Body composition estimation and nutritional status of African buffalo (*Syncerus caffer)* in the Kruger National Park

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TABLE OF CONTENTS

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ABSTRACT

Bovine tuberculosis (BTB) was first detected in the African buffalo (*Syncerus caffer*) in the Kruger National Park (KNP) in 1990. This study was initiated to investigate the effects and interactions of age, sex, region / habitat and tuberculosis status on the body condition and carcass composition of African buffalo in the KNP. Data from approximately 600 buffalo sampled in 1998 were analysed. It was found that gender, age and region where buffalo were sampled affected carcass composition. It was also found that body condition scores (BCS) and the percentage fat in the bone marrow (%BMF) of buffalo were poor predictors of proximate body composition. BTB did not influence body composition. Mineral levels in the liver of buffalo were also examined. There were regional differences in Se, Cu and Mn levels. It was also found that Cu levels were lower in buffalo that tested positive for BTB. Region, age and BTB status had an influence on carcass pH.

LIST OF TABLES

CHAPTER ONE

RESULTS AND DISCUSSION

LIST OF FIGURES

Acknowledgements

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CHAPTER 1

INTRODUCTION

Assessment of the physical condition of individual herds or sub-populations of African ungulates is useful as a measure to compare the effects of current and past management practices, stocking rates, weather, disease and other ecological conditions (Smith, 1970). The physical condition of an animal is a fairly accurate indicator of its nutritional status, but it does not always give an accurate indication of the mineral nutritional status of the animal. To quantify this aspect, tissue samples need to be analysed for mineral content. Certain tissues are better indicators of specific minerals than others (Van Ryssen, 2000). In this study samples of the liver were analysed.

The methodologies available for body condition estimation differ in terms of accuracy with which they predict body condition, composition or nutritional status. To accurately quantify the effects of environmental conditions on wildlife, it is necessary to evaluate the accuracy and feasibility of the available methodologies. Recently the effects of BTB on the African buffalo (*Syncerus caffer*) in the Kruger National Park has come under investigation in an extensive BTB monitoring programme involving a variety of researchers.

Bovine tuberculosis (BTB) is a chronic wasting disease caused by a complex of mycobacteria, *Mycobacterium tuberculosis* that include *M. tuberculosis*, *M. microti*, *M. africanum* and *M. bovis* (Kubica & Wayne, 1984). These mycobacteria can affect clinical disease symptoms in a wide range of mammalian hosts, including humans (Morris *et al.*, 1994). *Mycobacterium bovis*, known most commonly as a pathogen of livestock, was almost entirely eliminated from cattle and humans in the 1980's (Cosivi, Meslin, Daborn, & Grange, 1995). Recently it has become widespread in wildlife populations (Tessaro, 1986; Barlow, 1994; O'Reilly & Daborn, 1995; Schmitt, Fitzgerald, Cooley, Bruning-Fann, Sullivan, Berry, Carlson, Minnis, Payeur & Sikarskie, 1997; Bengis, 1999), and its prevalence has been increasing worldwide. Wildlife populations appear to

act as reservoir for the disease and it is accepted that they play a key role in its epidemiology.

Mycobacteriosis has been known to be present in the Kruger National Park (KNP) since 1967 (De Vos, McCully, & Van Niekerk, 1977), when it was described in an impala (*Aepyceros melampus*). No isolation of the causative organism was, however, attempted. Although BTB has been known to occur in free-ranging African buffalo (*Syncerus caffer*) since the middle 1960's (Woodford, 1982), it was not considered to be of practical importance to buffalo in the KNP and other areas in which buffalo occurred as late as the 1980's (Bengis & Erasmus, 1988). In 1990 it was however diagnosed in an African buffalo bull, which was found recumbent near the South-Western boundary fence of the KNP (Bengis, Kriek, Keet, Raath, De Vos & Huchzermeyer, 1996). Further investigation into the BTB status of the buffalo population in the KNP ensued. In 1992 the BTB prevalence was estimated to be 0%, 4.4% and 27.1% in the north, central, and southern regions of KNP respectively. By 1998 the BTB prevalence had increased to 1.5%, 16% and 38.2% for the three regions respectively (Rodwell, Kriek, Bengis, Whyte, Viljoen, De Vos & Boyce, 2001).

BTB was apparently first introduced into African buffalo in the southern region of the KNP during the 1960's or 1980's from domestic cattle (Bengis *et al.*, 1996). According to official reports, tuberculosis infected cattle had been identified on various farms south of the KNP in the period between 1955 and 1987 (Kloeck, 1998). Subsequent investigation has confirmed that BTB in the KNP had probably originated on a specific farm in the Barberton district of Mpumalanga Province where cattle and buffalo had reportedly mingled during the 1950's and 1960's (Vosloo, Bastos, Michel & Thomson, 2001).

The African buffalo, by reason of its high numbers, comparative high vulnerability to *M.bovis*, herd structure and characteristic behavioural patterns, serve as an effective maintenance host and reservoir of BTB. Once a buffalo herd becomes infected the prevalence can surpass 90% (Rodwell, 1999). Under these conditions, spillover of the infection to other species does occur and has been confirmed in a number of species (Bengis & Erasmus, 1998; Keet, Kriek,

Penrith, Michel & Huchzermeyer, 1996). When fences secluding wildlife and livestock areas are breached, infected buffalo accordingly pose a threat to cattle and also their owners.

Presently there are no practical methods for treating infected wildlife populations. Considering the risks these populations pose to livestock and humans, and as a result of growing concern for the health of infected wildlife populations, there is increased pressure to monitor and manage BTB in wildlife. The purpose of this study was to investigate a number of condition indices and to propose some in-vivo methods of condition / composition estimation not previously used in wild ungulates. The study also includes an investigation on the nutritional levels of micro minerals in the African buffalo (*Syncerus caffer*) and their influence on immune function as this may explain some of the variation in body composition. Although there are many variables that affect body composition, the hope is to gain a better understanding of the interaction between the animal and the various environmental factors, and to provide a reference base for future studies.

CHAPTER 2

EVALUATING METHODS FOR CONDITION ESTIMATION IN THE AFRICAN BUFFALO (*Syncerus caffer*)

2.1 Introduction

The assessment of body condition in wild African ungulates is important in view of their management, as it reflects not only their ability to survive under varying environmental conditions (Hirst, 1969; Sinclair, 1970) but also their potential as producers of meat (Monro, 1979). Assessment of the physical condition of individual herds or sub-populations is useful in the study of big game as the condition of game in one area can be compared with that of game in other areas. This would allow for evaluation of the effects of current and past management practices, stocking rates, weather and other ecological conditions (Smith, 1970). Body condition also serves to link the nutritional level of individuals and populations, in different seasons and under a variety of environmental conditions, with their growth and reproductive rates (Brooks, Hanks & Ludbrook, 1977).

Several body condition indices have been proposed for use with African ungulates (Smith 1970; Sinclair & Duncan 1972; Brooks *et al.,* 1977; Monro & Skinner, 1979). Kidney fat index (KFI) and bone marrow index (BMI), are the most frequently reported (Hanks, Cumming, Orpen, Parry & Warren, 1976; Brooks, 1978; Anderson, 1979; Dunham & Murray, 1982; Stelfox & Hudson, 1986; Shackleton & Granger, 1989; Van Rooyen, 1993).

The purpose of this chapter is to investigate a number of condition indices and to propose some in-vivo methods of condition / composition estimation not previously used in wild ungulates. Factors affecting suitability of the available methods will be discussed.

2.2 Justification for the use of fat as an indication of condition

It has been shown that in the earliest stages of normal growth of farm animals bone is the tissue, which develops at the fastest rate; later, muscle grows faster than bone, and finally fat becomes the fastest growing tissue. Fat is a metabolic tissue and primarily an energy store (Casey & Maree, 1993). This makes it a particularly useful index of the metabolic level and potential energy reserves of the animal. Implicit in the growth-rate principles, and metabolic function of fat, fat reserves within the body can thus be taken as a direct measure of condition, reflecting the animal's physiologic adjustment to its environment. In this paper, condition and total fat reserves are used synonymously.

Most wild animals are not as consistently well nourished as are farm animals, and their fat deposits are consequently not as well developed. Nevertheless, in general it is still possible to distinguish centres of location of their fats. The three main centres of location for body fats are the subcutaneous connective tissue, the abdominal cavity, and the inter-muscular connective tissue. The depot fats serve as the principal fuel storage reserves of the body. For this reason and because of the ease with which they can be observed in the carcass they serve as the principal criterion for assessment of condition (Riney, 1955).

The most obvious and straightforward way to determine fat reserves in the body is to dissect or render out all fat and express it as a percentage of the live weight or carcass weight. This is a tedious, expensive and time-consuming task, which generally results in various piles of lean, fat and bones, which are not as desirable for human consumption as is the intact carcass. We will consider other methods.

2.2.1 Order of fat deposition

The order of fat deposition is fundamental to the use of the amount of fat in various depots as an index to total fat reserved, or to condition. At any given time of change, fat is being laid on or taken off most of the depots at the same time. In other words, adipose tissue is in a dynamic state of continuous

synthesis, deposition, and utilisation. The order of deposition refers only to the start and finish of the process.

Various authors (Harris, 1945; Riney, 1955; Ransom, 1965; Trout & Thiessen, 1968) have indicated that the first fat depot to respond to a favourable metabolic change in various species is bone marrow. This is followed by the fat around the kidney, intestines, and stomach in that order, and, finally, by the subcutaneous fat on the back. Mobilisation of the fat depots observed, was in reverse order to that of deposition. This is in accordance with one of the characteristics of differential growth whereby fat deposition starts at different times in different depots. Wright & Russel (1984a) suggested that different fat depots play different roles in different physiological processes. In other words, fat depots are mobilised at varying degrees and at varying rates, depending on the physiological process involved.

2.2.2 Subcutaneous fat deposition

In animals, which are not subjected to cold stress, subcutaneous deposition of fat would probably serve as a physiological stressor during the summer season, which would be characterised in most cases by extreme heat. This assumption is supported by the examination of many East African ungulates, which have very little, if any fat under the skin (Smith, 1970). If fat was present, it would obstruct the dissipation of heat.

Minimum amounts of total fat found on the thinnest cattle (a temperate zone animal) raised in East Africa approximate those found on the fattest African game animals (Ledger & Smith, 1964; Ledger, Sachs & Smith, 1967). This suggests that fat is probably not the only major store of reserve energy in tropical ungulates or, conversely, that the wild animals are in poorer condition. Observations of other components such as lean percentage, which is from 10 – 16% higher in game animals than in Boran steers (Ledger & Smith, 1964), and higher dressing out percentages in game, tend to rule out that the animals are in poorer condition. This would rather indicate that they use protein as a stored reserve.

The fat related definitions of condition used might therefore not reflect the true physiological state of wild animals. Future research in this field would benefit from a better understanding of the metabolic reactions of tropical ungulates to nutritional stress, allowing "condition" to be more realistically defined.

2.2.3 Kidney fat index (KFI)

The KFI offers a simple, rapid and relatively objective quantitative measure of body condition. To obtain the data it is necessary to have only a knife and a balance capable of weighing to the nearest gram. The KFI is based on the mass percentage of the fat (capsula adiposa) surrounding the kidney. The KFI is determined as follows: The kidney together with the surrounding capsula adiposa is removed and weighed. The capsula adiposa is then peeled away from the surface and the kidney weighed without the fat. Condition is determined by the amount of fat around the kidney expressed as a percentage of the kidney weight, in order to place animals of different size on a comparable basis.

