Impact of season on the composition and quality of male and female blesbok (*Damaliscus pygargus phillipsi*) muscles

by
Jeannine Neethling

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Supervisor: Prof LC Hoffman
Co-supervisor: Prof TJ Britz

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DECLARATION

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SUMMARY

The objective of this study was to investigate the impact of season on the composition and quality of male and female blesbok (*Damaliscus pygargus phillipsi*) muscles (*Longissimus dorsi*, *Biceps femoris*, *Semimembranosus*, *Semitendinosus*, *Infraspinatus* and *Supraspinatus*). The latter was quantified on the chemical composition (moisture, protein, fat and ash contents), fatty acid profile, mineral composition and physical attributes (pH, colour, drip and cooking loss, water holding capacity and tenderness) of the selected muscles.

All of the blesbok muscles had higher ($p<0.01$) mean protein contents (20.6 g.100 g^{-1} to 23.1 g.100 g^{-1}) when the plane of nutrition was believed to be higher (spring of 2009). A strong negative correlation ($r = – 0.82$; $p<0.01$) existed between the moisture and protein contents of the blesbok muscles. The *Longissimus dorsi* muscle had the highest ($p<0.01$) mean intramuscular fat content (3.4 g.100 g^{-1}) when the plane of nutrition was higher. The chemical composition of the *Longissimus dorsi*, *Biceps femoris*, *Semitendinosus* and forequarter muscles (*Infraspinatus* and *Supraspinatus*) was affected least by the seasonal differences in the plane of nutrition and activity levels of the blesbok at the study area. However, season had a larger impact on the chemical composition of the *Semimembranosus* muscle.

Season did not have a significant impact on the fatty acid profile of blesbok muscles, but the difference in the fatty acid profiles between male and female muscles was significant. A Principal Component Analysis (PCA) bi-plot indicated that female blesbok muscles were associated with a higher saturated fatty acid (SFA) and mono-unsaturated fatty acid (MUFA) content. Male blesbok muscles had higher ($p<0.01$) proportions of total polyunsaturated fatty acids (PUFA) (40.15 ± 5.39) and polyunsaturated to saturated fatty acid ratios (P:S) (0.85 ± 0.18), in comparison to female muscles (27.18 ± 8.04 and 0.54 ± 0.20, respectively). Differences in the anatomical locations of the selected blesbok muscles furthermore influenced the fatty acid profiles. The less active *Longissimus dorsi* muscle had higher ($p<0.05$) total PUFA (38.34 ± 8.62), total omega-6 (ω6) PUFA (34.46 ± 7.83), total ω3 PUFA (3.44 ± 0.84) and P:S (0.85 ± 0.24) contents, in comparison to the *Infraspinatus* muscle (28.96 ± 8.65, 26.23 ± 7.86, 2.31 ± 0.70 and 0.56 ± 0.19, respectively) and *Supraspinatus* muscle (28.85 ± 9.23, 26.05 ± 8.24, 2.28 ± 0.76 and 0.55 ± 0.21, respectively). The hindquarter muscles (*Biceps femoris, Semimembranosus* and *Semitendinosus*) had intermediate fatty acid content.

Season had an impact on the calcium and zinc contents of blesbok muscles. The calcium content was higher ($p<0.05$) in the muscles of the animals harvested in spring (6.92 ± 1.94) compared to winter (5.61 ± 1.79). The zinc content was higher ($p<0.05$) in the muscles of male blesbok harvested in winter (4.04 ± 1.70) compared to spring (3.41 ± 1.67). The mineral composition was furthermore significantly different between the selected blesbok muscles. The *Biceps femoris* muscle had the highest ($p<0.05$) potassium (183.25 ± 12.79), phosphorus (180.21 ± 10.36) and magnesium (32.18 ± 1.72) content, while the sodium and calcium content was

highest in the forequarter muscles. The *Longissimus dorsi* muscle had the highest ($p<0.05$) iron (3.67 ± 0.51), but significantly lower zinc content (1.63 ± 0.28), in comparison to the forequarter muscles.

The pH value ≈24 h *post mortem* was higher ($p<0.05$) in the *Longissimus dorsi* muscle of the animals harvested in spring (5.60) compared to winter (5.54). The CIE a* (14.63 ± 0.86) and chroma (17.09 ± 0.63) values were higher ($p<0.05$) for winter than for male blesbok meat in spring (13.62 ± 1.08 and 16.10 ± 1.03, respectively). The latter values were also higher ($p<0.05$) for male compared to female (13.49 ± 0.88 and 16.22 ± 0.98) blesbok meat, at the end of the mating season (winter). The forequarter muscles had higher chroma values in comparison with the hindquarter muscles, which had higher ($p<0.01$) hue-angle values. Season had no influence ($p<0.05$) on the drip loss percentages and tenderness of blesbok muscles. The drip loss percentages were lowest ($p<0.05$) in the *Biceps femoris* and *Semimembranosus* muscles. The *Infraspinatus* and *Supraspinatus* muscles had the lowest ($p<0.01$) Warner Bratzler shear force values (20.89 ± 3.23 and 24.90 ± 5.35 N, respectively).

Seasonal differences in the chemical composition of blesbok muscles were statistically significant. However, these differences were numerically small and it is therefore debatable whether they are of any biological relevance relating to human nutrition. The differences in the fatty acid profile and mineral composition as well as the physical meat quality attributes of blesbok muscles were more attributed to differences in the anatomical locations of the selected muscles, as opposed to the impact of season or gender.

OPSOMMING

Die doel van die studie was om die impak van seisoen op die samestelling en kwaliteit van blesbok (*Damaliscus pygargus phillipsi*) spiere (*Longissimus dorsi*, *Biceps femoris*, *Semimembranosus*, *Semitendinosus*, *Infraspinatus* en *Supraspinatus*) te bepaal. Die seisoenale impak was gekwantifiseer op die chemiese samestelling (vog-, proteïen-, vet- en asinhoud), vetsuurprofiel, mineraal samestelling en fisiese eienskappe (pH, kleur, drup- en kookverlies, water houvermoë en taaiheid) van die geselekteerde spiere.

Met 'n hoër voedingspeil (lente 2009) het elkeen van die spiere gemiddeld 'n hoër ($p<0.01$) proteïeninhoud (20.6 g.100 g^{-1} tot 23.1 g.100 g^{-1}) gehad. 'n Sterk negatiewe korrelasie ($r = – 0.82$; $p<0.01$) het bestaan tussen die vog- en proteïeninhoud van die blesbokspiere. Met 'n hoër voedingspeil het die *Longissimus dorsi* spier die hoogste ($p<0.01$) gemiddelde intramuskulêre vetinhoud (3.4 g.100 g^{-1}) gehad. Seisoenale verskille in die voedingspeil en aktiwiteitsvlakke van dié blesbokke het minimale verskille in die chemiese samestelling van die *Longissimus dorsi*, *Biceps femoris*, *Semitendinosus* en voorkwartspiere (*Infraspinatus* en *Supraspinatus*) tot gevolg gehad. Daar was wel seisoenale verskille in die chemiese samestelling van die *Semimembranosus* spier.

Seisoen het nie 'n beduidende invloed op die vetsuurprofiel van die blesbokspiere gehad nie, maar daar was wel beduidende ($p<0.05$) verskille tussen geslagte. Soos aangedui deur 'n hoofkomponent-analise (PCA) bi-plot, was die spiere van die vroulike blesbokke meer geassosieer met hoër versadigde en mono-onversadigde vetsuursamestellings. Die spiere van die manlike diere het hoër ($p<0.01$) proporsies poli-onversadigde vetsure (PUFA) (40.15 ± 5.39) asook hoër poli-onversadigde tot versadigde vetsuur verhoudings (P:S) gehad (0.85 ± 0.18) in vergelyking met die spiere van die vroulike diere (onderskeidelik 27.18 ± 8.04 en 0.54 ± 0.20). Die vetsuurprofiel van blesbokspiere was ook beïnvloed deur die anatomiese ligging van die spiere. Die minder aktiewe *Longissimus dorsi* spier het 'n hoër ($p<0.05$) totale PUFA (38.34 ± 8.62), totale omega-6 (ω6) PUFA (34.46 ± 7.83), totale ω3 PUFA (3.44 ± 0.84) en P:S (0.85 ± 0.24) inhoud gehad in vergelyking met die *Infraspinatus* spier (onderskeidelik 28.96 ± 8.65, 26.23 ± 7.86, 2.31 ± 0.70 en 0.56 ± 0.19) en *Supraspinatus* spier (onderskeidelik 28.85 ± 9.23, 26.05 ± 8.24, 2.28 ± 0.76 en 0.55 ± 0.21). Die agterkwartspiere (*Biceps femoris*, *Semimembranosus* en *Semitendinosus*) het intermediêre vetsuursamestellings gehad.

Seisoen het 'n invloed op die kalsium- en sinkinhoud van die blesbokspiere gehad. In die lente het die spiere gemiddeld 'n hoër ($p<0.05$) kalsiuminhoud gehad (6.92 ± 1.94), in vergelyking met dié van winter (5.61 ± 1.79). Die manlike spiere van die blesbokke wat in winter geoes is, het weer 'n hoër ($p<0.05$) sinkinhoud (4.04 ± 1.70) in vergelyking met dié van die lente (3.41 ± 1.67) gehad. Verder het die mineraalinhoud van die geselekteerde blesbokspiere betekenisvol van mekaar verskil. Die *Biceps femoris* spier het die hoogste ($p<0.05$) kalium- (183.25 ± 12.79), fosfor- (180.21 ± 10.36) en magnesiuminhoud (32.18 ± 1.72) gehad. Die natrium- en kalsiuminhoud was

weer hoër in die voorkwartspiere. Die *Longissimus dorsi* spier het die hoogste ($p<0.05$) ysterinhoud (3.67 ± 0.51) gehad. Laasgenoemde het 'n beduidend laer sinkinhoud (1.63 ± 0.28) in vergelyking met die voorkwartspiere gehad.

Die *Longissimus dorsi* spiere van die blesbokke wat in die lente geoes is, het gemiddeld hoër pH-waardes by ≈24 uur *post mortem* gehad (5.60) in vergelyking met die pH-waardes van dié spiere in winter (5.54). Die CIE a*- (14.63 ± 0.86) en chroma-waardes (17.09 ± 0.63) van die manlike blesbokspiere was hoër ($p<0.05$) in die winter as in die lente (onderskeidelik 13.62 ± 1.08 en 16.10 ± 1.03). Aan die einde van die paartyd (winter) het die manlike blesbokke se spiere ook hoër ($p<0.05$) CIE a*- en chroma-waardes as die vroulike blesbokspiere (13.49 ± 0.88 en 16.22 ± 0.98) gehad. Die voorkwartspiere het gemiddeld hoër ($p<0.05$) chroma-waardes as die agterkwartspiere gehad, maar laasgenoemde het weer hoër ($p<0.01$) hue-angle waardes as die voorkwartspiere gehad. Seisoen het geen effek ($p<0.05$) op die drupverlies persentasies en taaiheid van die blesbokspiere gehad nie. Die *Biceps femoris* en *Semimembranosus* spiere het wel die laagste ($p<0.05$) drupverlies persentasies gehad. Die *Infraspinatus* en *Supraspinatus* spiere het weer die laagste ($p<0.01$) taaiheid (onderskeidelik 20.89 ± 3.23 en 24.90 ± 5.35) in vergelyking met die *Longissimus dorsi, Biceps femoris, Semimembranosus* en *Semitendinosus* spiere gehad (onderskeidelik 30.57 ± 6.69, 27.35 ± 3.42, 28.65 ± 4.48 en 31.51 ± 5.63).

Alhoewel daar in die studie statisties beduidende seisoenale verskille in die chemiese samestelling van die blesbokspiere was, is die verskille numeries klein en is dit debatteerbaar of dié verskille enigsins biologies van toepassing is op menslike voeding. Verder het die anatomiese ligging van die geselekteerde blesbokspiere in die studie 'n groter invloed op die verskille in die vetsuurprofiel, mineraal samestelling asook die fisiese eienskappe van die spiere gehad, in vergelyking met die impak van die oes-seisoen en die effek van geslag.

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LIST OF ABBREVIATIONS

LD	*Longissimus dorsi* muscle
BF	*Biceps femoris* muscle
SM	*Semimembranosus* muscle
ST	*Semitendinosus* muscle
IS	*Infraspinatus* muscle
SS	*Supraspinatus* muscle
mg	milligram
g	gram
kg	kilogram
ml	millilitre
cm	centimetre
ha	hectare
DFD	Dark Firm and Dry meat
DRI	Dietary Reference Intake
IMF	Intramuscular Fat
SFA	Saturated Fatty Acids
MUFA	Monounsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
P:S	Polyunsaturated to Saturated Fatty Acid Ratio
ω6:ω3	Omega-6 to Omega-3 Fatty Acid Ratio
ALA	Alpha-linolenic Acid
EPA	Eicosapentaenoic Acid
DHA	Docosahexanenoic Acid
MHC	Myosin Heavy Chain
pH_{45}	pH at ≈45 min *post mortem*
pH_{24}	pH at ≈24 h *post mortem*
$Temp_{45}$	Temperature at ≈45 min *post mortem*
$Temp_{24}$	Temperature at ≈24 h *post mortem*
WHC	Water Holding Capacity
LSMeans	Least Squares Means
SD	Standard Deviation
Anon.	Anonymous

NOTES

The language and style used in this thesis is in accordance with the requirements of the International Journal of Food Science and Technology. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between the chapters, especially in the Materials and Methods section, was therefore unavoidable.

Results from this study have been presented at the following symposiums:

7th International Wildlife Ranching Symposium (IWRS), 10 – 14 October 2011, Kimberley, South Africa.

Annual Congress of the South African Wildlife Management Association (SAWMA), 16 – 19 September 2012, Bela bela / Warmbaths, Limpopo Province, South Africa.

CONTENTS

CHAPTER 1

General Introduction

The potential value of South African game species was first recognised during the early 1950's (Carruthers, 2008). Consequently, the game meat export market developed during the search for alternative methods of utilising the surplus of game animals (Hoffman, 2003; Carruthers, 2008). Due to the spread of foot-and-mouth disease the South African game meat export market was unfortunately closed at the start of 2012, resulting in the industry focusing more on the local market.

The majority of the commercially harvested South African game species are found in free living populations (Conroy & Gaigher, 1982; Hoffman, 2003; Carruthers, 2008) and the hunting and/or harvesting of these species can frequently be very stressful (Hoffman, 2001). Game species usually have low energy stores in their muscles and this, together with the stressful harvesting conditions, can frequently lead to the formation of meat with higher ultimate pH (pH_u) values (>6.0) (Lawrie & Ledward, 2006b, d). Therefore, game meat can often be classified a as dark, firm and dry (DFD) meat (Hoffman, 2001; Lawrie & Ledward, 2006d). The pH_u of meat influences the shelf-life (Monin, 2004; Lawrie & Ledward, 2006c), flavour (Lawrie & Ledward, 2006b) and physical attributes, i.e. the water holding capacity (WHC), tenderness and colour of meat products (Honikel, 2004). Meat colour and the visible fat content are two of the major quality cues which assist consumers in predicting the healthiness (Hoffman *et al.,* 2005), quality (Mancini, 2009; Troy & Kerry, 2010) and freshness (Hoffman, 2001; Troy & Kerry, 2010) of meat products at the point of sale. However, consumers perceive game meat as being tough, dry and too dark in colour (Lawrie & Ledward, 2006a), as well as not being readily available (seasonal). Moreover, South African meat consumers are often ill-informed on the positive characteristics linked to game meat consumption (Hoffman *et al.,* 2005). The limited nutritional information available on South African game meat products (MRC, 2010) therefore negatively affects the marketing of these products (Issanchou, 1996).

Several authors have reported that game meat generally has a protein value of >20 g.100 g^{-1} and a fat content of ≤3 g.100 g^{-1} (Kroon *et al*., 1972; Aidoo & Haworth, 1995; Jansen van Rensburg, 2002; Du Buisson, 2006; Ramanzin *et al*., 2010; Van Schalkwyk & Hoffman, 2010). Game meat products are therefore high in protein and low in fat (Anon., 2010a) and can be marketed as such. In addition to the quantity of the fat in meat, the quality of the fat is also an important consideration. Three factors should be taken into consideration with regards to the nutritional information of meat products containing fat: the total fat content; the polyunsaturated to saturated fatty acid ratio (P:S); and the omega-6 to omega-3 fatty acid ratio (ω6:ω3) (Enser *et al.,* 1998). Since unsaturated fatty acids in the diet of ruminants are hydrogenated by rumen microorganisms to more saturated fatty acids (SFA) (Wood & Enser, 1997; Warriss, 2000), the

fatty acids present in the diet of game species are unfortunately not a representation of the subsequent fatty acid profiles in the meat. Nonetheless, the fatty acid profiles of game meat have some similarities with other commercially available red meat products, as palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1ω9) are usually the main fatty acids present (Aidoo & Haworth, 1995). In addition, female animals usually have higher quantities of intramuscular fat (IMF) (Lawrie & Ledward, 2006a) and may consequently have different fatty acid profiles, in comparison to meat from male animals.

The nature and quality of the forage consumed by game species can influence the chemical composition, fatty acid profile and mineral composition of the meat. The plane of nutrition and activity level of the animals also influences the muscle fibre type composition (Lawrie & Ledward, 2006b), consequently having an effect on the chemical composition (Doornenbal & Murray, 1982; Wood *et al.,* 2003) and quality (Mancini, 2009) of the meat products. Skeletal muscles therefore differ in composition and quality attributes.

In South Africa, seasonal rainfall patterns (Kruger, 2007) as well as vegetation types differ greatly between regions (Hanks, 2009). Game animals can therefore have seasonal variations in the quantity and quality of the forage available for consumption (plane of nutrition). Grass species for example, are generally divided into two categories, C_3 or C_4, according to their photosynthetic pathways. These are usually separated geographically in South Africa (Vogel *et al*., 1978), since the C_3 temperate grass species prefer moist, cool environmental conditions (Vogel *et al.*, 1978; Twiss, 1992; Owen-Smith, 2008) while the C_4 tropical grass species are more adapted to arid/semi-arid, warm and humid conditions (Vogel *et al.*, 1978; Feldhake & Boyer, 1986; Twiss, 1992).

Blesbok are seasonal breeders and highly selective in grazing short grass species (Du Plessis, 1972; Bothma *et al*., 2010). They usually have seasonal preferences to particular grass species (Skinner & Chimimba, 2005), but there are also seasonal variations in the grass species available to blesbok for consumption. Although South Africa has a distinct hunting season, during the winter months of May to August (Kroon *et al*., 1972; Hoffman, 2003), season is usually not taken into account during the commercial harvesting of game species (Anon., 2009, 2010b, 2011). Huntley (1971) found seasonal differences in the diet and consequently in the condition (fat reserves) of mature male blesbok, while Kroon *et al.* (1972) found seasonal differences in the total fat ($p<0.05$) and protein contents ($p<0.01$) of mature male (empty) blesbok carcasses. Each of these studies was, however, only conducted on male blesbok.

The objective of this study was therefore to investigate the impact of season on the chemical composition (moisture, protein, fat and ash content), fatty acid profile, mineral composition and physical meat quality attributes (pH, colour, drip loss, cooking loss, WHC and tenderness) of male and female blesbok (*Damaliscus pygargus phillipsi*) muscles (*Longissimus dorsi*, *Biceps femoris*, *Semimembranosus*, *Semitendinosus*, *Infraspinatus* and *Supraspinatus*).

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CHAPTER 2

Literature Review

BACKGROUND

Blesbok (*Damaliscus pygargus phillipsi*)

During the 17th century, the first documentation on the habitat of blesbok reported their presence in the plateau grasslands of the Highveld region, most likely due to the availability of water (Skinner & Chimimba, 2005). The distribution of blesbok included parts of western and north-western KwaZulu-Natal, the northern Karoo in the Northern and Eastern Cape, as well as the Highveld of the Free State and Gauteng (Lloyd & David, 2008). Blesbok are currently found widespread throughout Southern Africa but their current distribution is to a large extent artificial (Skinner & Chimimba, 2005). They are mainly present on privately owned farmlands in the summer rainfall regions. These regions include the Grassland Biome of the Free State, North West, Eastern Cape, Gauteng, Mpumalanga and the western edge of Kwazulu-Natal provinces. However, some blesbok are also present in the coastal, non-seasonal rainfall areas of South Africa (Smithers, 1983; Chase & Meadows, 2007; Watson *et al*., 2011).

Blesbok are not a concern for extinction (Lloyd & David, 2008). They are medium sized herbivores (± 55 kg live weight), seasonal breeders, tolerant of high ambient temperatures, highly selective in diurnal grazing of short grass species and easily held in captivity by regular livestock fencing (Du Plessis, 1972; Conroy & Gaigher, 1982; Skinner & Chimimba, 2005; Bothma *et al*., 2010). The colloquial name “blesbok” refers to the Afrikaans word *bles* for a blaze, in reference to the white facial markings from the nose up to the base of the horns, interrupted by a brown band just above the eyes (Smithers, 1983; Skinner & Chimimba, 2005).

Du Plessis (1972) identified the main grass species consumed by blesbok on unburnt areas at the Rietvlei Nature Reserve in the Gauteng Province (summer-rainfall region) as *Eragrostis pseudosclerantha* (footpath love grass), *Themeda triandra* (red grass), *Eragrostis curvula* (weeping love grass) and *Chloromelas sp.* Since blesbok are highly selective grazers, they will avoid eating some grass species (e.g. *Ctenium concinnum* (sickle grass), *Aristida junciformis* (ngongoni three-awn) and *Elionurus muticus* (wire grass)). They will consume selected grass species only during winter (e.g. *Seratia flabellataI*) and others only in summer (Skinner & Chimimba, 2005). It is therefore believed that blesbok will generally have a seasonal variation in their diet.

South African rainfall

The annual South African rainfall can be grouped into four main categories (Fig. 2.1), namely: winter rainfall; late summer rainfall; summer rainfall; and all year round rainfall (Kruger, 2007). The

western to eastern regions of the coastline vary greatly in seasonal and annual rainfall patterns (Chase & Meadows, 2007). Considering Fig. 2.1, the west coast regions have characteristic winter rainfall (>66% April to September), but moving more eastward, the summer rainfall patterns primarily predominate (>66% October to May). However, the areas situated between the west and east coastline (southern coast) may receive rain throughout the year, with a bias towards autumn as well as spring to early summer (Rebelo *et al.*, 2006; Chase & Meadows, 2007; Kruger, 2007).

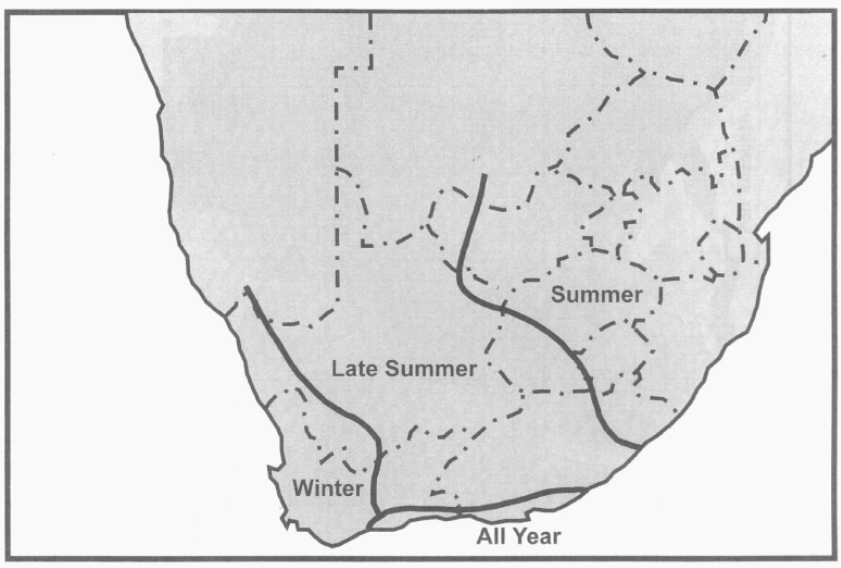

Figure 2.1 The maximum annual rainfall patterns for different areas in South Africa (Kruger, 2007). The location (34°18'24.0"S and 20°49'3.9"E; 93 m.a.s.l.) of the study area in this investigation was in the all year rainfall region.

Grass species (location, growth and nutritional value)

Grass species can be divided into two categories, C_3 or C_4, according to their photosynthetic pathways (Vogel *et al.*, 1978). The C_3 temperate grass species thrive in moist and cool environmental conditions (e.g. winter rainfall months) and become dormant once environmental temperatures increase, as with the onset of the warmer summer months (Vogel *et al.*, 1978; Twiss, 1992; Owen-Smith, 2008). The C_4 tropical grass species are more adapted to arid/semi-arid, warm and humid environmental conditions and therefore usually favour the summer months for growth (Vogel *et al.*, 1978; Feldhake & Boyer, 1986; Twiss, 1992). In South Africa the C_3 and C_4 plant species are generally separated geographically (Vogel *et al*., 1978), although a few areas can contain a combination of both (Twiss, 1992). The South African West Coast together with the peaks of the Drakensberg and selected mountain ranges in the Eastern Cape, are dominated by C_3 grass species (>75%). Moving eastward the C_3 grass species are increasingly replaced by C_4 grass species, whereby the regions east from Port Elizabeth are dominated by C_4 grass species (>75%). The distribution of the C_3 and C_4 grass species is not primarily correlated with regional difference in rainfall patterns (Vogel *et al.*, 1978).

The growth rate of grass species is determined by the environment, nutrient availability and the extent to which the leaf in the sward can intercept light (McDonald *et al.*, 2002). In cold to moderate climatic conditions, grass growth will commence after sufficient moisture has been supplied by winter rainfall patterns and when environmental temperatures rise sufficiently. The latter generally occurs with the onset of spring when soil temperatures reach 4° – 6°C (McDonald *et al*., 2002; Owen-Smith, 2008). Areas with rainfall more or less uniformly throughout the year will have fairly slow grass growth and maturation. In regions with warmer climatic conditions the soil temperatures can be high enough to enable the growth of grasses throughout the year; if there is no restriction in the water supply. However, grasses grown in warmer climatic conditions mature faster, bringing about an increase in fibre content and a corresponding decrease in protein content (McDonald *et al*., 2002). The anatomical structure of the leaves of C_3 grass species have a higher dry matter digestibility compared to the leaves of C_4 grass species (Wilson & Hacker, 1987). During the cool, wet winter months the C_3 grasses usually contain a higher crude protein content and are therefore more readily digestible than the C_4 grass species (Owen-Smith, 2008). Seasonal differences in rainfall patterns, therefore, result in variations in the abundance, composition and nutritional value of specific grass species (Radloff, 2008).

When soil and climatic conditions are unsuitable, grass species will have low nutritional quality. Moreover, the nutritional value of grasses change with grass growth and maturation (McDonald *et al.*, 2002). With the onset of growth the nutritional value and moisture content of grasses are high and result in the dilution of the dry matter, limiting the amount of dry matter ingested by grazers (Ruyle, 1993). Subsequently the moisture content decreases with grass maturation. The digestibility will initially remain relatively constant (at a 'plateau') for approximately a month into spring, where after it will decrease with maturation (McDonald *et al*., 2002). Immature grasses have high rumen degradability and overall digestibility, but with grass maturation the forage intake by ruminant's decreases and could lead to weight loss (Ruyle, 1993; Meissner, 1999; McDonald *et al*., 2002). The fibre content is inversely related to the protein content (energy) in grasses; grass maturation leading to the translocation of protein to the roots and a subsequent increase in fibrous tissues (Ruyle, 1993; McDonald *et al*., 2002).

Mineral deficiencies in soil will cause limited plant growth and reduced element concentration in the plant tissue or both. Phosphorus, magnesium, copper and cobalt are commonly deficient in grass forage. The calcium concentration in plants is low with high soil moisture, but accumulates in the plants during dry periods. However, the phosphorus content in plants is higher after high rainfall (McDonald *et al*., 2002). Diets lacking in minerals and sufficient amounts of energy will negatively affect the digestion and forage intake by ruminants (Meissner, 1999). Grass species normally have a low total lipid content (McDonald *et al.*, 2002) of which the main fatty acids are C18:3ω3 (α-linolenic acid, 60 – 75% of total fatty acids) and to a lesser extent C18:2ω6 (linoleic acid) and C16:0 (palmitic acid) (McDonald *et al.*, 2002; Khan *et al.,* 2012).

Fynbos biome (Coastal Renosterveld)

Climatic conditions, soil, landform and the prevalence of fires and/or frost determine the presence and growth of various plant species in different regions. The naturally occurring plant species established in the different regions of South Africa, have therefore been characterised into different biomes (Hanks, 2009). South Africa has seven of these unique biomes: Fynbos; Savannah (including the Bushveld, Lowveld kalahari); Grassland; Nama-Karoo; Succulent Karoo; Forest; and Albany Thicket (Rutherford *et al*., 2006; Hanks, 2009).

The 83 946 km^2 Fynbos biome (6% of country) is found in the south-western part of South Africa. This biome is relatively moist and typically classified in the winter rainfall region (Rutherford *et al*., 2006; Hanks, 2009). The Fynbos biome forms a curved band which fills most of the Western Cape Province, extends into the Eastern Cape Province and ends in Port Elizabeth. It has an average rainfall of 480 mm and average air temperatures are normally mild and do not exceed 30°C (except for some interior areas). Additionally, the Fynbos biome is unique as it is adapted to severe environmental conditions. This biome is beautiful, delicate and extremely diverse since it is made up of three primary low shrubby like vegetation types: Fynbos (67%); Renosterveld (29%); and Strandveld (4%), of which the Fynbos region is the focal point. The latter has biologically diverse vegetation types and the highest annual rainfall, followed by the Renosterveld and lastly the Strandveld regions (lowest rainfall).

