

## CHAPTER 4

### MATERIALS AND METHODS

#### 4.1 Description of the study area

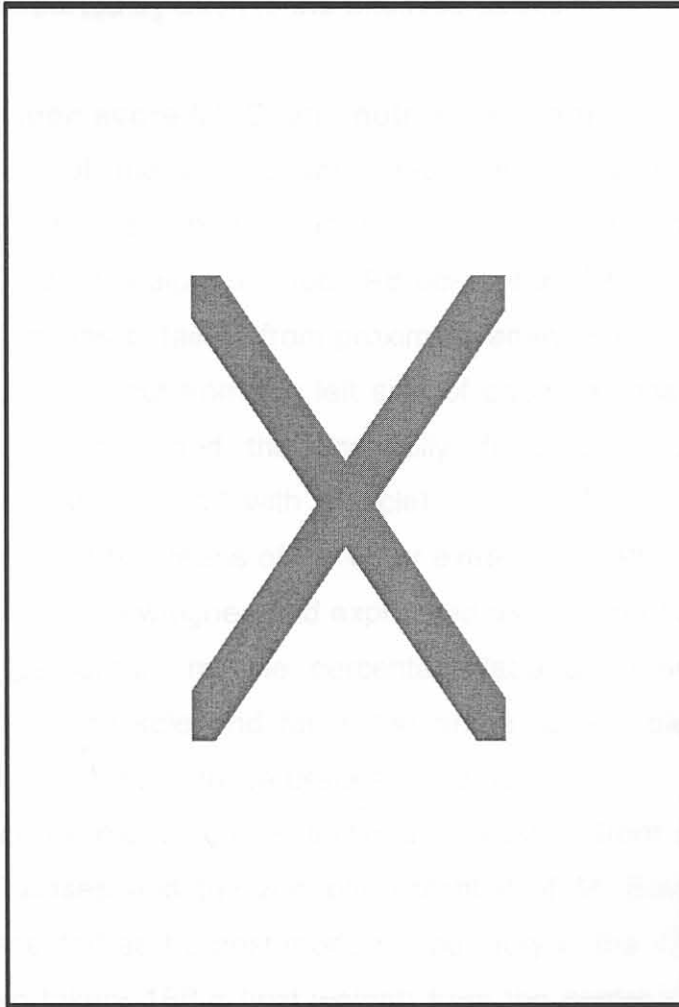
The KNP is an elongated nature reserve situated along the northeastern border of South Africa. The northern border of the KNP is with Zimbabwe, and in the east it borders with Mozambique. It embraces about 20 000 km<sup>2</sup> of undulating hills and doleritic dykes, grassy plains, parkland savannah, dry deciduous forest and thornbush. The KNP together with the private game farms surrounding much of the western boundary is one of the largest protected environments in Africa. KNP lies between latitudes 22°19' and 25°32' South and longitudes 30°52' and 32°03' East (Gertenbach, 1983).

The KNP was divided into three geographical regions (Figure 1), namely the northern region (between the Limpopo and Olifants rivers), the central region (between the Sabi and Olifants rivers), and the southern region (between the Sabi river and the southern border of the KNP, the Crocodile river) (Gertenbach, 1983). Vegetation in the southern region is dominated by mixed *Combretum* woodland and thickets, in the central region by mixed *Combretum* woodland (on granite and rhyolite) and *Acacia nigrescens* / *Sclerocarya birrea* savanna (on basalt), and in the northern region by *Colophospermum mopane* shrubland and sandveld vegetation (Gertenbach, 1983). There is a south-north variation in mean annual rainfall, ranging from ~700 mm in the extreme southwest to ~400 mm in the northern plains (Gertenbach, 1983). Gertenbach (1983) describes the vegetation and different environments in detail.

#### 4.2. Experimental animals

... buffalo were randomly selected from the north, central and southern regions of the park. These herds were then divided into two groups of 10 animals each. The procedure for handling the animals was as follows: (1) the animals were captured, (2) the animals were weighed, (3) the animals were fitted with a collar, (4) the animals were fitted with a radio collar, (5) the animals were fitted with a GPS collar, (6) the animals were fitted with a VHF collar, (7) the animals were fitted with a VHF collar, (8) the animals were fitted with a VHF collar, (9) the animals were fitted with a VHF collar, (10) the animals were fitted with a VHF collar. Approximately 100 animals were captured in each region.

**Figure 1.** Map of the Kruger National Park showing north (N), central (C) and southern (S) geographical regions (Gertenbach, 1983)



## 4.2 Experimental animals

Ten herds of buffalo were randomly selected from each region and a total of 660 buffalo from these herds were culled as part of the BTB monitoring program. The procedure for harvesting animals is well versed and involves the application of a lethal dose of scoline (succinylcholine chloride) from helicopter during the daytime. Approximately 20 animals from each herd were randomly culled, thus giving a random sample of the population of buffalo in the KNP. The animals were eviscerated (only the digestive system removed) in the veld and the carcasses transported by truck to the Skukuza abattoir.

## 4.3 Body condition score (BCS) and nutritional status

Visual BCSs of the buffalo were recorded using a five-point scale (Wildman *et al.*, 1982), the % bone marrow fat (% BMF) of the *os. humerus* was determined using the dry weight method of Brooks *et al* (1977), and an estimate of body composition was obtained from proximate analysis of prime-rib samples (T8-T10) (Naudé, 1972), cut from the left side of each carcass. Each prime-rib cut sample was weighed and then carefully dissected into bone, muscle (connective tissue was grouped with muscle) and fat. The fat content of the muscle was determined by means of the ether extraction method (AOAC, 1975). These fractions were then weighed and expressed as a percentage of the prime-rib cut weight i.e. percentage muscle, percentage fat and percentage bone. The percentages of bone, muscle and fat in the prime rib sample were used as estimates of the proportions of these tissues in the body.