In addition to being an accurate indication of the fat stored in the body, (Riney, 1955; Smith, 1970; Brooks *et al.,* 1977) the KFI has a wide range and can be duplicated by different workers. The KFI reveals differences between animals of different sexes and social status and seasonal changes in condition are clear as fat is mobilised from around the kidney before any other region. In terms of time, energy, and money expended for a comparable degree of accuracy, the KFI fulfils the needs of a workable technique, except in the case of hippopotamus and lesser kudu (Smith, 1970).

The KFI is influenced by various factors other than nutrition alone. Rainfall and habitat will influence the KFI as they influence body condition through their effect on nutrition. Sinclair & Duncan (1972) attributed fluctuations in KFI to physiological events related to reproduction. Hanks *et al.* (1976) indicated that changes in body weight and KFI of impala (*Aepyceros melampus*) above the age of three years are related to reproduction, and noted a reduction in KFI of males during the rut. Bear (1971) found significant seasonal changes in the KFI of male

and female pronghorns (*Antilocapra americana*) in Colorado. It was found that females generally maintained higher levels of kidney fat than did males. Mean fat indices for males exceeded those of females only in June and early July, at which time the mean fat index for females was at the lowest level. The differences between the trends of males and females may be attributed to stress on the females during pregnancy, parturition, and raising the young. Herding during late August and September likely placed an increased metabolic demand on males, at which time fat is utilised maximally.

The use of the kidney index as a measure of condition has limitations with younger animals, where very little body fat is deposited regardless of condition. Hanks *et al.* (1976) found a highly significant difference ($P<0.001$) in the mean KFI between impala under the age of three and impala above that age. Up to three years of age, the mean kidney index is considerably lower than the mean index for Impala above that age (Monro & Skinner, 1979).

Several authors have cautioned against the use of KFI for interpretation of seasonal trends in herbivore condition (Dauphine, 1975; Attwell, 1977). This stemmed from the detection of seasonal differences in the mass of the kidney itself, which in turn influenced values of the KFI. Suttie (1983) also reported seasonal weight changes in the kidneys of red deer (*Cervus elephas*). Scotcher (1982) and Shackleton & Granger (1989), however, produced conflicting results, where no significant seasonal change in kidney mass was detected. Brooks (1978) and Anderson (1979) also found no seasonal fluctuation in the mass of the kidney of impala and nyala respectively. This clearly requires thorough investigation before kidney fat indices can be employed in wildlife management with any degree of confidence.

2.2.4 Bone marrow index (BMI)

The BMI is a representation of the percentage fat in the bone marrow of animals. As bone marrow predominantly consists of fat, the BMI may conceivably be a fair appraisement of body condition.

It has been stated that the BMI is seasonally limiting in its range by not reflecting the full change in body condition of animals (Riney, 1955), thus being limiting in its prediction of fluctuations in body fat across its entire range. In the study of Bear (1971) the bone marrow showed no significant seasonal fluctuations, with very few specimens having values less than 70 percent. Monro, (1979) found that the bone marrow fat did not significantly correlate with body condition of impala. Both studies seem to indicate the inefficiency of the BMI in mirroring body condition.

A number of authors (Harris, 1945; Ransom, 1965; Bear, 1971; Sinclair & Duncan, 1972; Hanks *et al.*, 1976; Brooks *et al.*, 1977) have investigated the relationship between the KFI and the fat content of the bone marrow. They suggest that the perinephric fat, barring a small persistent quantity of residual fat (Brooks *et al,* 1977), is mobilised before that of the bone marrow fat. Thus indicating that the bone marrow fat is mobilised after the kidney fat and hence is a better measure of condition when the animal is suffering from more extreme environmental stresses. The finding that the BMI does not significantly deteriorate until the KFI declines below a certain level, and the poor relationship observed by Ransom (1965) between these two indices, suggesting that they reflect different responses of the animal to environmental and physiological stresses, support this.

Thus the KFI has little merit for condition estimation at the commencement of mobilisation of fat from the bone marrow, and the BMI has little merit when the KFI is above a certain level. This suggests the complementary use of the two measurements as a means of assessing physical condition over its entire range.

Although of value in older animals the study of Hanks *et al.* (1976) has demonstrated the limitations of the fat content of the bone marrow as measure of condition in young animals. In impala under two years of age the bone marrow was still very active in red blood cell formation and very little fat deposition had taken place.

It was found that bone marrow samples from a variety of bones in the body did not differ by more than 10% fat content, even in animals with very low

levels of marrow fat (Bear, 1971; Sinclair & Duncan, 1972). This is a useful finding for it allows an estimation of condition to be made from any of the bones, often predators and scavengers carry away the limb bones leaving only the vertebral column and pelvic girdle. Brooks *et al.* (1977), however found a very positive sequence of marrow fat mobilisation in the limb bones that is initiated in the femur and humerus, and suggested that for more sensitive comparative studies of condition, these two bones should be collected.

Estimates of condition of naturally dying wild animals must be obtained before primary and secondary causes of death can be identified (Sinclair & Duncan, 1972). In these cases bone marrow fat is a better measure of condition than the kidney fat, as often the carcass is only found after scavengers have eaten the soft parts of the carcass including the kidneys, the bone marrow however is often intact.

2.2.4.1 Chemical determination of percentage of marrow fat

Riney (1955) described a method whereby marrow is taken from the central section of the femur and weighed. The red blood-making ends of the marrow are not used in the analysis. The marrow is dropped into boiling 95 per cent alcohol in the field. The alcohol is removed *in vacuo* and the dried tissue continuously extracted with petroleum-ether (boiling point 40-60°C) in a Soxhlet apparatus. The weight of the petroleum-ether extract is used to give the percentage fat in the bone marrow.

The chemical determination of bone marrow fat is of little use as a field technique, however, because of the difficulty in collecting and in transporting specimens to the laboratory and the length of time involved before results can be obtained (Riney, 1955).

2.2.4.2 The visual description of bone marrow

Riney (1955) described a visual method of estimating the condition of bone marrow, from its texture and colour. Colour was determined and the rating (0-3) was judged on the following basis:

0 = Reddish or brownish in colour.

1 = Intermediate between 1 and 2.

2 = Light, but with faint wash of colour.

3 = White, or white streaked with small red vessels.

White, with or without red streaks, indicates marrow with high fat content. As the fat content decreases, the colour deepens, the lowest fat content marrow being reddish or brownish in colour.

The texture of the marrow was also rated:

0 = Gelatinous or watery.

1 = Slightly greasy.

2 = Soft and thickly greasy but not waxy.

3 = Firm and waxy.

As the fat content of the marrow increases, the texture, which in marrow of low fat content is watery or gelatinous, becomes firmer and increasingly waxy. Colour and texture judged separately in this way were combined to form the "visual estimate of fat content in femur marrow" with values ranging from 0 to 6.

Sinclair & Duncan (1972) describe another method of visually estimating the condition of bone marrow, from its texture and colour. The categories used were:

- Solid white fatty.
- White opaque gelatinous.
- Red-pink opaque gelatinous.
- Translucent yellow or pink gelatinous.

The mean percentage dry weight for each of the classes was calculated, and these means were then compared. With few exceptions the percentage dry weights in Class 1 were above 90 and the mean was significantly different from all other classes ($P < 0.05$). Class 4 was usually below 20% dry weight and the mean was also significantly different ($P < 0.05$) from all other classes. Classes 2 and 3 both showed a wide range of values between 90% and 10% dry weight and were not significantly different from each other; the colour difference did not reflect a difference in fat content. The samples from Class 3 were however

mostly from juvenile animals. In juvenile animals the marrow has a very opaque gelatinous appearance and is still very active in red blood cell formation (Hanks *et al.,* 1976), hence the colour difference. Thus Classes 2 and 3 were combined to make three categories.

Bear (1971) found the relationship between per cent bone marrow fat and colour to be very poor, there was however mention of a possible correlation between consistency ratings and percentage of fat content. The conclusion made was that visual estimates of fat content, using colour and consistency were reliable only for extreme fat values. Neiland (1970) was of the opinion that a four-step visual classification is not an especially sensitive method of comparison, particularly for individual specimens collected infrequently. Riney (1955) and Sinclair & Duncan (1972), however, found visual estimates of bone marrow to reflect true differences in bone marrow fat content. This classification is useful in circumstances where it is not possible to collect a sample of the marrow for further analysis; at least one can say with confidence at what end of the scale of condition the animal was when it died.

2.2.4.3 A compression method indicates fat content of bone marrow

Greer (1968) described a method using the extent of bone marrow compression to estimate the fat content in the marrow. Uniform samples of marrow were obtained with the aid of a jig or device. The jig was made from a plastic pipe with an inside diameter of 1½ cm with about one-third of the circumference removed. An open section in the tube allowed the marrow to be laid in a horizontal position and also provided access for holding marrow in a natural, not stretched or compacted position, when cutting measured sections. One end of the marrow was cut with a knife held against the end of the jig. The cut marrow and end of the jig were butted against a flat surface and the remaining marrow was cut off flush with the other end of the jig. If the marrow was not stretched or squeezed, it was the precise length of the jig (Greer, 1968).

When a known length of marrow sample is removed from the measuring jig and placed on end, its consistency is revealed by the immediate compression

under its own weight which can be measured with a scale. Jig lengths may be selected to coincide with existing graduated scales. Scales may be engraved or attached to the jig (Greer, 1968).

Extremely limp or flaccid marrow will not stand unaided for measurements but these can be easily handled inside the vertical jig. Although the tube may restrict complete basal expansion in some samples, an extensive compression of the marrow will be noted. Tubes should be at least twice as great in diameter as marrow to permit some area for limp marrow to expand at the base. If measured marrow sections will not stand unaided, a thin rod inserted through the centre of the marrow shaft can be used to hold a sample upright while compression is measured (Greer, 1968).

It appears that broad intervals of 10 or 20 percent fat content may be adequate to indicate an animal's condition. The compression method may not be as accurate, and is considerably more difficult to carry out than the visual estimate of bone marrow fat content. It nevertheless provides a general index to fat content in big game bone marrow that may be useful when chemical analyses are not feasible or not desired (Greer, 1968).

2.2.4.4 Weight of dried marrow as indicator of marrow fat

Neiland (1970) pursued an objective technique for estimating bone marrow fat content other than the visual procedure or the conventional extraction procedure. It was believed that extraction procedures were too tedious and more accurate than necessary for routine use on large numbers of marrow samples. As bone marrow is essentially a three component system comprised of water, fat, and residue, this component amounting to only a small fraction of the fresh weight, a convenient analytical method could be based on the inverse relationship, which exists in ruminants between body fat and water. The aqueous, the fat-soluble, and the non fat-soluble fractions were determined by weighing before and after drying and after extraction.

Several authors (Neiland, 1970; Sinclair & Duncan, 1972; Brooks *et al.,* 1977; Shackleton & Granger, 1989) came to the conclusion that bone marrow fat

content could be conveniently estimated with suitable accuracy and precision by simply drying the sample. In other words bone marrow dry weight expressed as a percentage of its fresh weight is a good indication of its fat content. The dry weight of a sample is the weight of fat and residue in the wet marrow sample. The error in the method can be reduced to a minimum by taking into account the average amount of residual material in marrow samples at various fat concentrations. Thus for maximum accuracy in estimating marrow fat with the dry-weight method, the residue value corresponding to a specific dry weight should be subtracted from that dry weight value to give the correct percentage of fat.

The relationship between the percentage marrow fat (y) and its comparative percentage dry weight (x) is given in Table 1 as linear regression equations from which it would be possible to calculate the percentage marrow fat if the percentage dry weight of the marrow were known.