The study area was situated in the Fynbos biome, specifically in the Renosterveld region. The Renosterveld is an evergreen shrub or grassland with the most fertile, fine-grained soils (Hanks, 2009). The Coastal Renosterveld primarily receives winter rainfall (300 – 500 mm per annum at an altitude between 0 – 300 m) as well as some rainfall patterns in parts of the summer and autumn months (Stindt & Joubert, 1979; Acocks & Momberg, 1988; Rebelo, 1996; Rutherford *et al*., 2006). The Coastal Renosterveld differs from the other Renosterveld regions due to the high amounts of grass species present in the former (Rebelo, 1996). The well developed grass section of the Coastal Renosterveld consists primarily of C_4 tropical grass species (Rebelo, 1996; Rebelo *et al.*, 2006) and to a lesser extent C_3 temperate grass species (Acocks & Momberg, 1988). A few of the C_3 grass species present are *Ehrharta calycin, Koeleria capensis, Fustuca scabra, Merxmuellera macowanii* and *Helictotrichon capense* (Russel *et al.*, 1990; Tainton, 1999). The *Koeleria* and *Helictotrichon* grass species are usually associated with cooler, winter months for growth, although they may frequently favour the summer months for growth instead (Tainton, 1999). A perennial C_4 grass species, *Themeda triandra*, is found scattered throughout the Renosterveld region and is prevalent throughout almost the entire South African winter rainfall region (Stindt & Joubert, 1979; Acocks & Momberg, 1988; Van Breda & Barnard, 1991; Morgan & Lunt, 1999; Van Rheede Van Oudtshoorn, 2007).

In South Africa blesbok are present in greater numbers in the summer rainfall regions which primarily (>75%) contain C_4 grass species (Vogel *et al.*, 1978). However, blesbok from this study

were harvested from a study area classified in the Coastal Renosterveld region, with all year round non-seasonal rainfall (Fig. 2.1) and predominately C_4 grass species (few C_3 grass species).

Commercial harvesting of game species

Although South Africa has a distinct hunting season during the winter months (May to August) (Kroon *et al*., 1972; Hoffman, 2003), season is usually not taken into account when harvesting game meat commercially (Fig. 2.2). In South Africa, the majority of the commercially harvested game species are found in free living populations, present on public or private land (Conroy & Gaigher, 1982; Hoffman, 2003; Hoffman & Wiklund, 2006; Carruthers, 2008). In recent years, blesbok was one of the three most commercially harvested and exported game meat species from South Africa (Anon., 2009, 2010, 2011a). However, due to the spread of foot-and-mouth disease, the South African game meat export market was unfortunately closed at the beginning of 2012 and has since not re-opened. Prior to this closure, the meat was exported as packaged, deboned, individual and/or aggregations of muscles or muscle cuts (steaks), such as the fillets (*M. psoas major*), hindquarter muscles (*Biceps femoris*, *Semimembranosus* and *Semitendinosus*) and forequarter muscles (*Infraspinatus* and *Supraspinatus*) (Paton *et al.*, 2009). Goulash was the exception, but this was used for the production of salami and other processed meat products.

The South African game meat industry has not yet reached its full potential, due to a lack of knowledge on the nutritional value and health benefits linked to game meat consumption. However, with the export market stil closed meat from the surplus game animals will have to be marketed and sold locally. Since springbok (*Antidorcas marsupialis*) is the most extensively harvested game species in South Africa (Hoffman & McMillin, 2009), thorough studies have been conducted on investigating the factors influencing the meat quality of this species (Hoffman, 2001; Hoffman *et al.,* 2007a, b, c, d; Hoffman & McMillin, 2009). Research on the extrinsic (such as season) and intrinsic (such as muscle) factors influencing blesbok meat quality is unfortunately more limited.

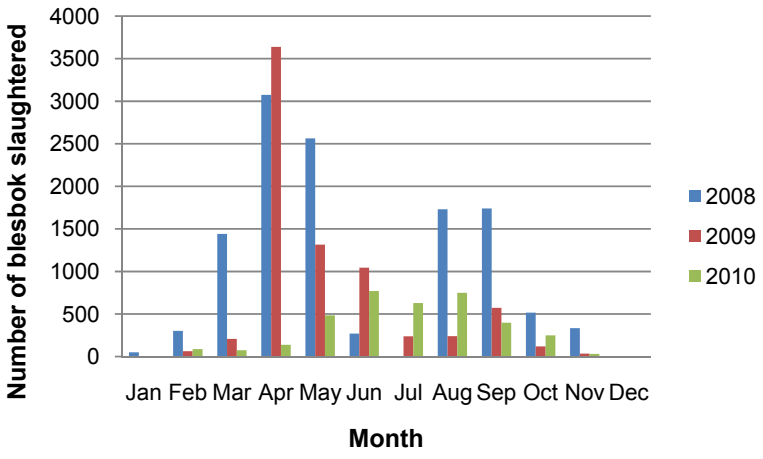

Figure 2.2 Commercial harvesting of blesbok during 2008, 2009 and 2010 (Anon., 2009, 2010, 2011a).

SKELETAL MUSCLE PROPERTIES

Skeletal muscles are primarily made up of contractile muscle fibres, arranged in bundles and held together by connective tissue (Swatland, 2004d; Taylor, 2004). Muscles are generally classified as 'red' or 'white', in relation to whether a sustained action (slow-twitch) is carried out or whether they function in short bursts (fast-twitch) (Cassens & Cooper, 1971; Lawrie & Ledward, 2006c).

The red and white muscles differ significantly in their biochemical characteristics and predominantly have aerobic and anaerobic metabolisms, respectively. Muscles are essentially a heterogeneous combination of red and white muscle fibres (Cassens & Cooper, 1971), randomly combined and prenatally produced under genetic control. Muscle fibres are therefore present in fixed quantities at birth and merely increase in size during growth (Frandson, 1966; Swatland, 2004d; Taylor, 2004). The plane of nutrition and degree of exercise to which muscles are exposed determines the extent to which longitudinal (increase in fibre length) and radial (increase in fibre thickness) muscle fibre growth will occur (Frandson, 1966; Swatland, 2004d; Lawrie & Ledward, 2006c). Animals on a restricted diet will have smaller muscle fibres compared to animals on a higher plane of nutrition (Frandson, 1966). When skeletal muscles become more active it results in a change in fibre type composition and metabolism (Mancini, 2009).

In general, muscle fibre types are classified as Type I, Type IIA or Type IIB, according to their metabolisms (Taylor, 2004). Type I are small (diameter), red, slow-twitch, oxidative fibres with low myosin ATPase activity (slow myosin type). They are suited for repetitive or endurance type of activities (contract for longer time periods), e.g. maintaining posture (Cassens & Cooper, 1971; Swatland, 2004f; Davies, 2004; Taylor, 2004). They are rich in mitochondria, sarcoplasm, myoglobin and fat droplets (Cassens & Cooper, 1971; Taylor, 2004); the latter which contributes to the juiciness and pleasant taste in final meat products (Hocquette *et al.,* 2010). However, Type I fibres can produce ATP aerobically from fat as well as from glycogen, although their glycolytic potential is low (Taylor, 2004; Kohn *et al.,* 2005). This fibre type is present closest to the blood supply (capillaries) within muscles, to maintain a constant oxygen and nutrient supply (Cassens & Cooper, 1971; Davies, 2004; Swatland, 2004f).

Type IIA fibres are also red in colour, but classified as fast-twitch muscle fibres which fatigue slowly. This fibre type has an intermediate metabolism, since it involves glycolytic and oxidative metabolisms and therefore enters rigor mortis faster than Type I. Type IIA fibres are also rich in myoglobin and mitochondria, but the myosin ATPase activity is high (Taylor, 2004). Type IIB are large (broad), white, very fast-twitch, anaerobic, glycolytic fibres (rich in glycogen and primarily use glucose as fuel), with high myosin ATPase activity. They are suited for situations requiring rapid, strong contractions and speed (such as sprinting), although they are easily exhausted (Swatland, 2004e; Taylor, 2004; Kohn *et al.,* 2005). Type IIB fibres change in size with increasing animal age and level of exercise. In these fibres, ATP is produced anaerobically from stored glycogen and glucose present in the blood (Kohn *et al*., 2005) and muscles with mainly Type IIB fibres will

therefore most probably have a low ultimate pH (pH_u) (Taylor, 2004). Type IIB fibres have few mitochondria, sarcoplasm, fat stores and myoglobin in comparison to Type I and IIA (Cassens & Cooper, 1971; Taylor, 2004). Type IIB has a small number of capillaries and relies on blood primarily for the removal of lactic acid (Cassens & Cooper, 1971). With rapid muscle growth, it is generally the fast-twitch muscle fibres which increase in size and so negatively affect the tenderness, taste and juiciness of meat products.

The metabolic characteristics of the three muscle fibre types are broadly linked to their different myosin heavy chain (MHC) isoforms expressed (Conley, 1994; Kohn *et al.,* 2005). Muscle fibre Type I, IIA and IIB express MHC I, IIa and IIb, respectively (Kohn *et al.,* 2005). The muscles of cattle and sheep, for example have higher quantities of fibre Types I and IIA (red muscle fibres) and thus express mainly MHC I and IIa (Taylor, 2004). The proportions of the different MHC isoforms are changed by various factors linked to the animals age and body weight (Kohn *et al.,* 2005). Kohn *et al.* (2005) found that four impala muscles (*Psoas major, Longissimus lumborum, Deltoideus* and *Semimembranosus*) expressed different quantities of the MHC isoforms (primarily MHC IIa). Impala *Psoas major, Longissimus lumborum* and *Semimembranosus* muscles were mainly linked to the endurance type of activities, such as walking, running and maintaining posture. The *Deltoideus* was the only muscle which expressed the fast-twitch MHC IIb isoform, due to its short term uses, primarily during fighting activities between rams. Impala generally move slowly during activities such as grazing, but once they feel threatened they move more rapidly by means of continuous leaps (Kohn *et al.,* 2005). The daily activity of blesbok is normally limited to standing and grazing (walking). When blesbok feel threatened they run, but only for a short distance (Lynch, 1971; Du Plessis, 1972). Blesbok therefore have similar daily activities when compared to impala.

The seasonal activities of blesbok influence the deposition, conservation and/or usage of the stored energy reserves. Blesbok usually become less active with the onset of the dry season (e.g. winter months in a summer rainfall region), when their food intake is reduced and they may lose weight (Du Plessis, 1972) due to the low quantity and quality of the available grass species (Mtimuni *et al*., 1983; Rebelo *et al.*, 2006; Chase & Meadows, 2007). Additionally blesbok will also walk slower and lie down more frequently during the day in the dry season. With the onset of the growing season (usually spring) and the presence of new grass sprouts, blesbok generally become more active. They will then gain back some body condition as they graze more frequently during the day, in addition to the early morning and late afternoon grazing intervals. Male blesbok are very active during the mating season (generally March to May), when they chase other rams away from their harem and/or out of their territories. Rams might also sometimes fight at random throughout the year (Du Plessis, 1972).

Muscle types

The musculature of the majority of South African game species has similarities with the general bovid, although some variations may be present for selected species (Hoffman & McMillin, 2009). In general the *Longissimus dorsi* (LD) muscle is the largest of the loin muscles (Frandson, 1966). This is a valuable muscle due to its relatively high protein content, tenderness and desirable taste in comparison with the other skeletal muscles (Swatland, 1994b; Lawrie & Ledward, 2006c). Consequently, the LD muscle demands the highest price on the retail market (Swatland, 1994b; Jones, 2004). It encompasses the area from the rib region, through the loin and generally ends on the anterior face of the ilium. The LD muscle is therefore located dorsal to the ribs in the thoracic region and to the transverse processes of the lumbar vertebrae. The LD muscle is a compound muscle, alternatively known as the *Longissimus thoracis et lumborum* (Swatland, 1994b), which assists in the maintenance of an animal's balance and stability during movement (Robert *et al.,* 2001). This muscle has a number of subunits which acts over the length of numerous vertebrae and it is involved in the neck and respiratory movements, along with assisting in the flexing of the vertebral column (Swatland, 1994b). The LD muscle has an overall 'good' texture, but larger muscle fibres are present in the posterior (bottom) portion in comparison to the top portion of this muscle. The tenderness of the LD muscle thus increases from the centre outwards towards both ends of this muscle (American Meat Institute Foundation, 1960).

Some of the finest muscle cuts are usually also found in the hindquarters (Von La Chevallerie, 1970; Swatland, 1994b). The *M. biceps femoris* (BF muscle), *M. semitendinosus* (ST muscle) and *M. semimembranosus* (SM muscle) are the main extensor muscles in the hip (*hamstring* muscles) (Frandson, 1966). The BF muscle is a large muscle with a fairly uniform tenderness (American Meat Institute Foundation, 1960; Swatland, 1994b). It is situated in the most lateral face of the posterior muscles (in the thigh) and together with the ST muscle, assists in extending the hock since a portion of these muscles enters into the tendon of the *Achilles* (Frandson, 1966; Swatland, 1994b, 1994c). The ST muscle is situated in the middle of the group of posterior thigh muscles (Frandson, 1966; Swatland, 1994c). This muscle has less muscle fibres of similar diameter per primary muscle fibre bundle (in comparison with the LD muscle) and larger muscle fibres situated in the top portion (anterior) compared to the bottom portion. The ST muscle therefore has a less desirable texture. It is usually seen as a 'white' muscle (higher quantity of Type IIB fibres) (Vestergaard *et al.,* 2000) and its tenderness increases when cooked between 58° and 67°C, but decreases when cooked between 67° and 75°C (American Meat Institute Foundation, 1960). The SM muscle is a large muscle situated on the posterior face of the hind limb, in a medial position to the ST muscle (Frandson, 1966; Swatland, 1994b, c). Its tenderness decreases considerably from the pelvic part of the muscle, outwards (American Meat Institute Foundation, 1960).

The *M. infraspinatus* (IS muscle) and *M. supraspinatus* (SS muscle) are two muscles situated in the front limb or forequarter. The IS muscle begins at the *infraspinous fossa* (Frandson, 1966), ventral to the spine (ridge) on the *scapula* (Swatland, 1994b). The IS muscle primarily acts as a very strong shoulder joint ligament and furthermore serves to flex, move (Frandson, 1966) and stabilise the shoulder (Totland & Kryvi, 1991). The SS muscle begins at the *supraspinous fossa* (Frandson, 1966), dorsal to the spine on the *scapula* (Swatland, 1994b). The SS muscle primarily performs as a ligament of the shoulder joint, although it may assist with the extension of the shoulder (Frandson, 1966). The SS muscle is generally classified as a 'red' muscle (higher oxidative muscle fibre content), with low total protein and high connective tissue contents (Lawrie & Ledward, 2006c).

Skeletal muscles thus differ in their anatomical location and consequently in their activity levels. The activity level of various skeletal muscles will also differ between game species, resulting in differences in the composition and possibly final meat quality.

PHYSICAL MEAT QUALITY

Meat production potential

Species differences

The meat production potential of game species is becoming progressively more important for the financial feasibility of game farms in South Africa (Hoffman *et al*., 2005a). Meat production potential is influenced by species, gender, age (chronological and physiological maturity), feeding habits and the plane of nutrition. The dressing percentage, calculated as a percentage of the carcass weight, gives a good indication of the meat production potential of a species (Van Zyl *et al.,* 1969). Ruminants are better equipped at utilising poor-quality nutrition, in comparison to single-stomached (non-ruminant) species. The digestive systems of game species are also better at utilising lower quality feeds (Von La Chevallerie, 1970). Game species often have higher dressing percentages compared to domesticated species (Table 2.1) (Ledger, 1963; Von La Chevallerie, 1970). However, the dressing percentage can vary with the presence/absence of horns (generally thicker for male animals), the presence and quantities of fat depots (the presence of viscera and kidney fat resulting in lower dressing percentages) (Swatland, 1994a) and the size and weight of the stomach and intestines (Van Zyl & Ferreira, 2002). The body composition of different species varies according to the forage consumed and their dependence upon water for survival (Ledger, 1963; Van Zyl & Ferreira, 2002).

Table 2.1 Dressing percentages of selected game and domesticated species

Species	Dressing percentage*
Game	
Blesbok[1,4,5]	49.5 – 53.7
Springbok[4]	56.2 – 57.6
Impala[4,6]	54.7 – 60.9
Kudu[1,6]	55.9 – 58.3
Red Hartebeest[7]	47.3 – 53.3
Domesticated	
Sheep[3]	47.0 – 50.0
Beef[2]	51

([1]Huntley, 1971; [2]Onyango *et al.,* 1998; [3]Cloete *et al.,* 2000; [4]Van Zyl & Ferreira, 2004; [5]Hoffman *et al.,* 2008; [6]Hoffman *et al.,* 2009a; [7]Hoffman *et al.,* 2010)
*Percentage carcass weight of live animal weight

Production region

Differences exist in the quality, quantity and suitability of vegetation available to various game species at different production regions. The production region can therefore have a considerable influence on the meat production potential of game species. Consequently, more favourable nutritional conditions can ensure that more energy is obtained through the diet, favouring the growth and development of game animals (Hoffman *et al.,* 2005a). Hoffman (2000) reported that the body weight of mature male and female impala (*Aepyceros melampus*) (older than four years) was higher for those from Maneze Wildlife Conservancy in central Zimbabwe (59.1 and 44.1 kg) in comparison to impala from the Kruger National Park (49.2 and 38.3 kg).

Season

In addition to region, the meat production potential of a species can also be influenced by the harvesting season. Since South Africa has varying annual rainfall regions (Fig. 2.1), the plane of nutrition will frequently differ between seasons. The regions with strictly winter or summer rainfall will thus have seasonal variations in the quantity and quality of nutritious vegetation. Although some game species are more adapted to poor veldt conditions, they may be more selective feeders during the dry season (Van Hoven, 2009). Gwynne & Bell (1968) noted seasonal differences in the grass species and parts thereof preferred and consumed by freely grazing African ungulates. Nieminen & Heiskari (1989) also reported seasonal variations in the forage consumed by freely grazing reindeer (*Rangifer tarandus tarandus L.*) of which the winter and early spring diets mainly contained energy rich lichens, poor in protein and mineral contents. Ramazin *et al.* (2010) noted seasonal variations in the vegetation consumed by wild ruminants and consequently seasonal differences in the fat content in these animals. Von La Chevallerie (1970) reported seasonal differences in the intramuscular fat (IMF) content of seven game species and

suggested that the seasonal influence on the availability of good quality forage should be thoroughly considered within a game harvesting scheme. Hoffman *et al.* (2009b), however, found no seasonal differences ($p>0.05$) (between spring and autumn) in the mean live weight, carcass weight and dressing percentage of black wildebeest (*Connochaetus gnou*). The latter study area (Maria Moroka Nature Reserve near Bloemfontein in the Free State Province, South Africa) was characterised by higher annual rainfall during late summer, but high amounts of rainfall occurred prior to the spring and autumn harvesting which might have resulted in smaller seasonal differences in the quality and quantity of the vegetation present.

Huntley (1971) found differences in the fat reserves (body condition) of male blesbok from a summer rainfall region, due to seasonal differences in the forage consumed. Consequently, it can be postulated that the IMF content and composition of blesbok meat will vary with seasonal variation in the quality, quantity and type of grass species consumed. Green grasses are present in abundance after summer rain (in a summer rainfall region) and blesbok are assumed to then be in their best condition (Du Plessis, 1972; Vogel *et al.*, 1978; Feldhake & Boyer, 1986; Twiss, 1992). Consequently, larger quantities of forage are available for the remainder of the blesbok population. The latter is particularly important for female blesbok during the gestation period (around April to December) and will subsequently increase the odds of yielding healthier offspring with an improved chance at survival (Bothma *et al.*, 1996).

Blesbok from a summer rainfall region will therefore generally gain weight during spring and summer (October to March) due to fat deposition, but then lose weight due to fat utilisation during autumn and winter (April to September) (Du Plessis, 1972). Blesbok can lose up to 12% of their total live weight during periods of food stress (less favourable nutritional conditions) (Van Hoven, 2009; Bothma *et al.,* 2010). The latter could be due to the utilisation of fat reserves (of which IMF is first) and the subsequent breakdown of muscle proteins to supply sufficient amounts of energy (Lawrie & Ledward, 2006a) to sustain normal metabolic rate as well as for daily activities (Van Hoven, 2009). The extent to which weight loss occurs in blesbok will thus depend on the nutritional quality of the diet and the amount of fat reserves present in each animal. Bothma *et al*. (1996) suggested that blesbok in summer rainfall regions should be harvested near the end of summer, through autumn towards early winter (February to June) for meat production purposes.

Blesbok from a non-seasonal rainfall area (rain throughout the year) will most likely not be exposed to as large a difference in the quality and quantity of forage available to them throughout the year. Since these areas most likely contain a combination of both C_3 and C_4 grass species (Twiss, 1992) it is believed that the winter and summer precipitation will favour the growth and nutritional quality of the C_3 and C_4 grass species, respectively. Therefore, blesbok present in an all year rainfall area (Fig. 2.1) may have good quality nutrition available all year round. These blesbok will possibly not lose live weight or body condition as drastically during the few months with lower precipitation and lower quantities of nutrition, as those present in the regions with a strictly summer or winter rainfall pattern.

Gender

In addition to the harvesting season, the mating season may also influence the meat production potential of game animals. Gender influences the growth and development of individual muscles (Lawrie & Ledward, 2006a) as well as the development of different muscle groups (e.g. forequarters and/or hindquarters) (Lawrie & Ledward, 2006b). In general, female animals will mature earlier and both immature and mature female game animals usually have higher hindquarter percentages (in relation to whole carcass weight) in comparison with mature male animals (Ledger, 1963). Male animals will generally be larger and heavier at maturity (Von La Chevallerie, 1970; Lawrie & Ledward, 2006a), have larger muscle fibres (Frandson, 1966) and more developed neck and thorax muscles. The latter two muscles are usually utilised whilst fighting for dominance (Lawrie & Ledward, 2006a). In the rutting (mating) season the male game animals will spend less time feeding and more time on mating and fighting to maintain their harem (Kohn *et al*., 2005). Consequently, male game animals loose condition (lower total fat content) during the rutting season (Smithers, 1983; Van Zyl & Ferreira, 2004).

Some researchers have not found gender differences in the mean live weight, carcass weight or dressing percentages of springbok (Van Zyl & Ferreira, 2004), while other researchers have reported that male springbok had higher ($p<0.05$) mean live weights and dressing percentages in comparison to female springbok (Kroucamp, 2004). No gender differences have been found in the mean live weight, carcass weight or dressing percentage of blesbok (Van Zyl & Ferreira, 2004) or kudu (*Tragelaphus strepsiceros*) (Mostert & Hoffman, 2007). Hoffman (2000) reported that male impala (49.4 kg) were heavier ($p<0.05$) than female impala (33.5 kg), yet their dressing percentages (as a percentage of the dressed weight) did not differ ($p>0.05$) (57.5% and 58.0%, respectively). Van Zyl & Ferreira (2004) reported that female impala had higher mean live and carcass weights compared to male impala. However, the latter was attributed to the age differences, since the male impala were 18 months and the female impala were 36 months of age. This is often found in the commercial game harvesting/hunting industry, as older male game animals are often utilised for trophy hunting.

Ultimate pH

The hunting of game species is often stressful and strenuous (Hoffman, 2001). In addition, free-living game species usually do not have enough energy in their diets to accumulate energy reserves (glycogen and lipids). The *post mortem* ultimate pH (pH_u) is determined by the lactic acid concentration (produced from glycogen) during anaerobic glycolysis (Lawrie & Ledward, 2006e). A 'normal' *post mortem* pH decline is from a physiological pH of 7.0 – 7.2 in the muscles of live animals, to an pH_u of 5.3 – 5.8 (Honikel, 2004a). However, when the initial glycogen concentrations in muscles are depleted by *ante mortem* stress (fear or wounding) or fatigue (Hoffman, 2001; Lawrie & Ledward, 2006e), the pH_u of game meat is often higher than normal

(pH_u>6) (Lawrie & Ledward, 2006d, g) resulting in dark, firm and dry (DFD) meat (Hoffman, 2001; Lawrie & Ledward, 2006g). Skeletal muscles differ in their functions and activity levels and therefore also differ in their susceptibility to *ante mortem* glycogen depletion due to stress (Lawrie & Ledward, 2006d). Muscle fibre diameter is inversely correlated to pH_u (r = – 0.76) (Brewer, 2004), since smaller muscle fibres have lower glycogen stores and correspondingly higher pH_u values. The rate of glycolysis is usually greater in muscles which cool down at a slower rate, i.e. those muscles which are situated deeper within the carcass (e.g. *M. infraspinatus* and *M. supraspinatus*) (Lawrie & Ledward, 2006c).

The pH_u of meat affects its colour, flavour, tenderness, water holding capacity (WHC) and shelf-life (Honikel, 2004a). Meat with a higher pH_u will therefore have: retarded oxidation of fat and myoglobin, resulting in more colour stable meat products with better flavour (Lawrie & Ledward, 2006d); a decrease in the extent of *post mortem* proteolysis (tougher meat products) (Warriss, 2000c; Lawrie & Ledward, 2006f); a higher WHC (Warriss, 2000d); and a higher susceptibility to bacterial spoilage (shorter shelf-life) (Hoffman, 2001; Monin, 2004; Lawrie & Ledward, 2006e). Negative characteristics which are specifically linked to DFD meat are: uneven colour development; poor processing attributes; only slight denaturation of proteins and therefore more strongly bound water (higher WHC); little or no exudate *post mortem* (low drip loss percentage) (Hoffman, 2001); more translucent (closed) muscle structure which absorbs more light (darker colour) (Hoffman, 2001; Monin, 2004); and lower cooking losses (exudate during cooking) (Lawrie & Ledward, 2006g). The darker colour is attributed to limited oxygen diffusion in the 'closed' structure whereby merely a thin bright red oxygenated myoglobin (MbO) surface layer can be formed. Consequently, the reduced purple myoglobin (Mb) core colour is more evident (Warriss, 2000d).

Meat colour

The colour of fresh red meat is of the highest importance, since it is the first meat quality attribute observed by consumers and used as an indication of meat freshness (Hoffman, 2001; Troy & Kerry, 2010). Red meat products should generally be bright pink or red in colour, as apposed to brown, grey or purple (Warriss, 2000d). The CIE L*a*b* colour system (Commission International De l'Eclairage, 1976) is a widely accepted system for the measurement of meat colour. Meat colour is usually measured on a bloomed cut meat surface (Honikel, 1998), after the fresh meat colour has changed from purple to a brighter red. The latter is attributed to the exposure of deoxymyoglobin (purple) to oxygen, resulting in the development of oxymyoglobin (bright red) (Mancini, 2009).

Higher quantities of IMF and/or collagen (which are normally coloured white) will cause a greater variability in meat colour measurements (Honikel, 1998). The meat from free-ranging game species will normally be darker in colour (lower CIE L* value) in comparison to the meat from domesticated livestock (Vestergaard *et al.,* 2000; Ramanzin *et al.,* 2010). The latter is attributed to

the higher activity level of free-ranging game species, resulting in higher quantities of the 'red', oxidative muscle fibres and consequently higher myoglobin concentrations (Lawrie & Ledward, 2006c). Additionally, Onyango *et al.* (1998) found a significant negative correlation ($r = -0.86$, $p<0.05$) between the myoglobin concentration and lightness (CIE L* value) of game meat.

The myoglobin content of muscles rapidly increase until an animal reaches an age of approximately 24 months (Lawrie & Ledward, 2006c). Muscles that are utilised more during the endurance type of activities (e.g. postural muscles) will contain a higher proportion of oxidative muscles fibres (Cassens & Cooper, 1971; Swatland, 1994b; Davies, 2004; Taylor, 2004) and will therefore be darker in colour.

Water holding capacity

Water holding capacity of meat can be defined as the ability of the meat to retain or hold water when a force (pressure or heat) is applied (Brewer, 2004). In living muscles, about 85% of the water in muscles is held within the myofibrils by capillary forces (Huff-Lonergan, 2009). Muscle WHC is thus located in the water which is present in intermolecular spaces amongst actin and myosin (salt soluble proteins). The WHC of muscle proteins will be at a minimum when the myofibrillar proteins reach their isoelectric point (5.4 ~ 5.5) (Brewer, 2004; Monin, 2004; Lawrie & Ledward, 2006g).

When skeletal muscles pass into *rigor mortis* the intermolecular spaces are reduced and the water is then expelled from the myofibrils to other regions in the muscle cells (Offer & Trinick, 1983). Meat therefore generally loose exuded fluids with the *post mortem* formation of actomyosin (Honikel, 2004b; Lawrie & Ledward, 2006d) due to the lower WHC of actomyosin in comparison to myosin and actin. The extent of *pre* rigor sarcomere shortening adds to the *post* rigor loss in WHC (Lawrie & Ledward, 2006g). Additionally, an increased rate of *post mortem* glycolysis is associated with a decrease in WHC (Brewer, 2004). A lower WHC in meat results in more drip formation which negatively affects the appearance and yield (loss in weight) of meat products. Consequently, a higher quantity of moisture is lost during cooking and the meat may be perceived as being dry (Warriss, 2000d).

