.080	0.470
C17:0	1.288±0.146		1.059±0.668	0.801±0.023	1.567±0.246	0.779±0.334	0.714
C18:0	10.793±6.009	6.517±3.379	10.613±6.177	10.146±2.687	14.267±7.087	9.571±3.973	0.342
C18:1	23.155±7.465	13.665±6.282	20.185±7.890	20.532±6.863	24.658±8.803	17.878±6.468	0.196
C18:2	2.460±1.868	1.366±0.609	1.900±0.491	2.184±0.508	2.099±0.816	2.092±0.716	0.388
C18:3	0.798±0.338	0.783±0.275	0.914±0.428	1.056±0.269	1.292±0.617	0.923±0.264	0.389
C20:1	0.092	0.519	0.328±0.250		0.122±0.090	0.425	0.797
UFA	28.931±7.376	17.609±8.119	25.278±9.186	25.464±8.741	31.401±10.989	22.633±8.011	0.217
SFA	71.051±7.395	82.178±8.173	74.558±9.228	74.536±8.741	68.496±10.991	77.306±8.037	0.239

Addendum VII *Interaction effects of area and gender on the long-chain fatty acid composition (Mean±SD; w/w %) of perirenal fat in the African buffalo.*

	MH		MLS		MD		P
	Female (n = 5)	Male (n = 5)	Female (n = 7)	Male (n = 6)	Female (n = 6)	Male (n = 7)	
C13:0	6.011±1.110	6.858±3.887	15.810±13.593	18.080±13.374	5.748±2.389	10.892±7.956	0.885
C14:0	3.703±2.419	7.577±5.742	4.225±1.976	19.047±24.505	10.845±10.970	4.057±3.678	0.102
C14:1	0.916±0.245	1.286±0.214	0.984±0.098	0.879±0.274	0.915±0.366	1.030±0.282	0.361
C15:0	0.803±0.195	0.864±0.218	1.440±0.732	0.818±0.279	0.963±0.210	0.863±0.238	0.004
C15:1	0.263±0.028	0.354±0.049	0.319±0.044	0.275±0.043	0.349±0.055	0.279±0.056	0.072
C16:0	24.070±5.934	21.373±4.986	20.649±7.461	14.700±8.593	18.452±7.329	19.169±4.941	0.258
C16:1	4.346±2.303	3.444±1.863	3.906±1.281	2.914±0.546	2.877±0.911	2.794±0.835	0.729
C17:0	2.185±0.113	2.205±0.213	2.079±0.160	1.827±0.440	1.632±0.536	1.855±0.235	0.229
C18:0	20.120±6.687	21.479±9.295	18.707±7.384	15.536±10.921	24.527±4.078	24.845±8.451	0.640
C18:1	34.337±3.190	31.257±3.121	30.262±3.008	25.064±14.013	29.165±4.679	31.102±2.884	0.336
C18:2	2.409±0.295	2.869±1.133	2.095±0.200	2.178±0.779	2.180±0.444	3.242±2.369	0.661
C20:0		1.156	2.181±1.200		0.680±0.369	0.738±0.249	
C18:3	1.722±0.450	1.782±0.323	1.592±0.144	1.660±0.723	2.325±0.686	1.796±0.414	0.179
UFA	43.993±2.603	40.007±4.235	37.451±5.768	30.868±17.079	37.541±5.715	38.514±5.266	0.480
SFA	56.007±2.603	59.533±3.936	62.543±5.773	69.125±17.085	62.459±5.715	61.486±5.266	0.492

Addendum VIII *Interaction effects of area and gender on the long-chain fatty acid composition (Mean±SD; w/w %) of omental fat in the African buffalo.*

	MH		MLS		MD		P
	Female (n = 5)	Male (n = 4)	Female (n = 7)	Male (n = 6)	Female (n = 4)	Male (n = 4)	
C13:0	8.379±3.833	13.738±8.144	16.708±9.543	25.472±16.177	12.806±8.465	15.518±2.461	0.771
C14:0	3.880±1.561	6.032±4.389	3.434±1.971	24.086±20.150	9.094±13.296	2.740±0.686	0.049
C14:1	0.866±0.223	0.726	0.922±0.256		1.063±0.163	0.745±0.230	
C15:0	0.788±0.345	1.138	1.385±0.491	0.386±0.122	0.997±0.155	0.663±0.141	0.001
C15:1	0.321±0.027	0.254	0.319±0.018		0.372±0.042	0.279	
C16:0	21.520±5.064	22.677±6.101	20.082±5.111	14.301±5.861	18.148±6.459	18.689±0.722	0.044
C16:1	3.060±1.449	3.394±1.264	2.962±1.126	2.446±1.324	2.503±0.684	1.968±0.609	0.444
C17:0	2.089±0.226	1.554±0248	1.846±0.311	1.494	1.835±0.363	1.576±0.229	0.747
C18:0	23.260±9.686	17.260±4.006	20.537±7.707	12.591±4.148	24.481±4.911	25.605±2.925	0.150
C18:1	32.122±3.458	30.868±1.841	29.619±3.261	21.064±7.563	26.549±3.545	27.837±1.284	0.055
C18:2	2.274±0.463	3.858±1.547	2.379±0.686	3.378±1.366	3.464±1.822	3.850±0.719	0.388
C18:3	1.679±0.621	0.961±0.378	1.210±0.569	0.786±0.098	1.865±0.226	1.092±0.517	0.884
UFA	40.258±3.651	38.844±2.296	36.747±3.980	27.004±9.620	33.540±5.399	35.376±2.448	0.088
SFA	59.691±3.666	61.156±2.296	63.234±3.988	72.813±9.599	66.404±5.452	64.625±2.448	0.101