The slopes of the regression lines are sufficiently close to unity to allow a more general formula of the form $y = x - a$ to be used, where 'a' is a constant for each species, representing the non – fat residue in the marrow. At the present time there is insufficient information to indicate how this changes with the different ruminant species. The purposes for which marrow fat estimates are needed, namely the estimation of condition in wild animals, allow the approximation using the mean of the residue constants, %marrow fat = %dry weight – mean of residue constants for at least the medium and large sized ruminants (Sinclair & Duncan, 1972).

Table 1.1. Regression formulae manifesting the highly significant correlation between the percentage fat content and the percentage dry mass of bone marrow in several species

Species	Formula for regression	r	Reference
Blesbok	Y = 1.13x – 19.15	0.975	Shackleton & Granger (1989)
Eland	Y = 1.05x – 13.30	0.983	
Gemsbok	Y = 1.08x – 19.30	0.993	
Impala	Y = 0.87x + 1.66	0.781	
Red hartebeest	Y = 1.03x – 13.30	0.987	
Blue wildebeest	Y = 1.02x – 12.73	0.957	
Blesbok	Y = 0.99x – 5.04	0.997	Brooks *et al.* (1977)
Buffalo	Y = 1.02x – 5.91	0.992	
Eland	Y = 1.02x – 5.41	0.999	
Impala	Y = 1.03x – 7.14	0.992	
Kudu	Y = 1.06x – 8.35	0.993	
Nyala	Y = 1.05x – 8.33	0.988	
Reedbuck	Y = 1.04x – 10.33	0.991	
Wildebeest	Y = 1.01x – 6.42	0.997	
Kongoni	Y = 1.0488x – 6.9483		Sinclair & Duncan (1972)
Wildebeest	Y = 1.0042x – 7.2829		
Buffalo	Y = 1.0045x – 3.4182		

Extraction procedures are theoretically as accurate as desired, but time consuming for large-scale use. The dry-weight method has the advantage of being as accurate, or nearly so, as the extraction method yet is substantially quicker and less expensive. The dry-weight method is slower than the visual or compression methods, but with experience it can be effectively speeded up (Neiland, 1970).

2.2.5 Back Fat Index (BFI)

The BFI is highly correlated with the kidney fat, the abdominal fat and marrow ratings (Riney, 1955). To measure the back fat a forward cut of approximately 12 in. is made starting at the base of the tail and at an angle of approximately 45 degrees from the spinal column. The greatest depth of fat observed along this cut is then measured to the nearest millimetre.

As back fat is the last to be deposited in prodigious times of the year, and is again the first to be mobilised in times of more severe stress, it points to seasonal limitations of back fat as an index to condition. This restricts its application to the upper part of the theoretical condition scale. Thus during times of under nourishment, back fat disappears and is consequently of little use on a year – round basis or in areas where the levels of nutrition are consistently low.

2.2.6 Abdominal Fat Rating

Riney (1955) described a method whereby stomach, intestines, and kidneys are exposed. A four-choice rating is assigned to each organ, based on the amount of fat obvious at first glance:

0 = No trace of fat obvious on the ventral side of the stomach, small intestine or around the kidney.

1 = Small amounts of fat on the membranes surrounding the above organs

2 = Moderate amounts of fat present and intermediate in quantity between 1 and 3

3 = Fat abundant on the organ in question, the extreme condition being represented by numerous broad, thick bands of fat on the stomach and lower intestine. In prime animals, the kidney is often totally hidden by surrounding fat.

The arbitrary numerical ratings thus obtained on stomach, small intestine, and kidney are combined to make a maximum rating of 9. Different sized animals can thus be rated on a comparable basis. However, the scale is narrow (0 – 9), causing it to be less agreeable as a scale in contrast to, for example, KFI.

2.2.7 Visceral fat

The visceral fat index is obtained by dividing the weight of the visceral fat (grams) by the eviscerated carcass weight (kilograms). Smith (1970) and Bear (1971) concluded that the correlation of visceral fat with total fat follows closely the pattern for KFI with total fat. This method, however, does not lend itself to usage in the field as much time and energy is spent in the separation of fat from the stomach and intestines.

2.2.8 Live weight ratio

The live weight ratio is calculated as live weight (minus uterus weight in females) divided by the hind foot length (*os calcis* to tip of hoof), to correct for skeletal size differences (Smith, 1970). The hind foot length is used as a correction factor as growth of the extremities are affected less by nutritional differences than are more proximal bones. Smith (1970) showed that of 10 species collected the only significant correlation between live weight ratio and total lean occurred in the waterbuck and wildebeest.

2.2.9 Girth measurement

Girth measurement is taken on a carcass, or live animal, within the first 2cm behind the posterior edge of the scapula. As the flesh is usually flabby, it is necessary for reproducible measurement always to hold the tape with the same degree of tautness (Riney, 1955).

In addition to the apparent operator inaccuracy and the necessity to restrain the animal, a further complication in the assessment of condition occurs when comparing the technique with known body fat reserves. As an example, two animals could have identical weight and girth measurements (and would look identical in the field) and yet at the same time they could differ substantially in their deposited fat reserves as demonstrated by KFI and the fat content of the bone marrow. It is only after a further mobilisation of fat reserves that the body weight falls significantly and the girth measurement is reduced (Hanks *et al.*, 1976).

2.2.10 Leg fat

Smith & Ledger (1965) found, in wild African ungulates, that the weight of the leg of a standard dressed carcass bears a constant relationship to the animal's live weight irrespective of the age or degree of fatness. Also the deposition of fat on the legs was in direct proportion to the total amount of fat on the body. Thus by knowing the weight of the leg fat and the relationship it bears with the live weight, the total amount of fat on the body could be calculated.

Smith (1970) found a significant correlation between the leg fat expressed as a percentage of the leg weight and the total body fat. Even though this method is an accurate index of total fat, its main drawback is that it is not as rapid or as easily obtained as, for example, the KFI.

2.2.11 Blood Constituents

Various researchers have examined the possibility of using blood constituents to describe or establish condition in ruminants. Their efforts have met with varying degrees of success. Rosen & Bischoff (1952) reported on the relationship between some blood constituents and condition in California deer. They found no correlation between bodyweight (their criterion of condition) and red blood cells, haemoglobin, or packed cell volume. Stewart, Norden, Wood & Cowan (1964) found that an increase in plasma cholesterol of black-tailed deer (*Odocoileus hemionus columbianus*) was associated with the point of maximum gain in body weight. In female deer there was an initial rise in cholesterol ensued by a decline as weight loss occurred. Males exhibited reduced levels throughout the time of weight loss. Bandy, Kitts, Wood & Cowan (1957) investigating white-tailed deer (*Odocoileus virginianus*) found that fibrinogen increased and blood sugar decreased on low plane nutrition. Taber, White & Smith (1959) suggested the use of serum protein in mg/100 ml serum as a measure of protein reserves in the body. Patterson (1965) associated the occurrence of xanthophyll in the plasma of sheep with the mobilisation of fat depots. He reported that the pigment xanthophyll is connected with the plasma non-esterified fatty acid concentration and appears in the blood after four days of starvation. The appearance of

xanthophyll gives a yellow to green colour to normally clear plasma. Franzmann & Leresche (1978), studying moose (*Alces americana*) in Alaska, found that a combination of packed red cell volume, total plasma proteins, haemoglobin, calcium, and phosphorus levels gave a reasonable indication of body condition. Monro, (1979), studying impala, found no significant correlation between blood constituents and body fat.

Smith (1970) commented on the unfortunate lack of information regarding the short-term effects on blood constituents, brought about by changes in the environment, in free-ranging African ungulates. Further research pertaining to blood constituents, and its correlation with body composition, may provide a rich source of information for describing the condition of these animals.

2.2.12 Adrenal Index

An index which would measure the sum of all factors: nutritional, physiological, behavioural, pathological and others, would be the ideal method of condition estimation. In search of this most descriptive method, Hughes & Mall (1958) and Taber *et al.* (1959) proposed the use of an adrenal index to measure the response of the adrenal cortex to environmental stresses. The adrenal index proposed by Taber *et al.* (1959) was corrected for individual body size by dividing the adrenal weight by the adrenal surface area, as organ weights increase in relation to surface area. Surface area was calculated from whole weight by a formula found in the work of Spector (1956). Hughes & Mall (1958) corrected for the effect of individual size difference by dividing adrenal weight by total length. Smith (1970) used the weight of the kidney as a correction factor, as it is not affected by stress in the same manner as the adrenal gland.

Hughes & Mall (1958), using estimated kidney fat as the measure of condition, found good correlation between adrenal weight and kidney fat, and adrenal weight/body length and kidney fat. They suggest adrenal weight as another index of condition, using it as the most convenient measure of adrenal cortex hypertrophy in response to body stresses. Taber *et al.* (1959) indicated that there was a time lag of several months between environmental stress and its

effects on the adrenal gland's weight. Smith (1970) however found that only in the warthog was there a significant correlation between adrenal weight and total fat. He also attempted to correlate adrenal weight/total body length, and found no evidence for this as an index to condition of the animals studied.

2.2.13 Body Condition Score (BCS)

Jefferies (1961) designed a system to outline body condition in sheep, based on a 6-point scale. Each point was described in terms of the amount of tissue cover over the lumbar region of the spine. Russel *et al.*, (1969) using an adaptation of the system of Jefferies (1961), quantified BCS in Scottish Blackface ewes and showed that it was related closely to the proportion of chemical fat in the body. The system used for sheep by Russel *et al.* (1969), was adapted for use with cattle by Lowmann, Scott & Somerville (1976). The System defines six grades (0 to 5), and describes each one in terms of the amount of tissue cover over the transverse processes of the lumbar vertebrae and around the tail head.

Several authors report on a five-point scale in which animals are ranked from 1 to 5. Emaciated cows are scored 1; thin cows, 2; average cows, 3; fat cows, 4; and obese cows, 5 (Wildman, Jones, Wagner, Boman, Troutt & Lesch, 1982; Otto, Ferguson, Fox & Sniffen, 1991). Often the scale is refined, by using plus or minus signs, in .25 to .5 units, or an augmented nine-point scale to evaluate more subtle changes in body fat than are permitted by unit increments. A BCS is allocated to a cow based on the appearance of tissue cover over the bony protrusions in the back and pelvic regions. Specific regions include the spinous and transverse processes of the lumbar vertebrae (loin), the ileal (hook bone) and ischeal (pin bone) tuberosities, the ileo-sacral and ischeal coccygeal ligaments, the tail head, and the thurl region (or rump). Tissue cover is assessed either by palpation, or by visual inspection, or by both. A single factor may be misleading, however, all factors considered together provide an accurate score.

Wildman *et al.* (1982), outlines the five point body condition scale as follows:

BCS = 1 Individual spinous processes have negligible flesh covering, are conspicuous, the ends are sharp to the touch and together the processes form a definite overhanging shelf effect to the loin region. Individual vertebrae of the chine, loin, and rump regions are prominent and distinct, hooks and pin bones are sharp with limited flesh covering, and exceedingly depressed causing the bone structure of the area to appear extremely sharp.

BCS = 2 Individual spinous processes are visible but are not conspicuous. Ends of processes are sharp to the touch although they have greater flesh covering. The processes do not have a particularly distinct overhanging shelf effect. Individual vertebrae of chine, loin, and rump regions are not visually prominent but are discernible by palpation. Hook and pin bones are prominent, but the depression between them is less severe. The area below the tailhead and between the pin bones is depressed, but the bone structure is not devoid of flesh covering.

BCS = 3 Spinous processes are perceivable by applying slight pressure. Together the processes appear smooth and the overhanging shelf effect is not distinguishable. Vertebrae of the chine, loin, and rump regions are seen as a rounded ridge. The hook and pin bones are rounded and smooth. The area between the pin bones and around the tailhead appears smooth without any sign of fat deposition.