Tenderness

Game meat is almost always more tender when compared to meat from domesticated animals (Ledger, 1963; Jansen van Rensburg, 2002). The *New Zealand Beef and Lamb Quality Mark Standards* deem a mean shear force value of 78.5 N or lower as acceptable, but most of the meat products should have shear force values of 107.9 N or lower (Slater, 2009), measured with a MIRINZ tenderometer and 117.7 N when Warner Bratzler measurements are performed (Johnson *et al*., 2005). The tenderness of skeletal muscles can be influenced by the IMF content, the

presence of muscle shortening, the connective tissue content and type as well as the enzymes involved in *post mortem* tenderisation (Swatland, 1994a).

The quantity and quality of connective tissue differs between animals of different ages. Younger animals tend to have a higher quantity of collagen which is of higher quality (Lawrie & Ledward, 2006c), whereas the degree of thermally stable cross-linkages (intra and intermolecular) between collagen polypeptide chains increases with age. Consequently, an increase in animal age has a negative impact on the quality of collagen and therefore the tenderness of meat from older animals (tougher meat) (Hoffman, 2001; Lawrie & Ledward, 2006c; Webb & O'Neill, 2008). However, the latter age differences in meat tenderness are not as evident in wild game species (Hoffman & McMillin, 2009).

With rapid muscle growth, it is generally the fast-twitch muscle fibres which increase in size negatively affecting meat tenderness (Swatland, 2004e). In general the muscle fibre type composition and connective tissue content differs between skeletal muscles and could be the reason for some muscles being less tender compared to others (Lawrie & Ledward, 2006c). Furthermore, the muscles from undernourished animals may have increased proportions of intramuscular collagen, possibly resulting in tougher meat (Lawrie & Ledward, 2006d).

Older animals usually have higher quantities of IMF when compared to younger animals (Warriss, 2000a). High amounts of IMF may have a diluting effect in the muscles and can result in more tender meat (Webb & O'Neill, 2008). However, due to the generally low quantity of IMF in meat from various game species, this phenomenon will not be as evident in game meat.

NUTRITIONAL QUALITY OF MEAT

In the past, food quality was linked to food safety, shelf-life and sensory attributes. Currently, food quality is related to background quality cues, such as the nutritional value and healthiness (Troy & Kerry, 2010). Since the South African game meat export market was closed in 2012 it is believed that an increase in the local commercial utilisation of game meat will follow. Unfortunately, the nutritional value of meat from South African game species is not as readily available as with some other established red meat products (e.g. beef). The former is of great importance so as to enable fair competition with the well-known red meat products on the local markets (Mostert & Hoffman, 2007). Furthermore, the availability of nutritional information on meat products assists consumers in making more informed decisions concerning their health (Harrington, 1994; Schönfeldt & Gibson, 2008). Research is often limited to the *Longissimus dorsi* (LD) muscle (loin), since the meat industry regards this muscle, especially that from the lumbar region, as the most representable of the total carcass composition and quality (Warriss, 2000a). However, the LD muscles of various game species may not be a good representation of the nutritional value of the other skeletal muscles. It is therefore important to investigate the nutritional and health characteristics for each of the indigenous game species (especially blesbok) and their individual muscles, so as to gain accurate information for the compilation of food compositional tables, e.g.

nutritional value of various foods as compiled by the South African Medical Research Council (MRC) (Jansen van Rensburg, 2002; Anon., 2011b).

Meat is a high quality, concentrated and easily digestible nutrient source which provides the human diet with essential amino acids and proteins of high biological value (American Meat Institute Foundation, 1960; Higgs, 2000; Jansen van Rensburg, 2002). Red meat is characterised as a high protein source as well as a high source of iron (Schönfeldt & Gibson, 2008). In general, meat primarily consists of five chemical constituents: moisture (± 75 – 80 g.100 g^{-1}); proteins (± 18 – 25 g.100 g^{-1}); IMF (± 1 – 13 g.100 g^{-1}); carbohydrates (± 1 – 2 g.100 $g^{-1}$of glycogen) and inorganic matter (± 1 g.100 g^{-1} minerals or ash) (Pearson & Young, 1989; Keeton & Eddy, 2004; Hocquette *et al.,* 2010). Game meat has higher protein content as well as significantly lower quantities of fat and energy (kJ), in comparison to meat from domesticated livestock (Jansen van Rensburg, 2002). The mean protein and IMF content of game meat is generally >20 g.100 g^{-1} and <3 g.100 g^{-1}, respectively (Aidoo & Haworth, 1995; Jansen van Rensburg, 2002; Ramanzin *et al*., 2010; Van Schalkwyk & Hoffman, 2010).

Animals on a higher plane of nutrition will have higher quantities of IMF and correspondingly lower moisture content (on a whole tissue basis) in the meat due to the inverse correlation between IMF and moisture content (Doornenbal & Murray, 1981; Pearson & Young, 1989; Keeton & Eddy, 2004; Lawrie & Ledward, 2006c). Since game meat has low quantities of IMF (high lean meat proportion) (Aidoo & Haworth, 1995; Ramanzin *et al.,* 2010; Van Schalkwyk & Hoffman, 2010), the moisture, protein and ash content will be higher in comparison to red meat from other species (Lawrie & Ledward, 2006a). Additionally, the inverse relationship will most likely not be between moisture and IMF content in game meat, but rather between moisture and protein content. The latter was found by Du Buisson (2006) in the LD muscle of blesbok, which had the highest ($p<0.05$) mean protein content and the lowest ($p<0.05$) mean moisture content, in comparison to four other blesbok muscles.

The meat from female animals have higher quantities of IMF when compared to male animals (Lawrie & Ledward, 2006c), especially during the gestation period. The mean IMF content in meat from female springbok (3.1 g.100 g^{-1}) was found to be higher ($p<0.05$) in comparison with the meat from male springbok (1.4 g.100 g^{-1}) (Hoffman *et al.*, 2007b). However, no gender differences have been found in the chemical composition of blesbok meat (Hoffman *et al.,* 2008).

The growth and development of animals results in an increase in most of the muscle constituents, except for the moisture content of muscle tissue (no increase). Different components in an animal's body will therefore be fully developed at different periods during growth (Lawrie & Ledward, 2006c). During normal or enhanced muscle growth, protein synthesis is favoured above fat deposition (until sufficient), whereafter a surplus of ingested energy is stored as fat (lastly IMF) (Lawrie & Ledward, 2006a). Fat is therefore the last tissue to mature during growth. Fat deposition usually starts with the onset of puberty (Warriss, 2000a). In young animals, fat is deposited at a slower rate compared to lean muscle, but as the animal gets older, fat is deposited

at a greater rate than lean muscle. It is thus inevitable that the amount of IMF will increase as an animal matures (Hocquette *et al.,* 2010). Consequently, older animals generally have higher quantities of IMF (Warriss, 2000a).

The function of animal fat is principally for the storage of surplus energy and/or insulation against cold environmental conditions (Davies, 2004). Free-living game species depend on the available vegetation in the environment for energy. Since the quantity and quality of forage may vary with season, game species do not always have good quality nutrition available to them throughout the year. Game species therefore often have little or no intramuscular and subcutaneous fat. The meat industry has for years been successful at reducing the fat content and modifying the fatty acid profile of red meats in accordance with the demands made by health conscious consumers (Higgs, 2000; Warriss, 2000d; Van Schalkwyk & Hoffman, 2010). However, reducing the fat content of game meat is not necessary, since the IMF content is known to be very low (2 – 3 g.100 g^{-1}) (Ramanzin *et al.,* 2010; Van Schalkwyk & Hoffman, 2010). The low IMF content is therefore an attractive characteristic for health conscious red meat consumers (Mostert & Hoffman, 2007).

In mammalian muscles, IMF mainly consists of phospholipids (within cell membranes) and triglycerides (reserved energy). Muscles rich in red, oxidative muscle fibres contain more triglycerides and phospholipids. The 'red' muscles specifically store IMF as fat droplets within the muscle fibres and consequently have higher quantities of IMF in comparison with the 'white' muscles. However, the redness of a muscle does not necessarily guarantee the development of IMF, since no strict association exists between oxidative metabolism of muscle fibres and its IMF content (Hocquette *et al.,* 2010). The fat content in game meat generally varies with age, gender, physiological condition and season (Cordain *et al.,* 2002; Ramanzin *et al.,* 2010). Kroon *et al.* (1972) established that mature male (empty) blesbok carcasses harvested in a summer rainfall region, showed a significant ($p<0.05$) increase in mean total fat content from spring (2.1 g.100 g^{-1}) to autumn (7.8 g.100 g^{-1}). They also found seasonal differences ($p<0.01$) in the mean total protein content between winter (23.0 g.100 g^{-1}) and summer (21.2 g.100 g^{-1}).

The fat content will usually increase from the head to the tail in an animal's body, but the deposition of IMF can be limited by the capacity (amount of adipocytes) of muscle tissue. Differences in the IMF content between species and muscle types are often as a result of differences in the muscle fibre type composition (Hocquette *et al.,* 2010). The presence or absence of IMF in meat products may have positive or negative effects on the final meat quality. Intramuscular fat and the fatty acid composition of meat has a direct impact on the sensory properties of meat products (e.g. flavour and juiciness) (Hocquette *et al.,* 2010) and an indirect impact on meat tenderness (Jeremiah *et al.,* 2003). Consequently, meat products with very low IMF will be less-tasty and perceived as being dry (Hocquette *et al.,* 2010). The meat industry is therefore challenged with the task of producing meat with enough IMF for a good eating

experience, but not too much so as to negatively affect the healthiness of the meat products (Swatland, 1994a; Issanchou, 1996; Warriss, 2000d; Hocquette *et al.,* 2010).

Fatty acid profile

Neutral lipids consist of one glycerol molecule and three even numbered long-chain fatty acids (Keeton & Eddy, 2004). Individual fatty acids can be classified as saturated fatty acids (SFA, no double bonds), mono-unsaturated fatty acids (MUFA, one double bond) or polyunsaturated fatty acids (PUFA, two or more double bonds) (Keeton & Eddy, 2004; Anon., 2008). The diet may offer a variety of fatty acids, but modification of some of the dietary fatty acids is required to preserve the unique fatty acid composition within bodily cells. Animal tissue is generally rich in unsaturated and saturated forms of long chain fatty acids (18 carbons or more) (Sul, 2006), although the polyunsaturated to saturated fatty acid ratio (P:S) is usually low. In addition, red meats generally contain very low quantities of omega-3 (ω3) fatty acids (Warriss, 2000b). Aidoo & Haworth (1995) identified C16:0 (palmitic acid), C18:0 (stearic acid) and C18:1ω9 (oleic acid) as the main fatty acids present in the IMF of various game species. Hoffman *et al.* (2008) identified C18:2ω6 (linoleic acid) in addition to palmitic acid, stearic acid and oleic acid, as the four main fatty acids in the LD muscle of blesbok. Mostert & Hoffman (2007) and Hoffman *et al.* (2007c) also identified the latter as the four main fatty acids in the LD muscles of kudu and springbok, respectively.

Three interrelated factors are important when considering the nutritional value of meat containing fat and the health consequences upon its consumption, these are: the total fat content; P:S; and the omega-6 to omega-3 fatty acid ratio (ω6:ω3) (Enser *et al.*, 1998; Cordain *et al*., 2002). Scientists have attempted to increase the P:S in the meat from ruminant animals, but this is very difficult seeing that PUFA from forage are hydrogenated by rumen microorganisms. Two major groups of rumen bacteria have been identified that isomerize either the *cis*-12 bond to *trans*-11, e.g. *Butyrivibrio fibrisolvens*, or the *cis*-9 bond to *trans*-10, e.g. *Megasphaera elsdenii* (Kim *et al.,* 2002). These rumen microorganisms hydrogenate the PUFA to unsaturated (with less double bonds) or SFA (Wood & Enser, 1997; Warriss, 2000b), although approximately a tenth of the unsaturated fatty acids can go through the rumen without being modified. Additionally, the unsaturated fatty acids are more sensitive to oxidation, limiting the extent to which meat with a higher P:S can be produced (Warriss, 2000b). As a result, the meat from ruminant species will generally have a lower P:S compared to single-stomached (monogastric) species (Wood & Enser, 1997; Enser *et al.*, 1998). The rumen microflora in young ruminant animals is not yet fully developed. Consequently, the dietary unsaturated fatty acids are not hydrogenised into more SFA. These thus go through the rumen unchanged and are deposited as such in animal tissue (Lawrie & Ledward, 2006c). Additionally, the fatty acid profile of meat may differ between game species (Webb & O'Neill, 2008). For example, the common duiker (*Sylvicapra grimmia*) is primarily a browser, feeding on forbs, shrubs, flowers and selected fruits (Kigozi, 2003), while springbok are mixed feeders and therefore tend to browse and graze and lastly blesbok are grazers (Du Plessis,

1972; Bothma *et al.,* 2010). Consequently, differences have been found in the fatty acid composition, P:S and ω6:ω3 of meat from the latter three species due to differences in their feeding habits and diets (Hoffman & Ferreira, 2004; Du Buisson, 2006).

Polyunsaturated fatty acids can be divided into two essential fatty acid classes, the ω6 and ω3 fatty acids, which can not be interconverted. These essential fatty acids are vital for the prevention of deficiencies and the maintenance of health, but should be consumed through dietary fat intake as they can not be completely synthesised in the body. The class names are derived from the position of the first double bond situated three and six carbons from the methyl end of the hydrocarbon chain in the ω3 and ω6 classes, respectively. Plants are able to synthesize the 18-carbon PUFA, such as C18:3ω3 (α-linolenic acid) and linoleic acid. Plants are therefore good sources of essential fatty acids in the diet (Spector, 2006). The main fatty acid in grass is α-linolenic acid (between 60% and 75% of the total fatty acids), followed by linoleic acid and C16:0 (palmitic acid) (McDonald *et al.*, 2002).

Linoleic acid, linolenic acid and C20:4ω6 (arachidonic acid) should be consumed in the diet, since they cannot be synthesised by animals due to a lack of the Δ12 and Δ15 desaturase enzymes (Warriss, 2000b; Roynette *et al*., 2004). Longer and more unsaturated fatty acids can be produced (Fig. 2.3) by the elongation and desaturation of ingested precursor ω6 and ω3 PUFA (linoleic acid and α-linolenic acid) (Roynette *et al*., 2004; Horton *et al.*, 2006). However, two similar enzymes (Δ12 and Δ15 desaturase) are present in the first steps of both the ω6 and ω3 PUFA metabolisms, although the enzymes have a greater affinity for the ω3 PUFA metabolism resulting in a competitive inhibition of the ω6 PUFA metabolism (Rose & Connolly, 1999; Roynette *et al*., 2004). The presence of higher concentrations of α-linolenic acid, C20:5ω3 (eicosapentaenoic acid; EPA) or C22:6ω3 (docosahexaenoic acid; DHA) might therefore result in a significant decrease in the desaturation of linoleic acid, consequently limiting the production of arachidonic acid (Rose & Connolly, 1999). Higher concentrations of α-linolenic acid in the diet, therefore inhibits the conversion of large amounts of linoleic acid for the production of some other essential ω6 fatty acids (Simopoulos, 2002). The ω6 and ω3 fatty acids are not essential for the maintenance of basic life processes in mammalian cells, but they are vital for lipid biomediator synthesis, the production of phospholipids in cell membranes, modulating membrane fluidity and cellular interactions and signalling (Roynette *et al.*, 2004; Spector, 2006). In human diets, the majority of foods contain more ω6 than ω3 fatty acids (Spector, 2006). The average dietary intake of ω6 fatty acids are 11 – 17 $g.day^{-1}$. The latter surpasses the minimum daily required amount for arachidonic acid (Spector, 2006) and so it is suggested that the daily human diet often includes enough ω6 fatty acids. However, meat generally contains low concentrations of arachidonic acid (Roynette *et al.*, 2004; Spector, 2006), while red meat normally contain low levels of ω3 PUFA (Warriss, 2000b).

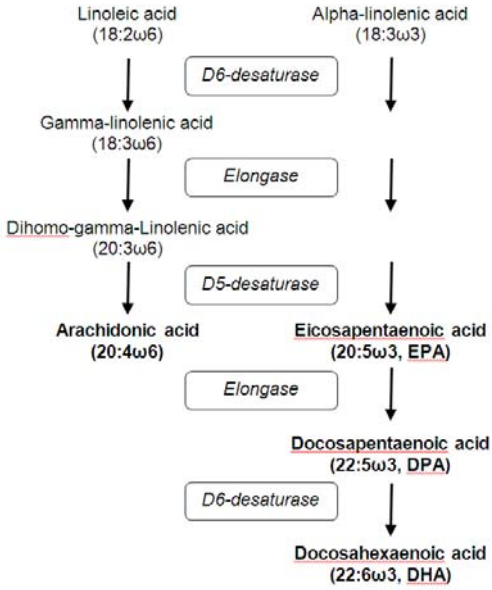

Figure 2.3 Pathway of the ω6 and ω3 PUFA metabolisms (as given by Roynette *et al*., 2004).

The 18-carbon ω3 PUFA precursor, α-linolenic acid (often called linolenic acid), is synthesised in small quantities by various terrestrial plants (Spector, 2006). It has structural similarities to linoleic acid, although an additional double bond is present at the 15th carbon. Alpha-linolenic acid does not have any unique functions in humans or animals, except for acting as a precursor for EPA and DHA synthesis (Roynette *et al.*, 2004; Spector, 2006; Stipanuk, 2006). Consequently, approximately 10% of the dietary α-linolenic acid is metabolised to EPA and DHA (often referred to as the fish oil fatty acids) (Spector, 2006). Docosahexaenoic acid is present at high concentrations in the phospholipids of the retina and brain and therefore is responsible for visual and cognitive activities (Kim & Edsall, 1999; Spector, 2006). Higher concentrations of EPA reduces the arachidonic acid content of phospholipids and replaces arachidonic acid with EPA and its elongation product, docosapentaenoic acid (DPA). The functions of arachidonic acid are therefore modulated by EPA (Spector, 2006).

It is generally believed that the majority of the SFA raise low-density lipoprotein (LDL) cholesterol and that PUFA usually slightly lowers the LDL cholesterol levels (Katan *et al.,* 1994; Raes *et al.*, 2004). However, some SFA (e.g. C18:0, stearic acid) are cholesterol neutral fatty acids since they do not exhibit negative or positive effects on cholesterol levels (Schönfeldt & Gibson, 2008). Other SFA (e.g. C12:0, lauric acid; C14:0, myristic acid; palmitic acid) raise the LDL serum cholesterol concentrations and therefore increase the risk for heart and coronary diseases (Katan *et al.,* 1994; Jansen van Rensburg, 2002; Schönfeldt & Gibson, 2008). Conversely, C18:1ω9 (oleic acid), linoleic acid and arachidonic acid are known for their cholesterol-lowering properties (Schönfeldt & Gibson, 2008). It has been anticipated that foods which are rich in ω3 fatty acids (e.g. fish and fish products) possibly provide protection against

cardiovascular disease, cataracts, immune function diseases, depression and cancer (Stipanuk, 2006). The positive characteristics of PUFA are therefore well established, although it is not yet known at what concentrations the essential fatty acids become detrimental to human health (Spector, 2006). Additionally, a P:S of >0.7 and ω6:ω3 of <5.0 has been found to contribute to the healthiness of meat products (Raes *et al.,* 2004).

Mineral composition

The smallest fraction of muscles consists of the inorganic matter, also referred to as the mineral or ash content. The mineral concentrations in meat is influenced by differences in species, hormones, gender, age, region and the mineral content in the diet (Doyle, 1980; Keeton & Eddy, 2004; Hocquette *et al.,* 2010). Meat is generally an essential source of phosphorus, potassium, iron, zinc and magnesium (Warriss, 2000d) of which potassium, followed by phosphorus are the most important (Lawrie & Ledward, 2006h). According to Keeton & Eddy (2004), muscle tissue is normally low in calcium (3 – 6 $mg.g^{-1}$), but rich in potassium (250 – 400 $mg.g^{-1}$), phosphorus (167 – 216 $mg.g^{-1}$), sodium (55 – 94 $mg.g^{-1}$), magnesium (22 – 29 $mg.g^{-1}$), zinc (1 – 5 $mg.g^{-1}$), iron (1 – 3 $mg.g^{-1}$) and copper (0.5 – 0.13 $mg.g^{-1}$). Dietary reference intake (DRI) for calcium and phosphorus (macro minerals) is set at >100 $mg.d^{-1}$ and for the trace elements (e.g. iron and zinc) at <100 $mg.d^{-1}$ (Higgs, 2000).

Game meat is generally a rich source of bioavailable haem iron (± 50 – 60%) (Higgs, 2000). The myoglobin content in red meats can account for approximately 95% of the iron present in the meat (American Meat Institute Foundation, 1960). Iron is essential as an O_2 carrier in cells; it ensures a healthy immune system and is responsible for providing energy to the body (Jansen van Rensburg, 2002). The iron in meat is more readily absorbed in comparison with non-haem iron present in plant foods. Additionally, the consumption of meat may almost double the absorption of iron from other food components (Higgs, 2000).

The essential mineral concentrations in forage may vary with differences in the plant species, growth stage (maturity), soil type, yield, climate and cultivation conditions (McDowell & Conrad, 1977; McDonald *et al*., 2002). Soil is a major source of minerals for plant growth (Nelson, 1998). Soil mineral concentrations influence the concentrations within the plants and consequently, the mineral concentrations in the muscle tissue of the animals feeding on the plants (Zomborszky *et al.*, 1996; McDowell & Conrad, 1977). Some researchers suggest that the absence of phosphorus is the most severe deficiency in plants throughout the whole of South Africa (Van Hoven, 2009).

CONSUMER PERCEPTION

The meat industry has changed from a once production driven industry to a consumer led one (Dransfield, 2003). Meat consumers have different quality cues which assist in the meat

purchasing decision. Extrinsic quality cues (meat origin and price) are not physically part of meat products, while intrinsic quality cues (e.g. colour and marbling) are (Troy & Kerry, 2010). Four meat quality cues are important at the point of sale: meat colour; packaged meat colour; visible drip loss percentage; and visible fat content. Alternatively, at the point of meat consumption the consumers tend to associate tenderness, juiciness, flavour and succulence with meat quality (Troy & Kerry, 2010). Also, modern consumers are more concerned with the healthiness of foods (Hocquette *et al.,* 2010).

A large percentage of consumers mainly consider the fat content of meat prior to purchase, providing game meat (IMF <3 g.100 g^{-1}) with an advantage above meat from most domesticated animals (Hoffman *et al*., 2005b), since a lower quantity of IMF (marbling) is generally preferred. However, upon consumption of cooked meat products with a higher quantity of IMF, consumers might prefer the higher IMF above the healthier meat options (since IMF is important in meat flavour development) (Aaslyng, 2009). The appearance (colour) of meat products is another very important meat quality attribute at the point of purchase. Meat colour allows consumers to make assumptions on the meat quality (Aaslyng, 2009; Mancini, 2009). Consumer perception on meat products is therefore related to the quality (Troy & Kerry, 2010), although the definition of quality might differ between different socio-demographic backgrounds (culture and health expectations) (Hocquette *et al.,* 2010).

Consumers generally expect meat products (especially red meat products) to be fresh, lean, nutritionally beneficial to their health, but still having good flavour, tenderness and juiciness (Dransfield, 2003; Hoffman & Wiklund, 2006). It is essential for consumers to have a positive perception towards a product; if not, they will not purchase or consume it (Troy & Kerry, 2010). South African meat consumers are generally uneducated about the positive attributes of game meat. This is partly due to a lack of good marketing as to the healthiness and benefits linked to the consumption of game meat products (Hoffman *et al.,* 2005b). Nonetheless, a number of South African consumers are aware of the low fat content of game meat, but the price of game meat, its dark red colour, taste and the lack of regular availability are some of the negative attributes which could prevent initial and further purchasing of game meat products. Consumers are also often not willing to pay more for game meat compared to other commercially produced meat products (e.g. chicken, pork or beef) (Hoffman, 2001; Hoffman *et al.,* 2005b). Some consumers also perceive game meat as being tough and dry, which may be ascribed to incorrect cooking methods, the low IMF content and/or a often relatively high WHC (associated with DFD meat) (Hoffman, 2001; Hocquette *et al.,* 2010). It is therefore important to correctly promote the positive attributes linked to game meat consumption.

CONCLUSIONS

Blesbok meat is well known by several South African consumers and many blesbok are hunted each year for local utilisation. Research on the nutritional value and meat quality of blesbok is

mainly limited to the *Longissimus dorsi* muscle (loin) (Hoffman *et al.,* 2008, 2010), since the meat industry regards this muscle as the most representable of the total carcass composition and quality (Warriss, 2000a). However, Du Buisson (2006) found differences in the chemical composition and physical attributes between five blesbok muscles (*Biceps femoris, Longissimus et lumborum, Rectus femoris, Semitendinosus* and *Supraspinatus*). The *Longissimus dorsi* muscles of game species may not be a representation of the nutritional value and meat quality of the other skeletal muscles. The *Longissimus dorsi* muscle is also not the only muscle sold commercially, other muscles (or meat cuts) are also of great importance, e.g. the fillets (*M. psoas major*), hindquarter muscles (*Biceps femoris*, *Semimembranosus* and *Semitendinosus*) and forequarter muscles (*Infraspinatus* and *Supraspinatus*). Additionally, some of the current meat consumers often desire smaller meat portions (Lawrie & Ledward, 2006c). Since the nutritional value, healthiness and meat quality may differ according to the selection of specific meat cuts or muscle portions, the labelling of the latter will differ.

The commercial harvesting of blesbok is not linked to any specific season (Fig. 2.2). Since blesbok have distinct seasonal activities (Du Plessis, 1972) in addition to the seasonal variations in the grass species preferred for consumption (Skinner & Chimimba, 2005), seasonal differences may be present in the chemical composition and quality of blesbok meat. Some researchers have found seasonal differences in blesbok carcass protein and fat content (Kroon *et al.,* 1972), but seasonal differences have not yet been quantified for individual blesbok muscles.

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CHAPTER 3

Impact of season on the chemical composition of male and female blesbok (*Damaliscus pygargus phillipsi*) muscles

ABSTRACT

The objective of this study was to investigate the impact of season on the chemical composition of six male and female blesbok muscles (*Longissimus dorsi, Biceps femoris, Semimembranosus, Semitendinosus, Infraspinatus* and *Supraspinatus*). Eight mature blesbok were harvested per season (spring of 2009, autumn of 2010, winter of 2010 and spring of 2010) on a farm near Heidelberg, Western Cape Province, South Africa. This area is classified as Coastal Renosterveld which primarily contains C_4 grass species and receives non-seasonal rainfall. The muscles were analysed for their chemical composition (moisture, protein, fat and ash contents) and there was a significant interaction between season and muscle type. The selected blesbok muscles had higher ($p<0.01$) mean protein contents (20.6 g.100 g^{-1} to 23.1 g.100 g^{-1}) when the plane of nutrition was higher (spring of 2009). There was a strong negative correlation ($r = – 0.82$; $p<0.01$) between the moisture and protein content in the blesbok muscles. The *Longissimus dorsi* muscle had the highest ($p<0.01$) mean intramuscular fat (IMF) content (3.4 g.100 g^{-1}) when the plane of nutrition was higher. The *Longissimus dorsi*, *Biceps femoris*, *Semitendinosus, Infraspinatus* and *Supraspinatus* muscles were the least affected by seasonal differences in activity levels and plane of nutrition, but the chemical composition of the *Semimembranosus* muscle was significantly different between seasons. Although the seasonal and muscle differences in the chemical composition of blesbok meat was statistically significant, these differences were numerically small and it is therefore debatable whether they are of any biological relevance relating to human nutrition.

INTRODUCTION

South Africa is well known for its game species and the hunting/harvesting thereof for local and international utilisation. It is therefore unfortunate that South African meat consumers are often uninformed about the positive characteristics of game meat, partly due to the insufficient marketing of these products. Game meat is often perceived as sought-after seasonal meat products which are not readily available; however, consumers are not willing to pay higher prices for game meat compared to commercially available meat products (Hoffman *et al.,* 2005). Limited information is available on the nutritional value of the meat from various South African game species (MRC, 2010), contributing to the difficulty in the marketing of game meat products.

South Africa has a distinct hunting season from May to August (winter months) (Hoffman, 2003), although season is generally not considered during the commercial harvesting of game species for meat export purposes (Anon., 2011). Blesbok is a popular game species in South Africa and numerous are hunted/harvested annually (Anon., 2011). They are medium sized herbivores and highly selective in grazing short grass species (Du Plessis, 1972; Bothma *et al.,* 2010). Blesbok are distributed widely throughout Southern Africa and flourish in both the summer and winter rainfall regions (Smithers, 1983). Blesbok from a summer rainfall region will usually be at their best condition during late summer/early winter (Bothma *et al*., 1996), whereas the blesbok from a winter rainfall region will most probably have a poorer condition during this period. These differences may be attributed to the presence/absence of the two categories of grass species, C_3 and C_4, classified according to their photosynthetic pathways. The C_3 and C_4 grass species are often separated geographically in South Africa (Vogel *et al.*, 1978), although some regions can contain combinations of both (Twiss, 1992). The C_3 temperate grass species thrive in the cooler seasons with moist weather conditions, but they become senescent once the temperatures increase during the summer months (Vogel *et al*., 1978; Twiss, 1992; Owen-Smith, 2008). The C_4 tropical grass species are more adapted to warm, arid or semi-arid, humid environments and therefore favour the summer months for growth (Vogel *et al*., 1978; Feldhake & Boyer, 1986; Twiss, 1992).