CHAPTER 5

CONCLUSIONS

5.1 DEPOT FAT

It can be concluded that SCF and LD of buffalo are more saturated than PRF, PCF and OMF. The dominant fatty acids present in depot fat of buffalo are C18:1, C18:0, C16:0 and C13:0. C13:0 were found to be the most abundant fatty acid in the external fat depots (SCF and LD) and C18:1 in the internal fat depots (PCF, PRF and OMF). Individual fatty acids differed between internal and external fat depots. No significant differences were found between the relative proportions of fatty acids in the internal fat depots.

SCF and LD were found to have similar fatty acid profiles, without any significant differences between the two depots.

The present results indicate a depletion of energy reserves for maintenance, growth and lactation, as a result of nutritional stress, during the dry season. During the winter months, animals were in a poor nutritional status where fat reserves were mobilised for physiological functions.

Buffalo fat and muscle contain high proportions of saturated fatty acids, mainly due to the high proportions of C13: present in LD and SCF. The proportions of C18:1 and C18:0 were higher than those of C16:0. High proportions of C16:0 in the human diet is associated with increased levels of LDL, the "bad" cholesterol (Mattson and Grundy, 1985, McNamara, 1991). The influence of high proportions of C13:0 on human lipoproteins, are unknown. Although the total UFA were proportionally low, the relative proportions of C18:1 and C18:0 were high, both being associated with reduced levels of

LDL in blood (McNamara, 1991). The indications that C16:0 and C18:0 were the main fatty acids mobilised in buffalo when in negative energy balance, may implicate the total fat content of buffalo meat to be very low and that the influence due to the intake of saturated fat, are negligible in the human diet.

5.2 INFLUENCE OF AGE

Age differences were noted for specific fatty acids, but the proportions of total saturated and unsaturated fatty acids in the internal and external fat depots were not significantly influenced except for LD where the proportion of saturated fatty acids decreased with age. The lack of an increase in the proportion of C16:0 is probably due to the mobilisation of C16:0 for the maintenance of a constant physiological state in the animal. Not all animals in the A age group reached puberty yet, and therefore they have not reached the stage of higher fat deposition in subcutaneous adipose tissues (Vernon, 1991).

Within SCF and LD, the proportions of C18:0 and C18:1 increased with age. Similarly, the proportions of C13:0 in LD, and C14:0 and C18:3 in SCF decreased with age. Within the internal fat depots (PRF, PCF and OMF) C15:0 and C16:0 decreased, while the proportion of C18:0 increased, although only significant in PRF.

Although the change in saturation level of SCF was not significant, adult buffalo of the C age appeared to have more unsaturated fat than animals in the A age. This is in agreement with previous research suggesting that saturated fatty acids are desaturated to unsaturated fatty acids with age (Banskalieva, 1996; Webb and Casey, 1995; Westerling and Hedrick, 1979; Zembayashi and Nishimura, 1996).

5.3 INFLUENCE OF GENDER

The fatty acid composition of SCF and LD was significantly influenced by gender. Not only was individual fatty acids affected, but also the proportions of total SFA present within these depots. Similarities were observed within SCF and LD for gender. Male buffalo had significantly more saturated fatty acids than females. In both depots, this appeared to be due to the higher proportions of C13:0 for males, although the difference were only significant for SCF.

Cramer and Marcello (1964) (as quoted by Webb, 1992) reported that females had larger amounts of fatty acids with 16 or more carbons and lower proportions of fatty acids with 16 or less carbons than males. Similar results were obtained in the present study where the proportions of C16:0, C16:1, C17:0, C18:0, C18:1, C18:2 and C18:3 were either significantly, or numerically higher in SCF and LD of females than observed in males.

The internal fat depots were, as expected, not significantly influenced by gender, except for C15:0 and C16:0 in PCF, and C15:0, C15:1, C17:0, C18:0 and C18:3 in OMF. These differences might be due to dietary differences between gender.

Although the reason for the difference in the domposition of pericardial fat between male and female buffalo is unclear, it appears that buffalo tend to deposit more fat pericardially than perirenally.