BCS = 4 Individual spinous processes are distinguishable by firm palpation and together, the processes appear flat or rounded with no overhanging shelf effect. The ridge formed by the vertebral column of the chine region is rounded and smooth, but loin and rump regions appear flat. Hook bones are rounded, and the span between the hooks is flat. The area around tailhead and pin bones is rounded, with evidence of subcutaneous fat deposition.

BCS = 5 Bone structure of the vertebral column, the spinous processes, and the hook and pin bone regions are not visually apparent. Evidence of subcutaneous fat deposition is prominent. The tail head appears to be buried in fatty tissue.

BCS is a non-invasive means of estimating fat stores in animals independent of frame size and body weight. Despite the fact that it is a subjective measure of body condition, it offers a very inexpensive method of assessing body composition with an acceptable degree of precision.

Wright & Russel (1984a) found that each of the four components, water, fat, protein and ash, and body energy were related to BCS. They concluded, in another paper (Wright & Russel, 1984b), that the precision of prediction of body composition from condition score compares favourably with other *in vivo* techniques used for estimating body composition. Otto *et al.* (1991), found BCS to be highly correlated with composition of the 9^{th} to 11^{th} rib section in cattle for dry matter (DM)(r^2 = 0.69), crude protein (CP) (r^2 = 0.61), ether extract (EE) (r^2 = 0.57) and ash content.

One problem associated with BCS is that there has been no standard system communicated among cattle producers, researchers, extension personnel and industry consultants. Condition scores have been expressed on a 17-point system (Gresham, Holloway, Butts & McCurley, 1986), a 9-point system (Whitman, 1975; Wagner, 1984) and a 5-point system (Lemenager, Nelson & Hendrix, 1980). Data compiled and published by Herd & Sprott (1986) indicated that percentage empty body lipid for BCS 1 through 9 was 0, 4, 8, 12, 16, 20, 24, 28 and 32, respectively. Data presented by Houghton, Lemenager, Moss & Hendrix (1990), indicated that percentage of empty body lipid for BCS 1 through 5 was 3.1, 8.7, 14.9, 21.5 and 27.2 respectively. These data suggest that condition scores 1, 3 and 5 on a 5-point system would be similar to condition scores 2, 5 and 8, respectively, on a 9-point system. Standardisation between operators is, therefore, essential to reduce variation.

Condition score assesses only subcutaneous fat cover (Wright & Russel 1984a). Thus variation in partitioning of fat among the main adipose tissue depots (subcutaneous, intermuscular and intramuscular, and abdominal) might be expected to influence the relationship between condition score and body fat. The outcome being that breeds differing in the partitioning of fat among the various depots will differ in the proportion of total body fat at the same condition

score. Wright & Russel (1984a+b) found that the proportion of fat contained in the major depots, however, remained relatively constant regardless of BCS, thus it probably correlates well with total body fat content.

BCS at lower ends of the scale (BCS = 1 to 2) may reflect changes in tissue water in addition to changes in CP and EE (Otto *et al.,* 1991). This provides evidence not only of fat depletion, but also of depletion of muscle in cows, with low BCS.

2.2.14 Weight to Height Ratio (WHR)

Weight to height ratio (WHR) has also been used as a predictor of mature cow body composition and is less subjective than BCS. The WHR is computed by dividing the weight of the animal with its hip height. Klosterman, Sanford & Parker (1968) and Nelsen *et al.* (1985) point out, however, that WHR is only dependable within a breed or biological type and only if the animals have a common nutritional background. Similar results were reported by Dunn *et al.* (1983), who observed that WHR was less significant as a forecaster of body composition (r = .56) than visual and palpated BCS (r = .86) were when a wide range of biological types with different nutritional histories were considered.

Houghton *et al.* (1990), using regression equations, concluded that BCS plus weight was a better predictor (r = .70 to .74) of percentage carcass lipid and total empty body lipid than were weight, weight height ratio or a combination of these factors. However, weight, weight transformations, WHR and BCS also were practical in predicting fat in the carcass and empty body (r = .62 to .70).

BCSs, even though they are subjective, can identify relative differences in cow body composition when a single person assigns condition scores to animals within a herd. Also, BCS has advantages over other body composition prediction methods such as WHR in that 1) cows do not need to be restrained, 2) no special equipment is needed and 3) evaluations can be made frequently. Visual BCS has been shown to be a reasonably good predictor of carcass and empty body lipid content when used in combination with weight (Houghton *et al.,* 1990).

2.3 Alternative Measures of Body Condition Estimation

Apart from considering the methods discussed above, any measurement, which might increase or decrease with changing condition could also be used if it were divided by any measurement which remained reasonably constant. Thus in the live individual, body mass and heart girth are changing parameters (Nelsen, Short, Reynolds & Urick, 1985). These could be divided by head length, metacarpal length, hind foot length, body length or shoulder height. In the slaughtered animal, carcass weight, buttock weight or buttock circumference could be divided by carcass length, metacarpal length, hind foot length or buttock length to give an index of condition (Monro, 1979).

Another method suggested by Monro (1979), which was in fairly good agreement with the assessment of body condition, was body fat divided by ash multiplied by 100, where fat and ash were both expressed as percentages of dry weight. As most indices have been used without defining what condition is in terms of body composition, some influence was given to non-fat reserves, which may play an important role in wild ungulates. Fat was therefore still the most important element, but the total amount of organic matter also played a role in determining condition.

To gauge the nutritional status of wild ungulates, Bandy, Cowan, Kitts, & Wood (1956), suggested a technique, which is based on the relationship of actual body weight to heart girth and body weight to hindfoot length. Assuming that the hindfoot length is little affected by nutritional status they estimated an optimum or ideal weight by a regression formula. The formula was derived by measuring increases in the length of hindfoot and body weight of penned black-tailed deer raised on a high plane nutritional diet. An estimated body weight based on the heart girth/body weight relationship (which is affected by nutritional status of the animal) was calculated and the ratio of this estimated weight and the ideal weight was used as an index of condition.

2.3.1 Probes To Measure Tissue Thickness

In pigs nearly 80% of the total carcass fat is located in the subcutaneous depot. Therefore an accurate method of determining subcutaneous fat thickness should provide a good prediction of total carcass fatness (Jones & Haworth, 1982). With cattle there is the problem of hide thickness and with sheep there are animal variations of fat depth (Kempster, Chadwick, Cue, & Grantley-Smith, 1984). Nevertheless probes are actively used on lamb and beef carcasses and form the basis of objective classification schemes in many countries (Jones & Haworth, 1982). In wildlife these methods have not yet to our knowledge been used.

2.3.2 The Back Fat Probe

To measure the actual fat depth, a small incision is made in the skin with a scalpel and a narrow ruler is forced through the fat layers or a ruler containing a needle point is forced directly through the skin and fat layers. Since nerve and vascular supplies in the skin and subcutaneous fat layers are minimal, pain and bleeding are not much of a problem in the live animal. The measuring device must penetrate the false lean or aponeurosis (sheet of fascial connective tissue separating the outer and middle layers of subcutaneous fat) and continue until there is a second resistance due to the epimysial connective tissue covering the muscle. The depth is usually verified and recorded (Kempster *et al.*, 1984)

Once the fat depth is known, it can be used in a previously developed regression equation with other variables such as live weight and muscling score to estimate composition. Fat depth alone usually accounts for most of the variation in composition but live weight and degree of muscling should improve the accuracy of the measurement (Fahey, Schaefer, Kauffman, Epley, Gould, Romans, Smith, & Topel, 1977).

The major advantages of this method are that it affords a reasonably accurate prediction of composition, it is easy to standardise, it makes a rapid measurement and it is inexpensive. On the other hand it requires that the animal

be restrained and it is too slow if a large number of animals or carcasses are involved (Fahey *et al*, 1977)

2.3.3 Reflectance (Optical) Probe

The optical probe (OP) or intrascope has a light source near its tip. Light is emitted through a lens carrying a clearly marked line at right angles to the probe shaft. The operator, by means of an internal mirror system, can observe this line through the top of the probe, adjacent to the handle. A graduated sliding barrel indicates the probe depth when the operator judges the line to coincide with the fat/lean interface. This is effectively a manually operated optical ruler.

The first automated detection of the tissue boundaries was based on the differential electrical conductivity of fat and lean tissues. This was the Danish probe, the KS meter (K= kod/meat; S= speak/fat) Fisher (1990). The automated version of this probe the KSA, sometimes referred to as the MFA (meat-fat automatic) in English texts, was based on a system where the electronic signals from the probe were interfaced to a microcomputer. The microcomputer calculated the lean meat percentage from fat and "meat" thickness based on data involving carcass dissections.

Other probe systems for estimating lean percentage via tissue thickness are based on the optical properties of lean and fat tissues. These are known as light reflectance probes and they may utilise visible light or light in the near infrared part of the spectrum. The mode of actions relies upon the fact that light emitted from an LED near the top of the probe is reflected in different ways by the different tissues. Thus with accurate measurement of the signal relative to the depth penetrated, the thickness of the fat at the predetermined position can be measured. Distance from the skin surface (depth) is measured by means of a spring loaded base plate which can move in relation to the probe but which always makes contact with the skin/carcass surface during operation.

The first probes to utilise this principle were the Ulster Probe (UP) and the fat depth indicator (FDI). Three more recent versions of the automatic light probes are the Hennessy Grading Probe (HGP) (Kempster, Chadwick, Jones, &

Cuthbertson, 1981; Kutsley, Murphey, Smith, Savell, Stiffler & Terrell, 1982; Fortin, Jones & Howorth, 1984; Kirton, Feist, Duganzich, Jordan, O`Donnel & Woods, 1987), which uses light in the green-yellow range (=570nm) The Fat-O-Meter (FOM) (Kempster *et al.*, 1981; Fortin *et al.*, 1984), which uses light in the near-infrared range (=915nm) and the Destron Probe (DST) which uses light in the near-infrared range. The FOM differs from the other two in that electronic signals are transmitted along a cable connecting the probe to a microprocessor, keyboard and display. In the HGP and DST, the microprocessor is an integral part of the probe and the relevant information, (tissue depth, lean percent) are displayed on the probes themselves. The DST also has a keyboard in the probe body.

2.3.4 Ultrasound Techniques

Ultrasound waves are sound waves with frequencies above the range of the human ear (Simm, 1983). The sound waves can be propagated through solids, liquids and gasses and behave in a similar fashion to light waves in that they displayed both refraction and reflection at boundaries between substances of different acoustic density (Terry, Savell, Recio, & Cross, 1989). The major tissues and organs of the body have characteristically different acoustic densities and there are at least three techniques, which utilise this principle: Pulse - echo ultrasound, velocity of sound (VOS) and real time imaging ultrasound (RTU) (Fortin, 1980; Simm, 1983).

2.3.5 Pulse-Echo Ultrasound Techniques

A pulse generator sends electrical pulses that are converted into sound (ultrasound) signals in the transmitter. These signals are then passed through the tissues until they are reflected at an interface (Fortin, 1980). The reflected signals are picked up by a receiver and can be amplified and shown in a visual form by an oscilloscope (Tong & Malcolm, 2002).

The A-mode ultrasonic machines display echo amplitude against time, which is shown on the screen as peaks super imposed on a time base line. The

distance between the peaks represents the thickness of the tissues being measured. The A-mode machines, initially developed for medical diagnostic purposes, were used to take leaner fat depth measurements, and are still in use today (Tong & Malcolm, 2002).