Huntley (1971) detected variations in the fat reserves of male blesbok due to seasonal differences in the forage consumed, whilst Kroon *et al.* (1972) detected seasonal differences in the mean total fat and protein content of empty mature male blesbok carcasses. However, these seasonal differences were noted with regards to the total body composition and not in the composition of specific blesbok muscles. The latter is of importance since game meat is generally exported as deboned individual and/or combinations of muscles such as the fillet, loin as well as the muscles from the forequarter and hindquarter (Paton *et al.,* 2009). Skeletal muscles are primarily made up of heterogeneous mixtures of contractile muscle fibres (oxidative, glycolytic or both) (Cassens & Cooper, 1971) of which the metabolic characteristics are broadly linked to different myosin heavy chain isoforms (MHC) (Conley, 1994; Kohn *et al.,* 2005). The combinations of muscle fibre types differ between species, the various skeletal muscles from one animal and the different regions within one muscle (Taylor, 2004). Similarly, Du Buisson (2006) reported differences ($p<0.05$) in the mean moisture and protein content of five blesbok muscles (*Biceps femoris; Longissimus et lumborum; Rectus femoris; Semitendinosus* and *Supraspinatus*). However, the latter investigation and muscle differences were limited to one harvesting period.

Since blesbok are seasonal breeders (Du Plessis, 1972; Bothma *et al.,* 2010) and the consumption of game meat in South Africa is not limited to any specific season, it is important to investigate the magnitude of the changes in the chemical composition between seasons. The aim of this study was therefore to quantify the impact of season on the chemical composition (moisture, protein, fat and ash contents) of male and female blesbok muscles.

MATERIALS AND METHODS

Harvesting

Blesbok were harvested on Brakkekuil farm (34°18'24.0"S and 20°49'3.9"E; 93 m.a.s.l.), near Witsand in the Western Cape Province, South Africa. The study area is classified as the Coastal Renosterveld and receives 300 – 500 mm of rainfall throughout the year (non-seasonal) (Rebelo *et al.*, 2006; Rutherford *et al*., 2006; Chase & Meadows, 2007; Kruger, 2007), although higher amounts of precipitation will generally occur in February and March (autumn) and again in September to November (spring) (Rebelo, 1996; Kruger, 2007). The rainfall patterns during the period of investigation at this study area (2009 and 2010) are indicated in Fig. 3.1. The arrows indicate the months in which the groups of blesbok were harvested (Fig. 3.1).

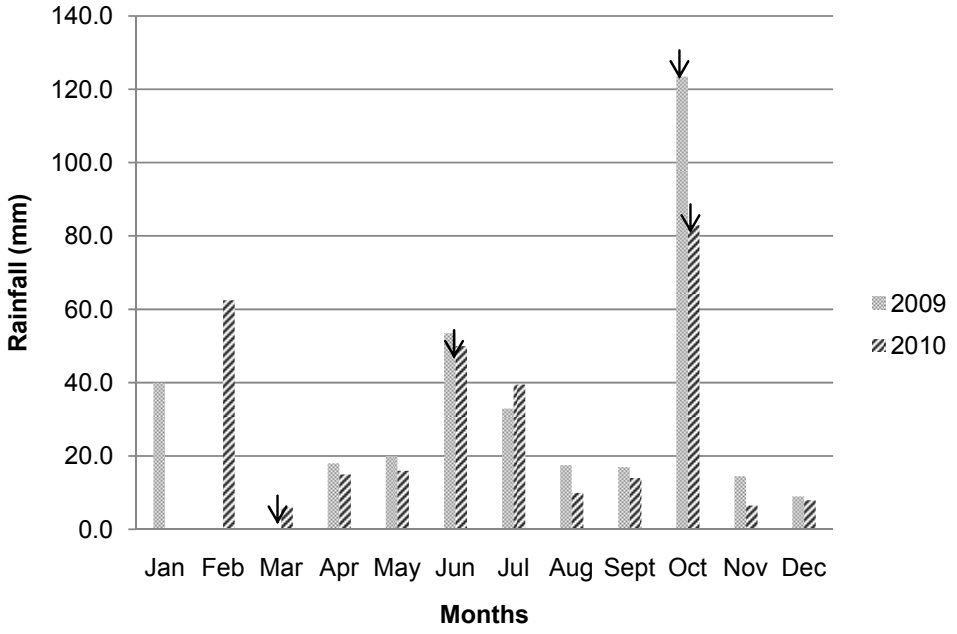

Figure 3.1 The 2009 and 2010 rainfall data (per month) for the study area indicating the period when blesbok were harvested.

The blesbok at Brakkekuil were confined by livestock fencing in a paddock of approximately 158 ha. Annual harvesting procedures were implemented for the reduction of the population size, so as to ensure sustainable yearly yields of animals and the positive growth of the population (Bothma, 2010). For this study, eight mature blesbok were harvested per season which occurred late in October of 2009 (spring), March of 2010 (autumn), June of 2010 (winter) and lastly in October of 2010 (spring). These harvesting periods formed part of the general management strategies of the farms and no preference was given to the selection of male or female blesbok (Table 3.1). The blesbok were harvested during the day and shot in the head or the high neck area with a .308 calibre rifle. Subsequently exsanguination occurred within two minutes, whilst in

the field (ethical clearance number: 10NP_HOF02, issued by *Stellenbosch University Animal Care and Use committee*). No unnecessary *ante mortem* stress was experienced by any of the animals.

Table 3.1 Number and gender of blesbok harvested during the 2009 and 2010 seasons

Year	Month	Male	Female	Total
2009	October	8	0	8
2010	March	5	3	8
2010	June	3	5	8
2010	October	4	4	8

Sample preparation

After harvesting, the undressed (bled) blesbok were transported to the slaughtering facilities where the head, legs and skin were removed and evisceration occurred according to the *Guidelines for the Harvesting of Game for Meat Export* (Van Schalkwyk & Hoffman, 2010).

The dressed carcasses were cooled (0° – 5°C) shortly after dressing (≈45 min *post mortem*). Subsequently, after at least 24 h of cooling, the *M. longissimus dorsi* (LD muscle), *M. biceps femoris* (BF muscle), *M. semimembranosus* (SM muscle), *M. semitendinosus* (ST muscle), *M. infraspinatus* (IS muscle) and *M. supraspinatus* (SS muscle) were removed completely from the left side of each carcass. Each muscle sample was weighed, homogenised, vacuum-packed and stored in a freezer at -20°C. Approximately four weeks after harvesting, the homogenised muscle samples were removed from the freezer (-20°C) and thawed for 12 h at ≈4°C, prior to the chemical analyses.

Chemical analyses

The moisture content (g.100 g^{-1}) of each muscle sample was determined at 100°C (24 h) on a 2.5 g portion, according to the AOAC official method 934.01 (AOAC, 2002a). The ash content (g.100 g^{-1}) was determined on the moisture free sample at 500°C (6 h), according to the AOAC official method 942.05 (AOAC, 2002b). The IMF content (g.100 g^{-1}) was determined on a 5 g portion of the muscle sample, by use of a rapid solvent extraction method as described by Lee *et al*. (1996) using chloroform/methanol (1:2 v/v). The filtrate from the latter extraction was consequently dried and analysed in a Leco Nitrogen/Protein Analyser (Leco Fp-528, Leco Corporation). The crude protein content (g.100 g^{-1}) was determined according to the Dumas combustion method 992.15 (AOAC, 2002c) from a dry, de-fatted, finely ground sample, encapsulated in a LecoTM foil sheet. The results from this method are given as the nitrogen content (% nitrogen), multiplied by a conversion factor of 6.25 (as meat protein is assumed to contain 16% nitrogen and therefore 100/16) to determine the total crude protein (g.100 g^{-1}) within each sample (McDonald *et al*., 2002).

An EDTA calibration sample (Leco Corporation, 3000 Lake View Avenue, St. Joseph, HI 49085-2396, USA, Part number 502-092, lot number 1038) was analysed in the Leco Nitrogen/Protein Analyser prior to each batch of protein samples, with the intention of ensuring the accuracy and recovery rate of each sample.

All of the above mentioned chemical analysis methods were tested bi-monthly for accuracy and repeatability by performing blind sample analyses as part of a National Inter-laboratory Scheme (AgriLASA: Agricultural Laboratory Association of South Africa).

Statistical analysis

Statistical analysis was performed using the Statistica 10 VEPAC module (STATISTICA, 2011). The mixed model repeated measures of analysis of variances (ANOVA's) were conducted with animal nested in season and gender taken as the random effect, and muscle treated as a within subject effect. Fisher LSD was used for post hoc testing. Normal probability plots were continuously checked for deviations from normality and possible outliers. A 5% significance level was used as a guideline for determining significant effects. The values are reported as the LSMeans and Standard Deviation (SD) of the mean.

RESULTS

A significant interaction ($p<0.01$) was present between gender and muscle type (gender x muscle) with regards to the mean moisture content of each muscle. The mean moisture content was higher ($p>0.05$) in the LD, BF and SM muscles, but lower ($p>0.05$) in the ST, IS and SS muscles of female blesbok (data not presented).

Table 3.2 depicts the differences in the chemical composition of the six blesbok muscles per season. There was a significant interaction ($p<0.01$) between the two main effects (season and muscle) on moisture, protein and IMF and the results are therefore discussed as such (Table 3.2). The data in Fig. 3.2 (a), (b) and (c) illustrates the significant interaction between season and muscle type (season x muscle) with regards to the mean moisture, protein and IMF contents. The differences in the plane of nutrition for each season are indicated in the figure legends.

Each of the six blesbok muscles had higher mean moisture content in winter of 2010 and spring of 2010, in comparison to the mean moisture content in spring of 2009 and autumn of 2010. The latter difference was the greatest ($p<0.01$) in the SM muscle. Conversely, the mean protein content of each blesbok muscle was higher ($p<0.01$) in both spring of 2009 and autumn of 2010, in comparison to the mean protein content in winter of 2010 and spring of 2010. The LD muscle had a higher ($p<0.01$) mean IMF content in spring of 2009, while the BF muscle had a higher ($p<0.01$) mean ash content in spring of 2009.

Spring 2009

The IS muscle had the highest mean moisture content, which differed ($p<0.01$) from the mean moisture contents in the LD and hindquarter muscles (BF, SM and ST). The SM muscle had the lowest mean moisture content, but the highest mean protein content which differed ($p<0.01$) from the mean protein content in the LD, ST, IS and SS muscles. The IS muscle had the lowest mean protein content, which differed ($p<0.01$) from the LD and hindquarter muscles. The LD muscle had the highest ($p<0.01$) mean IMF content. The BF muscle had the highest ($p<0.01$) mean ash content.

Autumn 2010

The SS muscle had the highest mean moisture content, which differed ($p<0.01$) from the mean moisture content in the LD and hindquarter muscles. The LD and BF muscles had the lowest mean moisture content, which differed ($p<0.01$) from the mean moisture content in the ST and SM muscles. The LD muscle had the highest ($p<0.01$) mean protein content and the SS muscle the lowest mean protein content. The LD muscle had the lowest ($p<0.01$) mean IMF content, while the BF and ST muscles had the highest ($p<0.01$) mean IMF contents. The LD and forequarter muscles had the lowest mean ash contents, which differed ($p<0.01$) from the mean ash contents in the hindquarter muscles.

Winter 2010

The SS and SM muscles had the highest mean moisture content, which differed ($p<0.01$) from the mean moisture content in the LD, BF and ST muscles. The LD muscle had the lowest ($p<0.01$) mean moisture content. The LD muscle had the highest mean protein content, which differed ($p<0.01$) from the mean protein contents in the BF, SM and forequarter muscles (IS and SS). The SS muscle had the lowest mean protein content. The BF muscle had the highest mean IMF content, which differed ($p<0.01$) from the mean IMF content in the SM muscle. The SS muscle had the lowest mean ash content, which differed ($p<0.01$) from the mean ash contents in the LD and hindquarter muscles.

Spring 2010

The forequarter muscles had the highest mean moisture contents, which differed ($p<0.01$) from the mean moisture contents in the LD, BF and ST muscles. Similar to the previous season, the LD muscle had the highest mean protein content, which differed ($p<0.01$) from the mean protein contents in the SM, ST and forequarter muscles. The forequarter muscles had the lowest mean protein contents. The SS muscle had the highest mean IMF content, which differed ($p<0.01$) from the mean IMF content in the SM muscle. The mean ash content of the IS muscle differed ($p<0.01$) from the mean ash contents in the LD and hindquarter muscles.

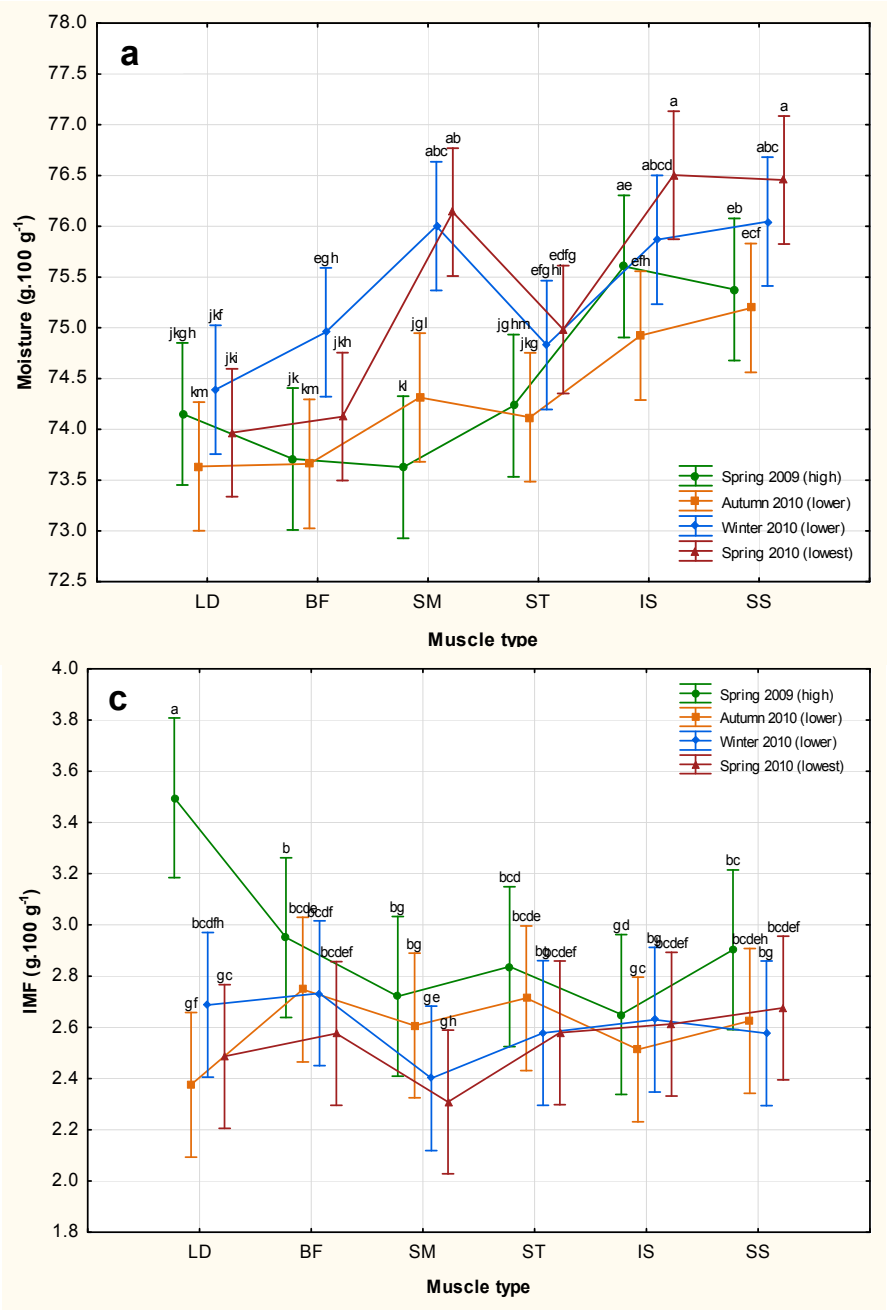

Figure 3.2 Interactions ($p<0.01$) between season and muscle type for the mean moisture (a), protein (b) and intramuscular fat (IMF) (c) contents (g.100 g^{-1}). Different alphabetical letters indicate significant differences in the mean values between muscles and seasons ($p<0.01$). LD, *M. longissimus dorsi*; BF, *M. biceps femoris*; SM, *M. semimembranosus*; ST, *M. semitendinosus*; IS, *M. infraspinatus*; SS, *M. supraspinatus.*

Table 3.2 The seasonal and muscle differences in the chemical composition (g.100 g^{-1}) of the six blesbok muscles from four seasons (LSMeans ± SD)

Season	LD	BF	SM	ST	IS	SS
Spring 2009						
Moisture	74.2^{jkgh} ± 0.71	73.7^{jk} ± 0.54	73.6^{kl} ± 0.86	74.2^{jghm} ± 0.68	75.6^{ae} ± 0.60	75.4^{eb} ± 0.64
Protein	21.7^{ge} ± 0.82	22.5^{abc} ± 0.53	23.1^{a} ± 0.74	22.4^{db} ± 0.56	20.6^{kilm} ± 0.70	21.0^{kh} ± 0.51
Fat	3.4^{a} ± 0.50	2.8^{b} ± 0.18	2.6^{bg} ± 0.20	2.7^{bcd} ± 0.28	2.5^{gd} ± 0.23	2.8^{bc} ± 0.34
Ash	1.2^{bcde} ± 0.05	1.6^{a} ± 0.30	1.2^{b} ± 0.05	1.2^{b} ± 0.04	1.1^{gie} ± 0.07	1.1^{gif} ± 0.05
Autumn 2010						
Moisture	73.6^{km} ± 1.15	73.7^{km} ± 0.93	74.3^{jgl} ± 1.37	74.1^{jkg} ± 1.04	74.9^{efh} ± 0.99	75.2^{ecf} ± 0.83
Protein	22.9^{ab} ± 1.04	22.1^{dce} ± 0.70	21.7^{dcef} ± 0.97	21.8^{dcef} ± 0.80	21.5^{dgh} ± 1.14	21.0^{ghij} ± 0.67
Fat	2.3^{gf} ± 0.36	2.7^{bcde} ± 0.29	2.6^{bg} ± 0.43	2.7^{bcde} ± 0.32	2.5^{gc} ± 0.31	2.6^{bcdeh} ± 0.47
Ash	1.1^{gie} ± 0.17	1.2^{bcd} ± 0.05	1.2^{bcde} ± 0.08	1.2^{bcd} ± 0.13	1.1^{gie} ± 0.06	1.1^{gie} ± 0.08
Winter 2010						
Moisture	74.4^{jkf} ± 0.92	75.0^{egh} ± 0.84	76.0^{abc} ± 0.59	74.8^{efghi} ± 0.86	75.9^{abcd} ± 1.00	76.0^{abc} ± 0.97
Protein	21.2^{geh} ± 0.94	20.5^{kil} ± 0.79	19.9^{nl} ± 0.69	20.9^{gfhij} ± 0.91	20.0^{kn} ± 0.48	19.6^{nmo} ± 0.92
Fat	2.7^{bcdfh} ± 0.37	2.8^{bcdf} ± 0.53	2.4^{ge} ± 0.37	2.6^{bg} ± 0.40	2.7^{bg} ± 0.52	2.6^{bg} ± 0.59
Ash	1.1^{gch} ± 0.09	1.2^{bcdef} ± 0.06	1.1^{gdh} ± 0.05	1.2^{bcdef} ± 0.05	1.1^{gi} ± 0.04	1.0^{i} ± 0.04
Spring 2010						
Moisture	74.0^{jki} ± 0.74	74.1^{jkh} ± 0.65	76.1^{ab} ± 0.89	75.0^{adfg} ± 1.23	76.5^{a} ± 0.66	76.5^{a} ± 1.06
Protein	21.5^{dgch} ± 0.58	21.1^{gfhi} ± 0.81	19.5^{no} ± 0.97	20.3^{kjlm} ± 1.26	19.1^{po} ± 0.42	18.7^{p} ± 0.53
Fat	2.5^{gc} ± 0.25	2.6^{bcdef} ± 0.41	2.3^{gh} ± 0.41	2.6^{bcdef} ± 0.63	2.6^{bcdef} ± 0.53	2.7^{bcdef} ± 0.61
Ash	1.2^{bcdef} ± 0.09	1.2^{bc} ± 0.06	1.1^{gc} ± 0.04	1.2^{bcde} ± 0.05	1.1^{ih} ± 0.08	1.1^{gie} ± 0.15

$^{a-m}$ Least square means in the same row with different superscripts are significantly different (p≤0.05) in the mean moisture contents between muscles and/or seasons (season x muscle)
$^{a-p}$ Least square means in the same row with different superscripts are significantly different (p≤0.05) in the mean protein contents between muscles and/or seasons (season x muscle)
$^{a-h}$ Least square means in the same row with different superscripts are significantly different (p≤0.05) in the mean intramuscular fat contents between muscles and/or seasons (season x muscle)
$^{a-i}$ Least square means in the same row with different superscripts are significantly different (p≤0.05) in the mean ash contents between muscles and/or seasons (season x muscle)
LD, *M. longissimus dorsi*; BF, *M. biceps femoris*; SM, *M. semimembranosus*; ST, *M. semitendinosus*; IS, *M. infraspinatus*; SS, *M. supraspinatus;* SD, Standard Deviation

DISCUSSION

Even though a significant second order interaction was present between gender and muscle type for the mean moisture content, the differences were not significant for gender. The latter may be attributed to an unequal quantity of male and female blesbok which were harvested per season in this investigation (Table 3.1). In general the meat from female animals can have higher quantities of IMF in comparison to male animals (Lawrie & Ledward, 2006c), especially during the gestation period. However, in this study no significant differences were found in the chemical composition due to the effect of gender. Mostert & Hoffman (2007) found no gender differences in the chemical composition of the LD muscle of kudu (*Tragelaphus strepsiceros*), while Hoffman *et al.* (2009b) also found no effect of gender on the chemical composition of the LD muscle of black wildebeest (*Connochaetus gnou*). In addition, Hoffman *et al.* (2008a) also found no effect of gender on the chemical composition of the LD muscle of blesbok.

The study area, the Coastal Renosterveld, consisted primarily of C_4 tropical grass species (Rebelo, 1996; Rebelo *et al.*, 2006) with smaller quantities of C_3 temperate grass species (Acocks & Momberg, 1988). In both the C_3 and C_4 grass species the digestibility and protein content decline with maturation (Owen-Smith, 2008). The decline in protein content occurs due to the translocation of nitrogen in grass to the "storage" components beneath the ground (Ruyle, 1993). The study area received non-seasonal rainfall throughout 2009 and 2010 (Fig. 3.1), being somewhat unique for each year. It was therefore believed that the winter rainfall in the Coastal Renosterveld region primarily favoured the growth of the C_3 grass species, while the summer rainfall primarily favoured the growth of the C_4 grass species. Consequently, the blesbok at this study area had seasonal variations in their plane of nutrition (quantity and nutritional value of the grasses consumed). In addition there could also have been seasonal variations in the activity level of the blesbok at this study area. With the onset of spring, a flush of new grass sprouts normally appear and the blesbok usually become more active and eat more frequently during the day. Conversely, when the quantity and quality of the nutrition is lower (generally during the colder months) blesbok usually become less active, resting more and eating less frequently during the day so as to preserve energy (Du Plessis, 1972).

The first harvesting season occurred near the end of October of 2009, after a substantial amount of rainfall (Fig. 3.1). Since the rainfall would most likely have favoured the sprouting and growth of the more abundantly present C_4 grasses, it was postulated that the blesbok had an adequate quantity of nutritious grass sprouts available for consumption. It was therefore believed that the first group of blesbok were on a relatively high plane of nutrition and were more active. With the onset of summer, the grasses would have matured resulting in a decline in the protein content and digestibility of the C_4 grasses. It was therefore believed that the blesbok which were harvested in autumn of 2010 were on a lower plane of nutrition. The blesbok which were harvested in winter of 2010, a cold and wet season, were believed to be on an even lower plane of

nutrition and less active during the day. The blesbok from the final harvesting season (spring of 2010) presumably had the lowest plane of nutrition. Although the first and last harvesting seasons were both characterised as spring, there was a clear difference in the amount of rainfall prior to these harvesting periods (Fig. 3.1). The latter difference could have resulted in a difference in the plane of nutrition between the two groups of blesbok.

General differences in the composition of skeletal muscles are rather complex and can be attributed to several intrinsic factors. These include: the species; breed; gender; age; anatomical location; level of exercise as well as; the plane of nutrition (Lawrie & Ledward, 2006c). Since muscle differences were linked to the season in which they were harvested (season x muscle), it may be postulated that the proposed seasonal differences in the plane of nutrition and activity level of each muscle may have influenced the chemical composition of these blesbok muscles (Table 3.2). Since the blesbok from this study area were all considered mature, the moisture content (on a fat free basis) in the skeletal muscles should have remained relatively constant (Lawrie & Ledward, 2006c). However, animals on a higher plane of nutrition will have higher quantities of IMF and correspondingly lower moisture contents in their muscles, as a result of the inverse correlation between IMF and moisture content (Doornenbal & Murray, 1981; Pearson & Young, 1989; Keeton & Eddy, 2004; Lawrie & Ledward, 2006c). Also, animals on a lower plane of nutrition will utilise their IMF (Jones, 2004; Taylor *et al*., 2005; Lawrie & Ledward, 2006a), resulting in a noticeable increase in the moisture content in the muscles (Lawrie & Ledward, 2006c). The latter was noted in the blesbok muscles from this study, since the mean moisture content in the six blesbok muscles was higher for winter of 2010 and spring of 2010 (Fig. 3.2 (a), Table 3.2), when the animals were on a lower plane of nutrition. Even though there was a significant negative correlation ($r = – 0.34$; $p<0.01$) between the mean moisture and IMF contents, the inverse correlation was not evident in the results (Fig. 3.2 (a) and (c), Table 3.2). This can be attributed to the low IMF content in the muscles of game species in general (Van Schalkwyk & Hoffman, 2010). Game muscle can therefore have a stronger negative correlation between the mean moisture and protein contents, which was noted in the blesbok muscles from this study ($r = – 0.82$; $p<0.01$). Each of the selected blesbok muscles therefore had a higher mean protein content in spring of 2009 and autumn of 2010 (Fig. 3.2 (b), Table 3.2), in comparison to the mean protein contents in winter of 2010 and spring of 2010. Game species generally have a higher proportion of lean meat due to the low quantities of fat in their carcasses (Lawrie & Ledward, 2006a). Age and plane of nutrition are two of the factors which determine the amount of fatty tissue in the body (Lawrie & Ledward, 2006b). However, when animals have reached maturity, the plane of nutrition can have a larger impact on the chemical composition of game meat as a result of the low quantities of stored energy reserves.

Skeletal muscles are primarily made up of a heterogeneous mixture of the different contractile muscle fibres (Swatland, 1994a; Taylor, 2004) and the differences in the function and activity level of muscles result in differences in the muscle fibre type composition (Taylor, 2004).

However, histochemical studies on muscle fibre typing of different game species is limited (Goldspink, 1996). Kohn *et al.* (2005) established that four commercially important impala (*Aepyceros melampus*) muscles (*Psoas major, Longissimus lumborum, Deltoideus* and SM muscle) expressed various proportions of the three MHC isoforms (I, IIa and IIx). However, the LD and SM muscles primarily expressed MHC IIa and to a lesser extent MHC I isoforms and therefore only contained muscle fibre Types I and IIA. As a result, impala LD and SM muscles had highly oxidative metabolisms and were most probably utilised during the endurance type of activities, e.g. maintaining the posture, standing and walking (Cassens & Cooper, 1971; Swatland, 1994b; Davies, 2004; Taylor, 2004). Since the daily activities of blesbok and impala are quite similar (Lynch, 1971; Du Plessis, 1972; Kohn *et al.,* 2005) it was postulated that the LD and SM muscles of blesbok also have highly oxidative metabolisms. Furthermore, blesbok are also not accustomed to jumping and therefore utilise the LD muscle even less in comparison to impala. In addition, the blesbok from this study area were not familiar with feeling threatened and were therefore even less active. A higher quantity of oxidative muscle fibres could therefore have been present in the LD and SM muscles as well as in the other skeletal muscles of these blesbok (Swatland, 1994a).