Considering the movement restrictions on tissues from animals infected with controlled diseases and the zoonotic potential of *M. Bovis*, prime-rib cut samples were dissected at the post-mortem laboratory in the KNP. Liver biopsy samples were also taken, 150 g (wet weight) from the centre of the liver. They were placed in 200 ml buffered analytical grade formalin and refrigerated for transport and subsequent analysis.

From the available liver samples, 311 were stratified according to geographical region of origin and randomly selected for analysis. The numbers

from each region were proportionate to the population density of that region and also reflected the gender and age distribution. Selenium analyses were however performed on all available liver samples.

Each liver sample was cut into pieces using a sterile stainless steel scalpel. It was dried to a constant weight (for at least 24h) at a temperature of 100 °C. The liver was then milled to uniform consistency in an IKA 10 analytical mill (Kika Werke, Germany) and the dry matter percentage determined. Samples of 50 g were measured for digestion. After digestion in duplicate with analytical grade nitric acid followed by perchloric acid (Heckman, 1971), the Cu, Mn and Co concentrations were measured on a Varian SpectrAA 50 atomic absorption spectrometer (AAS).

After wet digestion, in duplicate, for 18 h with analytical grade nitric and perchloric acids, in a temperature-controlled digestion block (Gelman, 1985), the Se concentration in liver samples was measured using a PerkinElmer 2380 AAS with a hydride generator attachment. Bovine liver (National Institute of Standards and Technology, standard reference material 1577a) served as laboratory control.

#### **4.4 Carcass characteristics**

Carcass mass (including skins, excluding heads), head mass, sex and age were recorded as soon as the carcasses arrived at the abattoir. Muscle pH was taken 2 h after slaughter and again 26-48 h later, using a Russell combination pH electrode, type CMSW711 / KNIpHE. Drip loss of the *m. longissimus* dorsi taken between lumbar vertebra 1 and lumbar vertebra 6 was determined using the method of Naudé (1972).

#### **4.5 Bovine tuberculosis (BTB) diagnosis**

BTB was diagnosed by necropsy of the entire buffalo, with detailed macroscopic inspection of intestinal and thoracic organs and lymph node sections. Buffalo were determined to be positive for BTB if they had lesions that were consistent with *M. bovis* infection (Croner, 1994). Gross lesions that were

positive or suspicious (possibly not caused by *M. bovis*) were confirmed with histopathology. Diagnostic methods are described in detail in Bengis *et al.* (1996).

Bacterial cultures were also prepared from lymph node sections of all buffalo sampled in 1998. *Mycobacterium bovis* was identified in the samples by the Onderstepoort Veterinary Institute, South Africa, using standard techniques (Bengis *et al.*, 1996).

#### 4.6 Data analysis

Data were analysed by multi-factor analysis of variance using the general linear model (GLM) procedure (SAS, 1992) and where applicable multiple range analysis was performed by means of the Bonferroni test method. The factors that were included were: Region, Age, Sex, Tuberculosis status and their interactions. The dry matter mineral levels are given as Least Squares means and standard deviations. Pearson product moment correlations were calculated between BCSs, bone marrow fat content and body composition based on prime-rib cut. Analyses were calculated by means, tuberculosis status and mineral content.

## CHAPTER 5

## RESULTS AND DISCUSSION

## 5.1 Body composition

The effects of region (please refer to Figure 1 for a description of the regions), as well as gender and age, on the carcass composition of African buffalo are presented in Tables 1 to 6. Both the percentages of muscle and bone did not differ significantly between genders. The results suggest that female buffalo had significantly greater body fat reserves ( $P < 0.05$ ) compared to males, despite the fact that their bone marrow fat contents did not differ significantly (Table 1).

In general, males had greater body mass than females but the difference was not significant. This may be due to the fact that old lone roaming bulls were not sampled and that the masses represented are the average masses across all age groups. Different genders also had significantly different head weights ( $P < 0.05$ ), male buffalo having heavier heads.

**Table 1.** The influence of gender between all ages on the carcass composition (means and SE) of the African buffalo in the Kruger National Park

Sex	Carcass mass mean (SE)	% Bone mean (SE)	% Muscle mean (SE)	% Fat mean (SE)	% Drip loss mean (SE)	% Head weight mean (SE)	% BMF mean (SE)
F	178.49 (4.51)	30.40 (0.27)	57.83 (1.66)	11.99 (0.29) <sup>a</sup>	1.76 (0.07)	8.76 (0.56) <sup>b</sup>	90.889 (5.166)
M	185.71 (5.43)	30.42 (0.33)	57.16 (2.04)	10.80 (0.36) <sup>b</sup>	1.85 (0.08)	9.55 (0.55) <sup>a</sup>	90.442 (7.514)

<sup>a,b</sup>Means within the same column with different superscripts differ significantly ( $P \leq 0.05$ ); % BMF= percentage bone marrow fat; F=female; M=male; SE= standard error

In female buffalo 15.79% of the variation in the percentage body fat was explained by the percentage of fat in the bone marrow ( $P=0.01$ ) (Table 2). There is probably a continual mobilisation and synthesis of fat in female buffalo, according to the physiological requirements. This postulate is supported by the

smaller and less significant correlation between bone marrow fat content and carcass fat content in male buffalo (Table 2).

Although the pooled results suggest a significant correlation ( $P < 0.01$ ) between % fat (% fat in the body) and % BMF (% fat in the bone marrow), % fat only explained 13 % of the variation in % BMF (Table 2). It, therefore, seems that % BMF is a poor predictor of proximate body composition in the buffalo.