For the B-mode machines, the signals are shown on a cathode ray tube as a series of bright spots. The distance between successive bright spots represents the thickness of the tissue. These machines were developed to produce two-dimensional scans either by movement of the probe along a curved track (as in the scanogram) or by firing an array of transducers in sequence (as in the Danscanner). From these two-dimensional scans, eye muscle and fat areas in addition to leaner fat depth may be calculated (Alliston, Barker, Kempster, & Arnall, 1981).

2.3.6 Velocity of Sound

Miles & Fursey (1974) reported an ultrasound technique, which overcomes some of the problems in applying pulse echo techniques to predicting carcass compositional trades in live sheep and cattle. Compared with pigs, sheep and cattle deposit relatively less of the fat subcutaneously and relatively more in the intramuscular fat depot. This is probably the main reason for the lower correlations found in sheep and cattle between subcutaneous fat depths and area measurements and carcass lean or fat content. Correlations can be improved by using the inter- and intramuscular depots in addition to the subcutaneous fat by propagating a wave of ultrasound completely through the body and measuring its velocity. The principle described by Miles, Fursey & York (1984), is that ultrasound waves travel slower through adipose tissue than through lean. The time taken to travel a known distance through a mixture of lean and fat is therefore related to the proportions of the two tissues in the line of flight.

The equipment consists of a transmitter and receiver held facing each other by a steel frame. The distance between the two is adjustable to accommodate various animal sizes. Having selected a site where the passage

of the wage will be unimpeded by bone an ultrasound pulse is propagated and the time taken to travel a known distance between the two transducers is recorded electronically and the reciprocal of the velocity is computed and displayed. The precision of this technique was compared by a visual assessment of fatness in the carcass as a predictor of the composition by McKenzie, Rafferty & Beckett (1998). Measurement of the speed of ultrasound at two locations in the hind limb was almost as good a predictor of carcass composition as the mean fat score given by two experienced judges, and was more highly correlated with total fat content of the carcass than was the mean of three fat depths taken on the carcass over *M. Longissimus dorsi* (r=0.60).

In another study measurements of the speed of ultrasound at two sites gave an RSD for adipose tissue proportion in the side comparable to that achieved with a scanogram pulse echo technique (0.0227 compared to 0.0223) and a further significant reduction in the RSD was achieved when the two were used together to predict fatness (Miles, Fursey & Pomeroy, 1983b). The speed of ultrasound through hind leg gave as precise a prediction of carcass lean weight and almost as precise a prediction of carcass lean percentage as two B-mode real time scanners (technicare and vetscan).

Despite its apparent benefits, it is yet to receive much acceptance in the abattoir, because of its cost (approximately double that of the probe) with no significant increase in performance. It is prone to error due to sight location although in the hands of a skilled operator, it is a very effective instrument. To achieve optimum results, the manually operated ultrasound technique takes a considerable period of time, 30 to 60 seconds, to accurately locate the site, activate the device and take the readings. Good coupling, between the device and the surface of the animal, is also required for the system to work efficiently. Warm soft tissues at the surface of the lambs are mobile and this may interfere with coupling. Also air pockets in the surface fat layers of lambs and cattle may impair coupling (Miles *et al.*, 1983b).

2.3.7 Real Time Imaging Ultrasound (RTU)

Real time machines produce a practically instantaneous picture by rapid electronic switching from element to element. The principle involved is similar to that described in pulse echo ultrasound, except that movement of the tissue can be seen because of the continuous nature of the picture. The Dan scanner that was already referred to under B-mode scanners is an example of a real time imaging ultrasound system (RTU).

RTU creates cross-sectional and longitudinal images at various carcass/body locations to facilitate the measurement of fat depth, muscle depth, or muscle area without cutting the carcass (Forrest, 1995). The interpretation of results for B-mode and RTU machines usually requires the tracing of depths and areas of pictures. The disadvantages of ultrasound imaging include the skill required to obtain a good image, the skill required to interpret and measure the image and the speed with which all of this can be accomplished in an on line situation. There are, however, microprocessors for computers, which may facilitate interpretation, and light pen and mouse techniques for establishing area measurements, which should speed up the process, although no fully automatic systems, are commercially available yet. It has been stated, "the human is still the best interpreter of current ultrasound images" (Forrest, 1995).

Miller, Cross, Smith, Baker, Beyers & Recio (1986) and Recio, Savell, Cross & Harris (1986) showed that RTU measurements obtained by very experienced operators could accurately predict carcass composition traits. The RTU live measures of *M. Longissimus dorsi* area, 12th rib, and shoulder fat thickness were significantly correlated to comparable carcass measurements. Adjusted fat thickness was the single most useful carcass measurement for predicting percentage carcass fat.

2.3.8 Electrical Methods

2.3.8.1 Electromagnetic Scanning

Electromagnetic scanning often referred to as TOBEC (total body electrical conductivity) is a method of compositional analysis by means of an

instrument called the electronic meat measuring equipment (EMME). This equipment measures the fat and lean content in live pigs (model SA-1). EMME SA1 was later modified for the measurement of packaged, boxed and boneless meat. The EMME/TOBEC HA-1 also obtained in-vivo measurements in humans, which is the prototype to the new HA-2 (Domermuth, Veum, Alexander, Hedrick, Clark, & Eklund, 1976).

The theoretical basis of the method is that conductivity of electricity through lean tissue or the fat free body (FFB) exceeds that through fat tissue by about 20 fold (Lenkins, Leymaster & Turbington, 1988). This is due to the high water electrolyte content found in the tissue and extra cellular water making up the FFB. Current flow induced in a biological system is a function of conductive and dielectric properties. The conductive properties are related to the intra- and extracellular ionic content, and the dielectric effect is associated primarily with capacitance related to cell membranes. Impedance to current flow in the system revolves in an irreversible loss of energy as heat. This energy is related to the conductive mass. The dielectric or capacitance properties of current flow represent the reactive part of impedance in which energy transfer is reversible due to temporary storage of electrical energy. Capacitance is partly determined by the geometry of the conductor, which may produce an effect whereby capacitance increases as cross-sectional area, length, or both, increase. While theoretically both electrical properties define the flow of current in a conductive mass, the conductive properties appear to exert a more dominant effect in estimating FFB mass (Lenkins *et al.*, 1988).

Electromagnetic scanners consists of a Cu wire, solenoid coiled around a glass fibre (Plexiglas) tube which forms a scanning chamber large enough to accommodate pork and lamb carcasses or beef hind quarters. When current is applied it induces an electromagnetic field in which the body is statically situated (HA-1) or scanned (HA-2). The conducting mass passing through the electromagnetic field absorbs heat energy, thereby perturbing the electrical field of the coil. The loss of energy detected in the coil is an index of the conductive mass of the body. The oscillating current frequency applied to the coil is an

important aspect of the measurement, since the degree of separation in the conductivities of FFB and fat is frequency dependent. This first TOBEC model (HA-1) used 5-Mhz oscillating coil current and required a 0.5 second measurement on a statically situated carcass/body. The new TOBEC HA-2 instrument uses 2.5-Mhz and subjects move through the coil at a constant rate. It requires about 40 seconds for one measurement. The change in coil energy as the body moves through the length of the coil is detected as change in conductance and capacitance relative to an empty coil. The measured conductance and capacitance of the conductor (subject) is reflected in a phased angle/distance curve. The area under the curve is an index of total body conductivity (Domermuth *et al.*, 1976)

The precision of TOBEC appears to be excellent relative to that of other techniques for the assessment of body composition. Domermuth *et al.* (1976) measured 12 pigs 14 times a day for two days and found an average coefficient of variation (CV) among the animals of 4 percent. Bracco, Yang, Segal, Hashim, & Van Itallie (1983) observed a high association between TOBEC values and 30 live rats and FFB estimated by densitrometry and by chemical analysis of the carcass. High linear correlations were also observed between TOBEC and total protein (r=0.95) and total body water (r=0.98) (Bracco *et al.*, 1983)

Domermuth *et al.* (1976) reported on the relationship between TOBEC and other body composition estimation methods including total potassium and carcass analysis in pigs. Two experiments were conducted, one with 42 pigs and the other with 35 pigs, in which the animals were fasted for 16 to 18 hours to obtain a empty body weight before being measured by TOBEC and total potassium. The animals were killed and their carcasses analysed for specific gravity and fat, water, and protein content. The linear correlations between live animal TOBEC readings and potassium measurements were 0.75 and 0.81 for experiments 1 and 2 respectively.

Electromagnetic scanning of the full pork carcass side under laboratory conditions accurately measured lean mass in the carcass (r=0.90 RSD = 1.64

kg), dissected lean meat percentage (r=0.70, RSD=2.38 %), and lean mass within individual primal cuts from full carcass scans (Domermuth *et al.*, 1976).

Electromagnetic scanning can be applied to uneviscerated warm carcasses, eviscerated warm carcasses, or chilled carcass sides with comparable accuracy. However, scanning warm, pre-rigor, whole carcasses may be the most practical procedure.

In a study where beef hind- and forequarters were scanned separately, the correlation for predicted dissected lean weight in the hind quarter ranged from 0.91 to 0.94, with RSD=1.6 to 1.3 kg. Prediction of dissected lean in the forequarter ranged from r=0.86 to r=0.91 with RSD=2.27 to 1.81 kg. Full beef carcasses could be scanned if the chamber size were enlarged. In a study in which 22 lamb carcasses were scanned to predict total lean in the pre-rigor carcass resulted in a correlation of 0.98 and a RDS of 0.35 kg, with warm carcass weight, carcass length, and 1 TOBEC reading as the independent variables.

The system suffers from a number of practical drawbacks. It is considerably more expensive compared to probes and is relatively slow, even the current fastest throughput speed would limit carcass numbers to approximately 200 to 250 carcasses per hour. The system also requires the object to pass through in a horizontal position, whereas most abattoirs are built around a vertical hanging system. The technique gives no indication of fat depths, which for practical purposes could be important in determining individual carcass applications. Each EMME machine also has to be calibrated and a formula established for this specific machine. Temperature and humidity can also influence the results. The optimum application of this technology requires constant sample positioning, constant sample temperature, a constant feed through the system and calibration that takes account of sample geometry (Domermuth *et al.*, 1976).

2.3.8.2 Bioelectrical Impedance Analysis (BIA)

Bioelectrical impedance analysis (BIA) operates on the same principle as Electromagnetic Scanning in that there is a difference in conductivity between fat tissue and the fat free mass/body (FFB). As carcass fatness increases, the impedance to the flow of electricity increases. The current is applied through an array of electrodes placed on the surface of the body.

BIA has been shown to be highly accurate in predicting the fat free soft tissue content of lamb carcasses (Swantek, Marchello, Crenshaw, Lukaski, & Lewis, 1989). Fat free mass in warm lamb carcasses was predicted using warm carcass weight, carcass length, resistance, reactance and carcass temperature. The best four variable equation utilising BIA measurements on chilled carcasses included warm carcass weight, carcass length, and two bioelectrical impedance parameters (Forrest, 1995).

When a single fat depth measurement was placed in a four variable equation with warm carcass weight and two bioelectrical impedance parameters, the precision of prediction increased while the RSD decreased. This result suggests that, where practical, combinations of technologies could/should be explored. The relatively low cost of bioelectrical impedance instruments makes it a good candidate for combination with optical probe or ultrasound systems (Forrest, 1995).

2.3.8.3 X-Ray Computed Tomography (CT)

CT makes use of the differential that exists between the rates at which the tissues of the body attenuate x-rays, in much the same way as conventional film based radiography. In CT however, a two-dimensional image is produced by a 360-degree rotation of an x-ray source around the body or carcass of the animal. An arc-array of highly sensitive detectors measures the attenuation of the radiation beam as they rotate in synchrony with the source. During the rotation pulses of radiation are fired at discrete intervals (usually every degree or half degree of rotation) and each detector signals to the computer the amount of radiation received. The computer processes the data from the large number of

crossing pathways to produce a matrix of attenuation values for the target body. The matrix is displayed as an image on a monitor. By this technique, the density (CT number) of different body tissues at different distances from the x-ray source can be calculated (Groeneveld, Kallweit, Hemming, & Pfau, 1984).