Type I and IIA oxidative muscle fibres mainly utilise IMF and to a lesser extent glycogen as energy sources (Taylor, 2004; Kohn *et al.,* 2005), i.e. when the plane of nutrition is low. Once all lipids except for the structural lipids have been utilised, the muscle proteins can be catabolised as an additional energy supply (Lawrie & Ledward, 2006a, c; Hoffman *et al*., 2009b). The chemical composition of muscles with a higher proportion of oxidative muscle fibres may therefore be more adversely affected by a lower plane of nutrition. The content of oxidative muscle fibres may have been highest in the LD muscle (Taylor, 2004; Lefaucheur, 2010); since the blesbok uses this muscle the least in comparison to the activity levels of the five other selected blesbok muscles. In addition, the LD muscle is situated in the region of the body which is developed last and the body will deposit IMF last as well as first utilise the stored energy reserves from this region (Lawrie & Ledward, 2006a). As a result of a decrease in the plane of nutrition after spring of 2009, the mean IMF content in the LD muscle significantly decreased after spring of 2009 (Fig. 3.2 (c), Table 3.2). The SM muscle, however, had a significant decrease in its mean protein contents after spring of 2009; consequently being the lowest in spring of 2010 (Fig. 3.2 (b), Table 3.2). The latter may be attributed to a relatively high proportion of the oxidative muscle fibres and no additional IMF, except for the structural lipids. Therefore, with the decrease in the plane of nutrition and the need for additional energy, the SM muscle proteins were most probably catabolised to provide an additional energy supply (Lawrie & Ledward, 2006c). Furthermore, a decrease in the activity level of the hindquarter and forequarter muscles, in addition to the decrease in the plane of nutrition, could have resulted in some muscle fibres becoming narrower (Frandson, 1966) and therefore more oxidative in metabolism. For each season, the forequarter muscles had the lowest mean protein content (Fig. 3.2 (b, Table 3.2) and correspondingly the highest mean moisture contents (Fig. 3.2 (b), Table 3.2) in comparison to the other blesbok muscles. When the plane of nutrition

was higher (spring of 2009 and autumn of 2010), the chemical composition of the hindquarter muscles (BF, SM and ST) were relatively similar (Table 3.2). However, with the reduction in the plane of nutrition the chemical composition of the SM muscle differed from the other hindquarter muscles (BF and ST) andbecame similar ($p>0.01$) to the chemical composition of the forequarter muscles (Table 3.2). It may therefore be postulated that the chemical composition of the SM muscle will be similar to that of the muscles present in the same region of the body when the plane of nutrition is relatively high, but as the latter decreases the chemical composition of the SM muscle could become more similar to that of the forequarter muscles. The chemical composition of the BF, ST and forequarter muscles was not affected as drastically by the seasonal differences in the nutritional value and quantity of the grass species at this study area. Although Kohn *et al.* (2005, 2007) completed some preliminary studies on the MHC isoforms expressed in the muscles of selected game species, further investigation into the fibre typing of specific blesbok muscles is essential so as to give more insight into the chemical differences between blesbok muscles.

Even though there were significant seasonal differences in the chemical composition of selected blesbok muscles, it is questionable whether these differences are of biological value. The chemical composition of game and other red meat types can vary from 70 – 75 g.100 g^{-1} for the moisture content (Keeton & Eddy, 2004; Sebranek, 2004; Hoffman *et al*., 2007, 2009a), 20 – 24 g.100 g^{-1} for the protein content (Chan, 2004; Hoffman *et al*., 2008a, b, 2009a), 0.2 – 2.5 g.100 g^{-1} for the IMF content (Keeton & Eddy, 2004; Lawrie & Ledward, 2006c; Hoffman *et al*., 2008a, 2009a) and 1.0 – 2.4 g.100 g^{-1} for the ash content (Keeton & Eddy, 2004; Sebranek, 2004; Hoffman *et al*., 2007, 2008a, b, 2009b). It is debatable whether seasonal differences in the chemical composition of the blesbok muscles from this region validate the seasonal classification of the meat. However, regardless of this the individual muscles can still be marketed as being high in protein and low in fat (Anon., 2010). Furthermore, the seasonal impact on the chemical composition of blesbok muscles may be amplified if the blesbok are harvested in a study area with specific grass species (only C_3 or C_4), in addition to definite seasonal rainfall patterns (wet and dry seasons).

CONCLUSIONS

Gender did not have a large influence on the chemical composition of blesbok meat from this study area. There was a strong negative correlation between the moisture and protein content of each of the blesbok muscles. The seasonal changes in the plane of nutrition greatly influenced the IMF content in the LD muscle. When the blesbok were on a higher plane of nutrition, the chemical composition of the LD muscle differed from the other muscles, but the hindquarter and forequarter muscles had similar chemical compositions according to their anatomical locations. When the plane of nutrition was lower the chemical composition of the SM muscle differed from the other hindquarter muscles, but was similar to the forequarter muscles. The chemical composition of the BF, ST and forequarter muscles was the least affected by seasonal differences. Seasonal

differences in the chemical composition of these blesbok muscles were of small magnitude and it is debateable whether it validates the seasonal classification of blesbok meat from this region. The selected blesbok muscles were high in protein and low in fat and may be marketed as such. It may be argued that the impact of season was limited since the study area received non seasonal rainfall. The latter therefore warrants further research, since the seasonal impact on the chemical composition of blesbok meat may be greater in a region with more definite seasonal rainfall and/or specific grass species.

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CHAPTER 4

Impact of season on fatty acid and mineral composition of male and female blesbok (*Damaliscus pygargus phillipsi)* muscles

ABSTRACT

The objective of this study was to quantify the impact of season on the fatty acid profile and mineral composition of six male and female blesbok muscles (*Longissimus dorsi, Biceps femoris, Semimembranosus, Semitendinosus, Infraspinatus* and *Supraspinatus*). Eight mature blesbok were harvested per season (winter and spring). The results showed that season did not have a significant impact on the fatty acid profile of blesbok muscles, but the difference in the fatty acid profiles between male and female muscles was significant. A Principal Component Analysis (PCA) bi-plot indicated that female blesbok muscles were associated with a higher saturated fatty acid and mono-unsaturated fatty acid content. Male blesbok muscles had higher ($p<0.01$) proportions of total polyunsaturated fatty acids (PUFA) (40.15 ± 5.39) and polyunsaturated to saturated fatty acid ratios (P:S) (0.85 ± 0.18), in comparison to female muscles (27.18 ± 8.04 and 0.54 ± 0.20, respectively). Differences in the anatomical location of the selected blesbok muscles furthermore influenced the fatty acid profiles. The less active *Longissimus dorsi* muscle had higher ($p<0.05$) total PUFA (38.34 ± 8.62), total omega-6 (ω6) PUFA (34.46 ± 7.83), total ω3 PUFA (3.44 ± 0.84) and P:S (0.85 ± 0.24) contents, in comparison with the *Infraspinatus* muscle (28.96 ± 8.65, 26.23 ± 7.86, 2.31 ± 0.70 and 0.56 ± 0.19, respectively) and *Supraspinatus* muscle (28.85 ± 9.23, 26.05 ± 8.24, 2.28 ± 0.76 and 0.55 ± 0.21, respectively). The hindquarter muscles (*Biceps femoris, Semimembranosus* and *Semitendinosus*) had intermediate fatty acid contents.

Season also had an impact on the calcium and zinc contents of blesbok muscles. The calcium content was higher ($p<0.05$) in spring (6.92 ± 1.94) compared to winter (5.61 ± 1.79). The zinc content was higher ($p<0.05$) in the male muscles from winter (4.04 ± 1.70) compared to spring (3.41 ± 1.67). The mineral composition was furthermore significantly different between the selected blesbok muscles. The *Biceps femoris* muscle had the highest ($p<0.05$) potassium (183.25 ± 12.79), phosphorus (180.21 ± 10.36) and magnesium (32.18 ± 1.72) contents, while the sodium and calcium contents were highest in the *Infraspinatus* and *Supraspinatus* muscles. The *Longissimus dorsi* muscle had the highest ($p<0.05$) iron (3.67 ± 0.51), but significantly lower zinc contents (1.63 ± 0.28) in comparison to the *Infraspinatus* and *Supraspinatus* muscles. Differences in the fatty acid and mineral composition of blesbok muscles were therefore more attributed to differences in the anatomical location of the selected muscles, as apposed to the impact of season.

INTRODUCTION

Red meat consumers primarily use the visible fat of meat products (intra- and intermuscular) (Hoffman *et al.*, 2005b) as well as the nutritional claims on the packaging, as an indication of the healthiness of meat products (Issanchou, 1996). Three factors are important when considering the nutritional value of meat containing fat: the total fat content; the P:S; and the omega-6 to omega-3 fatty acid ratio (ω6:ω3) (Enser *et al.*, 1998). The fatty acid profile of meat is an important consideration with regards to the healthiness and sensory properties of the meat products (Hocquette *et al.,* 2010).

The meat industry has been successful in reducing the fat content and modifying the fatty acid profile of red meat products in accordance with the demands by health conscious consumers (Higgs, 2000; Warriss, 2000a; Van Schalkwyk & Hoffman, 2010). However, reducing the fat content of game meat is not necessary as the fat content is known to be very low (2 – 3 g.100 g^{-1}) (Aidoo & Haworth, 1995; Van Schalkwyk & Hoffman, 2010). Difficulty exists in modifying the fatty acid profile of meat from ruminant animals, since polyunsaturated fatty acids (PUFA) from forage are hydrogenated to unsaturated (with less double bonds) or saturated fatty acids (SFA) (Wood & Enser, 1997; Warriss, 2000c). Approximately a tenth of the unsaturated fatty acids go through the rumen unchanged (Warriss, 2000c). Meat from ruminants will therefore generally have a correspondingly lower P:S (Wood & Enser, 1997; Enser *et al.*, 1998) and lower ω6:ω3 (especially in the strictly grazing ruminants) (Enser *et al.,* 1998). The fatty acid profile of game meat has similarities with other types of red meat, since the main fatty acids in the meat are palmitic acid (C16:0) and stearic acid (C18:0), both saturated, as well as oleic acid (C18:1ω9) a mono-unsaturated fatty acid (MUFA) (Aidoo & Haworth, 1995). The muscles from female animals often have higher quantities of intramuscular fat (IMF) (Lawrie & Ledward, 2006b), resulting in difference in the fatty acid profile between genders.

One of the popular game species hunted and consumed in South Africa is blesbok. They are a free-living game species and graze selectively on short grass species (Du Plessis, 1972; Bothma *et al.*, 2010). In addition to regional and seasonal differences in the grass species available to them, blesbok can also have a seasonal preference for specific grass species (Skinner & Chimimba, 2005). In ruminant animals, the composition of the forage consumed influences the quantity and quality of the fat and minerals present in the meat. The mineral content in grass varies with the grass species, growth stage, type of soil and the conditions of cultivation, the latter being strongly influenced by rainfall patterns. Nonetheless, meat is generally an essential source of phosphorus, potassium, iron, zinc and magnesium (Warriss, 2000c).

Differences in the activity level and plane of nutrition are known to influence the muscle fibre type composition of different skeletal muscles (Lawrie & Ledward, 2006b). Subsequently the fatty acid profile (Wood *et al.,* 2003) and mineral composition of the meat can also vary (Doornenbal & Murray, 1982). Du Buisson (2006) found differences ($p<0.05$) in the stearic acid, P:S, calcium, magnesium and zinc contents of five selected blesbok muscles (*Biceps femoris;*

Longissimus et lumborum; Rectus femoris; Semitendinosus and *Supraspinatus*). However, studies on the factors influencing the chemical composition of the meat from various game species are usually limited to the *M. longissimus dorsi* (LD muscle) (Hoffman *et al.,* 2007, 2008a, b, 2009a, b; Purchas *et al.,* 2010), since the commercial red meat industry considers the LD muscle the most representable muscle in carcasses (Warriss, 2000d).

This study was therefore aimed at quantifying the impact of season on the chemical composition of six blesbok muscles (*Longissimus dorsi, Biceps femoris, Semimembranosus, Semitendinosus, Infraspinatus* and *Supraspinatus*) from male and female animals.

MATERIALS AND METHODS

Harvesting

Blesbok were harvested on Brakkekuil farm (34°18'24.0"S and 20°49'3.9"E; 93 m.a.s.l.), near Witsand in the Western Cape Province of South Africa. The study area is classified as the Coastal Renosterveld and receives 300 – 500 mm of non-seasonal rainfall (Rebelo *et al.*, 2006; Rutherford *et al*., 2006; Chase & Meadows, 2007; Kruger, 2007). Higher volumes of precipitation usually occur in February and March (autumn) as well as in September to November (spring) (Rebelo, 1996; Kruger, 2007).

The blesbok at Brakkekuil were confined by livestock fencing in a paddock of ≈158 ha. Annual harvesting procedures have been implemented for the reduction of the population size, so as to ensure sustainable yearly yields of animals and the positive growth of the population (Bothma, 2010). For this study, eight mature blesbok were harvested per season in June of 2010 (winter) and October of 2010 (spring). The harvesting periods formed part of the general management strategies of the farms and no preference was given to the selection of male or female blesbok (Table 4.1). The blesbok were harvested during the day and shot in the head or the high neck area with a .308 calibre rifle. Subsequently, exsanguination occurred within two minutes, whilst in the field (ethical clearance number: 10NP_HOF02, issued by *Stellenbosch University Animal Care and Use committee*). No unnecessary *ante mortem* stress was experienced by any of the animals.

Table 4.1 Number and gender of blesbok harvested for this study

Year	Month	Male	Female	Total
2010	June	3	5	8
2010	October	4	4	8

Sample preparation

See Chapter 3 (Materials and Methods)

Intramuscular fatty acid composition

Frozen muscle samples were thawed (at ≈4°C) overnight after which 2 g samples were extracted (Folch *et al*., 1957) with a chloroform:methanol (2:1; v/v) solution containing 0.01% butylated hydroxytoluene (BHT) as antioxidant. Samples were homogenised for 30 s in the extraction solvent, by use of a polytron mixer (WiggenHauser, D-500 Homogenizer). To enable quantification of the individual fatty acids in the original muscle sample, heptradecanoic acid (C17:0) was used as internal standard. A sub-sample was taken from the extracted fats and transmethylated for 2 h at 70°C with a methanol:sulphuric acid (19:1; v/v) solution as the transmethylating agent. The sub-sample was then cooled to room temperature after which the resulting fatty acid methyl esters (FAME) were extracted with the use of water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen. Fifty µl hexane was added to the dried sample of which 1 µl was injected.

The FAME were analysed by gas-liquid chromatography (Varian Model 3300 equipped with a flame ionisation detector) using a 60 m BPX70 capillary column of 0.25 mm internal diameter (SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134, Australia). The hydrogen gas flow rate was 25 $ml.min^{-1}$ and the hydrogen carrier gas flow rate was 2 – 4 $ml.min^{-1}$. Temperature programming was linear at 3.4°$C.min^{-1}$ with the following temperature settings: initial temperature of 60°C; final temperature of 160°C; injector temperature of 220°C; and detector temperature of 260°C. The run time was ≈45 min with an injection volume of 1 µL. The FAME in the total lipids of each sample ($mg.g^{-1}$ sample) were identified by comparing the retention times with those of a standard FAME mixture (Supelco™ 37 Component FAME Mix, 10 $mg.ml^{-1}$ in CH_2Cl_2, Catalogue Number 47885-U. Supelco™, North Harrison Road, Bellefonte, PA 16823-0048, USA).

Mineral composition

The mineral composition was determined on a 0.5 g dry, defatted, finely ground meat sample. The samples were ashed at 460° – 480°C for 6 h, cooled; where after 5 ml of 6 M HCl was added and then placed in an oven for 30 min at 50°C. Consequently 35 ml distilled water was added, then filtered into a brown bottle and made up to 50 ml with distilled water (Method 6.1.1 Dry Ashing, AgriLASA, 2007). Elements were quantified on an iCAP 6000 Inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 auto sampler. Samples were analysed for: phosphorus; potassium; calcium; magnesium; sodium; iron; copper; zinc; manganese; boron; and aluminium. Element concentrations were calculated using iTEVA Analyst software.

Argon gas flow rate was 2 – 5 $ml.min^{-1}$ and the instrument settings were as follows: camera temperature -27°C; generator temperature 24°C; optics temperature 38°C; RF power 1150 W; pump rate 50 rpm; auxiliary gas flow 0.5 $L.min^{-1}$; nebulizer 0.7 L min^{-1}; coolant gas 12 L min^{-1}; and a normal purge gas flow. Wavelengths for the elements were as follows: phosphorus at 177.495

nm; potassium at 766.490 nm; calcium at 317.933 nm; magnesium at 285.213 nm; sodium at 589.592 nm; iron at 259.940 nm; copper at 324.754 nm; zinc at 213.856 nm; manganese at 257.610 nm; boron at 249.773 nm; and aluminium at 167.079 nm. After 11 samples, standards with a high, medium and low range was analysed for quality control.

Statistical analysis of data

Statistical analyses were performed using the Statistica 10 VEPAC module (STATISTICA, 2011). The mixed model repeated measures of analysis of variances (ANOVA's) were conducted with animal nested in season and gender taken as a random effect, and muscle treated as a within subject effect. Fisher LSD was used for post hoc testing. Normal probability plots were continuously checked for deviations from normality and possible outliers. A 5% significance level was used as guideline for determining significant effects. The values are reported as the LSMeans and Standard Deviation (SD) of the mean. Pearson correlations were used to test for relationships between measured variables.

A Principal Component Analysis (PCA) and Discriminant Analysis (DA) was conducted to indicate the relationship between the fatty acid profiles as well as the mineral composition of six male and female blesbok muscles (Rencher, 2002). The multivariate statistical analysis was performed using XL STAT™ statistical software (Version 2011, Addinsoft, New York, USA).

RESULTS

Fatty acid profile

The significant interactions between the main effects (season, gender and muscle) and the impact of the main effects on the fatty acid profile of blesbok meat are presented in Table 4.2. Muscle type and gender had the largest effect on the fatty acid profile of blesbok meat.

The significant interaction between season, gender and muscle (season x gender x muscle) for the mean C20:3ω6 content and ω6:ω3 content of blesbok muscles are presented in Table 4.3. For both harvesting seasons, the male muscles had higher ($p<0.05$) mean C20:3ω6 contents in comparison to the female muscles. In winter, the ω6:ω3 was higher in the forequarter muscles (*Infraspinatus* and *Supraspinatus*) of male blesbok in comparison with the ω6:ω3 in the forequarter muscles of female blesbok. For both seasons and genders, the forequarter muscles had the highest ω6:ω3 in comparison with the ω6:ω3 of the other blesbok muscles.

The significant interaction between gender and muscle (gender x muscle) for the mean oleic acid and total MUFA contents of blesbok is presented in Table 4.4. Both the mean oleic acid and total MUFA contents were highest ($p<0.01$) in the female muscles. In male blesbok, the IS muscle had the highest ($p<0.01$) mean oleic acid and total MUFA contents in comparison with the LD, *Biceps femoris* (BF) and *Semitendinosus* (ST) muscles. In female blesbok, the ST muscle had the highest ($p<0.01$) mean oleic acid and total MUFA contents in comparison with the LD, BF,

Semimembranosus (SM) and *Supraspinatus* (SS) muscles. The significant interaction between season and muscle (season x muscle) for the mean oleic acid and total MUFA contents of blesbok is presented in Table 4.5. For both seasons, the *Infraspinatus* (IS) muscle had the highest ($p<0.01$) oleic acid and total MUFA content.

The effect ($p<0.05$) of muscle type on the fatty acid profile of blesbok meat is presented in Table 4.6. The SM and forequarter muscles had higher ($p<0.01$) mean C15:0 and palmitic acid contents, while only the forequarter muscles had higher ($p<0.01$) mean stearic acid contents. The total SFA was therefore also higher ($p<0.01$) in the forequarter muscles. The SM and forequarter muscles had higher ($p<0.01$) mean C14:1 contents, while the SM and SS muscles had higher ($p<0.01$) mean C15:0 contents. The SS muscle had the highest ($p<0.01$) mean C18:1ω9t (elaidic acid) content. The LD and BF muscles had higher ($p<0.01$) mean C22:1ω9 (erucic acid) contents, while the LD had the highest ($p<0.05$) mean C24:1ω9 content. The LD muscle also had the highest ($p<0.01$) mean C18:2ω6c (linoleic acid), C18:3ω6 (gamma-linoleic acid), C22:5ω3 and C22:6ω3 (docosahexanenoic acid, DHA) contents. The LD muscle therefore had the highest ($p<0.01$) mean PUFA, ω3 PUFA and ω6 PUFA contents. The LD muscle had a low total SFA content and the highest ($p<0.01$) P:S, while the forequarter muscles had the lowest ($p<0.01$) P:S.

The significant effect of gender on the fatty acid profile of blesbok meat is presented in Table 4.7. The meat from female blesbok had a higher ($p<0.05$) mean palmitic acid content, while the mean C15:1, C16:1ω9, erucic acid, C24:1ω9, linoleic acid, C20:4ω6 (arachidonic acid), C20:5ω3 (eicosapentaenoic acid, EPA), C22:5ω3, DHA, total PUFA, total ω6 PUFA, total ω3 PUFA contents and P:S were higher ($p<0.05$) in the meat from male blesbok.

The significant impact of season on the fatty acid profile of blesbok meat is presented in Table 4.8. The mean C15:1, C20:1ω9, C24:1ω9 and EPA contents were higher ($p<0.05$) in the meat from the winter, while the mean erucic acid, C18:2ω6t and gamma-linoleic acid contents were higher ($p<0.05$) in the meat from spring.

Table 4.2 Impact of season on the fatty acid profiles (g.100 g^{-1} total fatty acids) of male and female blesbok muscles

Fatty acid composition	SxGxM	GxM	SxM	SxG	Muscle	Gender	Season
C14:0	*	ns	ns	ns	**	**	ns
C15:0	ns	ns	ns	ns	**	ns	ns
C16:0	ns	ns	ns	ns	**	*	ns
C18:0	ns	ns	ns	ns	**	ns	ns
C20:0	ns	*	ns	ns	**	ns	ns
C21:0	ns	ns	ns	*	ns	ns	**
C22:0	**	*	*	ns	**	**	ns
C14:1	ns	ns	ns	ns	**	ns	ns
C15:1	ns	ns	ns	ns	**	*	*
C16:1ω9	ns	ns	ns	ns	ns	**	ns
C18:1ω9c	ns	**	**	ns	**	**	ns
C18:1ω9t	ns	ns	ns	ns	**	*	ns
C20:1ω9	ns	*	ns	ns	**	**	**
C22:1ω9	ns	ns	ns	ns	**	*	*
C24:1ω9	ns	ns	ns	ns	*	**	*
C18:2ω6c	ns	ns	ns	ns	**	**	ns
C18:2ω6t	ns	ns	ns	ns	**	ns	**
C18:3ω3	ns	ns	ns	ns	ns	ns	ns
C18:3ω6	ns	ns	ns	ns	**	ns	**
C20:2	ns	*	ns	ns	**	**	ns
C20:3ω3	ns	**	**	ns	ns	**	ns
C20:3ω6	*	*	ns	ns	**	**	ns
C20:4ω6	ns	ns	ns	ns	ns	**	ns
C20:5ω3	ns	ns	ns	ns	ns	**	**
C22:2	ns	*	ns	ns	*	**	ns
C22:5ω3	ns	ns	ns	ns	**	**	ns
C22:6ω3	ns	ns	ns	ns	**	*	ns
Fatty acid totals							
SFA	ns	ns	ns	ns	**	ns	ns
MUFA	ns	**	**	ns	**	**	ns
PUFA	ns	ns	ns	ns	**	**	ns
ω3 PUFA	ns	ns	ns	ns	**	**	ns
ω6 PUFA	ns	ns	ns	ns	**	**	ns
Fatty acid ratios							
P:S	ns	ns	ns	ns	**	**	ns
ω6:ω3	*	ns	ns	ns	**	ns	ns

ns, p>0.05; *, p<0.05; **, p<0.01; SxGxM, interaction between harvesting season (S), gender (G) and muscle type (M); GxM, interaction between gender (G) and muscle type (M); SxM, interaction between harvesting season (S) and muscle type (M); SxG, interaction between harvesting season (S) and gender (G); SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω3 PUFA, omega-3 polyunsaturated fatty acids; ω6 PUFA, omega-6 polyunsaturated fatty acids; P:S, polyunsaturated to saturated fatty acids ratio; ω6:ω3, omega-6 to omega-3 polyunsaturated fatty acids ratio

SFA = sum of C14:0, C15:0, C16:0, C18:0, C20:0, C21:0 and C22:0; MUFA = sum of C14:1, C15:1, C16:1ω9, C18:1ω9c, C18:1ω9t, C20:1ω9, C22:1ω9 and C24:1ω9; PUFA = sum of C18:2ω6c, C18:2ω6t, C18:3ω3, C18:3ω6, C20:2, C20:3ω3, C20:3ω6, C20:4ω6, C20:5ω3, C22:2, C22:5ω3 and C22:6ω3; ω3 PUFA = sum of C18:3ω3, C20:3ω3, C20:5ω3, C22:5ω3 and C22:6ω3; ω6 PUFA = sum of C18:2ω6c, C18:3ω6, C20:3ω6 and C20:4ω6; P:S = [(sum of C18:2ω6c, C18:2ω6t, C18:3ω3, C18:3ω6, C20:2, C20:3ω3, C20:3ω6, C20:4ω6, C20:5ω3, C22:2, C22:5ω3 and C22:6ω3)/(sum of C14:0, C15:0, C16:0, C18:0, C20:0, C21:0 and C22:0)]; ω6:ω3 = [(sum of C18:2ω6c, C18:3ω6, C20:3ω6 and C20:4ω6)/(sum of C18:3ω3, C20:3ω3, C20:5ω3, C22:5ω3 and C22:6ω3)]

Table 4.3 The C20:3ω6 content (g.100 g^{-1} total fatty acids) and ω6:ω3 of six male and female blesbok muscles from two harvesting seasons (LSMeans ± SD)

	LD	BF	SM	ST	IS	SS
C20:3ω6						
Winter 2010						
Male	11.21^{abc} ± 0.94	11.44^{ab} ± 2.13	9.94^{fcg} ± 1.58	12.31^{a} ± 0.89	9.61^{fdgh} ± 2.05	11.02^{abc} ± 0.76
Female	6.93^{jim} ± 1.51	6.14^{jkm} ± 1.78	6.95^{jim} ± 1.26	6.10^{jkm} ± 1.89	5.45^{nk} ± 1.79	4.86^{nl} ± 1.60
Spring 2010						
Male	10.58^{acde} ± 0.82	10.71^{acd} ± 1.47	9.49^{fbe} ± 1.38	10.64^{acd} ± 1.07	8.89^{jg} ± 1.28	8.09^{fbghi} ± 0.91
Female	7.04^{jhkl} ± 2.37	7.56^{fghik} ± 2.27	6.20^{jn} ± 2.39	6.63^{jikl} ± 2.66	5.19^{nm} ± 1.73	5.13^{nm} ± 2.14
ω6:ω3						
Winter 2010						
Male	10.40^{dgh} ± 2.15	10.23^{dgh} ± 1.30	10.00^{dgh} ± 1.57	10.11^{dgh} ± 1.04	13.18^{a} ± 0.99	12.39^{ab} ± 1.63
Female	10.48^{ge} ± 0.84	10.00^{ghe} ± 0.72	9.76^{ghf} ± 0.63	9.59^{hfi} ± 0.80	11.37^{db} ± 0.64	11.95^{abc} ± 0.40
Spring 2010						
Male	10.03^{dhe} ± 1.09	9.37^{gh} ± 0.94	9.20^{gi} ± 0.86	9.19^{gi} ± 1.07	10.46^{def} ± 0.71	10.72^{dcef} ± 0.66
Female	9.62^{ghf} ± 1.73	9.62^{ghf} ± 0.78	9.38^{ghf} ± 0.60	8.95^{hi} ± 1.11	11.16^{dbe} ± 0.83	11.18^{dbe} ± 1.03

[a-n] Different superscripts at the least square means indicate significant differences ($p<0.05$) between seasons, genders and/or muscle types for a fatty acid/fatty acid ratio
LD, *M. longissimus dorsi*; BF, *M. biceps femoris*; SM, *M. semimembranosus*; ST, *M. semitendinosus*; IS, *M. infraspinatus*; SS, *M. supraspinatus*
ω6:ω3 = [(sum of C18:2ω6c, C18:3ω6, C20:3ω6 and C20:4ω6)/(sum of C18:3ω3, C20:3ω3, C20:5ω3, C22:5ω3 and C22:6ω3)]

Table 4.4 The oleic acid and total MUFA contents (g.100 g^{-1} total fatty acids) of six muscles from male and female blesbok (LSMeans ± SD)

	LD	BF	SM	ST	IS	SS
C18:1ω9c						
Male	8.30^{e} ± 1.57	8.76^{e} ± 1.57	9.23^{de} ± 1.45	8.51^{e} ± 2.05	10.67^{d} ± 1.84	9.29^{de} ± 3.10
Female	16.71^{c} ± 4.53	19.06^{b} ± 5.85	17.42^{c} ± 4.06	21.41^{a} ± 7.31	20.82^{a} ± 5.17	19.04^{b} ± 4.31
Total MUFA						
Male	10.45^{e} ± 1.45	10.95^{e} ± 1.55	11.66^{de} ± 1.47	10.83^{e} ± 1.91	12.93^{d} ± 1.77	11.49^{de} ± 2.91
Female	18.59^{c} ± 4.40	20.87^{b} ± 5.83	19.24^{c} ± 4.00	23.23^{a} ± 7.40	22.73^{a} ± 5.04	20.95^{b} ± 4.27

[a-h] Different superscripts at the least square means indicate significant differences ($p<0.05$) between genders and/or muscle types for a fatty acid/fatty acid total
LD, *M. longissimus dorsi*; BF, *M. biceps femoris*; SM, *M. semimembranosus*; ST, *M. semitendinosus*; IS, *M. infraspinatus*; SS, *M. supraspinatus;* C18:1ω9c, oleic acid
MUFA = sum of C14:1, C15:1, C16:1ω9, C18:1ω9c, C18:1ω9t, C20:1ω9, C22:1ω9 and C24:1ω9

Table 4.5 The oleic acid and total MUFA contents (g.100 g^{-1} total fatty acids) of six blesbok muscles from two harvesting seasons (LSMeans ± SD)

	LD	BF	SM	ST	IS	SS
C18:1ω9c						
Winter 2010	13.15^{ec} ± 5.49	14.77^{ae} ± 7.10	14.07^{eb} ± 5.25	14.86^{ae} ± 8.33	15.90^{ad} ± 6.49	13.51^{ec} ± 6.58
Spring 2010	12.92^{ed} ± 5.94	14.34^{ed} ± 7.10	13.61^{ed} ± 5.55	16.67^{ab} ± 9.33	16.86^{ab} ± 6.99	16.03^{abc} ± 5.99
Total MUFA						
Winter 2010	15.20^{db} ± 5.33	16.79^{ad} ± 7.02	16.22^{ad} ± 4.99	16.85^{ad} ± 8.14	18.02^{ab} ± 6.27	15.64^{db} ± 6.41
Spring 2010	14.85^{dc} ± 5.71	16.27^{dc} ± 6.85	15.63^{dc} ± 5.23	18.76^{a} ± 9.17	18.87^{a} ± 6.78	17.99^{ab} ± 5.85