5.4 INFLUENCE OF AREA

The fatty acid profiles of buffalo sampled in different areas differed significantly. Internal fat depots appeared to be more stable compared to the external depots, which were more susceptible to environmental influences.

Significant differences ($P<0.05$) were found in the total unsaturated fatty acid (UFA) content of the subcutaneous fat of buffalo sampled in the different areas. The area and therefore the diet of the animals significantly influenced the fatty acid composition of the subcutaneous fat of buffalo. These differences are probably due to different veld-types and possibly different plant species selected by buffalo in the different areas.

A higher proportion of unsaturated fatty acids was observed in animals from the Mopane/Bushwillow woodlands (MH) than animals from the thorn thickets (MLS), while that of animals from the Marula savannah (MD) was intermediate. This may indicate that animals from MLS were in poorer condition than MD, with animals from MH in the best condition. Although no data was available on the conditions of the animals or the veld condition, animals from MLS were visually observed to be leaner (de Vos, personal communication).

It can be concluded that male buffalo, especially from MD, were in poorer body condition that females, although all animals appeared to be in a poor condition due to the depletion of stores of C16:0, C18:0 and C18:1, resulting consequently in a relative increase in proportions of especially C13:0 and other fatty acids within the depot fat of buffalo.

Due to the mobilisation of C16:0, C18:0 and C18:1 in animals from MLS, the proportion of C13:0 was relatively higher in these samples. The concentration of C13:0 may have remained constant, but the proportions, relative to the other fatty acids, increased due to the depletion of adipose tissue reserves. This may indicate that C13:0 is not easily mobilised from adipose tissue stores after deposition from the diet (without being modified).

The present results indicate that animals were in a poor nutritional status during the winter months, where fat reserves were mobilised for physiological functions. Parasitic infections and diseases in buffalo may also result in decreased productivity and poor condition of buffalo (Young and van den Heever, 1969). The original datasheet indicated that 6 buffalo were infected with TB. This type of work need to be repeated in different seasons to quantify the effect of season and fatty acid profiles as well as correlations with the condition scores of buffalo.

5.5 CRITICAL EVALUATION

Fatty acid mobilisation is part of a complex system, especially in free ranging buffalo where it is significantly influenced by environmental and dietary variations.

Results obtained in this study did not always agree with that reported for domestic ruminant species. This is probably due to the fact that animals were exposed to a harsh environment with significant fluctuating environmental conditions (e.g. feed quality and quantity, temperature etc).

The proportions of C13:0 observed in especially SCF and LD of buffalo, were unexpectedly high and caused concern. The first reaction was to ignore it, because it has never been reported to be a significant fatty acid in animal tissue.

The prominent peak of C13:0 could have been residues due to the addition of BHT during the extraction method. Therefore, similar samples were prepared with and without the addition of BHT but without any difference in the C13:0 peak. The standard was injected in-between samples and all relevant peaks occurred at the correct retention times, which rules out any deficiency of the column. Sample extraction and preservation were changed

(see Chapter 3) without any change in the peak of C13:0, the only difference being a better separation of the peaks of C13:0 and C14:0.

Before and after analysis of the buffalo samples, adipose tissue samples from steers and sheep were analysed, using the same extraction methods. C13:0 were not present to the same extend in these samples compared to buffalo samples. The lower proportions of C13:0 in PCF, OMF and PRF than in SCF and LD indicated that it could not be ignored.

In the present study, the peak has been identified and reported as C13:0, but the possibility that it may be wrong, can not be ignored. In future studies, other extraction methods need to be used and samples will need to be referred to other laboratories for confirmation of results.

Odd-numbered and branched-chain fatty acids can be synthesised *de novo* by certain rumen microbes (Christie, 1981a) and are deposited in adipose tissue without further metabolism. It can also be present in the diet. The rumen microbial population vary between species, breeds and even individual animals within herds (Noble, 1981) and is also influenced by the diet of the animal.

The proportion of C13:0 was influenced by the area and gender, suggesting that it could have been due to a specific plant pecies present in all areas, and consumed by all animals. It may be something like the common reed, *Phragmites austalis*, found not only in the riverine areas of KNP, but all over the world (Marks *et al.*, 1993). It is suspected that reeds are grazed during the dry winter months when herds utilise the riverine areas more readily (Funston *et al.*, 1994). Fatty acid profiles of reeds are not available. Fatty acid analyses of the common reed need to be included in future studies.

Before C13:0 are condemned as 'impossible to be present in ruminant fat in the proportions reported', it is important to note that the body condition of the animals, appeared to be poor and mainly due to the fact that the proportions of C16:0, a fatty acid known to be mobilised extensively during periods of negative energy balance, were very low in especially LD and SCF. Concentrations of the different fatty acids were not calculated, but might have given a completely different view and probably a better explanation for the observed results.