All available machines have been designed for use in human diagnostics. The patient is laid on a table, which is then moved through an aperture housing the radiation source and detectors. The size of the aperture restricts the technique to small-to-medium sized animals such as poultry, goats, sheep and pigs. Even if the financial incentives existed for a larger purpose built machine to scan cattle there are technical barriers to such a scaling-up. As it is important to reduce movement to a bare minimum during scanning, all animals have to be restrained in a cradle, and pigs must be anaesthetised, thereby reducing the speed of the throughput. A typical scanning procedure begins by transporting the animal through the aperture with the x-ray source stationary, but firing constantly. This results in a topogram, a longitudinal image of the body in which the skeleton is readily identifiable. By this method anatomical locations for tomograms (slices through the body) may be located precisely. Once a procedure has been established, requiring for example two tomograms, 20-30 sheep or 10-15 pigs per hour could be scanned. The amount of various tissues (muscle, bone, fat and water) can be predicted from these cross sections (Allen, & Vagen, 1984).

Radiation is, of course highly dangerous, but since the equipment has been designed for use on humans the dose levels are low and can be considered harmless even for breeding animals. Operators are protected, by having the operating console in a room separate from the lead-shielded room housing the source (Groeneveld *et al.*, 1984).

2.3.8.4 Nuclear Magnetic Resonance Imaging (NMR) and Spectroscopy

The NMR method for estimating body composition is based on a strong static magnetic field and pulsed radio waves that induce resonance of protons in the tissues of the measured carcass or living body (Allen, 1990). When the

subject is placed in the strong magnetic field, atomic nuclei with an odd number of protons and/or neutrons - the hydrogen atom with its single proton being the most common of these in the body - align with the field and spin at resonant frequencies determined by the type of nuclei and the field strength (Mitchell, Wang & Elsasser, 1987). The electromagnetic signals emitted from the body, yield information on the concentration and distribution of these nuclei. The signals are produced as a reaction of the body to the high-frequency disturbance, and are therefore a product of the matter itself. The intensities of the signals will depend on the proton spin densities and the molecular structure of the tissue (Fuller, Foster & Hutchison, 1984).

In NMR systems a strong magnetic field is produced by a large annular electromagnet or superconducting electromagnet with an aperture large enough for the subject to pass through. A secondary changing field is superimposed on the main field by electric currents passing through coils near the subject. The strength and orientation of the magnetism is changed in a regular pattern in order to map the locations of the spinning nuclei in the desired plane, cross-sectional, longitudinal, transverse, or oblique. A full cross-sectional image can be made on any plane in the carcass or live animal. Discrimination between muscle and adipose tissue results from their different proton densities (Fuller, Foster & Hutchison, 1984).

As the superimposed changing tilts the magnetic field the angular momentum of the protons delays the return of the field to its equilibrium position. The delay is known as relaxation of which two components can be measured. The “spin-lattice” time (T1) is the longitudinal component due to the interaction of the nuclear spin system with the surrounding “lattice”. The transverse component due to the interactions of neighbouring spins is known as the “spin-spin” relaxation time (T2). The magnitude of these components depends on the chemical structure of the tissue, in particular the relative amounts of water and triglyceride. The high water content of muscle results in a moderate relaxation time (T1) whereas high triglyceride content gives fatty tissue a shorter T1 value. A range of images may be formed by combining the T1 and T2 measurements

with proton density information, and with different weightings given to the relaxation times (Mitchell *et al.*, 1987).

A few seconds are required to produce an image with NMR, so that the technique is relatively "immune" to movement. As with x-ray CT, however, animals would have to be anaesthetised and strapped to some form of cradle or table. Unlike x-ray CT, there are no moving parts with NMR and the technique does not use ionising radiation. There is no known health hazard from the magnetic fields of the strength employed, except to human patients with certain types of cardiac pacemaker. The equipment is very expensive and the method is very complex; its future will depend on the amount of resources available for its development as an agricultural tool (Mitchell *et al.*, 1987).

2.4 Comparison and Selection of Techniques

There are several problems in making definitive statements about the relative merits of the techniques studied to predict body composition. Firstly, only a small number of trials were studied where a range of techniques has been compared on the same group of animals. Secondly, in many trials relatively complex methods have not even been compared with simple, readily available indicators of composition such as live weight, sex and growth rate. Many trials, particularly those involving new techniques, have been carried out using animals with much wider variation in age, weight and composition than would be the case in practice. Making comparisons between techniques across different studies is also complicated by factors such as differences in the experience of operators, the choice of dependant variables, the size and variability of the sample and the presentation of the results (Fahey *et al.*, 1977).

The main criteria for selection of a technique are cost, practicability, precision and accuracy. Total cost includes running, as well as capital costs. Practicability includes factors such as mobility, physical requirements such as size, power supply, shielding (both operators and animal/carcass), simplicity of operation and the speed of throughput. Public acceptability is also included in the list. Precision refers to the RSD of the predicted characteristic and accuracy is

the lack of bias in predicting carcass characteristics, when different groups of animals are to be evaluated by different investigators (Fahey *et al.*, 1977). The data thus obtained must be of comparable accuracy in order to validate comparisons of populations from different areas and at different times. The various techniques are listed below, and "scored" for the important criteria.

Table 1.2. Summary of techniques to determine body composition in animals

Method	Cost	Portability	Simplicity	Potential precision	Species availability	Potential application
Ultrasonic scanning	4	5	4	4	C,P,S	F,M,B,E
VOS	5	5	5	3	C,P,S	F,M,B,E
X-Ray CT	1	1	1	5	P,S	B,E
NMR	1	1	1	5	P,S	B,E
Electrical conductivity	2	2	2	3	P,S	B,E
Probes	6	6	6	4	C,P,S	F,M,B,E
KFI	6	6	6	2	C,P,S	F,M,B,E
BMI	6	5	6	2	C,P,S	F,M,B,E
Back fat	6	6	6	2	C,P,S	F,M,B,E
Abdominal fat	6	6	6	2	C,P,S	F,M,B,E
Visceral fat	6	6	6	2	C,P,S	F,M,B,E
Live weight ratio	6	6	6	1	C,P,S	F,M,B,E
Girth	6	6	6	1	C,P,S	F,M,B,E
Leg fat	6	6	6	1	C,P,S	B,E
Blood	6	2	5	1	C,P,S	B,E
Adrenal index	6	6	6	1	C,P,S	B,E
BCS	6	6	6	2	C,P,S	F,M,B,E
Weight height ratio	6	6	6	1	C,P,S	F,M,B,E

Scores: 1=least favourable, 6=most favourable
F= on farm; M= market and slaughter houses; B= breeding programs;
E=experimental stations
VOS = Velocity of Sound
X-Ray CT = X-Ray Computed Tomography
NMR = Nuclear Magnetic Resonance Imaging and Spectroscopy
KFI = Kidney Fat Index
BMI = Bone Marrow index
BCS = Body Condition Score (Fahey *et al.*, 1977; Eveleigh, Thwaites, Hassab, Paton, Smith, & Upton, 1985)

Table 1.3. The advantages and disadvantages of different methods of body composition estimation

METHOD	ADVANTAGES	DISADVANTAGES
KFI	Enables different sized animals to be compared on a uniform basis. Measurement over a wide range.	Does not measure complete range of condition.
BMI	Condition assessment can be done on animals dead several weeks or months.	Poor for measuring changes in the upper parts of the condition scale.
Back fat	Changes in condition can be detected in top condition animals.	Different sized animals not placed on a comparable basis. Measurement of condition restricted seasonally due to inability to measure the lower part of the condition scale. The scale has a narrow range
Abdominal fat	Measures entire range of condition. Rates different sized animals on a comparable basis.	Scale is narrow. Rating is subjective.
Visceral fat	Simple affordable objective rating.	Time and energy consuming.
Live weight ratio	Affordable objective rating.	Animals need to be restrained
Girth	Simple and affordable.	Animals need to be restrained More a measure of size than of condition.
Leg fat	Accurate measure of condition.	Time consuming.
Blood	Could prove to be ingenious in condition estimation	Lack of information pertaining to blood constituents compared with condition.
Adrenal index	Convenient measure of adrenal cortex hypertrophy.	Time lag between environmental stress and its effect on adrenal gland.
BCS	Animals do not need to be restrained. Non invasive, easily	Subjective estimate of condition. No standard system has

	obtainable estimate of fat stores, allowing for frequent evaluation.	been devised.
Weight height ratio	Affordable objective rating.	Only dependable within breed or biological type.
Probes	Affordable, easy to standardise Reasonably accurate, rapid.	Animals need to be restrained Too slow for large numbers of animals
Ultrasonics	Relatively affordable, portable Simple to use, high precision	Need direct contact with sample surface through sound conducting medium Need efficient operators for max. Contact Need interpretation of images Needs 30-60 sec. For reading
VOS	Affordable, simple, precise	Bone interferes with measurement All disadvantages listed for ultrasonics
X-Ray CT	High precision	High cost, slow throughput, animals need to be anaesthetised.
NMR	High precision	High cost, slow throughput, complex, animals need to be anaesthetised.
Electrical conductivity	Relatively precise	High cost, constant sample positioning, constant temperature required.

KFI = Kidney Fat Index
BMI = Bone Marrow index
BCS = Body Condition Score
VOS = Velocity of Sound
X-Ray CT = X-Ray Computed Tomography
NMR = Nuclear Magnetic Resonance Imaging and Spectroscopy
(Fahey *et al.*, 1977; Eveleigh *et al.*, 1985)

ADDENDUM
CV = Variance / Population average
r = Covariance of x and y / (variation of x *variation of y)
SD = √ Variance
RSD = Standard deviation after treatment effects have been removed.

CHAPTER 3

MINERALS AND THE IMMUNE SYSTEM

3.1 Introduction

The body's defence mechanism is a function of the mineral status. Optimum nutrition assures optimum tissue integrity, an increased immune response, and other benefits, which alleviate stress. Erosive nutritional imbalances wear out livestock, which then succumb easily to challenges of an otherwise manageable nature.

Skin, its appendages, and the mucous membranes are an effective first line of defence. Adequate protein levels and amino-acid balance in the diet are essential for an intact epithelium. Antibody synthesis and phagocyte activity are, in turn, dependent on several vitamins and minerals, which serve as a second line of defence against infectious agents.

Immuno-competence is the resistance to infectious agents, foreign particles, cells, toxins and neopalastic cells, which ensures the survival of the host. Deficiencies within the host may contribute towards an inadequate response to the above challenges.

Many trace elements influence the immune system of the body (Boyazoglu, 1997), and although common deficiency signs are seen in deficient animals, marginal deficiencies could affect the immune system, without showing any signs of deficiency. It therefore seems that to ensure an adequate defence system, dietary requirements of some minerals are much higher than the levels prescribed in conventional recommendations. This emphasises the importance of preventing trace element deficiencies in the diet.

Some trace elements form part of the anti-oxidant defence capacity of the body to combat reactive oxygen and other free radicals in the body. Laboratory methods have been developed to measure the possible magnitude of the reactive oxygens in the body. Based on enhanced immunocompetence at adequate trace element intakes, a technique has been developed to challenge

the body with a pathogen and measure at what level of mineral intake no further increase in antibody titre production occurs. This would signify an adequate trace mineral intake (Van Ryssen, 1997).