**Table 2.** The correlation ( $r$ ) between % body fat and % fat in the bone marrow in the overall population, as well as in different genders of buffalo in the Kruger National Park

Sex	% Fat mean (SE)	% BMF mean (SE)	Pearson correlation (significance)
M	10.885 (3.990)	90.889 (5.166)	0.1062 (0.1708)
F	12.152 (4.975)	90.442 (7.514)	0.1579 (0.0095) Significant
Ave.	11.661 (4.649)	90.626 (6.674)	0.1387 (0.0036) Significant

F=female; M=male; %BMF=% Bone marrow fat; SE= standard error.

As the body condition of buffalo increased so the % muscle, % fat and the % fat in the bone marrow increased while the % bone decreased. Also established is the negative correlation between % fat and % bone. There was a significant negative correlation between % bone and % muscle and between % bone and % drip loss, with a significant positive correlation between % muscle and % drip loss (Table 3).

The general tendency, although not statistically significant, was that the total body fat and % fat in the bone marrow increased as the BCS increased (Table 3). BCS was therefore a poor indicator of proximate body composition in the buffalo.

**Table 3.** Body composition of African buffalo within different body condition score (BCS) categories

BCS	% Fat (mean $\pm$ SD)	% Bone (mean $\pm$ SD)	% Muscle (mean $\pm$ SD)	% Drip loss (mean $\pm$ SD)	% BMF (mean $\pm$ SD)
1	11.66 $\pm$ 4.65	30.33 $\pm$ 4.21	56.35 $\pm$ 6.24	1.81 $\pm$ 1.10	90.63 $\pm$ 6.67
2	10.53 $\pm$ 2.04	33.80 $\pm$ 3.51	54.22 $\pm$ 3.78	1.35 $\pm$ 0.60	84.48 $\pm$ 10.85
3	11.26 $\pm$ 4.28	30.67 $\pm$ 4.11	56.37 $\pm$ 6.13	1.69 $\pm$ 1.01	90.10 $\pm$ 6.53
4	12.27 $\pm$ 5.18	29.60 $\pm$ 4.28	56.57 $\pm$ 6.18	2.06 $\pm$ 1.23	91.74 $\pm$ 6.54

SD=Standard deviation; %BMF= % bone marrow fat.

Body fat percentage is a function of maturity, because the fat depots generally increase with ageing (Casey, 1993). This was also confirmed in buffalo (Table 4). Body fat percentage increased from 9% in juvenile buffalo to 14% in adult buffalo. In buffalo aged between 7-11 years there was a significant correlation ( $P = 0.02$ ) between the % fat in the body and the % fat in the bone marrow, although only 24% of the variation in % body fat is explained (Table 4).

**Table 4.** The correlation and significance levels between % body fat and % fat in the bone marrow of buffalo in different age groups in the Kruger National Park

Age	% Fat (mean $\pm$ SD)	% BMF (mean $\pm$ SD)	Pearson correlation coefficient (significance)
0	9.494 $\pm$ 4.093	90.750 $\pm$ 5.675	0.1310 (0.2265)
1-6	11.784 $\pm$ 4.262	90.621 $\pm$ 7.015	0.1069 (0.0971)
7-11	12.946 $\pm$ 5.264	90.658 $\pm$ 6.455	0.2449 (0.0200) Significant
12+	14.412 $\pm$ 5.531	89.701 $\pm$ 7.378	0.2567 (0.3199)

%BMF= % Bone marrow fat; SD=standard deviation

Buffalo sampled in different regions had significantly different carcass masses ( $P=0.0436$ ). The lowest carcass mass found in the central region of the KNP. A significant correlation was observed between region and the percentage of bone ( $P=0.0001$ ) in buffalo carcasses. The highest percentage of bone was found in the southern region (Table 5). Neither gender nor region had a



significant effect on the % muscle in the buffalo carcass, although female buffalo in the central and northern regions had significantly lower % muscle than female buffalo in the southern region (Table 6).

There was a significant positive correlation between region and the percentage of carcass fat ( $P=0.0107$ ). In the southern and northern regions the percentage of total body fat was relatively lower than in the central region (Table 5), with buffalo from the southern region having the lowest body fat reserves. Buffalo of the same gender had significantly different percentages of carcass fat in the different regions (Table 6).

Buffalo sampled from different regions had significantly different head weights ( $P=0.0069$ ) (Table 5). There was also a significant difference in head weight between genders within the southern region (Table 6).

Buffalo sampled in the northern region had a significantly ( $P=0.0001$ ) lower percentage of bone marrow fat than animals in the rest of the KNP (Table 5).

**Table 5.** Carcass composition of the African buffalo in the different regions of the Kruger National Park

Region	Carcass mass mean (SE)	% Bone mean (SE)	% Muscle mean (SE)	% Fat mean (SE)	% Drip loss mean (SE)	% Head weight mean (SE)	% BMF mean (SE)
Southern	176.25 (5.22)	31.95 (0.42) <sup>a</sup>	61.37 (2.56)	9.91 (0.46) <sup>c</sup>	1.84 (0.09)	9.65 (0.17) <sup>a</sup>	92.04 (4.85) <sup>a</sup>
Central	169.59 (5.48) <sup>b</sup>	29.59 (0.31) <sup>b</sup>	55.65 (1.93)	12.49 (0.34) <sup>a</sup>	1.88 (0.08)	9.31 (1.56)	91.79 (7.03) <sup>a</sup>
Northern	188.82 (5.61) <sup>a</sup>	30.17 (0.32) <sup>b</sup>	57.00 (1.96)	11.45 (0.35) <sup>b</sup>	1.74 (0.08)	8.50 (0.33) <sup>b</sup>	87.80 (7.22) <sup>b</sup>

<sup>a,b,c</sup>Means within the same column with different superscripts differ significantly ( $P \leq 0.05$ ); % BMF= % bone marrow fat; SE= standard error.