Since C16:0, C18:0 and C18:1 are mobilised for maintenance during growth and lactation, they are proportionally decreased as compared to C13:0, although the concentration of C13:0 probably remained constant in SCF and LD. The proportions of C13:0 in the internal depots (PRF, PCF and OMF) remained relatively uninfluenced by age or gender, with only PRF being influenced by area. This implicated that either the animals from MLS were in a poorer condition (PRF were also in the process of being mobilised) or there was more C13:0 in the diet of these animals.

Future research may involve rumen microorganisms and ruminal fermentation of dietary fatty acids in buffalo, plasma concentrations of fatty acids, seasonal variation in the long-chain fatty acid composition (the diet of buffalo are reported to be changing throughout the year). A research project was recently conducted in the KNP to study the correlations between body condition and composition of free-ranging buffalo and the incidence of TB. This research will also provide valuable information and could be combined with the present results to provide excellent baseline data for future projects on the African buffalo.

CHAPTER 6

BIBLIOGRAPHY

ADROUNI, B. AND KHACHADURIAN, A.K., 1968. Metabolism of individual fatty acids by sheep depot adipose tissue in vivo and in vitro. *Comp. Biochem. Physiol.*, 26:321-327.

AOAC, 1975. Official methods of Analysis of the Association of Official Analytical Chemists. 28.057: 479-498.

ASCN/AIN, 1996. Position paper on trans fatty acids. Special Task Force report. *Am J. Clin Nutr.* 63: 663-670.

BANSKALIEVA, V., 1996. Effect of age, physiological state and nutrition on fatty acid composition in depot fat and ruminal volatile fatty acids in sheep. *Small Rumin. Res.* 24: 37-42.

BAS, P., GALLOUIN, F. AND MORAND-FEHR, P., 1992. Changes in lipid content, fatty acid composition and lipoprotein lipase activity in dry goat omental adipose tissue according to tissue site. *Lipids* 27(6): 470-473.

BAUMAN, D.E. AND CURRIE, W.B., 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63: 1514-1529.

BEEKMAN, J.H. AND PRINS, H.H.T., 1989. Feeding strategies of sedentary large herbivores in East Africa with emphasis on the African buffalo, *Syncerus caffer*. *Afr.J.Ecol.* 27(2): 129-147.

BELL, A.W., 1981. Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. In: *Lipid metabolism in ruminants.* Edited by W.W. Christie First edition. Pergamon Press, Oxford. 363-410.

BERTSCHINGER, H.J., 1996. Reproduction in the African Buffalo: A Review. In: *Proceedings of a Symposium on the African Buffalo as a Game Ranch Animal, Onderstepoort, 26 October 1996.* Edited by B.L. Penzhorn. RSA. 62-74.

BONANOME, A. AND GRUNDY, S.M., 1987. Effects of dietary fats on plasma cholesterol in humans. American Meat Science Association Reciprocal Meat Conference Proceedings 40: 89-92.

BROWN, W.H., 1988. Introduction to Organic Chemistry. 4th edition. Brooks/Cole Publishing Company. Pacific Grove California. (Chapter 12: Lipids) 415-442.

CASEY, N.H. AND WEBB, E.C., 1995. Influence of dietary energy levels and form of diet on composition of fatty acids in subcutaneous tissue of wethers. *Small Rumin. Res.* 18: 125-132.

CASEY, N.H., AND VAN NIEKERK, W.A., 1988. Fatty acid composition of subcutaneous and kidney fat depots of Boer goats and the response to varying levels of maize meal. *S. Afr. J. Anim. Sci.* 15 (2): 60-62.

CASEY, N.H., VAN NIEKERK, W.A. AND SPREETH, E.B., 1988. Fatty acid composition of subcutaneous fat of sheep grazed on eight different pastures. *Meat Sci.* 23: 55-63.

CHRISTIE, W.W., 1981a. The composition, structure and function of lipids in the tissue of ruminant animals. In: *Lipid metabolism in ruminants*. First edition. Edited by W.W. Christie. Pergamon Press, Oxford.

CHRISTIE, W.W., 1981b. The effects of diet and other factors on the lipid composition of ruminant tissues and milk. In: *Lipid metabolism in ruminants*. First edition. Edited by W.W. Christie. Pergamon Press, Oxford.

CRAWFORD, M.A., GALE, M.M., WOODFORD, M.H. AND CASPERD, N.M., 1970. Comparative studies on fatty acid composition of wild and domestic meats. *Int. J. Biochem.* 1 (3): 295-305.

DE FRANCIS, G. AND MORAN, J.B., 1991. Meat Production from River Buffalo. *World Review of Animal Production*. 26 (4): 65-71

DE VOS, V., BENGIS, R.C. AND COETZEE, H.J., 1983. Population control of large mammals in the Kruger National Park. In: *The management of large mammals in African conservation areas*. Edited by R.N Owen-Smith, Pretoria: HAUM Publishers. 213-231.