3.2 Free radicals, antioxidants and immune function

Ordinarily antioxidant defences, which are available intra- and extracellularly are adequate to safeguard against oxidative damage. The balance can however be tipped as a result of overproduction of free radicals or by exposure to sources which overwhelm the antioxidant defences (Bendich, 1990). An active immune reaction stimulates the manifold production of cells, proteins and hormones, which necessitates an immense supply of energy. Metabolic pathways supplying this energy lead to the production of reactive oxygen species. In addition phagocytic cells produce free radicals in the respiratory burst to invalidate foreign organisms (Nockels, 1996).

a. Diet

Individual components in the diet can alter the proportions of free radical production in the body. High levels of dietary polyunsaturated fatty acids (PUFA) can be immuno suppressive. The unsaturated double bonds found in these PUFA are primary targets for free radical damage with consequent chain reactions that lead to the formation of lipid peroxides. Lipid peroxides and aldehides can impede normal cellular function and even lead to the breakdown of oxidised cell membranes. Lipoproteins in the plasma can also be oxidised and the resultant molecules are lymphotoxic (Bendich, 1990)

The fluidity of cellular membranes is partially dependent on the degree of unsaturation of its fatty acids. As the proportion of PUFA increases, so the potential for membrane lipid peroxidation also increases. Lipid peroxidation results in forfeiture of membrane fluidity. Metabolites of lipid peroxidation can furthermore influence immune reaction. Reduced membrane fluidity is directly coupled to a lowered ability of the lymphocyte to react to an immunological challenge (Bendich, 1990).

b. Stress

According to Nockels (1996), stress is often a precursor to infection in animals. Throughout a stress period free radicals are produced during corticoid synthesis. Superoxide radicals are produced during the reactions between catecolamines and oxygen. The resultant depletion of antioxidant reserves impedes an effective immune reaction.

3.2.1 Leukocyte usage of free radicals

Individual cells of the immune system employ free radicals for the destruction of intrusive pathogens. The two main types of leukocytes, which produce reactive oxygen intermediates, are neutrophills and macrophages (Bendich, 1990). The respiratory burst associated with phagocytosis by leukocytes produces $O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, ClO^- and possibly singlet oxygen. These free radicals damage the cell walls of target organisms (Hill, 1981).

3.2.1.1 Neutrophylls

Neutrophylls are the most common circulating phagocytic cells in the body. These cells are attracted by chemical signals produced at regions of infection and move in the direction of the signal (chemotaxis). Stimulated neutrophills have the ability to appropriate molecular oxygen and produce reactive oxygen containing molecules (respiratory burst). Free radicals and singlet oxygen, in addition to other reactive molecules, can directly invalidate bacterial pathogens or can react with granulocyte specific enzymes to produce highly toxic compounds (Bendich, 1990). These reactive molecules can however cause mutations, can break down normal cells like other neutrophylls, erythrocytes and thrombocytes, can cause inflammation of surrounding tissue, inactivate protected enzymes and inhibit lymphocyte proliferation. Lymphocytes are however guarded against neutrophyll oxidative damage by monocytes since they contain catalase. Neutrophylls are also capable of breaking down tumour cells (Bendich, 1990).

3.2.1.2 Macrophage

Where neutrophills are primarily associated with acute inflammatory reactions, macrophages are associated with chronic inflammation. In contrast to neutrophylls, macrophages are central to the development of specific immune reactions. They process antigens for presentation to lymphocytes. Both these phagocytic cells posses the cell membrane associated NADPH oxidation system, which allows the synthesis and secretion of reactive oxygen species. Prostaglandines, leucotrines, interleukin 1 and interferons are also produced by macrophages (Bendich, 1990).

3.2.1.3 Lymphocytes

The leukocytes, which are primarily involved in the production of specific immune reactions, are the T and B-lymphocytes. Individual essential nutrient deficiencies influence many aspects of T and B-lymphocyte function, and are associated with increased infection in domestic animals (Bendich, 1990). It has been confirmed that Se and Cu deficiencies influence T-lymphocytes to a larger extent than B-lymphocytes. As laboratory animals age, the performance of T-lymphocytes is reduced to a larger extent than that of B-lymphocytes. T-lymphocyte membranes are more fluid than B lymphocyte membranes in young mice. As mice age, T-cells lose their fluidity, while B-cells maintain the same level of fluidity. T-cell lipids are more sensitive to peroxidation than B-cell lipids. The ability of T-lymphocytes to form rosettes is significantly inhibited after exposure to oxygen radicals, while B-lymphocyte rosette formation is not influenced to a large degree (Bendich, 1990).

The vitamin C and E content of lymphocytes and mononuclear cells are normally slightly higher than that in the thrombocyte and erythrocytes. When splenocytes of mice receiving standard diets are exposed to substances causing peroxidative damage via the production of free radicals, the multiplication of both T and B-lymphocytes as a result of exposure to mitogens (causative species) is inhibited. In vitro antigen induced antibody production by B-lymphocytes is further

more also inhibited. When additional vitamin E or other antioxidants are added to this culture, the immuno suppression is overcome (Bendich, 1990).

3.3 Minerals

3.35.1 Co

In ruminants, Co is needed for the synthesis of vitamin B_{12} (cyanocobalamine). Although bacteria produce vitamin B_{12}, higher plants or animals cannot synthesise it. Co deficiency in ruminants is therefore a vitamin B_{12} deficiency, brought about by the inability of the rumen micro-organisms to synthesise enough vitamin B_{12}, when dietary Co is inadequate (Underwood *et al.*, 1999).

Co is essential to mammals in two distinct coenzyme forms of vitamin B_{12}, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl) (Furguson, Mitchell, & MacPherson, 1989). As MeCbl, Co assists a number of methyltransferase enzymes by acting as a carrier of methyl groups, obtained from N^5 – methyltetrahydrofolate (Armstrong, 1989; Dawn *et al.*, 1996), and is thus involved in the build-up of carbon chains. An example would be the methylation of homocysteine to produce methionine via methionine synthase (Armstrong, 1989).

As AdoCbl, Co influences energy metabolism. It facilitates conversion of propionate to succinate by methylmalonyl-coenzymeA (CoA) mutase during the formation of glucose. This reaction is part of the metabolic route for the conversion of carbons from valine, isoleucine, threonine, thymine, and the last 3 carbons of odd chain fatty acids, all of which form propionyl CoA, to the tricarboxylic acid (TCA) cycle intermediate succinyl CoA (Dawn *et al.*, 1996).

Ruminants make extremely inefficient use of dietary Co. The rumen microbes partition Co between active (cobalamins) and physiologically inactive vitamin B_{12}-like compounds (corrinoids) that the ruminant can neither absorb nor use. Furthermore, vitamin B_{12} is poorly absorbed from the digestive tract (McDonald, Edwards & Greenhalgh, 1988). Seemingly, there is therefore an

interconversion of the two coenzymes. In addition Co is recycled at the tissue level and by way of biliary secretion (Underwood *et al.*, 1999).

In keeping with their reputation of tolerance when compared with sheep, cattle show little or no evidence of MeCbl or AdoCbl dysfunction when subject to severe Co-deprivation (Kennedy, Young, Kennedy, Scott, Molloy, Weir & Price, 1995).

Co-deficiency leads to defective lipid metabolism, which is essential to offset loss of appetite. Consequently, there is an accumulation of triglycerides providing a pool of readily peroxidizable, unsaturated fats. A further metabolic defect, accumulation of homocysteine, initiates a chain of peroxidation, which leads to accumulation of the oxidation product, lipofuscein, depletion of the antioxidant, vitamin E, and damage to mitochondrial structure (Kennedy, McConnel, Anderson, Dennedy, Young & Blanchflower, 1997).

Lack of appetite and weight loss, have been used as parameters to determine the dietary Co and vitamin B_{12} requirements of animals. The concentration of vitamin B_{12} in liver and plasma is a useful index of the dietary status of an animal, but it includes inactive analogues of cobalamin. The concentration of methylmalonic acid (MMA) in plasma seems to be a more reliable index. Furthermore the Co concentration in tissues has little merit to indicate the vitamin B_{12} status of an animal (Van Ryssen, 1997). The Co concentration in feed and that extractable form soil have been used for diagnostic purposes (Van Ryssen, 1997). The mineral concentration of soils is however a poor indicator of mineral uptake by plants and thus their availability to animals.

Disease and parasite resistance may decline during Co deficiency. Furguson *et al.* (1989) reported increased sensitivity to infection by the abomasal parasite *Ostertagia circumcincta* in the Co-deficient lamb. In the same experiment, 7 out of 8 lambs in a severely deficient, unchallenged group succumbed to miscellaneous microbial infections. An account by MacPherson, Gray, Mitchel & Taylor (1987), suggested that immune responses were compromised in Co deficient cattle in response to a challenge by *Ostertagia ostertagii*.

Reductions in the ability of neutrophils to destroy foreign cells in both the ovine (Fisher & MacPherson, 1986) and bovine (MacPherson *et al.*, 1987) have been reported in Co deficiency. It is noteworthy that the reported changes in the ovine neutrophil became evident before plasma vitamin B_{12} concentrations had fallen below "normal" and before there was any increase in serum methylmalonic acid (MMA) values. In other words, before the first acknowledged signs of functional deficiency in propionate metabolism. If these results reflect a functional impairment in phagocytic killing ability *in vivo*, then the interpretation of conventional (vitamin B_{12}) and new (MMA) biochemical criteria of Co status must be questioned (Suttle, Brebner, Munro & Herbert, 1989).

3.3.2 Cu

Cu is an essential element necessary for the routine functioning of all living systems. It is thought to be present in all body cells (McDonald *et al.*, 1988) and is particularly concentrated in the liver, which acts as the main storage organ of the body.

Plasma concentrations of Cu are used to indicate Cu status of animals. Other indices are SOD activity in whole blood, plasma cerruloplasmin activity, and Cu concentrations in various tissues, for example kidney (Van Ryssen, 1997) and liver (Stabel, Reinhardt & Nonnecke, 1991).

Cu-deficiency may be expressed in the conventional ways (swayback, anaemia, diarrhoea and growth retardation). Of greater economic importance, however, is the increased susceptibility to fatal infections. The ideal should therefore be to predict the probability of any individual showing "immune dysfunction" (Suttle, 1988).

Cu is a cumulative poison as the animal body is unable to excrete it efficiently. Continuous ingestion in excess of nutritional requirements therefore leads to an accumulation of the element in the body tissues and eventual toxicity (McDonald *et al.*, 1988). There are various published recommendations for the concentration of Cu in the diet of ruminants. In practice, however, tolerance levels depend on interactions of Cu with other minerals (Underwood, 1977).

Another complicating factor, which would influence the ability of a feed to meet the Cu requirements of ruminants or to pose a risk of Cu poisoning, is the absorbability of the Cu source in the diet (Underwood *et al.*, 1999).

Cu's necessity is expressed primarily as specific cupro-enzymes (Prohaska & Lukasewycz, 1990). In animals there are approximately ten proteins that are commonly accepted as cupro-enzymes. They range in size from small single polypeptides to complex tetramers with up to 8 Cu atoms per mol. Some cupro-enzymes have additional co-factor requirements like Zn, haem Fe and pirroloquinolien guinine. The manifold characteristics of the cupro-enzymes are responsible for the diversity of specialised functions of Cu (Prohaska & Lukasewycz, 1990). When Cu levels in the environment are varied, the levels of a number of these cupro-enzymes are likewise altered. Cu is surpassed only by Zn in the number of enzymes that it activates (Underwood *et al.*, 1999), making it difficult to determine the precise reason why particular abnormalities arise in livestock deprived of Cu.