$^{a-e}$ Different superscripts at the least square means indicate significant differences ($p<0.05$) between seasons and/or muscle types for a fatty acid/fatty acid total
LD, *M. longissimus dorsi*; BF, *M. biceps femoris*; SM, *M. semimembranosus*; ST, *M. semitendinosus*; IS, *M. infraspinatus*; SS, *M. supraspinatus*; C18:1ω9c, oleic acid
MUFA = sum of C14:1, C15:1, C16:1ω9, C18:1ω9c, C18:1ω9t, C20:1ω9, C22:1ω9 and C24:1ω9

Table 4.6 The effect of muscle type on the fatty acid contents (g.100 g^{-1} total fatty acids) of blesbok meat (LSMeans ± SD)

Fatty acids	LD	BF	SM	ST	IS	SS
C15:0	0.40^{c} ± 0.08	0.49^{b} ± 0.12	0.59^{a} ± 0.13	0.45^{b} ± 0.09	0.57^{a} ± 0.10	0.62^{a} ± 0.14
C16:0	19.55^{c} ± 2.20	19.57^{c} ± 1.86	21.75^{a} ± 1.72	20.39^{b} ± 1.66	21.33^{a} ± 1.66	21.43^{a} ± 2.39
C18:0	23.90^{d} ± 2.39	25.42^{bc} ± 2.88	26.15^{b} ± 3.49	24.87^{dc} ± 3.09	27.70^{a} ± 2.58	29.07^{a} ± 2.92
C14:1	0.14^{c} ± 0.04	0.19^{b} ± 0.06	0.22^{a} ± 0.08	0.18^{b} ± 0.05	0.23^{a} ± 0.04	0.26^{a} ± 0.06
C15:1	0.34^{c} ± 0.06	0.37^{b} ± 0.06	0.41^{a} ± 0.06	0.37^{b} ± 0.08	0.38^{ab} ± 0.06	0.40^{a} ± 0.06
C18:1ω9t	0.20^{c} ± 0.07	0.21^{c} ± 0.05	0.22^{cb} ± 0.06	0.20^{c} ± 0.04	0.24^{ab} ± 0.06	0.26^{a} ± 0.06
C22:1ω9	0.14^{a} ± 0.04	0.13^{a} ± 0.04	0.13^{ab} ± 0.03	0.12^{ac} ± 0.04	0.11^{cb} ± 0.04	0.11^{c} ± 0.05
C24:1ω9	0.18^{a} ± 0.05	0.16^{b} ± 0.05	0.16^{ab} ± 0.05	0.15^{b} ± 0.06	0.15^{b} ± 0.05	0.16^{b} ± 0.06
C18:2ω6c	20.04^{a} ± 4.66	17.50^{b} ± 4.93	15.98^{dc} ± 4.18	16.53^{bc} ± 5.45	14.84^{d} ± 4.73	14.75^{d} ± 4.98
C18:2ω6t	0.12^{e} ± 0.03	0.15^{cd} ± 0.04	0.16^{cb} ± 0.06	0.14^{d} ± 0.04	0.17^{ab} ± 0.04	0.19^{a} ± 0.05
C18:3ω6	5.56^{a} ± 1.66	5.08^{b} ± 1.74	4.89^{bc} ± 1.54	4.60^{c} ± 1.63	4.20^{d} ± 1.48	4.24^{d} ± 1.41
C22:5ω3	2.40^{a} ± 0.65	2.35^{a} ± 0.73	2.20^{b} ± 0.58	2.31^{a} ± 0.77	1.55^{c} ± 0.50	1.52^{c} ± 0.52
C22:6ω3	0.45^{a} ± 0.20	0.39^{cb} ± 0.14	0.36^{c} ± 0.11	0.42^{ab} ± 0.19	0.29^{d} ± 0.11	0.29^{d} ± 0.11
Fatty acid totals						
SFA	46.31^{c} ± 3.87	48.08^{c} ± 4.37	51.19^{b} ± 4.85	48.39^{c} ± 4.17	52.18^{ab} ± 3.47	53.89^{a} ± 4.61
PUFA	38.34^{a} ± 8.62	35.03^{b} ± 9.67	32.50^{c} ± 7.88	33.46^{bc} ± 10.59	28.96^{d} ± 8.65	28.85^{d} ± 9.23
ω3 PUFA	3.44^{a} ± 0.84	3.23^{ab} ± 0.95	3.05^{b} ± 0.75	3.20^{ab} ± 1.06	2.31^{c} ± 0.70	2.28^{c} ± 0.76
ω6 PUFA	34.46^{a} ± 7.83	31.38^{b} ± 8.67	29.03^{c} ± 7.10	29.86^{bc} ± 9.48	26.23^{d} ± 7.86	26.05^{d} ± 8.24
Fatty acid ratios						
P:S	0.85^{a} ± 0.24	0.75^{b} ± 0.25	0.65^{c} ± 0.19	0.71^{b} ± 0.26	0.56^{d} ± 0.19	0.55^{d} ± 0.21

$^{a-e}$ Least square means in the same row with different superscripts are significantly different ($p<0.05$)
LD, *M. longissimus dorsi*; BF, *M. biceps femoris*; SM, *M. semimembranosus*; ST, *M. semitendinosus*; IS, *M. infraspinatus*; SS, *M. supraspinatus*; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; ω3 PUFA, omega-3 polyunsaturated fatty acids; ω6 PUFA, omega-6 polyunsaturated fatty acids; P:S, polyunsaturated to saturated fatty acids ratio
SFA = sum of C14:0, C15:0, C16:0, C18:0, C20:0, C21:0 and C22:0; PUFA = sum of C18:2ω6c, C18:2ω6t, C18:3ω3, C18:3ω6, C20:2, C20:3ω3, C20:3ω6, C20:4ω6, C20:5ω3, C22:2, C22:5ω3 and C22:6ω3; ω3 PUFA = sum of C18:3ω3, C20:3ω3, C20:5ω3, C22:5ω3 and C22:6ω3; ω6 PUFA = sum of C18:2ω6c, C18:3ω6, C20:3ω6 and C20:4ω6; P:S = [(sum of C18:2ω6c, C18:2ω6t, C18:3ω3, C18:3ω6, C20:2, C20:3ω3, C20:3ω6, C20:4ω6, C20:5ω3, C22:2, C22:5ω3 and C22:6ω3)/(sum of C14:0, C15:0, C16:0, C18:0, C20:0, C21:0 and C22:0)]

Table 4.7 The effect of gender on the fatty acid contents (g.100 g^{-1} total fatty acids) of blesbok meat (LSMeans ± SD)

Fatty acids	Male	Female
C16:0	19.61^{b} ± 1.92	21.49^{a} ± 1.83
C15:1	0.41^{a} ± 0.07	0.35^{b} ± 0.05
C16:1ω9	1.21^{a} ± 0.16	0.97^{b} ± 0.20
C22:1ω9	0.14^{a} ± 0.03	0.11^{b} ± 0.03
C24:1ω9	0.20^{a} ± 0.04	0.13^{b} ± 0.03
C18:2ω6c	20.20^{a} ± 3.48	13.81^{b} ± 4.25
C20:4ω6	0.22^{a} ± 0.04	0.15^{b} ± 0.05
C20:5ω3	0.27^{a} ± 0.07	0.19^{b} ± 0.15
C22:5ω3	2.49^{a} ± 0.53	1.71^{b} ± 0.67
C22:6ω3	0.46^{a} ± 0.13	0.30^{b} ± 0.14
Fatty acid totals		
PUFA	40.15^{a} ± 5.39	27.18^{b} ± 8.04
ω6 PUFA	36.06^{a} ± 4.95	24.40^{b} ± 7.14
ω3 PUFA	3.54^{a} ± 0.62	2.44^{b} ± 0.88
Fatty acid ratios		
P:S	0.85^{a} ± 0.18	0.54^{b} ± 0.20

[a,b] Least square means in the same row with different superscripts are significantly different ($p<0.05$)
PUFA, polyunsaturated fatty acids; ω3 PUFA, omega-3 polyunsaturated fatty acids; ω6 PUFA, omega-6 polyunsaturated fatty acids; P:S, polyunsaturated to saturated fatty acids ratio
PUFA = sum of C18:2ω6c, C18:2ω6t, C18:3ω3, C18:3ω6, C20:2, C20:3ω3, C20:3ω6, C20:4ω6, C20:5ω3, C22:2, C22:5ω3 and C22:6ω3; ω3 PUFA = sum of C18:3ω3, C20:3ω3, C20:5ω3, C22:5ω3 and C22:6ω3; ω6 PUFA = sum of C18:2ω6c, C18:3ω6, C20:3ω6 and C20:4ω6; P:S = [(sum of C18:2ω6c, C18:2ω6t, C18:3ω3, C18:3ω6, C20:2, C20:3ω3, C20:3ω6, C20:4ω6, C20:5ω3, C22:2, C22:5ω3 and C22:6ω3)/(sum of C14:0, C15:0, C16:0, C18:0, C20:0, C21:0 and C22:0)]

Table 4.8 Impact of season on the contents (g.100 g^{-1} total fatty acids) of individual fatty acids in blesbok meat (LSMeans ± SD)

Fatty acids	Winter 2010	Spring 2010
C15:1	0.40^{a} ± 0.07	0.36^{b} ± 0.05
C20:1ω9	0.10^{a} ± 0.02	0.08^{b} ± 0.02
C22:1ω9	0.11^{b} ± 0.03	0.14^{a} ± 0.04
C24:1ω9	0.17^{a} ± 0.06	0.15^{b} ± 0.05
C18:2ω6t	0.13^{b} ± 0.03	0.18^{a} ± 0.05
C18:3ω6	3.62^{b} ± 0.95	5.91^{a} ± 1.28
C20:5ω3	0.26^{a} ± 0.07	0.19^{b} ± 0.16

[a,b] Least square means in the same row with different superscripts are significantly different ($p<0.05$)

Multivariate analysis

Fig. 4.1 (a) depicts the Principal Component Analysis (PCA) bi-plot of the fatty acid profile of the six male and female blesbok muscles from the different harvesting seasons. The two principle components of the bi-plot explain 64.02% of the total variation, while the first Factor (F1) explains 51.98% of the total variation and F2 explains 12.03% of the total variation. Important information may be extracted from a table and displayed by a PCA bi-plot and therefore the correlations between different variables (i.e. fatty acids) and the association of variables with the main effects may be observed. Furthermore, the correlation coefficient (r) may be used to establish whether there are significant correlations between different variables. Fig. 4.1 (b) depicts the Discriminant Analysis (DA) plot of the differences in the fatty acid profiles of six blesbok muscles from two harvesting seasons. The two components of the DA plot explain 81.01% of the total variation, while F1 explains 62.56% of the total variation and F2 explains 18.44% of the total variation. The DA indicates the presence of groups of data and what the relationship is between the different groups.

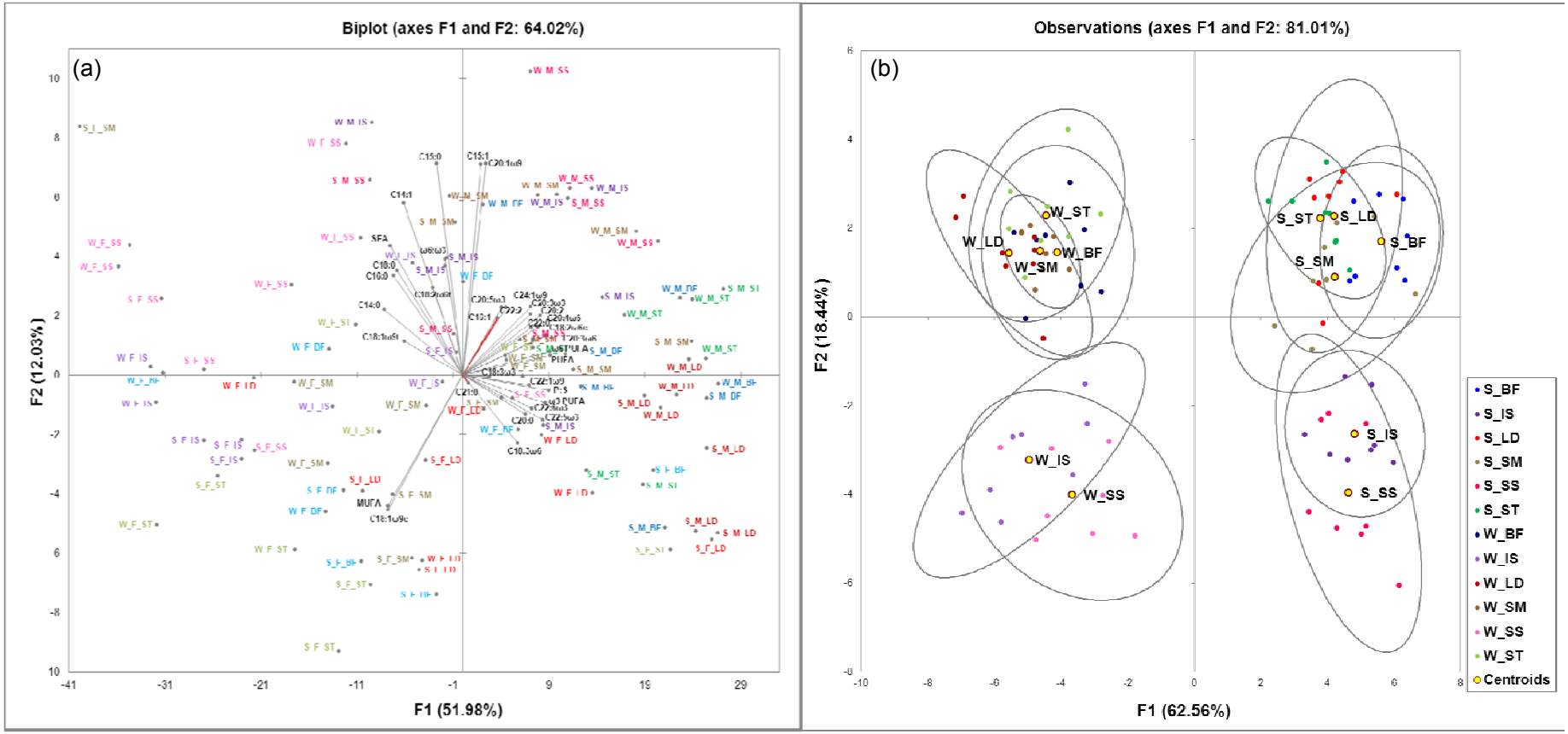

Figure 4.1 (a) PCA bi-plot of the fatty acid profiles of six male (M) and female (F) blesbok muscles (LD, *Longissimus dorsi*; BF, *Biceps femoris*; SM *Semimembranosus*; ST, *Semitendinosus*; IS, *Infraspinatus*; SS, *Supraspinatus*) from different harvesting seasons (W, winter; S, spring); (b DA plot of the fatty acid profiles of six blesbok muscles from two harvesting seasons.

Mineral composition

The significant interactions between the main effects (season, gender and muscle) and the impact of one of the main effects on the mineral composition of blesbok meat are presented in Table 4.9. Muscle type had the largest effect on the macro and micro mineral contents of blesbok muscles.

Table 4.9 Impact of season on the mineral composition (mg.100 g^{-1} dry base) of male and female blesbok muscles

Mineral composition	SxGxM	GxM	SxM	SxG	Muscle	Gender	Season
Macro minerals							
Potassium	ns	ns	ns	ns	**	*	ns
Phosphorus	ns	ns	ns	ns	**	ns	ns
Magnesium	ns	ns	ns	ns	**	ns	ns
Sodium	ns	ns	ns	ns	**	ns	ns
Calcium	ns	ns	ns	ns	*	*	**
Macro minerals							
Iron	ns	ns	ns	ns	**	ns	ns
Zinc	ns	ns	ns	**	**	*	**
Aluminium	ns	ns	ns	ns	ns	ns	ns
Copper	ns	*	ns	ns	ns	ns	ns
Manganese	ns	ns	ns	ns	**	ns	ns
Boron	ns	ns	ns	ns	ns	ns	ns

ns, p>0.05; *, p<0.05; **, p<0.01; SxGxM, interaction between harvesting season (S), gender (G) and muscle type (M); GxM, interaction between gender (G) and muscle type (M); SxM, interaction between harvesting season (S) and muscle type (M); SxG, interaction between harvesting season (S) and gender (G)

The significant interaction between gender and muscle (gender x muscle) for the mean copper content of blesbok muscles is presented in Table 4.10. There were no differences (p>0.05) in the copper content between female muscles, but in male muscles the BF muscle had the highest (p<0.05) and the ST muscle the lowest (p<0.05) mean copper contents. Between genders, the ST muscle of females had higher (p<0.05) mean copper content in comparison the ST muscle of the males.

Table 4.10 The copper content (mg.100 g^{-1} dry base) in male and female blesbok muscles (LSMeans ± SD)

	LD	BF	SM	ST	IS	SS
Copper						
Male	0.21^{ab} ± 0.10	0.25^{a} ± 0.03	0.19^{ad} ± 0.07	0.12^{d} ± 0.11	0.13^{db} ± 0.07	0.20^{ad} ± 0.08
Female	0.16^{dbc} ± 0.09	0.17^{ad} ± 0.08	0.18^{ad} ± 0.07	0.22^{ac} ± 0.07	0.21^{ad} ± 0.08	0.17^{ad} ± 0.07

$^{a-d}$ Different superscripts at the least square means indicate significant differences (p<0.05) in copper content between genders and/or muscles

There was a significant interaction between season and gender (season x gender) for the zinc content of blesbok meat (Table 4.11). There was no seasonal difference ($p>0.05$) in the mean zinc content of the meat from female blesbok, but the meat from male blesbok had higher ($p<0.01$) mean zinc content in winter. There was no gender difference ($p>0.05$) in the mean zinc content of blesbok meat from spring, but in winter the meat from male blesbok had a higher ($p<0.01$) mean zinc content.

Table 4.11 The zinc content (mg.100 g^{-1} dry base) in the meat of male and female blesbok from two harvesting seasons (LSMeans ± SD)

	Winter 2010	Spring 2010
Zinc		
Male	4.04^{a} ± 1.70	3.41^{b} ± 1.67
Female	3.37^{b} ± 1.58	3.50^{b} ± 1.65

[a,b]Different superscripts at the least square means indicate significant differences ($p<0.05$) in the zinc content between seasons and/or genders

The significant effect of muscle type on the mineral composition of blesbok meat is presented in Table 4.12. The BF muscle had the highest ($p<0.01$) mean potassium, phosphorus and magnesium contents. The forequarter muscles had the highest ($p<0.01$) mean sodium and zinc contents, while the IS muscle had the highest ($p<0.05$) mean calcium content. The LD muscle had the highest ($p<0.01$) mean iron and manganese contents.

Table 4.12 The effect of muscle type on the mineral composition (mg.100 g^{-1} dry base) of blesbok meat (LSMeans ± SD)

Minerals	LD	BF	SM	ST	IS	SS
Macro minerals						
Potassium	169.43^{cd} ± 17.92	183.25^{a} ± 12.79	175.09^{cb} ± 11.70	179.39^{ab} ± 14.19	167.78^{cd} ± 8.73	165.24^{d} ± 11.66
Phosphorus	172.92^{ab} ± 15.17	180.21^{a} ± 10.36	163.67^{c} ± 6.98	172.81^{b} ± 11.38	146.13^{d} ± 6.77	145.48^{d} ± 8.85
Magnesium	30.28^{b} ± 2.68	32.18^{a} ± 1.72	29.89^{b} ± 1.43	30.23^{b} ± 2.36	27.40^{c} ± 1.67	27.17^{c} ± 2.01
Sodium	16.23^{c} ± 2.68	18.82^{b} ± 1.87	18.83^{b} ± 1.95	19.02^{b} ± 3.50	24.73^{a} ± 3.52	23.48^{a} ± 3.37
Calcium	5.51^{b} ± 1.17	5.71^{b} ± 1.87	6.33^{b} ± 2.16	6.05^{b} ± 1.14	7.43^{a} ± 2.99	6.60^{ab} ± 1.53
Macro minerals						
Iron	3.67^{a} ± 0.51	3.58^{ab} ± 0.25	3.55^{ab} ± 0.76	2.85^{c} ± 0.47	3.27^{cb} ± 0.50	3.49^{ab} ± 0.83
Zinc	1.63^{e} ± 0.28	2.52^{c} ± 0.31	3.83^{b} ± 0.42	2.14^{d} ± 0.33	5.61^{a} ± 0.47	5.53^{a} ± 0.43
Manganese	0.04^{a} ± 0.01	0.03^{b} ± 0.00	0.03^{b} ± 0.01	0.03^{bc} ± 0.01	0.02^{d} ± 0.00	0.03^{dc} ± 0.01

[a-d] Least square means in the same row with different superscripts are significantly different ($p<0.05$)
LD, *M. longissimus dorsi*; BF, *M. biceps femoris*; SM, *M. semimembranosus*; ST, *M. semitendinosus*; IS, *M. infraspinatus*; SS, *M. supraspinatus*

The significant effect of gender on the mineral composition of blesbok meat is presented in Table 4.13. The mean potassium and calcium contents were higher ($p<0.05$) in the meat from male blesbok.

Table 4.13 The effect of gender on the potassium and calcium contents (mg.100 g^{-1} dry base) of blesbok meat (LSMeans ± SD)

Minerals	Male	Female
Potassium	179.01^{a} ± 14.02	168.86^{b} ± 13.09
Calcium	6.87^{a} ± 2.36	5.80^{b} ± 1.46

[a,b] Least square means in the same row with different superscripts are significantly different ($p<0.05$)

The significant impact of season on the mineral composition of blesbok meat is presented in Table 4.14. The mean calcium content was higher ($p<0.01$) in the meat from spring.

Table 4.14 The effect of season on the calcium content (mg.100 g^{-1} dry base) of blesbok meat (LSMeans ± SD)

Minerals	Winter 2010	Spring 2010
Calcium	5.61^{b} ± 1.79	6.92^{a} ± 1.94

[a,b] Least square means in the same row with different superscripts are significantly different ($p<0.05$)

Multivariate analysis

Fig. 4.2 (a) depicts the PCA bi-plot of the mineral composition of male and female blesbok muscles from different harvesting seasons. The two principle components of the bi-plot explain 56.55% of the total variation, while F1 explains 33.88% of the total variation and F2 explains 22.67% of the total variation. Fig. 4.2 (b) depicts the DA plot of the differences in the mineral composition of six blesbok muscles. The two components of the DA plot explain 98.63% of the total variation, while F1 explains 96.70% of the total variation and F2 explains 1.93% of the total variation.

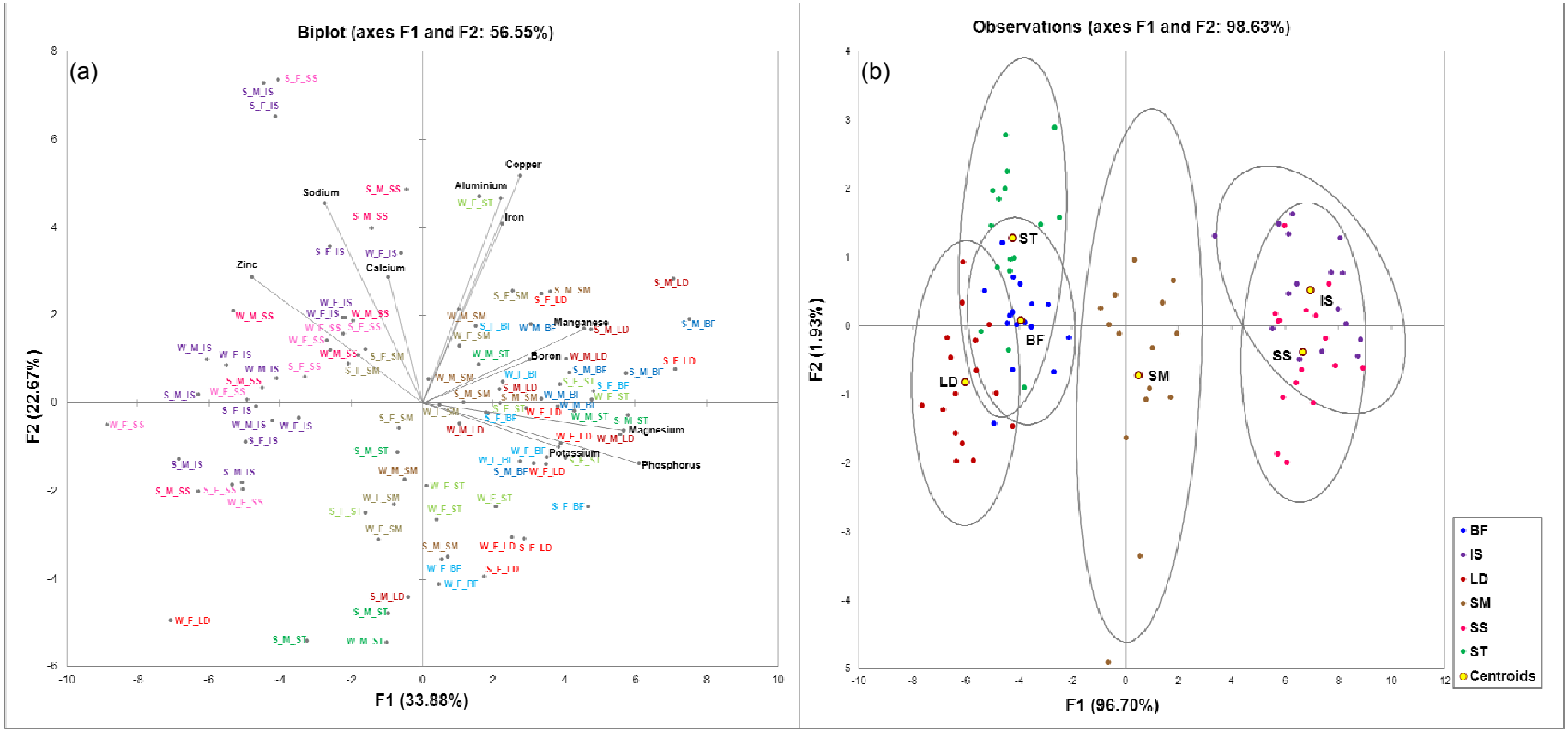

Figure 4.2 (a) PCA bi-plot of the mineral composition of male (M) and female (F) blesbok muscles (LD, *Longissimus dorsi*; BF, *Biceps femoris*; SM, *Semimembranosus*; ST, *Semitendinosus*; IS, *Infraspinatus*; SS, *Supraspinatus*) from different harvesting seasons (W, winter; S, spring); (b) DA plot of the mineral composition of six blesbok muscles.

DISCUSSION

Fatty acid profile

There were no interactions between the main effects on the C18:3ω3 (α-linolenic acid; ALA) content of blesbok meat (Table 4.2). The latter can be attributed to the primary function of ALA in the body, which is to serve as a substrate for the synthesis of EPA and DHA (Spector, 2006). This also explains why the ALA content (g.100 g^{-1} total fatty acids) in blesbok meat from this study area was so low (0.14 ± 0.05: LSMeans ± SD).

Seasonal differences

The growth of grass species in colder to moderate climatic conditions usually commences after an adequate amount of precipitation and a sufficient rise in environmental temperatures. In general, the latter will occur with the onset of spring when the soil temperatures reach 4° – 6°C (McDonald *et al*., 2002; Owen-Smith, 2008). Grass species normally have a low total lipid content (McDonald *et al.*, 2002) of which the main fatty acids are ALA (60 – 75% of total fatty acids) and to a lesser extent linoleic acid and palmitic acid (McDonald *et al.*, 2002; Khan *et al.,* 2012). However, different grass species can have unique fatty acid profiles (Dewhurst *et al.,* 2001). Nevertheless, the ALA content and so the total lipid content in grass is high during the primary growth stage (spring), but decreases with maturation (Khan *et al.,* 2012). Since blesbok are strict grazers (Du Plessis, 1972), it is expected that the blesbok which were harvested at this study area in spring, would have a higher total lipid and so ALA, linoleic acid and palmitic acid contents in their diet. Similar results were found in a study comparing the total lipid content of winter and spring samples of three ryegrass species (Dewhurst *et al.*, 2001).

Alpha-linolenic acid and linoleic acid are precursors for the longer chained ω6 and ω3 PUFA. In the ω6 PUFA metabolism, a double bond is added to linoleic acid to create gamma-linoleic acid. Consequently arachidonic acid, normally the most abundant product from the ω6 PUFA metabolism, is created by adding two carbons (elongase) and a double bond (desaturase) (Spector, 2006). Docosahexaenoic acid is the most abundant ω3 PUFA. Since there are two similar enzymes (Δ12 and Δ15 desaturase) in the first steps of the ω3 and ω6 PUFA metabolisms and these enzymes have a greater affinity for the ω3 PUFA metabolisms, there is a competitive inhibition of the ω6 PUFA metabolism (Rose & Connolly, 1999; Roynette *et al*., 2004). In addition, the formation of longer chain ω6 PUFA from arachidonic acid is only possible with a deficiency of ω3 PUFA in the diet (Spector, 2006). It is difficult to establish the impact of a seasonal difference in the nutrition of ruminant animals on the fatty acid profile of the meat, since the PUFA in the diet are hydrogenated by the rumen microorganisms to more saturated fatty acids (Wood & Enser, 1997). It is postulated that the higher quantity of ω3 PUFA in the diet of blesbok in spring, could have favoured the ω3 PUFA metabolism resulting in lower ($p<0.01$) EPA quantities in the meat

from spring (Table 4.8). Consequently, due to the competitive inhibition of the ω6 PUFA metabolism with the presence of sufficient amounts of ω3 PUFA in the diet, the gamma-linoleic acid content was higher ($p<0.01$) in spring (Table 4.8). Normally the changes in the dietary intake of fatty acids may result in changes in fatty acid composition of the membrane phospholipids, characterised by variations in the unsaturated fatty acid contents of the membranes. However, the seasonal difference in the diet of blesbok at this study area did not have a major impact on the fatty acid profile of the meat.