DEVENDRA, C., 1992. Nutrition of Swamp Buffaloes. In: *Buffalo Production. World Animal Science C, Production-system approach 6.* Chapter 6. Edited by N.M. Tulloh and J.H.G. Holmes. Amsterdam: Elsevier. 135-151.

DHANDA, J.S., TAYLOR, D.G., MURRAY, P.J. AND McCOSKER, J.E., 1999. The Influence of goat genotype on the production of capretto and chevon carcasses. 4. Chemical composition of muscle and fatty acid profiles of adipose tissue. *Meat Sci.* 52(4): 375-379.

DUNCAN, W.R.H. AND GARTON, G.A., 1967. The fatty acid composition and intramolecular structure of triglycerides derived from different sites in the body of the sheep. *J. Sci. Fd Agric.* 18: 99-102.

EGAN, A.R., 1976. Lipid metabolism. In: *Veterinary Physiology*. Edited by J.W. Phyllis, Bristol: Wright-Scientechnica. 593.

EICHHORN, J.M., BAILEY, C.M. AND BLOMQUIST, G.J., 1985. Fatty acid composition of muscle and adipose tissue from crossbred bulls and steers. *J. Anim. Sci.* 61(4): 892-904.

EICHHORN, J.M., COLEMAN, L.J., WAKAYAMA, E.J., BLOMQUIST, G.J., BAILEY, C.M. AND JENKINS, T.G., 1986. Effects of breed type and restricted versus *ad libitum* feeding on fatty acid composition and cholesterol content of muscle and adipose tissue from mature bovine females. *J. Anim. Sci.* 63: 781-794.

ENSER, M., HALLETT, K., HEWITT, B., FURSEY, G.A.J. AND WOOD, J.D., 1996. Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Sci.* 42(4): 443-456.

FARID, A., 1991. Carcass Physical and chemical composition of three fat-tailed breeds of sheep. *Meat Sci.* 29: 109-120.

FISCHER, H. AND BODHIPAKSHA, P., 1992. Reproduction in Swamp Buffaloes. In: *Buffalo Production. World Animal Science C, Production-system approach 6*. Chapter 7. Ed. N.M. Tulloh and J.H.G. Holmes. Amsterdam: Elsevier. 171-183.

FOLCH, J., LEES, M. AND STANLEY, H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chemistry* 226: 497-509.

FUNSTON, P.J., SKINNER, J.D. AND DOTT, H.M., 1994. Seasonal variation in movement patterns, home range and habitat selection of buffalo in semi-arid habitat. *Afr. J. Ecol.* 32(2): 100-114.

GREENWOOD, M.R.C., KAVA, R., WEST, D.B. AND SAVARD, R., 1988. Fat distribution and metabolism in animal studies. *In Fat Distribution during Growth and Later Health Outcomes*. Alan R Liss, Inc. 285-295.

GROBLER, D.G., 1996. The potential utilization of the African Buffalo with regard to hunting and meat production. In: *Proceedings of a symposium on the African Buffalo as a Game Ranch Animal, Onderstepoort, 26 October 1996*. Edited by B.L. Penzhorn. RSA 37-42.

HARFOOT, C.G., 1981. Lipid metabolism in the rumen. In: *Lipid metabolism in ruminants*. First edition. Edited by W.W. Christie. Pergamon Press, Oxford. 1-56.

HILL, D., 1988. Cattle and buffalo meat production in the tropics. (Intermediate Tropical Agricultural Series). Harlow: Longman Scientific and Technical.

HORNICK, J.L., VAN EENAEME, C., CLINQUART, A., DIEZ, M. AND ISTASSE, L., 1998. Different periods of feed restriction before compensatory growth in Belgian blue bulls: I.

Animal performance, nitrogen balance, meat characteristics, and fat composition. *J. Anim. Sci.* 76: 249-259.

HUERTA-LEIDENZ, N.O., CROSS, H.R., SAVELL, J.W., LUNT, D.K., BAKER, J.F., PELTON, L.S. AND SMITH, S.B., 1993. Comparison of the fatty acid composition of subcutaneous adipose tissue from mature Brahman and Hereford cows. *J. Anim. Sci.* 71: 625-630.

HUERTA-LEIDENZ, N.O., CROSS, H.R., SAVELL, J.W., LUNT, D.K., BAKER, J.F. AND SMITH, S.B., 1996. Fatty acid composition of subcutaneous adipose tissue from male calves at different stages of growth. *J. Anim. Sci.* 74: 1256-1264.

ISHIDA, M., BARRY, T.N., PURCHAS, R.W. AND ALLSOP, T.F., 1989. Comparison of lipid synthesis metabolism in sheep, deer and goats. *AJAS* 2(3): 542-543.