**Table 6.** Effect of Region and gender on the carcass composition of African buffalo in the Kruger National Park

Region	Sex	Carcass mass mean (SE)	% Bone mean (SE)	% Muscle mean (SE)	% Fat mean (SE)	% Head weight mean (SE)	% Drip loss mean (SE)
Southern	F	167.85 (6.85) <sup>1</sup>	32.11 (0.53) <sup>A1</sup>	64.85 (3.29) <sup>A</sup>	10.18 (0.59) <sup>a</sup>	9.18 (0.23) <sup>B</sup>	1.83 (0.11)
	M	186.35 (7.98)	31.69 (0.65) <sup>A</sup>	56.31 (4.02)	9.79 (0.72) <sup>A</sup>	10.14 (0.26) <sup>A</sup>	1.84 (0.13)
	Â	177.10 (5.26)	31.90 (0.42) <sup>a</sup>	60.58 (2.60)	9.99 (0.46) <sup>1</sup>	9.66 (0.17) <sup>1</sup>	1.83 (0.09)
Central	F	169.40 (6.90)	29.53 (0.39) <sup>B</sup>	54.87 (2.43) <sup>B</sup>	13.38 (0.43) <sup>b</sup>	Non-est	1.78 (0.10)
	M	167.58 (8.79)	29.66 (0.50) <sup>2</sup>	57.11 (3.09)	11.42 (0.55) <sup>B</sup>	9.71 (1.55)	2.02 (0.13)
	Â	168.49 (5.59) <sup>A</sup>	26.59 (0.32) <sup>b</sup>	55.99 (1.96)	12.40 (0.35) <sup>2</sup>	Non-est	1.90 (0.08)
Northern	F	186.38 (7.09)	30.06 (0.40) <sup>B</sup>	56.70 (2.47) <sup>B</sup>	11.96 (0.44) <sup>c</sup>	8.33 (0.41) <sup>B</sup>	1.73 (0.11)
	M	190.50 (8.98) <sup>2</sup>	30.34 (0.51) <sup>B</sup>	57.67 (3.11)	10.99 (0.55) <sup>1</sup>	8.50 (0.55) <sup>B</sup>	1.73 (0.13)
	Â	188.44 (5.72) <sup>B</sup>	30.20 (0.32) <sup>b</sup>	57.19 (1.99)	11.48 (0.35) <sup>2</sup>	8.41 (0.34) <sup>2</sup>	1.73 (0.09)

<sup>A,B,1,2,a,b,c</sup> Means within the same column with different superscripts differ significantly ( $P \leq 0.05$ ); F= female; M= Male; SE= standard error; Â= mean of the region.

The effect of BTB status on the carcass composition of the African buffalo is represented in Tables 7 – 9. Although not indicated in the tables, the percentage bone and percentage muscle of buffalo were not affected by the occurrence of the disease. From the tables it is clear that BCS cannot be used as an index of the tuberculosis status of buffalo, because the correlation between BCS and the tuberculosis status of buffalo was not statistically significant. The tuberculosis status of the buffalo also did not significantly affect the % fat in the carcass. Unfortunately no data were available on the extent of BTB (varying from small primary lesions to extensive caseous pneumonia). A more detailed description on the interactions between BTB and body condition was therefore not possible.

**Table 7.** The influence of Bovine tuberculosis (BTB) status on the body condition scores (BCSs) and carcass fat percentage of buffalo in the Kruger National Park (KNP)

Condition	BTB	% Fat (mean $\pm$ SD)
2	N	10.13 $\pm$ 2.07
	Y	11.03 $\pm$ 2.32
3	N	11.50 $\pm$ 0.33
	Y	10.39 $\pm$ 0.62 <sup>b</sup>
4	N	12.26 $\pm$ 0.37 <sup>a</sup>
	Y	10.30 $\pm$ 1.16

<sup>a, b</sup> Means within the same column with different superscripts differ significantly. ( $p \leq 0.05$ ); N = No BTB detected; Y = Yes, BTB detected

BCSs were not significantly influenced by the BTB status of buffalo within the central region of the KNP. However approximately 80 % of the BTB negative animals had condition scores of 3 and 4 whereas only ca. 20 % of BTB positive animals had condition scores of 3 and 4 (Table 8a). Based on the multifactorial analysis of variance results this tends toward significance.

**Table 8a.** Table of Bovine tuberculosis (BTB) by condition within the central region of the Kruger National Park.

BTB		Condition 2	Condition 3	Condition 4
N	No. of animals	7	90	67
	Cumulative %	4.27	54.88	32.52
Y	No. of animals	2	22	18
	Cumulative %	0.97	10.68	8.74

N = No BTB detected; Y = Yes, BTB detected

Within the southern region of the KNP the BTB status of buffalo significantly influenced their body condition ( $P=0.017$ ;  $P=0.012$ ;  $P=0.005$ ) (Table 8b).

**Table 8b.** Table of Bovine tuberculosis (BTB) by condition within the southern region of the Kruger National Park.

BTB		Condition 2	Condition 3	Condition 4
N	No. of animals	1	120	20
	Cumulative %	0.48	49.28	9.66
Y	No. of animals	3	77	4
	Cumulative %	1.45	37.20	1.93

N = No BTB detected; Y = Yes, BTB detected

No significant differences in the % fat in the body were observed between BTB positive and BTB negative animals (Table 9).