Gender differences

On the PCA bi-plot (Fig. 4.1 (a)) the muscles from female blesbok were associated with the variables on the left side of F1, while most of the muscles from male blesbok were associated with the variables on the right side of F1. It is therefore argued that gender had a more prominent effect on the fatty acid profile of the selected muscles from blesbok at this study area. Consequently, the muscles from female blesbok were more associated with the total SFA and total MUFA contents, whereas the muscles from male blesbok were more associated with the total PUFA content. Aidoo & Haworth (1995) identified palmitic acid, stearic acid, and oleic acid as the main fatty acids present in red meat. Palmitic acid was present at a significantly higher content in the muscles from female blesbok (Table 4.7). Palmitic acid together with C14:0 (myristic acid) are the main SFA responsible for raising the low-density lipoprotein (LDL) serum cholesterol concentrations in humans (Katan *et al.,* 1994; Schönfeldt & Gibson, 2008; Daley *et al*., 2010), ultimately increasing the risk for coronary diseases (Jansen van Rensburg, 2002). The oleic acid and the total MUFA contents were also higher ($p<0.01$) in the muscles from female blesbok, at almost double the quantity present in the muscles from male blesbok (Table 4.4). Oleic acid is usually the most abundant MUFA and therefore makes out the greatest portion of the total MUFA content in meat (Spector, 2006). The latter was also true for blesbok meat from this study area, since the oleic acid content was very strongly correlated with the total MUFA content ($r = 1.00$, $p<0.01$). There was a strong negative correlation between the total MUFA and total PUFA content in the blesbok muscles ($r = – 0.88$, $p<0.01$). The latter was supported by the significantly higher mean individual and total PUFA content in the muscles from male blesbok from both harvesting seasons (Tables 4.3 and 4.7).

Similar results for gender differences in the fatty acid profile of other game species have been established; the LD muscles from female kudu (*Tragelaphus strepsiceros*) have higher ($p>0.05$) total SFA and total MUFA contents, while the LD muscles from male kudu have higher total PUFA contents (Mostert & Hoffman, 2007). The LD muscles from grazing female impala (*Aepyceros melampus*) have higher total SFA ($p<0.05$) and total MUFA ($p>0.05$) contents, whereas the total PUFA content is higher ($p<0.05$) in the LD muscles from grazing male impala (Hoffman *et al.,* 2005a). Furthermore, the meat from male game species often has a lower IMF content (Lawrie & Ledward, 2006b) and therefore fewer triglycerides (Hoffman *et al.,* 2005a). The

meat from male blesbok therefore had lower total SFA content in comparison to the females (Fig. 4.1 (a)). Since the essential PUFA are primarily present in the phospholipids (structural lipid components) (Spector, 2006), the meat from male blesbok had a higher proportion of ω3 and ω6 PUFA (Table 4.7, Fig. 4.1 (a)). Subsequently, the P:S was also higher in the meat from male blesbok (Table 4.7).

Muscle type differences

Skeletal muscles are generally unique, heterogeneous combinations of different muscle fibre types (Cassens & Cooper, 1971; Taylor, 2004). Oxidative muscle fibres (Type I) primarily utilise fat as an energy source, while the glycolytic muscle fibres (Type IIB) primarily utilise stored glycogen or glucose as energy sources (Cassens & Cooper, 1971; Taylor, 2004; Kohn *et al.,* 2005). In blesbok, the LD muscles are mostly utilised for the maintenance of posture (balance and stability) (Robert *et al.,* 2001), whereas the hindquarter muscles (*Biceps femoris, Semimembranosus* and *Semitendinosus*) and forequarter muscles are also utilised during the former, in addition to walking whilst grazing and running when threatened (Lynch, 1971; Du Plessis, 1972; Kohn *et al.,* 2005). Differences in the muscle fibre type composition of the six blesbok muscles were expected and consequently these muscles would have differences/similarities in their fatty acid profiles according to their anatomical locations (Wood *et al.,* 2003).

The significant difference in the fatty acid profiles of the selected blesbok muscles is illustrated on the DA plot (Fig. 4.1 (b)). A clear separation can be seen between the forequarter muscles and the other blesbok muscles (*Longissimus dorsi, Biceps femoris, Semitendinosus* and *Semimembranosus*). The major muscle differences can also be seen on F2 of the PCA bi-plot (Fig. 4.1 (a)). The forequarter muscles were associated with the ω6:ω3 (Fig. 4.1 (a)). This was confirmed by the significantly higher mean ω6:ω3 in the forequarter muscles from both genders and both harvesting seasons (Table 4.3). The SS muscles and to a lesser extent the IS muscles, were associated with the total SFA and some of the shorter chain MUFA, while the LD muscles were associated with the longer chain MUFA and the PUFA (Fig. 4.1 (a)). Consequently, the fatty acid profile of the forequarter muscles was very different to that of the other blesbok muscles (Fig. 4.1 (b)). Muscles with a higher proportion of oxidative fibres will generally have a higher quantity of phospholipids and thus higher quantities of total PUFA (Wood *et al.,* 2003; Spector, 2006). It was believed that the blesbok LD muscle had the highest proportion of oxidative muscle fibres, as a result of its lower activity level in comparison to the other selected blesbok muscles (Chapter 3). The latter was confirmed by the higher ($p<0.01$) total PUFA content and P:S in the LD muscles from blesbok at this study area (Table 4.6). The total PUFA content is also normally characteristically higher in the meat from wild ruminant animals (Cordain et al., 2002). Since the ω3 and ω6 PUFA are more beneficial for human health in comparison with SFA (Lawrie & Ledward, 2006a), meat products with a higher P:S is preferred, therefore adding value to the LD muscles (Table 4.6). A P:S of ≥0.45 and an ω6:ω3 of ≤4.0 is recommended in the UK (Warriss,

2000d), while other researchers recommend that red meat should have a P:S of ≥0.70 and an ω6:ω3 of ≤5.0 (Raes *et al*., 2004). The P:S of the LD, BF and ST muscles were above the recommended values (Table 4.6). Hoffman *et al*. (2008a) reported a slightly higher mean P:S at 0.93 for the LD muscles from blesbok. Unfortunately, all the selected male and female blesbok muscles from both harvesting seasons had ω6:ω3 values well above the above mentioned recommended values (Table 4.4). However, ω3 PUFA is normally present at low levels in red meat products (Warriss, 2000b; Lawrie & Ledward, 2006d). Since blesbok consume high quantities of ω3 PUFA the competitive inhibition of the ω6 PUFA metabolisms might be responsible for the resulting high ω6:ω3 values in the blesbok meat from this study area. Despite the latter, oleic acid (C18:1ω9), linoleic acid (C18:2ω6) and arachidonic acid (C20:4ω6) are known for their cholesterol-lowering properties (Schönfeldt & Gibson, 2008). In addition, the ω6 PUFA are also of importance since they have essential functions in membrane phospholipids, i.e. in sphingolipids for the prevention of water loss through the skin as well as for signal transduction.

Mineral composition

Seasonal differences

A low protein content and increased maturity of grass species could significantly reduce the food intake and consequently the mineral intake in grazing animals (McDowell, 1985). Spring was presumed to be the growing season when the nutritional value (i.e. protein content) of the grass species would have been higher in comparison with those present in winter. Calcium was the only mineral present at different concentrations in the blesbok meat from the two different harvesting seasons (Table 4.13). This may be attributed to the higher intake of nutritious grass species prior to the harvesting in spring, resulting in a higher calcium intake by the blesbok from this study area.

Muscle type differences

Blesbok generally have a much lower copper content in comparison with more domestic species (Quan, 2001), such as cattle (23 – 409 $mg.kg^{-1}$ DM) and sheep (186 – 1374 $mg.kg^{-1}$ DM) (Grace, 1994). However, the copper content can vary widely in healthy animals (Grace, 1994). Even though there were differences in the copper content of the selected muscles from male and female blesbok (Table 4.10), these were of a very small magnitude and not considered biologically relevant.

The mineral composition of muscles are affected by the animal species, hormones, gender, age, production region and the mineral content in the diet (Doyle, 1980). The mineral composition can also be different in skeletal muscles at different anatomical locations (Zarkadas *et al.,* 1987), as a result of different physical activities and consequently fibre type compositions (Doornenbal & Murray, 1982). The DA plot of the mineral composition (Fig. 4.2 (b)) displays the significant difference in the mineral composition of selected blesbok muscles. The forequarter muscles were

more associated with sodium, calcium and zinc (Fig. 4.2 (a)), which was confirmed by the significantly higher mean contents of these minerals in the forequarter muscles (Table 4.12). The LD and hindquarter muscles were more associated with the potassium, phosphorus, magnesium, manganese and boron contents (Fig. 4.2 (a)). Difference in the activity level of each of the selected blesbok muscles could have resulted in a variation in the mineral distribution between the muscles (Doornenbal & Murray, 1982). The majority of protein sources available in the diet of ruminant animals provide large amounts of potassium and phosphorus (McDowell, 1985) and these are the two most important minerals in meat (Lawrie & Ledward, 2006d). In this study, potassium followed by phosphorus, were present at the highest quantities in all of the blesbok muscles (Table 4.6). Du Buisson (2006) also established that potassium and phosphorus were the minerals present at the highest quantity in the LD, BF, ST and SS muscles from blesbok.

It has been suggested that muscles which are used more during movement will have higher zinc content (Doornenbal & Murray, 1982). The latter might be the reason for the significantly higher zinc content in the postulated more active forequarter muscles in comparison to the low zinc content in the less active LD muscle (Table 4.12). Du Buisson (2006) also found higher ($p<0.05$) mean zinc contents in the SS muscle of blesbok, in comparison with the mean zinc contents in the LD, BF and ST muscles. Meat generally contains large quantities of bioavailable zinc (Wyness *et al.,* 2011), essential for the repair and growth of tissue (i.e. healing wounds; protein synthesis) and the maintenance of a healthy immune system (Doyle & Spaulding, 1978; Wyness *et al.,* 2011). In contrast to the suggestions made for the mean zinc content, it has been suggested that more active muscles will have lower calcium contents (Doornenbal & Murray, 1982). However, the latter was not the case in this study since the calcium content was highest in the forequarter muscles and lowest in the LD and hindquarter muscles (Table 4.12). In a previous study on the mineral content of blesbok muscles, the mean calcium content was found to be highest ($p<0.05$) in the less active LD muscle (Du Buisson, 2006). Calcium and phosphorus have significant functions in muscles and for reproduction as well as important roles in the formation of healthy teeth and bones (McDowell, 1985; Gill *et al.,* 2004). The mean manganese content was highest ($p<0.05$) in the less active LD muscle. Manganese is an essential micro-mineral required for growth and a deficiency can result in problems with the nervous system (Demirezen & Uruc, 2006).

Meat contains bioavailable iron, present in the haem form for better absorption and use in the body (Gibson & Ashwell, 2002; Wyness *et al.,* 2011). The haem iron is important as it is utilised in the formation of haemoglobin in red blood cells (Wyness *et al.,* 2011) and is therefore vital for the maintenance of oxygen transport in the blood (Gibson & Ashwell, 2002). The iron content in the diet is proportional to the myoglobin concentration in the muscles, a lower iron content resulting in a lower myoglobin concentration (Lawrie & Ledward, 2006c). However, the red, oxidative muscle fibres are rich in myoglobin (Lawrie & Ledward, 2006b). It was therefore believed that a higher mean iron content would be present in the muscles with a larger proportion of oxidative muscle fibres (i.e. blesbok LD muscles). The LD muscle from blesbok had significantly

higher mean iron content (Table 4.12). The iron content is also different between various beef muscles, the less active fillet muscle (*Psoas major*) for example, having the highest iron content due to its higher proportion of slow, oxidative muscle fibres (Lombardi-Boccia *et al*., 2005).

From the results obtained in this study, it appears that the different muscles or carcass cuts from blesbok will have different mineral compositions according to their activity level and anatomical locations. Limited information is, however, available on the effect of muscle type on the mineral composition of various skeletal muscles derived from wild animals, warranting further research.

CONCLUSIONS

Seasonal changes in the diet of blesbok at this study area did not have a marked influence on the fatty acid composition of the selected muscles. Gender and to a lesser extent muscle type, had a significant effect on the fatty acid profile of the selected blesbok muscles. Female blesbok muscles were more associated with a higher SFA and MUFA content, while male blesbok muscles had a higher proportion of total PUFA and P:S. The meat from male blesbok at this study area can therefore be considered healthier when compared to the meat from female blesbok. Blesbok LD muscles were generally considered more healthy for human consumption due to its higher total PUFA content and P:S, while the forequarter muscles were less healthy with a higher total SFA content and ω6:ω3. The blesbok diet is high in ω3 PUFA, which may have favoured the ω3 PUFA metabolism and resulted in the unfavourably high ω6:ω3 in the meat. The anatomical location and activity level of blesbok muscles therefore influenced the total fatty acid content. Muscle type also had a great influence on the mineral composition of blesbok muscles. Limited information is available on the effect of muscle type on the mineral composition of various skeletal muscles, warranting further research.

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CHAPTER 5

Impact of season on the physical attributes of male and female blesbok (*Damaliscus pygargus phillipsi)* muscles

ABSTRACT

The influence of harvesting season on the physical meat quality attributes of six muscles (*Longissimus dorsi, Biceps femoris, Semimembranosus, Semitendinosus, Infraspinatus* and *Supraspinatus*) derived from male and female blesbok was investigated. Eight mature blesbok were harvested per season (winter and spring) in a non-seasonal rainfall area in the Western Cape Province, South Africa. The pH value ≈24 h *post mortem* was higher ($p<0.05$) in the *Longissimus dorsi* muscle of the animals harvested in spring (5.60) compared to winter (5.54). The CIE a* (14.63 ± 0.86) and chroma (17.09 ± 0.63) values were higher ($p<0.05$) for winter compared to the values for male blesbok meat from spring (13.62 ± 1.08 and 16.10 ± 1.03, respectively). The latter values were also higher ($p<0.05$) for male compared to female (13.49 ± 0.88 and 16.22 ± 0.98) blesbok meat at the end of the mating season (winter). The forequarter muscles (*Infraspinatus* and *Supraspinatus*) had higher chroma values in comparison with the hindquarter muscles (*Biceps femoris, Semimembranosus* and *Semitendinosus*), which had higher ($p<0.01$) hue-angle values. Season had no influence ($p<0.05$) on the drip loss percentages and tenderness of blesbok muscles. The *Infraspinatus* and *Supraspinatus* muscles had the lowest ($p<0.01$) Warner Bratzler shear force values (20.89 ± 3.23 and 24.90 ± 5.35 N, respectively) and were therefore the most tender of the selected blesbok muscles. Differences in the physical meat quality attributes of blesbok muscles were greater between muscles than between harvesting seasons or genders.

INTRODUCTION

Many South African and international meat consumers are familiar with game meat. In recent years, blesbok was known to be the third most commonly commercially harvested and exported game meat species from South Africa (Anon., 2009, 2010, 2011). However, due to the spread of foot-and-mouth disease, the South African game meat export market was unfortunately closed at the start of 2012 and has since not been opened, thus necessitating the commercial game meat industry to focus on local marketing of their products.

South African meat consumers are usually uneducated on the positive attributes linked to game meat consumption (Hoffman *et al.,* 2005). Additionally, consumers perceive game meat to be tough, dry and too dark in colour (Lawrie & Ledward, 2006a). The latter can be attributed to an overall low intramuscular fat (IMF) content in game meat, often also to a high water holding capacity (WHC) and/or the use of incorrect or extensive cooking procedures (Hoffman, 2001;

Hocquette *et al.,* 2010). The hunting of game species is often relatively stressful and strenuous (Hoffman, 2001). As a result of low energy stores naturally present in game meat, the ultimate pH (pH_u) of game meat is often higher than normal (pH_u>6) (Lawrie & Ledward, 2006b, d) resulting in dark, firm and dry (DFD) meat (Hoffman, 2001; Lawrie & Ledward, 2006d). Consequently, the pH_u affects the shelf-life (Monin, 2004; Lawrie & Ledward, 2006c), flavour (Lawrie & Ledward, 2006b) and physical attributes of meat products, i.e. the colour, WHC and tenderness (Honikel, 2004a). Meat with a high pH_u will have a high WHC (Warriss, 2000d); but regardless of the WHC, meat normally loose exuded fluids with the *post mortem* formation of actomyosin (Honikel, 2004b; Lawrie & Ledward, 2006b). A low WHC and thus higher moisture loss through drip, negatively affects consumer perceptions as they relate drip loss in meat packaging to a lower product quality. Furthermore, a low WHC also influences the quantity of moisture lost during cooking, which often results in the meat being perceived as dry (Warriss, 2000d).

A number of quality cues assist consumers in making good decisions on the quality of meat products at purchase. The extrinsic quality cues (e.g. meat origin and price) are not physically part of the meat products, while intrinsic quality cues are (e.g. colour and marbling) (Troy & Kerry, 2010). However, consumers from different social-demographic backgrounds could have different perceptions on meat quality (Hocquette *et al.,* 2010). Nonetheless, consumers expect meat products to be fresh, lean and beneficial to their health, but still having good flavour, juiciness and tenderness (Dransfield, 2003; Hoffman & Wiklund, 2006).

In the past, retail meat cuts were relatively large and included sections of more than one muscle type. Nowadays, the latter is still true, although meat consumers increasingly desire smaller meat portions (e.g. individual muscle cuts) (Lawrie & Ledward, 2006a). Skeletal muscles usually have unique characteristics; however, the possibility exists for the latter to be impacted by seasonal differences in the animal's activity level and the plane of nutrition. Blesbok generally become less active with the onset of the dry season; when the plane of nutrition is usually lower. Blesbok will again become more active with the onset of the growing season (usually spring) and the subsequent presence of new grass sprouts (higher plane of nutrition) (Du Plessis, 1972). Consequently, an increased activity level can result in changes in the muscle fibre type, composition and metabolism of skeletal muscles (Mancini, 2009).

South Africa has a specific hunting season during the winter months (May to August) (Kroon *et al*., 1972; Hoffman, 2003), but season is usually not taken into account when harvesting game meat commercially. This study was therefore aimed at quantifying the impact of harvesting season (differences in the plane of nutrition) on the physical attributes (pH, colour, drip loss, cooking loss, WHC and tenderness) of six commercially important blesbok muscles.

MATERIALS AND METHODS

Harvesting

Blesbok were harvested on Brakkekuil farm (34°18'24.0"S and 20°49'3.9"E; 93 m.a.s.l.), near Witsand in the Western Cape Province of South Africa. The study area is classified as the Coastal Renosterveld and receives 300 – 500 mm of rainfall throughout the year (all seasons) (Rebelo *et al.*, 2006; Rutherford *et al*., 2006; Chase & Meadows, 2007; Kruger, 2007), although higher amounts of precipitation will generally occur in February and March (autumn) and again in September to November (spring) (Rebelo, 1996; Kruger, 2007).

The blesbok at Brakkekuil were confined by livestock fencing in a paddock of ≈158 ha. Annual harvesting procedures were implemented for the reduction of the population size, so as to ensure sustainable yearly yields of animals and the positive growth of the population (Bothma, 2010). For this study, eight mature blesbok were harvested per season in June of 2010 (winter) and October of 2010 (spring). The harvesting periods formed part of the general management strategies of the farm; therefore no preference was given to the selection of male or female blesbok (Table 5.1). The blesbok were harvested during the day and shot in the head or the high neck area with a .308 calibre rifle. Subsequently, exsanguination occurred within two minutes whilst in the field (ethical clearance number: 10NP_HOF02, issued by *Stellenbosch University Animal Care and Use committee*). No unnecessary *ante mortem* stress was experienced by any of the animals.

Table 5.1 Number and gender of blesbok from both harvesting seasons

Year	Month	Male	Female	Total
2010	June	3	5	8
2010	October	4	4	8

Sample preparation

The undressed (bled) blesbok were transported to the slaughtering facilities where they were weighed and the head, legs and skin were removed and evisceration occurred according to the *Guidelines for the Harvesting of Game for Meat Export* (Van Schalkwyk & Hoffman, 2010).

The dressed carcasses were cooled (0° – 5°C) and ≈24 h *post mortem*; the following muscles were removed entirely from the right side of each carcass: *Longissimus dorsi* (LD); *Biceps femoris* (BF); *Semimembranosus* (SM); *Semitendinosus* (ST); *Infraspinatus* (IS); and *Supraspinatus* (SS). The physical analyses (pH, colour, cooking and drip loss percentages, WHC and Warner Bratzler shear force) of each muscle sample commenced immediately after removal

from the carcasses. Each muscle sample was cut into ≈1.5 cm thick steaks (starting from the anterior side).

Physical analysis

pH

The pH_{45} and $temperature_{45}$ values were measured at ≈45 min *post mortem* with a calibrated portable Crison PH25 pH meter with a glass electrode (with an automatic paired temperature reading). Before the *M. longissimus dorsi* (LD muscle) was removed from each carcass the pH was measured through a small incision at the fourth and fifth lumbar vertebrae. The pH_{24} and $temperature_{24}$ values were measured ≈24 h *post mortem* at the same location as used for previous pH measurements.

Surface colour

The colour measurements were performed on the freshly cut second and third steaks of each of the six blesbok muscles. The samples were allowed to bloom (oxygenate) for ≈1 h after which the surface colour was measured according to the CIE L*a*b* colour system (Honikel, 1998). The CIE L* (lightness), CIE a* (green-red value) and CIE b* (blue-yellow value) values were determined in triplicate (at random locations) with a Color-guide 45°/0° colorimeter (BYK-Gardner GmbH, Gerestried, Germany). Consequently, the CIE a* and CIE b* values were used for the calculation of the chroma value (saturation/colour intensity) and hue-angle (colour definition). The calculations were:

$$\text{Hue-angle } (°) = \tan^{-1}(b^*/a^*)$$

$$\text{Chroma } (C^*) = (a^{*2} + b^{*2})^{-0.5}$$

Moisture loss

The WHC was determined in duplicate on the first muscle steak by use of the press method (Trout, 1988). A small portion was cut out of the middle section of each muscle steak and subsequently diced with a scalpel, care being taken to ensure that the sample did not lose any moisture (prevent drying out). A 500 mg sub-sample was then placed on a Whatman #2 filter paper. The filter paper containing the sub-sample was then pressed between two Perspex disks for 60 s at a standard pressure (588 N). After pressing, a photo was taken of each filter paper after which the *ImageJ* computer program (Version 1.41, 2009, http://rsbweb.nih.gov/ij/) was used to determine the area of the expressed moisture (outer area, A_1) and pressed meat (inner area, A_2). The WHC of each muscle samples was then calculated as the ratio of the expressed moisture area to the pressed meat area. The calculation was:

$$\text{WHC (ratio water:meat)} = A_1 / A_2$$

The drip loss percentage was determined on the second and third muscle steaks. The muscle steaks were suspended together in an inflated polyethylene bag (without touching each other or the side of the bag) for 24 h at ≈4°C. The two steaks were weighed together prior to the colour measurements (initial weight; W_1) and again after the 24 h suspension, after removal from the bag and a quick blot with absorbent paper to dry each steak (final weight, W_2). The drip loss percentage was calculated as (Honikel, 1998):

$$\text{Drip loss (\%)} = W_1 – W_2 / W_1 \times 100\%$$

The cooking loss percentage of each muscle sample was determined by weighing the fourth muscle steak prior to placing it into a polyethylene bag (W_1) and cooking it at 80°C in a water bath for 60 min. The bags with the cooked samples were drained of all water and cooled in ice (whilst in the bag). Once cooled, the samples were removed from the bag, lightly blotted dry and weighed (W_2). The cooking loss percentage was calculated as (Honikel, 1998):

$$\text{Cooking loss (\%)} = W_1 – W_2 / W_1 \times 100\%$$

Warner Bratzler shear force

The cooked samples (as described in previous paragraph) were cut perpendicular to the longitudinal axis of the muscle using a Warner Bratzler shear attachment (with a circular cross section of 1.27 cmø blade) fitted to an electrical scale programmed to measure maximum weight (force). Maximum shear force values to shear a cylindrical core (1.27 cmø) of cooked meat (at a crosshead speed of 3.33 $mm.s^{-1}$) were recorded for each sample and a mean was calculated for each individual animal (Honikel, 1998). Mean values of the four shear force measurements for each of the six muscles were used for the statistical analysis of the data. The unit for the Warner Bratzler shear force measurement (kg.1.27 cm) was subsequently converted to Newton as follows:

$$\text{Shear force (N)} = \text{kg.1.27 cm} * 9.81 / \text{Area}$$

$$\text{where Area} = \pi(1.27/2)^2$$

Statistical analysis of data

Statistical analysis was performed by use of a Statistica 10 VEPAC module (STATISTICA, 2011). The mixed model repeated measures of analysis of variances (ANOVA's) were conducted with animal nested in season and gender taken as a random effect, and muscle treated as a within subject effect. Fisher LSD was used for post hoc testing. Normal probability plots were

continuously checked for deviations from normality and possible outliers. A 5% significance level was used as guideline for determining significant effects. The values are reported as the LSMeans and Standard Deviation (SD) of the means. Pearson correlations were used to test for relationships between measured variables.

RESULTS

Meat production potential

The mean undressed (bled) blesbok weights, dressed carcass weights and dressing percentages of the blesbok harvested in winter and spring are presented in Table 5.2. No statistically significant seasonal differences were present ($p>0.05$).

Table 5.2 The undressed (bled) blesbok weights, dressed carcass weights and dressing percentages of blesbok from two harvesting seasons (LSMeans ± SD)

	Winter	**Spring**
Live weight (kg)	54.06 ± 8.48	49.28 ± 8.62
Carcass weight (kg)	27.06 ± 3.89	25.24 ± 4.62
Dressing %	50.15 ± 1.91	51.74 ± 2.11

[a,b] Least square means in the same column with different superscripts are significantly different ($p<0.05$)
Dressing % = Percentage carcass weight of live animal weight

pH

The seasonal differences in the initial (≈45 min *post mortem*) and ultimate pH and temperature values (≈24 h *post mortem*) are presented in Table 5.3. The mean pH_{45}, $Temp_{45}$ and $Temp_{24}$ did not differ ($p>0.05$) between seasons. The mean pH_{24} was higher ($p<0.01$) in the LD muscles from spring.

Table 5.3 The impact of season on the pH of blesbok *M. longissimus dorsi* (LSMeans ± SD)

Season	**pH_{45}**	**$Temp_{45}$**	**pH_{24}**	**$Temp_{24}$**
Winter	6.11 ± 0.22	26.18 ± 6.28	5.54^{b} ± 0.06	8.14 ± 0.82
Spring	6.09 ± 0.28	23.63 ± 3.31	5.60^{a} ± 0.02	6.70 ± 2.38

[a,b] Least square means in the same column with different superscripts are significantly different ($p<0.05$)
pH_{45} and $Temp_{45}$ are the pH and temperature values measured ≈45 min *post mortem*
pH_{24} and $Temp_{24}$ are the pH and temperature values measured ≈24 h *post mortem*

Surface colour

The mean colour coordinate values of the blesbok muscles are presented in Table 5.4. The CIE L*, CIE a* and CIE b* values as well as the hue-angle values differed ($p<0.01$) between blesbok muscles. The hindquarter muscles (BF, SM and ST) had significantly higher mean CIE L* values

in comparison with the LD and forequarter muscles (IS and SS). The forequarter muscles had significantly higher mean CIE a* values. The IS and hindquarter muscles had significantly higher mean CIE b* values. The BF muscle had the highest ($p<0.01$) mean hue-angle value in comparison with the LD, SM and forequarter muscles.

There was a significant interaction ($p = 0.04$) between season and muscle (season x muscle) for the mean chroma values (Fig. 5.1). The LD and SM muscles had higher ($p<0.05$) mean chroma values in winter. However, the IS muscle had the highest ($p<0.05$) mean chroma value in comparison with the other muscles from winter, while both forequarter muscles had higher ($p<0.05$) mean chroma values in comparison with the muscles from spring.

There was a significant interaction ($p = 0.02$) between season and gender (season x gender) for the mean CIE a* and chroma values (Table 5.5). Male blesbok meat had higher mean CIE a* and chroma values in winter compared to spring. Female blesbok meat had no seasonal differences in the mean CIE a* and chroma values. In winter, the mean CIE a* and chroma values were higher for male blesbok in comparison with female blesbok. No gender differences ($p>0.05$) were present in the mean CIE a* and chroma values for spring.