KEMP, J.D., MAHYUDDIN, M. AND ELY, D.G., 1981. Effect of feeding systems, slaughter weight and sex on organoleptic properties and fatty acid composition of lamb. *J. Anim. Sci.* 51(2): 321-330.

KOTZ, J.C. AND PURCELL, K.F., 1987. Chemistry and chemical reactivity. Saunders College Publishing, United States. 890.

KRUGER NATIONAL PARK, 1993. Make the most of Kruger. Jacana, Johannesburg.

KRÜGER, J., 1996. Socio-ecology of the African buffalo (*Syncerus caffer*): implications for game ranching. In: *Proceedings of a Symposium on the African Buffalo as a Game Ranch Animal, Onderstepoort, 26 October 1996.* Edited by B.L. Penzhorn. RSA. 1-5.

KURBANOV, YU.R., RAKHMANOV, R.R., ISMAILOV, A.I. AND KAMALOV, S.H., 1975. Fatty acid composition of mutton fat of hybrids of fat-tailed sheep. *Uzb. Biol. Zh.* 3: 34-37.

LINK, B.A., BRAY, R.W., CASSENS, R.G. AND KAUFFMAN, R.G., 1970. Fatty acid composition of bovine skeletal muscle lipids during growth. *J. Anim. Sci.* 30: 726-731.

LUDDY, F.E., BRADFORD, R.A. AND RIEMENSCHNEIDER, R.W., 1960. Direct Conversion of Lipid Components to their Fatty Acid Methyl Esters. *J. Am. Oil Chem.* 37: 447-451.

MAHADEVAN, P., 1992. Distribution, Ecology and Adaptation. In: *Buffalo Production. World Animal Science C, Production-system approach 6.* Chapter 1. Edited by N.M. Tulloh and J.H.G. Holmes. Amsterdam: Elsevier. 1-12.

MALAU-ADULI, A.E.O., SIEBERT, B.D., BOTTEMA, C.D.K. AND PITCHFORD, W.S., 1997. A comparison of the fatty acid composition of triacylglycerol in adipose tissue from Limousin and jersey cattle. *Aust. J. Agric. Res.* 49: 715-722.

MARCELLO, M.J., COOK, NANCY, K., SLANGER, W.D., JOHNSON, V.K., FISCHER A.G. AND DINUSSON, W.E., 1983. Fatty acid composition of lean and fat tissue of swine fed various dietary levels of sunflower seed. *J. Food. Sci.* 48: 1331.

MARKS, M., LAPIN, B. AND RANDALL, J., 1993. Element Stewardship abstract for *Phragmites australis*, common reed. The Nature Concervancy, Virginia.

MARTINN, C.R.M., 1995. Dictionary of endocrinology and related biomedical sciences. Oxford University Press, New York, Oxford.

MATHEWS, C.K. AND VAN HOLDE, K.E., 1990. Biochemistry. The Benjamin/Cunnings Publishing Company, Inc.

MATTSON, F.H. AND GRUNDY, S.M., 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid Res.* 26:194-202.

MCNAMARA, D.J., 1991. Relationship between fat, cholesterol and coronary heart disease. *AMSA Reciprocal Meat Conference Proceedings* 44: 147-152.

MOORE, J.H. AND CHRISTIE, W.W., 1981. Lipid metabolism in the mammary Gland of Ruminant Animals. In: *Lipid metabolism in ruminants.* First edition. Ed. W.W. Christie. Pergamon Press, Oxford. 227-278.

MORAN, J.B., 1992. Growth and Development of Buffaloes. In: *Buffalo Production. World Animal Science C, Production-system approach 6.* Chapter 10. Edited by N.M. Tulloh and J.H.G. Holmes. Amsterdam: Elsevier. 191-221.

MUDGAL, V.D., 1992. Reproduction in River Buffaloes. In: *Buffalo Production. World Animal Science C, Production-system approach 6.* Chapter 8. Edited by N.M. Tulloh and J.H.G. Holmes. Amsterdam: Elsevier. 171-183.

NAGARCENKAR, R. AND LUDRI, R.S., 1992. Lactation in River Buffaloes. In: *Buffalo Production. World Animal Science C, Production-system approach 6.* Chapter 9. Edited by N.M. Tulloh and J.H.G. Holmes. Amsterdam: Elsevier. 183-190.

NOBLE, R.C., 1981. Digestion, absorption and transport of lipids in ruminant animals. In: *Lipid metabolism in ruminants*. First edition. Edited by W.W. Christie. Pergamon Press, Oxford. 57-94; 411-448.

NÜRNBERG, K., GRUMBACH, S., PAPSTEIN, H.J., MATTHES, H.D., ENDER, K. AND NÜRNBERG, G., 1996. Fatty acid composition of lamb meat. *Fett/Lipid* 98: 77-80.