**Table 9.** The influence of the Bovine tuberculosis status (BTB) on carcass fat percentage of buffalo in the Kruger National Park

	Pooled	Central region	Southern region
BTB	% Fat (mean $\pm$ SD)	% Fat (mean $\pm$ SD)	% Fat (mean $\pm$ SD)
N	11.30 $\pm$ 0.71%	12.84 $\pm$ 5.43%	9.90 $\pm$ 2.91
Y	11.24 $\pm$ 0.89%	11.64 $\pm$ 4.36%	10.18 $\pm$ 2.33

N = No BTB detected; Y = Yes, BTB detected; SD=standard deviation

## 5.2 Carcass characteristics

After death there is very little oxygen available for cellular respiration. Muscle fibers may survive for some time through anaerobic glycolysis that releases energy from stored glycogen (Lawrie, 1984). Anaerobic glycolysis leads to the formation of lactate (lactic acid) and a resultant decline in meat pH. Sooner or later, however, either the primary store of carbohydrate, glycogen, is depleted,

or the end product of anaerobic glycolysis, lactate, deactivates biochemical systems by its acidity. When energy is no longer available, muscle fibres begin to lose their cellular integrity. The lack of energy prevents resynthesis of protein molecules. Those present begin to denature and become susceptible to attack by endogenous proteinases. This leads to tenderization (Lawrie, 1984).

Inadequate lactate formation may leave the meat dark, firm, and dry (DFD), while too much lactate, formed too quickly while muscles are still warm, may leave the meat pale, soft, and exudative (PSE). If the pH of meat drops to the point at which a meat protein bears no net charge, the protein exhibits its lowest solubility and water-binding capacity. This is called the isoelectric point of the protein. The isoelectric point of muscle proteins is near a pH of 5.5. When meat approaches the isoelectric points of its various proteins, the ease with which it will release water by evaporation or as drip loss is increased.

The effect of age, gender and region on the carcass characteristics of buffalo sampled in the KNP, are presented in Tables 10-12. A significant difference ( $P < 0.05$ ) in meat pH values of buffalo sampled from different regions of the KNP was noted (Table 10). The highest pH values are found in carcasses of animals sampled from the northern region of the KNP. The differences could be attributed to varying environmental conditions that may have an influence on the condition of animals and therefore on the meat quality. Availability of forage in the different areas is one example, but other environmental stressors may also have an influence on meat pH (Lawrie, 1984).

**Table 10.** The influence of region on the pH of buffalo carcasses in the Kruger National Park

Region	pH – 2h (mean±SE)	pH 26 – 48h (mean±SE)
Northern	5.97±0.04 <sup>a</sup>	6.12±0.06 <sup>a</sup>
Southern	5.77±0.02 <sup>b</sup>	5.89±0.03 <sup>b</sup>

<sup>a,b</sup>Means within the same column with different superscripts differ significantly ( $P \leq 0.05$ )

No difference exists in the initial pH of meat from different age groups of buffalo. The final pH however shows a significant difference ( $P < 0.05$ ) in the different age groups (Table 11). Older animals are stated to produce meat of lower ultimate pH value (Swatland, 1984; Varnam & Sutherland, 1995). The results from this study concur.

**Table 11.** The effect of age on the pH of buffalo carcasses in the Kruger National Park

Age	pH – 2h (mean±SE)	pH 26 – 48h (mean±SE)
0	5.89±0.05	6.16±0.08 <sup>a</sup>
1-6	5.88±0.02	5.98±0.03 <sup>b</sup>
7+	5.84±0.05	5.87±0.08 <sup>b</sup>

<sup>a,b</sup>Means within the same column with different superscripts differ significantly ( $P \leq 0.05$ )

There was a significant difference in meat pH between BTB positive and BTB negative buffalo (Table 12). Stressors, including disease, affect meat quality by lowering the stored glycogen levels in the muscle (Varnam & Sutherland, 1995). The lower glycogen levels precipitate a lower production of lactate, and therefore a higher pH value. This is confirmed by the higher pH of meat from diseased buffalo (Table 12).

**Table 12.** The effect of Bovine tuberculosis (BTB) on the carcass pH of buffalo in the Kruger National Park

BTB	pH – 2h (mean±SE)	pH 26 – 48h (mean±SE)
N	5.82±0.02 <sup>b</sup>	5.93±0.03 <sup>b</sup>
Y	5.92±0.04 <sup>a</sup>	6.07±0.06 <sup>a</sup>

<sup>a,b</sup>Means within the same column with different superscripts differ significantly. ( $P \leq 0.05$ )

From the tables above it is evident that the pH decreases in the first two hours, but increases slightly thereafter. This slight increase may be attributed to the effect of temperature. Bendall (1973) showed that the pH of meat decreases when the meat is warmed, and increases when the meat is cooled. It is suspected that cooling of carcasses after slaughter was responsible for the anomaly seen in the Tables.

### **5.3 Effects of region, gender, age and Bovine tuberculosis (BTB) status on the mineral status of African buffalo**

The effects of region as well as gender, age and tuberculosis status of buffalo, on the mineral status of African buffalo are presented in Tables 10 to 13. Significant differences ( $P < 0.05$ ) were observed in the concentrations of Cu and Se in liver samples of buffalo sampled in different regions (Table 13). Highest concentrations of Se and Cu were observed in the northern and central regions of KNP. The finding of high Cu levels in these areas is concurrent with findings of other investigators (Grobler & Swan, 1999), who observed Cu toxicity in domestic and wild ruminants in areas downwind of opencast Cu mining and refining operations (Grobler & Swan, 1999). Significantly lower concentrations of both Se and Cu were found in samples from buffalo sampled in the southern region of KNP. Based on the standards for cattle (Puls, 1994), the Se concentrations from livers sampled in these regions suggest a marginal Se deficiency. The concentrations of Mn also differed significantly ( $P < 0.05$ ) between buffalo sampled from the central and southern regions (Table 13b). It should, however, be noted that the Mn concentration in the liver is a poor indicator of the Mn status of the animal, except in situations of exceedingly high or low intake (Hurley & Keen, 1987), which seemingly do not occur in the KNP. Although the liver concentration of Co is a poor indication of the vitamin B<sub>12</sub> status of the animal, it does reflect differences in Co intake (Van Ryssen, Miller, Gentry & Neathery, 1987). The Co concentrations in the livers of the buffalo were within the range classified as "high" for cattle, but well below toxic concentrations (Puls, 1994). This suggests

that the buffalo in the KNP received adequate quantities of Co but were not exposed to excessively high intakes of the element.