Moisture loss

There was a significant interaction ($p = 0.02$) between season, gender and muscle (season x gender x muscle) for the ratio of expressed moisture over the area of pressed meat (WHC) of the selected blesbok muscles (Fig. 5.2). The mean WHC ratio was significantly higher in the BF and IS muscles of male blesbok from winter, while the mean WHC ratio was significantly higher in the ST muscle of female blesbok for spring. The IS muscle of male blesbok had the highest mean WHC ratio in winter, while the SS muscle had the highest mean WHC ratio in comparison with the other male muscles in spring. The BF muscle of female blesbok had the highest mean WHC ratio in winter, while the SS muscle also had the highest mean WHC ratio in comparison with the other female muscles in spring. The male IS muscle had a significantly higher WHC ratio in winter compared to the female IS muscle. The female BF, ST and IS muscles had significantly higher WHC ratios in spring compared to the WHC ratio of similar muscles from male blesbok.

The mean drip loss percentage from blesbok muscles differed significantly between seasons ($p = 0.03$) as well as between the selected muscles ($p = 0.00$) (Fig. 5.3). The mean drip loss percentage from blesbok meat in spring (2.36 ± 1.18%) was higher ($p<0.05$) in comparison with the mean drip loss percentage from blesbok meat in winter (1.58 ± 0.39%). The muscle differences in Fig. 5.3 shows that the BF and SM muscles had significantly lower mean drip loss percentages in comparison with the other muscles.

Table 5.4 The differences in colour measurements of selected blesbok muscles (LSMeans ± SD)

	LD	BF	SM	ST	IS	SS
CIE L*	$31.05^{c} \pm 1.33$	$33.70^{a} \pm 1.12$	$33.92^{a} \pm 0.98$	$33.61^{a} \pm 1.29$	$32.11^{b} \pm 1.52$	$31.60^{bc} \pm 1.12$
CIE a*	$13.35^{b} \pm 0.86$	$13.19^{b} \pm 0.94$	$13.48^{b} \pm 0.81$	$13.49^{b} \pm 1.08$	$14.59^{a} \pm 0.87$	$14.48^{a} \pm 0.87$
CIE b*	$8.07^{c} \pm 0.93$	$9.44^{a} \pm 1.15$	$8.89^{ab} \pm 0.89$	$9.36^{a} \pm 0.82$	$9.13^{a} \pm 0.90$	$8.47^{cb} \pm 0.81$
Hue-angle	$31.12^{d} \pm 2.76$	$35.53^{a} \pm 3.41$	$33.38^{cb} \pm 3.02$	$34.81^{ab} \pm 3.26$	$32.03^{cd} \pm 3.11$	$30.32^{d} \pm 2.63$

[a-d] Least square means in the same row with different superscripts are significantly different (p<0.01)
LD, *M. longissimus dorsi*; BF, *M. biceps femoris*; SM, *M. semimembranosus*; ST, *M. semitendinosus*; IS, *M. infraspinatus*; SS, *M. supraspinatus*

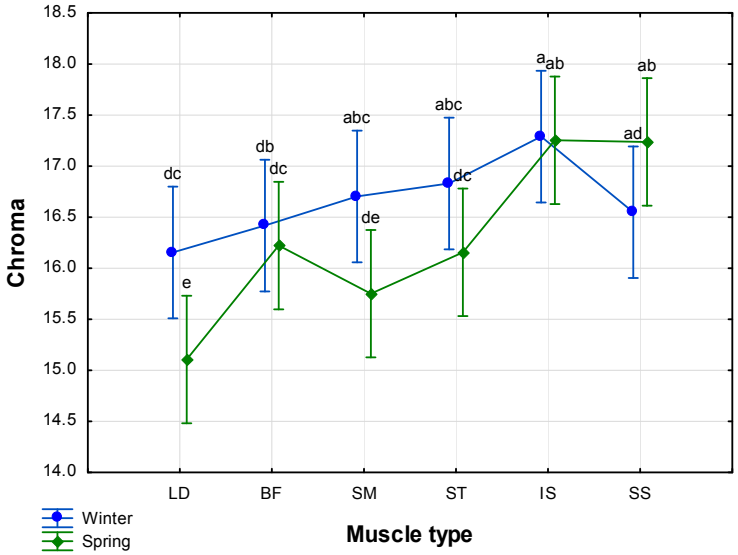

Figure 5.1 The chroma value of blesbok muscles (LD, *Longissimus dorsi*; BF, *Biceps femoris*; SM, *Semimembranosus*; ST, *Semitendinosus*; IS, *Infraspinatus*; SS, *Supraspinatus*) from different harvesting seasons. Different letters indicate significant differences (p<0.05).

Table 5.5 The impact of season and gender on the colour of blesbok meat (LSMeans ± SD)

	Male	Female
CIE a*		
Winter	14.63[a] ± 0.86	13.49[b] ± 0.88
Spring	13.62[b] ± 1.08	13.61[b] ± 1.05
Chroma		
Winter	17.09[a] ± 0.63	16.22[b] ± 0.98
Spring	16.10[b] ± 1.03	16.47[ab] ± 1.24

[a,b] Different superscripts at the least square means indicate significant differences (p<0.05) between seasons and/or genders

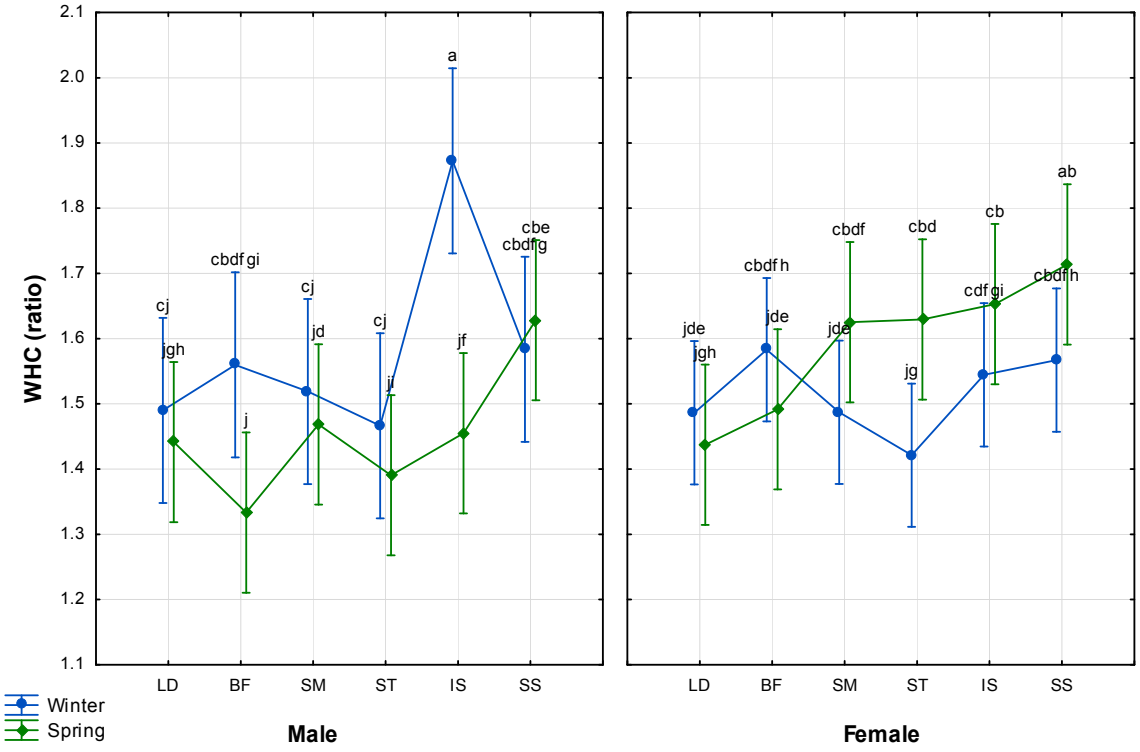

Figure 5.2 The water holding capacity (ratio water:meat) of male and female blesbok muscles (LD, *Longissimus dorsi*; BF, *Biceps femoris*; SM, *Semimembranosus*; ST, *Semitendinosus*; IS, *Infraspinatus*; SS, *Supraspinatus*) from different harvesting seasons. Different letters indicate significant differences (p<0.05) between seasons, genders and/or muscles.

There was a significant interaction (p = 0.00) between season, gender and muscle (season x gender x muscle) for the mean cooking loss percentage from blesbok muscles (Fig. 5.4). No seasonal differences were present in the mean cooking loss percentages of female blesbok muscles; while the male IS muscle had a significantly higher mean cooking loss percentage in winter and the male SS muscle had a significantly higher mean cooking loss percentage in spring. No gender differences were present in the mean cooking loss percentages of male and female muscles in spring. In winter the male IS muscle had significantly higher and the male SS muscle significantly lower mean cooking loss percentages in comparison with similar muscles of female blesbok in winter.

Warner Bratzler shear force

The mean Warner Bratzler shear force values differed significant (p = 0.00) between the selected blesbok muscles (Table 5.6). The ST muscle had the highest (p<0.01) mean Warner Bratzler shear force value and the IS muscle had the lowest mean Warner Bratzler shear force value.

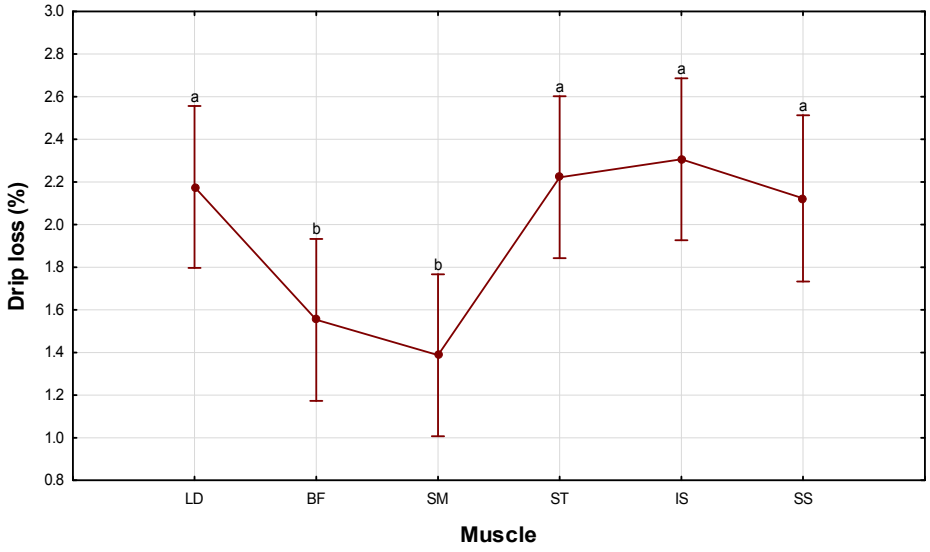

Figure 5.3 The drip loss percentage of selected blesbok muscles (LD, *Longissimus dorsi*; BF, *Biceps femoris*; SM, *Semimembranosus*; ST, *Semitendinosus*; IS, *Infraspinatus*; SS, *Supraspinatus*). Different letters indicate significant differences (p<0.05).

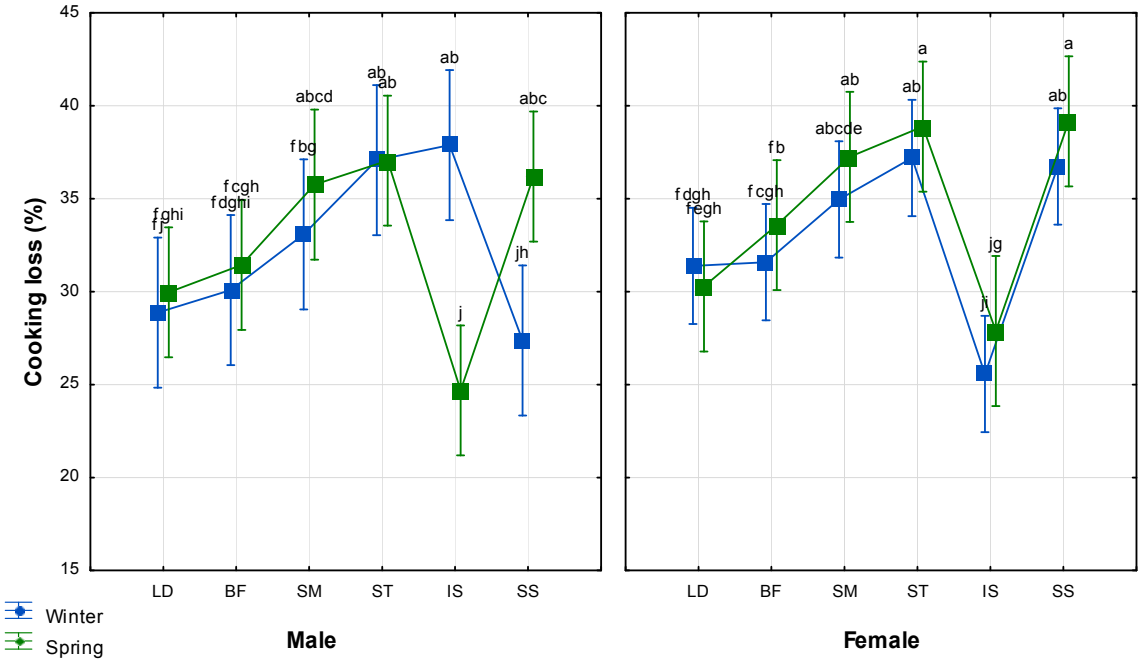

Figure 5.4 The cooking loss percentage of male and female blesbok muscles (LD, *Longissimus dorsi*; BF, *Biceps femoris*; SM, *Semimembranosus*; ST, *Semitendinosus*; IS, *Infraspinatus*; SS, *Supraspinatus*) from different harvesting seasons. Different letters indicate significant differences ($p<0.05$) between seasons, genders and/or muscles.

Table 5.6 The differences in Warner Bratzler shear force values between blesbok muscles (LSMeans ± SD)

	LD	BF	SM	ST	IS	SS
Shear force (N)	30.57^{ab} ± 6.69	27.35^{bc} ± 3.42	28.65^{b} ± 4.48	31.51^{a} ± 5.63	20.89^{d} ± 3.23	24.90^{dc} ± 5.35

[a-d] Least square means with different superscripts are significantly different ($p<0.01$)
LD, *M. longissimus dorsi*; BF, *M. biceps femoris*; SM, *M. semimembranosus*; ST, *M. semitendinosus*; IS, *M. infraspinatus*; SS, *M. supraspinatus*

DISCUSSION

Since no significant differences were present in the mean undressed (bled) blesbok weights, dressed carcass weights and dressing percentages of blesbok meat from both harvesting seasons (Table 5.2), it was accepted that the meat production potential of blesbok from this study area is similar for these two seasons.

Post mortem pH decline is generally from a physiological pH of 7.0 – 7.2 in skeletal muscles of live animals, to a pH_u of 5.3 – 5.8 in meat (Honikel, 2004a). Moreover, the quantity of lactic acid produced from glycogen during anaerobic glycolysis determines the extent of *post mortem* pH decline and consequently the pH_u of meat (Lawrie & Ledward, 2006c). Skeletal muscles are heterogeneous combinations of red and white muscle fibres (Cassens & Cooper, 1971) which store lower and higher quantities of glycogen, respectively (Taylor, 2004; Kohn *et al.,* 2005). Hence, skeletal muscles usually differ in glycogen concentrations and consequently in the rate and extent of the *post mortem* pH decline (Lawrie & Ledward, 2006a). Unfortunately the pH_u (pH_{24}) was only measured on the LD muscles of blesbok in this study.

Even though the pH_{24} of blesbok LD muscles differed significantly between seasons (Table 5.3), the mean pH_{24} values for both seasons were still within the biologically 'normal' range. It is believed that the blesbok harvested at this study area in winter had a slightly higher plane of nutrition in comparison to those harvested in spring (Chapter 3). The blesbok LD muscles therefore might have had slightly higher glycogen stores in winter, resulting in the slightly lower ($p<0.05$) mean pH_{24} in winter.

In turn, the pH_u of meat affects fresh meat colour, flavour, tenderness, WHC and shelf-life (Honikel, 2004a). As the colour of fresh red meat products is the first physical quality attribute observed at the purchasing point, it is also considered the most important meat quality attribute with regards to consumer perception. The latter is of utmost importance, since the meat industry has changed from a once production driven industry to being increasingly more consumer led (Dransfield, 2003). When consumers are unsatisfied with meat products, it can lead to no further purchasing and consumption thereof (Troy & Kerry, 2010). Consumers frequently make use of the colour of fresh meat as an indication of meat product freshness (Hoffman, 2001; Troy & Kerry, 2010) and quality (Mancini, 2009). In general consumers expect red meat products to have a bright pink or red surface colour, as opposed to brown, grey or purple (Warriss, 2000d). Meat colour is mainly attributed to myoglobin (Mancini & Hunt, 2005) and the appearance of red meat is influenced by the myoglobin quantity (Mancini, 2009) as well as the quality (Lawrie & Ledward, 2006d).

Moreover, meat colour is usually measured on a cut and bloomed meat surface (Honikel, 1998), since it allows purple deoxymyoglobin to be oxygenated, resulting in the development of oxymyoglobin which is responsible for the desirable bright red colour (Mancini & Hunt, 2005; Mancini, 2009). The bloomed time and muscle type can cause differences in meat colour

measurements (Brewer *et al.,* 2001). However, the muscles used in this study were bloomed at comparable times and so differences in colour measurements are mainly attributed to muscle type.

The forequarter muscles (IS and SS) had higher ($p<0.01$) mean CIE a* (Table 5.4) as well as higher mean chroma values (Fig. 5.1). The CIE a* and b* values are both used for the calculation of the chroma value, consequently the chroma value of blesbok muscles had a moderate correlation with the CIE b* value ($r = 0.55$, $p<0.01$) and a strong correlation with the CIE a* value ($r = 0.84$, $p<0.01$). An increase in the CIE a* value will therefore have the largest contribution to an increase in the chroma value of blesbok meat, and *vice versa*. Moreover, the CIE a* value is generally positively correlated with the myoglobin concentration in meat (Vestergaard *et al.,* 2000). The forequarter muscles of blesbok could therefore have a more saturated red colour. It can therefore be postulated that blesbok forequarter muscles contained higher concentrations of myoglobin in comparison with the other muscles used in this study. However, this warrants further research.

In addition, differences in the function and activity level of skeletal muscles usually result in differences in the muscle fibre type composition (Taylor, 2004). For example, the muscles used for endurance type of activities (e.g. postural muscles) will contain higher amounts of the oxidative muscle fibre types (Cassens & Cooper, 1971; Swatland, 1994a; Davies, 2004; Taylor, 2004). The SS muscle, for example is generally referred to as a 'red' muscle as it usually has higher amounts of the Type I and IIA, oxidative muscle fibres, whereas the ST muscle is more of a 'white' muscle due to a higher content of glycolytic, Type IIB muscle fibres (Vestergaard *et al.,* 2000). Consequently, skeletal muscles with higher activity levels will contain higher quantities of myoglobin, resulting in a darker red colour (Cassens & Cooper, 1971; Taylor, 2004).

The mean chroma values of each of the blesbok muscles, except for the SS muscle, was higher in winter (Fig. 5.1). The first group of blesbok were harvested late in the mating season (end of June 2010), the latter usually being between March and June (Bothma *et al.,* 2010). The blesbok will typically have a higher activity level during the mating season, which could have resulted in higher myoglobin contents in the muscles and consequently higher mean chroma values. In addition, male blesbok generally spend less time feeding and more time mating as well as fighting to maintain their harem (Kohn *et al*., 2005). Therefore, the higher activity level of male blesbok may have attributed to the higher mean CIE a* and chroma values of male blesbok muscles (Table 5.5).

The CIE a* value was negatively correlated with the hue-angle value ($r = – 0.54$, $p<0.01$), while the CIE b* value had a strong correlation with the hue-angle value ($r = 0.83$, $p<0.01$). Consequently, blesbok meat with a higher hue-angle value will have a more yellow/brown meat colour (Anon., 1998). Moreover, the CIE b* value is primarily influenced by the myoglobin structure and not at all by myoglobin concentrations (Lindahl *et al.*, 2001) and so a higher CIE b* value is associated with the presence of higher amounts of metmyoglobin on the meat surface (Mancini & Hunt, 2005). The blesbok hindquarter muscles (BF, SM and ST) had high mean CIE b* values and

consequently higher hue-angle values in comparison with the other muscles (Table 5.4). The hindquarter muscles will therefore have a less red/more brown meat surface colour after being bloomed. As the LD muscle had a lower ($p<0.01$) mean hue-angle value compared to the hindquarter muscles, this muscle was more red/less brown (Table 5.4). Since consumers might only notice the brown meat colour when ≈60% of the myoglobin is in the metmyoglobin form (Lawrie & Ledward, 2006d), it is questionable whether the significant differences in the colour measurements between selected blesbok muscles will be noticeable by red meat consumers.

The visible drip loss in meat packaging at the point of sale is also an important consideration, in addition to the fresh meat colour. Drip loss definitely has a negative effect on the appearance of meat products and therefore on consumer acceptability (Troy & Kerry, 2010), since consumers dislike the presence of exuded fluids in meat packaging (Issanchou, 1996). Additionally, the meat industry strives at keeping the drip loss percentage at a minimum, as valuable proteins (sarcoplasmic proteins) are lost from the meat structure. Myoglobin is usually one of the major proteins lost, resulting in the formation of a red/pinkish fluid which might be mistaken for blood (Huff-Lonergan, 2009). The BF and SM, two large hindquarter muscles, had significantly lower mean drip loss percentages in comparison with the other muscles (Fig. 5.3). This was unexpected, as these two large muscles have higher surface area to volume ratios which normally leads to increased drip loss. The blesbok BF and SM muscles could therefore have much lower weight losses when packaged as fresh meat products.

The WHC is the ability of the meat to retain/hold water when a force (e.g. pressure or heat) is applied. Muscles with an increased rate of *post mortem* glycolysis are associated with a lower WHC (Brewer, 2004). The rate of glycolysis is usually greater in muscles that tend to cool down at a slower rate, i.e. those muscles which are situated deeper within the carcass (Lawrie & Ledward, 2006a). Since the ST, IS and SS muscles are situated slightly deeper within the blesbok carcass, it was postulated that these cooled down at a slightly slower rate and that the rate of glycolysis may have been greater in these muscles. The latter could therefore have contributed to the higher mean drip loss percentages in these three muscles.

Furthermore, a higher ratio of expressed water to pressed meat (WHC ratio) is associated with a lower WHC. The IS muscle of male blesbok from winter had an abnormally high mean WHC ratio and thus a very low WHC (Fig. 5.2). The latter may be attributed to much higher glycogen stores in the IS muscles of male blesbok in winter, although it was believed that the glycogen stores would have been much lower in the forequarter muscles of the more active male blesbok during the mating season (winter). In addition, the IS muscles of winter male blesbok had significantly higher mean cooking loss percentages (Fig. 5.4). This is caused by the very low WHC and therefore more moisture could be lost during cooking (Warriss, 2000d). However, the IS muscle of blesbok generally had the lowest cooking loss percentage, followed by the LD muscle (Fig. 5.4). A higher cooking loss percentage can cause meat to be perceived as being dry

(Warriss, 2000d) in addition to being less juicy. Consumers may often perceive meat with a high cooking loss, as being tough rather than dry.

As previously mentioned, the ST muscle is generally referred to as the 'white' muscle (Vestergaard *et al.,* 2000), although muscles with higher proportions of the larger Type IIB muscle fibres will generally have lower tenderness (Swatland, 1994b). Tenderness is a major factor influencing the eating quality of meat products (Lawrie & Ledward, 2006d; Aaslyng, 2009). The *New Zealand Beef and Lamb Quality Mark Standards* consider shear force values of 78.5 or less as ideal (Slater, 2009). However, Destefanis *et al.* (2008) found that Warner Bratzler shear force values of <42.87 N are an indication of tender beef and >52.68 N indicates tough beef.

In this study, the Warner Bratzler shear force values of blesbok muscles were all far below the above suggested value for tender meat (Table 5.6). The forequarter muscles were the most tender, while the ST muscle had the highest and the SM muscle the second highest mean Warner Bratzler shear force values. The selected blesbok muscles from this study can therefore be arranged from least to more tender: ST; LD; SM; BF; SS; and IS muscles. The ST muscle has less muscle fibres of similar diameter per primary muscle fibre bundle, in addition to larger muscle fibres situated in the top portion (anterior) compared to the bottom portion (American Meat Institute Foundation, 1960). The ST muscle therefore has a less desirable texture. Additionally, due to the size and anatomical location of the SM muscle, variability can be present in the meat quality attributes of this muscle, e.g. muscle colour (Sawyer *et al.,* 2007) and tenderness (American Meat Institute Foundation, 1960; Sawyer *et al.,* 2007).

The larger blesbok skeletal muscles (e.g. BF and SM) may therefore have a great deal of variation within the muscles. The location of the sub-samples taken from these muscles for the analysis of the physical attributes may thus influence the outcome of blesbok muscle comparisons. Therefore, further research is required for the investigation of the variability in physical meat quality within blesbok skeletal muscles, in addition to between blesbok skeletal muscles.

CONCLUSIONS

The pH_{24}, CIE a* value, chroma value, WHC and cooking loss percentage of selected blesbok muscles was influenced by the harvesting season. The most significant of the seasonal difference was in the bloomed meat surface colour. Blesbok forequarter muscles had a more desirable and intense red colour, while the hindquarter muscles had more of an undesirable brown colour. The LD muscle had an intermediate colour in comparison with the fore- and hindquarter muscles. Nevertheless, these muscle colour differences might not even be noticeable by red meat consumers. Blesbok muscles differed significantly in tenderness, although all muscles were considered tender. The differences between blesbok skeletal muscles were greater compared to the seasonal differences in the physical meat quality.

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CHAPTER 6

GENERAL CONCLUSIONS

The study area (Coastal Renosterveld) from which blesbok were harvested during this investigation received non-seasonal annual rainfall. Consequently the quantity and quality of the grass species available to the blesbok at this study area varied between seasons. Therefore, the plane of nutrition for example in spring of 2009 was not similar to the plane of nutrition in spring of 2010.

Limited seasonal differences were present in the chemical composition (moisture, protein, fat and ash contents), fatty acid profile, mineral composition and physical attributes of blesbok meat harvested from this study area. When the plane of nutrition of blesbok was assumed to be higher, the chemical composition of the *Longissimus dorsi* muscle, hindquarter muscles (*Biceps femoris, Semimembranosus* and *Semitendinosus*) and forequarter muscles (*Infraspinatus* and *Supraspinatus*) differed significantly from each other. Yet when the plane of nutrition at this study area was believed to be lower, the chemical composition of the *Semimembranosus* muscle differed from the chemical composition of the other hindquarter muscles (*Biceps femoris* and *Semitendinosus*). In addition, the intramuscular fat content of the *Longissimus dorsi* muscle differed significantly between seasons. These seasonal differences in the chemical composition of the *Longissimus dorsi* and *Semimembranosus* muscles of blesbok were of a small magnitude and should therefore not create difficulties with the nutritional information on the labelling of meat products containing these two muscle types.

The fatty acid profile of the blesbok muscles did not differ between seasons. However, gender and to a lesser extent muscle type, had a significant effect on the fatty acid profile of the selected blesbok muscles from this study area. The *Longissimus dorsi* muscle of blesbok can be considered more healthy due to its higher total polyunsaturated fatty acids (PUFA) contents and higher polyunsaturated to saturated fatty acid ratio (P:S). The forequarter muscles can be considered the least healthy of the selected blesbok muscles, since these contained higher omega-6 to omega-3 fatty acid ratios (ω6:ω3) and higher total SFA contents. Furthermore, the meat from male blesbok can be considered healthier due to higher proportions of total PUFA and higher P:S.

Seasonal differences were present in the calcium and zinc content of blesbok meat. Since limited research is available in the literature on the mechanisms involved in the distribution of minerals in the muscles of ruminant animals, it is not easy to make any postulations on the causes of these differences – this area (mineral deposition and metabolism in wild ungulates) warrants further research.

Although seasonal differences were found in the pH value at ≈24 h *post mortem*, CIE a* values, chroma values, water holding capacity and cooking loss percentages of the selected

blesbok muscles, the most significant of these were the seasonal differences in the CIE a* and chroma values. The forequarter muscles of blesbok from this study area had a more desirable and intense red colour, while the hindquarter muscles had more of an undesirable brown colour. Since consumers use meat colour as a prediction of the freshness and quality of meat products at the point of purchase, meat consumers might perceive the forequarter muscles of blesbok to be fresher and of higher quality compared to the other muscles. The selected blesbok muscles from this study can be arranged from least to more tender: ST; LD; SM; BF; SS; and IS muscles, although, all of the muscles were considered tender.

Some significant differences in the chemical composition and physical attributes of blesbok muscles from this study area were present, although these seasonal differences do not validate the seasonal classification of the blesbok meat from the study region since the muscles from each harvesting season can be considered high in protein and low in fat. However, the impact of season in this study was most likely limited due to the non-seasonal annual rainfall patterns of the study area. Season might have a greater impact on the chemical composition and physical attributes of blesbok meat from a study area with more prominent seasonal differences, for example, an area receiving mainly summer rainfall patterns. The latter study area will therefore have more prominent wet and dry seasons and consequently very high and very low quality and quantities of forage available to blesbok for consumption during different seasons. Further research is therefore required to evaluate the effect of more extreme weather patterns on the forage quality as well as the effect of the latter on the meat quality. The possible meat quality differences can then be quantified sensorically, to establish whether consumers will be able to detect meat quality differences and to what extent. Another aspect that could prove interesting is to evaluate the chemical composition of the forage, especially the fatty acid profile, and then establish how this influences the fatty acid profile of the muscles. Similarly, a more in-depth study on muscle fibre type and its effect on the muscle chemical composition and quality will also provide interesting information towards understanding the role of muscle fibre types in the meat quality of ungulates.