NÜRNBERG, K., WEGNER, J. AND ENDER, K., 1998. Factors influencing fat composition in muscle and adipose tissue of farm animals. *Livest. Prod. Sci* 56(2): 145-156.

PARK, Y.W. AND WASHINGTON, A.C., 1993. Fatty acid composition of goat organ and muscle meat of Alpine and Nubian breeds. *J. Food Sci.* 58 (2) 245-248.

PAYNE, E. AND MASTERS, C.J., 1971. The in vitro incorporation of 14C-labelled fatty acids into ovine liver and omental adipose tissue. *Int. J. Biochem.* 2: 623-643.

PEARSON, A.M. AND DUTSON, T.R., 1991. Growth regulation in farm animals. Advances in Meat Research Vol. 7. Elsevier Applied Science. London and New York.

PEARSON, A.M. AND DUTSON, T.R., 1991. Meat and Health. Advances in Meat Research Vol. 6. Elsevier Applied Science. London and New York.

PERRY, D., NICHOLLS, P.J. AND THOMPSON, J.M., 1998. The effect of sire breed on the melting point and fatty acid composition of subcutaneous fat in steers. *J. Anim. Sci.* 76:87-95.

PHYLLIS, J.W., 1976. Veterinary Physiology. Wright-scientechnica. Bristol.

PRINS, H.H.T., 1996. Ecology and Behavior of the African Buffalo: Social inequality and decision making. 1st edition. London: Chapman and Hall.

PRINS, H.H.T. AND BEEKMAN, J.H., 1989. A balanced diet as a goal for grazing: The food of the Manyara buffalo. Afr. J. Ecol. 27(3): 241-259.

RANJHAN, S.K., 1992. Nutrition of river buffaloes in Southern Asia. In: *Buffalo Production. World Animal Science C, Production-system approach 6*. Chapter 5. Edited by N.M. Tulloh and J.H.G. Holmes. Amsterdam: Elsevier. 111-134.

ROWE, A., MACEDO, F.A.F., VISENTAINER, J.V., SOUZA, N.E. AND MATSUSHITA, M., 1999. Muscle composition and fatty acid profile in lambs fattened in drylot or pasture. *Meat Sci.* 51(4): 282-288.

RULE, D.C., MACNEIL, M.D. AND SHORT, R.E., 1997. Influence of sure growth potential, time on feed, and growing-finishing strategy on cholesterol and fatty acids of the ground carcass and longissimus muscle of beef steers. *J. Anim. Sci.* 75: 1525-1533.

RUMSEY, T.S., OLTJEN, R.R., BOVARD, K.P. AND PRIODE, B.M., 1972. Influence of widely diverse finishing regimens and breeding on depot fat composition in beef cattle. *J. Anim. Sci.* 35:1069-1075.

SAS, 1992. SAS/STAT User's guide. Statistical Analysis System Institute, Inc., Cary, NC, USA.

SEKHON, K.S. AND BAWA, A.S., 1996. Effect of muscle-type, stage of maturity and level of nutrition on the quality of meat from male buffalo calves. *Food Res. Int.* 29(8): 779-783.

SIEBERT, B.D., DELAND, M.P. AND PICHFORD, W.S., 1996. Breed differences in the fatty acid composition of subcutaneous and intramuscular lipid of early and late maturing, grain-finished cattle. *Aust. J. Agric. Res.* 47:943-952.

SINCLAIR, A.R.E., 1977. The African buffalo: a study of resource limitation of populations. Chicago: University of Chicago Press.

SINNET-SMITH, P.A. AND WOOLLIAMS, J.A., 1988. Genetic variations in subcutaneous adipose tissue metabolism in sheep. *Anim. Prod.* 42: 263-270.

STARK, M.A., 1986. Daily movement, grazing activity and diet of savanna buffalo, *Syncerus caffer* brachyceros, in Benoue National Park, Cameroon. *Afr.J.Ecol.* 24(4): 255-262.

STEENKAMP, K., WEBB, E.C., DE VOS, V AND VAN VUUREN C.J., 1999a. Long-chain fatty acid composition of different fat depots in the African buffalo (*Syncerus caffer*). *SA. J. Anim. Sci.* 29(ISRP). 223-224.

STEENKAMP, K., WEBB, E.C., DE VOS, V AND VAN VUUREN C.J., 1999b. The effect of age, sex and habitat on long-chain fatty acid composition of fat depots in the African buffalo (*Syncerus caffer*). *SA. J. Anim Sci.* 29(ISRP). 218-220.

STURDIVANT, C.A., LUNT, D.K., SMITH, G.C. AND SMITH, S.B., 1992. Fatty acid composition of subcutaneous and intramuscular adipose tissue and M. longissimus dorsi of Wagyu cattle. *Meat Sci.* 32(4): 449-458.

TICHENOR, D.A., KEMP, J.D., FOX, J.D., MOODY, W.G AND DEWEESE, W., 1970. Effect of slaughter weight and castration on ovine adipose fatty acids. *J. Anim. Sci.* 31:671.