**Table 13.** The effect of region in the Kruger National Park on the average trace mineral concentration in the liver of African buffalo

Region	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)
Northern	1.183 (0.039) <sup>a</sup>	138.13 (10.63) <sup>a</sup>
Central	0.630 (0.037) <sup>b</sup>	131.54 (9.98) <sup>a</sup>
Southern	0.414 (0.034) <sup>c</sup>	85.26 (9.14) <sup>b</sup>

<sup>a,b,c</sup>Means within the same column with different superscripts differ significantly.

( $P \leq 0.05$ ), DM = dry material; SE= standard error

**Table 13a.** The effect of region in the Kruger National Park on the average trace mineral concentration in the liver of African buffalo

Region	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
Northern	1.24 (0.04) <sup>a</sup>	146.61 (5.59) <sup>a</sup>	4.04 (0.46)	7.06 (1.38)
Central	0.68 (0.02) <sup>b</sup>	132.39 (6.30) <sup>a</sup>	4.01 (0.44)	7.02 (1.34)
Southern	0.51 (0.01) <sup>c</sup>	83.20 (5.49) <sup>b</sup>	4.32 (0.41)	7.69 (1.56)

<sup>a,b</sup>Means within the same column with different superscripts differ significantly ( $P$

$\leq 0.05$ ), DM = dry material; SE= standard error

**Table 13b.** The effect of region on the average trace mineral concentration in the liver of African buffalo sampled in the central and southern regions of the Kruger National Park

Region	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
Central	0.641(0.027) <sup>a</sup>	135.14 (13.66) <sup>a</sup>	3.91 (0.09)	6.75 (0.25) <sup>a</sup>
Southern	0.424(0.026) <sup>b</sup>	64.54 (16.97) <sup>b</sup>	3.82 (0.08)	7.52 (0.24) <sup>b</sup>

<sup>a,b</sup>Means within the same column with different superscripts differ significantly. ( $P$

$\leq 0.05$ ), DM = dry material; SE= standard error

The Se concentration of liver samples from buffalo bulls was significantly higher ( $P < 0.05$ ) compared to buffalo cows (Table 14), particularly in the northern



part of KNP (Table 14a). This suggests a greater storage capacity in buffalo bulls or higher Se requirements for physiological processes such as pregnancy and lactation in cows.

**Table 14.** The effect of gender on the average trace mineral concentration in the liver of African buffalo in the Kruger National Park

Sex	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
F	0.70 (0.03) <sup>a</sup>	113.30 (6.69)	4.14 (0.39)	7.29 (1.41)
M	0.82 (0.05) <sup>b</sup>	118.39 (5.95)	4.16 (0.51)	7.50 (1.49)

<sup>a,b</sup>Means within the same column with different superscripts differ significantly ( $P \leq 0.05$ ), F= female; M= male; DM = dry material; SE= standard error

**Table 14a.** The effect of gender on the average trace mineral concentration in the liver of African buffalo sampled within the northern region of the Kruger National Park

Sex	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
F	0.83 (0.14) <sup>a</sup>	180.10 (21.93)	3.96 (0.16)	7.34 (0.53)
M	1.15 (0.13) <sup>b</sup>	165.34 (19.62)	3.99 (0.15)	7.48 (0.48)

<sup>a,b</sup>Means within the same column with different superscripts differ significantly ( $P \leq 0.05$ ), F= female; M= male; DM = dry material; SE= standard error

**Table 14b.** The effect of gender on the average trace mineral concentration in the liver of African buffalo sampled within the combined central and southern region of the Kruger National Park

Sex	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
F	0.530 (0.025)	102.50 (11.53)	3.87 (0.08)	7.20 (0.23)
M	0.534 (0.028)	97.18 (12.90)	3.86 (0.09)	7.07 (0.26)

F= female; M= male; DM = dry material; SE= standard error

**Table 14c.** The effect of gender on the average trace mineral concentration in the liver of African buffalo sampled within the central region of the Kruger National Park

Sex	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
F	0.629 (0.036)	129.10 (18.37)	3.90 (0.11)	6.88 (0.29)
M	0.666 (0.038)	138.59 (19.44)	3.93 (0.12)	6.35 (0.31)

F= female; M= male; DM = dry material; SE= standard error

**Table 14d.** The effect of gender on the average trace mineral concentration in the liver of African buffalo sampled within the southern region of the Kruger National Park

Sex	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
F	0.444 (0.039)	71.44 (14.23)	3.85 (0.13)	7.51 (0.39)
M	0.415 (0.045)	53.90 (16.42)	3.82 (0.15)	7.79 (0.46)

F= female; M= male; DM = dry material; SE= standard error

Minor differences in Se concentrations were observed between age groups, with highest concentrations in buffalo of between 1 and 11 years of age (Table 15). Differences were also observed in Co concentrations within the central region of the KNP, the Co concentration in the liver decreasing with age (Table 15).