TROLLOPE, W.S.W., POTGIETER, A.L.F. AND ZAMBATIS, N., 1989. Assessing veld condition in the Kruger National Park using key grass species. *Koedoe* 32 (1): 67-93.

TULLOH, N.M. AND HOLMES, J.H.G., 1992. Buffalo Production. World Animal Science C, Production-system approach 6. Amsterdam: Elsevier.

VAN OUDTSHOORN, F.P., 1992. Guide to grasses of South Africa. First edition. BRIZA Publications cc.

VERNON, R.G., 1981. Lipid metabolism in the adipose tissue of ruminant animals. In: *Lipid metabolism in ruminants*. First edition. Edited by W.W. Christie. Pergamon Press, Oxford. 279-362.

WAYS, P. AND HANAHAN, D.J., 1964. Characterization and quantification of red cell lipids in normal man. *J. Lipid Res.* 5: 318-328.

WEBB, E.C. AND CASEY, N.H., 1995. Genetic differences in fatty acid composition of subcutaneous adipose tissue in Dorper and SA Mutton Merino wethers at different live weights. *Small Rum. Res.* 18:81-88.

WEBB, E.C. AND CASEY, N.H., 1995. Fatty acids in carcass fat of steers treated with a ß-adrenergic agonist individually or in combination with trenbolone acetate + oestradiol-17ß. *Meat Sci.* 41(1):69-76.

WEBB, E.C., 1992. The influence of dietary energy levels on subcutaneous fatty acid profiles and meat quality in sheep. M.Sc (Agric) (Meat Science) thesis, University of Pretoria, Pretoria.

WEBB, E.C., 1994. Synthesis of long chain fatty acids in ruminants and their effects on meat quality. Ph.D. thesis, University of Pretoria, Pretoria.

WEBB, E.C., BOSMAN, M.J.C. AND CASEY, N.H., 1994. Dietary influences on subcutaneous fatty acid profiles and sensory characteristics of Dorper and SA Mutton Merino wethers. *SA. J. Food Sci. Nutr.* 6(2): 45-50,

WEBB, E.C., CASEY, N.H. AND VAN NIEKERK, W.A., 1994. Fatty acids in the subcutaneous adipose tissue of intensely fed SA Mutton Merino and Dorper wethers. *Meat Sci.* 38:123-131.

WEBB, E.C., DE SMET, S., VAN NEVEL, C., MARTENS, B. AND DEMEYER, D.I., 1998. Effect of anatomical location on the composition of fatty acids in double-muscled Belgian Blue cows. *Meat Sci.* 50(1): 45-53.

WENTZEL, J.J., DU P. BOTHMA J. AND VAN ROOYEN, N., 1991. Characteristics of the herbaceous layer in preferred grazing areas of six herbivore species in the south-eastern Kruger National Park. *Koedoe.* 34(1): 51-59.

WESTERLING, D.B. AND HEDRICK, H.B., 1979. Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. *J. Anim. Sci.* 48(6): 1343-1348.

WHYTE, I.J., 1996. The management of large buffalo populations. In: Proceedings of a Symposium on The African Buffalo as a Game Ranch Animal, Onderstepoort, RSA, 26 October 1996. Edited by B.L. Penzhorn 21-36.

WOOD, J.D., ENSER, M. AND WARRIS, P.D., 1991. Reducing fat quantity: implications for meat quality and health aspects. In: *Animal biotechnology and the quality of meat production*. Edited by L.O. Fiems, B.G. Cottyn and D.I. Demeyer. Elsevier, Amsterdam. 69-84.

WU, FU-YU AND SAVELL, J.W., 1992. Effects of breed and sex on the fatty acid composition of subcutaneous fat from sheep and goats. *J. Chin. Soc. Anim. Sci.* 21(3): 299-305.

XIE, Y.R., BUSBOOM, J.R., GASKINS, C.T., JOHNSON, J.J., WRIGHT, R.W. AND CRONRATH, J.D., 1996. Effects of breed and sire on carcass characteristics and fatty acid profiles of crossbred Wagyu and Angus steers. *Meat Sci.* 43(2): 167-177.

YOUNG, E. AND VAN DEN HEEVER, L.W., 1969. The African buffalo as a source of food and by-products. *J. S. Afr. Vet. med. Ass.* 40(1): 83-88.

ZEMBAYASHI, M. AND NISHIMURA, K., 1996. Genetic and nutritional effects on the fatty acid composition of subcutaneous and intramuscular lipids of steers. *Meat Sci.* 43(2): 83-92.

ZEMBAYASHI, M., NISHIMURA, K., LUNT, D.K. AND SMITH, S.B., 1995. Effect of breed type and sex on the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers and heifers. *J. Anim. Sci.* 73: 3325-3332.