**Table 15a.** The effect of age on the average trace mineral concentration in the liver of African buffalo sampled within the northern region of the Kruger National Park

Age	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
0	0.951 (0.161)	176.74 (24.49)	4.02 (0.18)	7.77 (0.59)
1-6	1.007 (0.138)	167.86 (21.00)	4.14 (0.16)	7.19 (0.51)
7-11	1.171 (0.133)	156.17(20.34)	3.69 (0.15)	7.39 (0.49)
12+	0.824 (0.223)	190.10 (33.96)	4.06 (0.25)	7.29 (0.82)

DM = dry material; SE= standard error

**Table 15b.** The effect of age on the average trace mineral concentration in the liver of African buffalo sampled within the combined central and southern region of the Kruger National Park

Age	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
0	0.492 (0.033) <sup>a</sup>	101.40 (15.63)	3.92 (0.11)	7.37 (0.31)
1-6	0.589 (0.015) <sup>b</sup>	103.36 (6.73)	4.08 (0.05)	7.66 (0.14)
7-11	0.611 (0.023) <sup>b</sup>	99.67 (10.12)	3.92 (0.07)	7.13 (0.21)
12+	0.437 (0.082) <sup>a</sup>	94.91 (38.53)	3.54 (0.27)	6.37 (0.77)

<sup>a,b</sup> Means within the same column with different superscripts differ significantly. ( $p \leq 0.05$ )

DM = dry material; SE= standard error

**Table 15c.** The effect of age on the average trace mineral concentration in the liver of African buffalo sampled within the central region of the Kruger National Park

Age	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
0	0.651 (0.060)	104.96 (30.95)	4.07 (0.19)	6.43 (0.50)
1-6	0.696 (0.023)	123.93 (12.14)	4.22 (0.07) <sup>a</sup>	7.23 (0.19)
7-11	0.735 (0.036) <sup>a</sup>	127.41 (18.42)	3.82 (0.11) <sup>b</sup>	6.81 (0.29)
12+	0.507 (0.101) <sup>b</sup>	179.08 (52.22)	3.55 (0.32) <sup>b</sup>	5.99 (0.84)

DM = dry material; SE= standard error

**Table 15d.** The effect of age on the average trace mineral concentration in the liver of African buffalo sampled within the southern region of the Kruger National Park

Age	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
0	0.353 (0.040)	90.78 (14.58)	3.83 (0.13)	8.11 (0.40)
1-6	0.488 (0.021)	82.92 (7.54)	3.97 (0.67)	8.03 (0.21)
7-11	0.494 (0.030)	69.73 (11.20)	4.04 (0.10)	7.43 (0.31)
12+	0.384 (0.142)	72.5 (5.25)	3.51 (0.46)	7.02 (1.45)

DM = dry material; SE= standard error

Within the central and southern regions of the KNP, BTB positive animals had significantly lower liver Cu concentrations than did animals in which BTB was not detected (Table 16). This may point to an influence on the immune function of

animals, which leads to a higher incidence of BTB. Conversely, it may imply altered Cu-intake and or -absorption in infected animals. If there is an influence on the immune system it may indicate that requirements of buffalo are greater than those of cattle. In the northern region of the KNP there is a tendency for animals with BTB to have lower Se concentrations ( $P=0.0638$ ) (Table 16a).

**Table 16a.** Comparison of the trace mineral concentration in the liver of BTB positive and BTB negative African buffalo within the central and southern region of the Kruger National Park

BTB	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
N	0.528 (0.025)	113.14 (11.35) <sup>a</sup>	3.85 (0.08)	6.99 (0.23)
Y	0.537 (0.028)	86.56 (13.14) <sup>b</sup>	3.88 (0.09)	7.28 (0.26)

N = No BTB detected; Y = Yes, BTB detected; DM = dry material; SE= standard error.

**Table 16b.** Comparison of the trace mineral concentration in the liver of BTB positive and BTB negative African buffalo within the northern region of the Kruger National Park

BTB	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
N	1.214 (0.065)	144.83 (9.92)	4.05 (0.07)	7.00 (0.24)
Y	0.762 (0.235)	200.61 (35.78)	3.91 (0.27)	7.82 (0.87)

N = No BTB detected; Y = Yes, BTB detected; DM = dry material; SE= standard error.

**Table 16c.** Comparison of the trace mineral concentration in the liver of BTB positive and BTB negative African buffalo within the central region of the Kruger National Park

Macro BTB	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
N	0.633 (0.033)	151.41 (17.05)	3.84 (0.10)	6.54 (0.27)
Y	0.662 (0.041)	116.28 (21.28)	4.00 (0.13)	6.69 (0.34)

N = No BTB detected; Y = Yes, BTB detected; DM = dry material; SE= standard error.

**Table 16d.** Comparison of the trace mineral concentration in the liver of BTB positive and BTB negative African buffalo within the southern region of the Kruger National Park

Macro BTB	Se (SE) (mg / kg DM)	Cu (SE) (mg / kg DM)	Co (SE) (mg / kg DM)	Mn (SE) (mg / kg DM)
N	0.431 (0.039)	73.32 (14.51)	3.87 (0.13)	7.43 (0.40)
Y	0.428 (0.044)	52.01 (16.06)	3.80 (0.14)	7.87 (0.45)

N = No BTB detected; Y = Yes, BTB detected; DM = dry material; SE= standard error.

## 5.4 Discussion

### 5.4.1 Effect of gender on carcass composition

In cattle, both heifers and cows are generally fatter than bulls, and this is associated with the time of onset of fat deposition (Swatland, 1984). This is also confirmed in buffalo as can be seen from Table 1. This observation can be explained by the fact that androgens in the male cause their carcasses to be leaner with higher muscle to bone ratios (Casey, 1993). Another explanation could be that females require fat reserves that can be mobilised readily to ensure survival of the species through channelling of energy to the reproductive pathways / functions in the body. This theory is supported by the data in Table 2, where we see a significant correlation between the % fat in the body and the % fat in the bone marrow of female buffalo. This correlation suggests that all the energy reserves in the female are in a state of constant fluctuation according to physiological and environmental requirements. In male buffalo it appears that the percentage of fat in the bone marrow remains at a higher, relatively more constant level, while the body fat fluctuates according to fluctuating environmental conditions and physiological requirements. Lower body fat reserves in the male buffalo do not necessarily signify poorer condition, as protein may play a greater role as an energy reserve. Bone marrow fat reserves may therefore not be utilised as readily in males as in females, explaining the lower correlation between % Fat and % BMF in males (Table 2).

The Se concentration of liver samples from buffalo bulls was significantly higher ( $P < 0.05$ ) compared to buffalo cows (Table 14), particularly in the northern part of KNP (Table 14a). This suggests a greater storage capacity in buffalo bulls or higher Se requirements for physiological processes such as pregnancy and lactation in cows.

#### 5.4.2 Effect of age on carcass composition

Animal age is the dominant factor determining muscle to bone ratios (Preston & Willis, 1974). As animals grow older or fatter, muscle to bone ratios increase, since longitudinal bone growth slows down in older animals and muscles start to accumulate appreciable amounts of intra-muscular fat (Swatland, 1984). In the buffalo we see that as body condition increased, also signifying an ageing process, % muscle and % Fat increased while % bone decreased (Table 3). This is expected from growth principles, which dictate these results as an animal ages (Swatland, 1984).

Minor differences in Se concentrations were observed between age groups, with highest concentrations in buffalo of between 1 and 11 years of age (Table 15). The very young and very old animals had lower liver Se concentrations. This may point to differences in foraging activity or differences in the digestive ability of young and old animals.

#### 5.4.3 Effect of region on carcass composition

As seen in Table 5 buffalo sampled in different regions had significantly different carcass masses ( $P = 0.0436$ ). This difference could be attributed to real differences in the size of animals sampled from different areas, or it could indicate that animals sampled in the central region was of an overall younger age than animals sampled elsewhere. The significantly lower percentage bone and higher percentage fat in these animals, compared to other regions, however point to the conclusion that these animals are mature and that the observed difference in size is real.

The highest percentage of bone was found in the southern region, which coincided with a relatively high % muscle, indicating that animals were not young, and contained a significantly lower % body fat. The % bone marrow fat was again however, higher, suggesting that animals mobilise body fat reserves before bone marrow fat reserves. This finding is in accordance with that of other authors (Brooks *et al.*, 1977). The significant differences in fat reserves found in animals sampled in different regions could be attributed to differences in the environment, which include nutrition, stress and disease.

Significant differences ( $P < 0.05$ ) were observed in the concentrations of Cu and Se in liver samples of buffalo sampled in different regions (Table 13). Highest concentrations of Se and Cu were observed in the northern and central regions of KNP. The finding of high Cu levels in these areas is concurrent with findings of other investigators (Grobler & Swan, 1999), who observed Cu toxicity in domestic and wild ruminants in areas downwind of opencast Cu mining and refining operations (Grobler & Swan, 1999). Significantly lower concentrations of both Se and Cu were found in samples from buffalo sampled in the southern region of KNP. Based on the standards for cattle (Puls, 1994), the Se concentrations from livers sampled in the southern region suggest a marginal Se deficiency. The concentrations of Mn also differed significantly ( $P < 0.05$ ) between buffalo sampled from the central and southern regions (Table 13b). It should, however, be noted that the Mn concentration in the liver is a poor indicator of the Mn status of the animal, except in situations of exceedingly high or low intake (Hurley & Keen, 1987), which seemingly do not occur in the KNP. Although the liver concentration of Co is a poor indication of the vitamin B<sub>12</sub> status of the animal, it does reflect differences in Co intake (Van Ryssen, Miller, Gentry & Neathery, 1987). The liver is useful only in determining the copper, selenium and vitamin B12 status of animals (Van Ryssen, 2000). The Co concentrations in the livers of the buffalo were within the range classified as "high" for cattle, but well below toxic concentrations (Puls, 1994). This suggests that the buffalo in the KNP received adequate quantities of Co but were not exposed to excessively high intakes of the element.

#### 5.4.4 Body condition and tuberculosis status

In the southern region of the KNP the occurrence of BTB had a significant ( $p < 0.05$ ) effect on the BCS (Table 8b). From Table 5 it is apparent that animals sampled from this region also had significantly lower body fat reserves. This is in accordance with the findings of Caron *et al.* (2003) who provide evidence that body condition of buffalo in the KNP is significantly related to BTB prevalence. It must, however also be considered that the different regions fall in different landscapes (Gertenbach, 1983) with different vegetative and forage values for herbivores. There are therefore many variables between and within regions that may affect condition and carcass composition of these animals. It may be advisable to compare the various regions or vegetative zones within the KNP to have a more realistic approach to determine the influence of nutrition on BTB.

From Tables 7 – 9 it is clear that very few animals that were BTB positive had low BCSs. It is possible that the body condition of BTB positive animals only deteriorates once the disease has progressed for some time or perhaps that affected animals die before their body condition is affected. Unfortunately no data were available on the extent of BTB. A more detailed description on the interactions between BTB and body condition was therefore not possible. It may be advisable to initiate a study that could compare the extent of BTB lesions with the condition of animals. This would provide an idea when during the course of the disease body condition begins to be affected.

Within the central and southern regions of the KNP, BTB positive animals had significantly lower liver Cu concentrations than did animals in which BTB was not detected (Table 16). This may point to an influence on the immune function of animals, which leads to a higher incidence of BTB. Conversely, it may imply altered Cu-intake and or -absorption in infected animals. If there is an influence on the immune system it may indicate that requirements of buffalo are greater than those of cattle.