3.3.2.1 The function of Cu in the erythrocyte and O_2 metabolism

Although Cu is not actually a constituent of haemoglobin it is present in certain other plasma proteins such as ceruloplasmin, which are concerned with the release of Fe from the cells into the plasma. A deficiency of Cu impairs the animal's ability to absorb Fe, mobilise it for the tissues and utilise it in haemoglobin synthesis. Cu is also a component of other proteins in blood. One of these, erythrocuprein, occurs in erythrocytes where it plays a role in oxygen metabolism (McDonald *et al.*, 1988). The element is also known to play a vital role in many enzyme systems; for example, it is a component of cytochrome c oxidase (CCO), responsible for the terminal electron transfer in the respiratory chain in oxidative phosphorylation. Thus, during infection the greatly increased metabolism of cells of the immune system might make them vulnerable to depletion of the energy-generating CCO.

3.3.2.2 The function of Cu in the immune system

Cu plays an important role in the immune system, as can be seen during a Cu-deficiency in both man (Kelley, Daudu, Taylor, Mackey, & Turnlud, 1995) and animal. It has been demonstrated that resistance of cattle (Brazle & Stokka 1994) and sheep (Woolliams, Woolliams, Suttle, Jones & Wiener, 1986) to infectious organisms is reduced with a Cu-deficiency. Protection against common pathogens in the Cu-deficient calf (Stabel, Spears & Brown, 1993) and heifer (Arthington, Corah, & Blecha, 1996; Gengelbach, Ward & Spears, 1997) do not, however, seem to be compromised. Stabel *et al.* (1993) suggested that Cu-deficient animals are at a greater risk than non-deficient animals for infection. The authors nevertheless failed to observe a consistent immune response. In immune cells from the Cu-deficient bovine, killing ability is reduced before leukocyte SOD activity declines; this is attributed to lowered intracellular concentration of the superoxide anion (Boyne & Arthur 1986). In the Cu-deficient lamb, prevalence of microbial infection was not increased, but mortality was (Suttle & Jones 1986). Chirase, Hutcheson & Thompson (1991) reported that calves injected with Cu glycinate before shipping had lower dry matter intake (DMI) and body weight (BW) change after a challenge with IBRV than calves not injected with Cu. Serum Cu concentrations have been reported to increase with market and transit stress and after inoculation with IBRV (Orr, Hutcheson, Grainger, Cummins, & Mock, 1990; Chirase *et al.*, 1991; Stabel *et al.*, 1993), possibly to increased Cu availability for immune modulation. Cu levels below optimum in sheep nutrition resulted in lambs having a reduced immuno-competence (Boyazoglu, 1997). Various biochemical and cellular hypotheses exist, which can explain the limitations in the immune system during a Cu-deficiency

3.3.2.3 Antioxidant

Changes in the intracellular redox potential and energy metabolism can impair the immune system. Increased lipid peroxidation, as a result of Cu-deficiency, not only influences membrane composition, but can also influence redox potential so that changes in glutathion (GSH/GSSG) occur. This can influence both cell formation and antigen production. In Cu-deficient rats, changes in GSH levels as well as changes in piridine nucleotide ratios in the brain were found. It was shown that lactate / pyruvate and ∀-glycerol phosphate / dehydroxy acetone increases phosphate which can predict a rise in $NADH/NAD^+$ (Prohaska & Lukasewycz 1990).

Cu may protect tissues from oxidant stress via two distinct pathways, one involving impaired Fe metabolism, the other a Cu-Zn SOD enzyme (CuZnSOD).

Hepatic activity of the Fe-containing, haem enzyme catalase is reduced in Cu-deficiency, despite a rise in tissue Fe concentrations (Taylor, Bettger & Bray, 1988). This rise in tissue Fe concentration promotes free-radical generation and the reduced catalase leads to increased tissue damage by hydrogen peroxide and hydroperoxide (Golden & Ramdath, 1987).

Hepatic defences against another free radical, superoxide, may have been reduced in Taylor *et al.*'s (1988) study by a fall in CuZnSOD, which accompanies liver Cu depletion in all species.

Ceruloplasmin may contribute to antioxidant defences by scavenging free Fe and free radicals (Saenko, Yaroplov & Harris, 1994). There is thus scope for interactions with other nutrients with antioxidant properties, such as Mn, Se and vitamin E (Underwood *et al.*, 1999).

3.3.2.4 Immune cells

Immunity can be lowered because of changes in the plasmamembrane due to lipid peroxidation in lymphocyte walls during a Cu-deficiency (Prohaska & Lukasewycz, 1990).

Lymphocytes contain CuZnSOD and cytochrome C oxidase, two Cu dependant factors. They have serruloplasmin receptors and therefore have a

specific mechanism to take up Cu from the blood. Lymphocytes may contain unique Cu-dependent enzymes. During a Cu-deficiency changes in these enzymes may directly influence the immune system. One such enzyme is possibly the sulphydril oxidase occurring in the plasma membrane of lymphocytes. This enzyme is important in the formation of IgM and thus B-cell differentiation

There are several reports of dysfunction *in vitro* in immune cells from Cu-deficient ruminants. Xin, Waterman, Hemken & Harmon (1991) indicate that steers given a *Cu-deficient* diet, showed reductions in neutrophil Cu, neutrophil CuZnSOD and neutrophil killing capacity (Suttle *et al.*, 1989). Saker, Swecker & Eversole (1994) reported that Cu lysine-supplemented calves had increased plasma Cu concentrations, monocyte phagocytic activity, and monocyte oxidative burst measurements compared with calves fed the basal diet. Boyne & Arthur (1986) noted that neutrophil phagocytic ability decreased with feed restriction and with Cu-deficiency. Other authors have however, found no impairment of phagocytic ability (Boyne & Arthur, 1981; Jones & Suttle 1981), but the capacity to kill the engulfed organism was invariably reduced (Boyne & Arthur, 1981; Jones & Suttle, 1981; Boyne & Arthur, 1986). Arthington, Corah, & Blecha, 1995) found that Cu-deficiency induced by Mo and S did not affect *in vitro* or in vivo measurements of neutrophil chemotaxis.

Reductions in SOD activity in leukocytes from Cu-deficient sheep accompanied increased release of superoxide anion, suggesting that superoxide might accumulate and weaken the phagocyte after the pathogen triggers the respiratory burst (Jones & Suttle, 1981). The physiological role of superoxide may, however, vary between species and with the severity of Cu-deficiency. In addition experimental results may be influenced by the stage of the phagocytic process at which biochemical measurements are made.

Proliferative responses of lymphocytes to mitogenic stimulation *in vitro* are also affected in Cu-deficient ruminants. Cu-supplementation in lambs produced enhanced responses to mitogens (Woolliams *et al.*, 1986). However, other workers (Young, Edwards & Hucker, 1985) could find no effects of Cu depletion

on peripheral blood lymphocyte responses to antigens in lambs. Ward, Gengelbach & Spears, (1997) found that Lymphocyte viability did not differ among treatments, nor did lymphocyte blastogenic responses. The authors concluded that Cu-deficiency had inconsistent effects on immune function in calves.

In severely deficient small laboratory animals, Cu-deficiency can affect the numbers of cells mediating immunity, increasing mast cells (i.e. non-specific immune cells) in muscle (Schuschke, Saari, West, & Miller, 1994) and decreasing some subpopulations of T cells (Mulhern & Koller, 1988). It would appear as if the functions of macrophages are also influenced by a change in Cu-status, seeing that Cu-deficient mice have greater amounts of interleukin-1 (a monokine produced by macrophages) (Prohaska & Lukasewycz, 1990).

Some authors have, however expressed concern about the usefulness of *in vitro* tests and their ability to predict *in vivo* responses (Suttle *et al.*, 1989; Underwood *et al.*, 1999).

3.3.2.5 Antibody production

Cu may also be involved in intra and inter molecular disulphide formation in proteins like immunoglobulin (Prohaska & Lukasewycz, 1990). Studies with mice and rats have shown that Cu is important in both antibody and cell mediated immunity and inflammatory reactions (Prohaska & Lukasewycz, 1990; Windhauser, Dappel, McClure & Hegsted, 1991). Ward *et al.* (1997), however, found that the effect of a Cu-deficiency on specific immune functions was minimal and changeable in cattle. In contrast to this, Cerone, Sansinanea, & Auza (1995) found that total levels of IgG and total complement concentrations are restricted by a low Cu status. Kill *et al.* (1991) also reported increased humoral immune response to Cu supplementation. These results show that immune reactions toward an intracellular pathogen may be restricted by a Cu-deficiency (Cerone *et al.*, 1995)

3.3.2.6 Neuroendocrine function

Changed neuro-endocrine functions can also not be excluded when considering the effects of Cu-deficiency on immunity. There exists a complex interaction between immune, nervous and reproductive systems. The synthesis of norepinephrine, for example, is dependent on the cupro-enzyme, dopamine--∃-mono-oxygenase. It is known that the transformation of norepinephrine decreases during an immune reaction. It appears however that norepinephrine transition is increases by a Cu-deficiency. Furthermore it has been shown that norepinephrine levels in the spleen of mice are lower with a Cu-deficiency. Norepinephrine metabolism may thus provide an explanation for the changes in the immune system during a Cu-deficiency

Lymphokines are proteins produced by lymphocytes to serve as molecular communicators among cells of the immune system and are important in the co-ordination of immune-inflammatory reactions. *In vitro* – studies in Cu-deficient media have shown that splenocytes have a lowered production of interleukin-1, originating from monocytes, and the T-cell replacement factor (interleukin-5). Furthermore there is a decrease in production of interleuckin-2 (a limphokine from type I helper T cells) (Prohaska & Lukasewycz, 1990). Gengelbach *et al.* (1997) found that Cu-supplemented calves had greater plasma tumor TNF concentrations than Mo-supplemented calves at weaning, and Cu-supplemented calves tended to have higher TNF after IRBV inoculation than calves given Mo or Fe. The authors interpreted their findings as evidence of an improved immune response in the groups given Cu and Fe. They suggested that dietary levels of Mo and Cu can alter body temperature and feed intake responses to disease by affecting TNF, and possibly other cytokines (Gengelbach *et al.*, 1997).

Another region of the neuroendocrine system, which may react to changes in dietary Cu, is the hypothalamus. The emission of lutenizing hormone (LH) releasing hormone is greatly increased in the presence of Cu and prostaglandine E_2. Emission of LH from the pituitary gland has a great effect on gonadal hormone emission, which in turn affects the thymus.

3.3.3 Mn

Mn is widely distributed in very low concentrations in the cells and tissues of the animal body, the highest concentrations occurring in the bones, liver, kidney, pancreas and pituitary gland (McDonald *et al.*, 1988). It is essential for the normal development of bone and proper functioning of reproductive processes (Egan, 1972) in both males and females (Underwood *et al.*, 1999). Mn is also important in the animal body as an enzyme activator, activating a number of metalloenzymes, chiefly phosphate transferases and carboxylases (McDonald *et al.*, 1988; Underwood *et al.*, 1999).

Although deficiencies of Mn in grazing ruminants are likely to be rare (McDonald *et al.*, 1988), severe deprivation may impair immunity and central nervous system function (Underwood *et al.*, 1999).

Mn forms part of a SOD enzyme, MnSOD that was first isolated from chicken liver mitochondria (Gregory & Fridovich, 1974). The superoxide dismutases (SOD) protect cells from damage by the free oxygen radical O_2^-. Malecki & Greger (1996) found that Mn deficiency lowers MnSOD activity in the heart and increases the peroxidative damage caused by high dietary levels of PUFA. Compensatory increases in CuZnSOD suggested overlapping roles for the two forms of SOD and possible interactions between dietary Cu and Mn. Bell & Hurley (1974) related structural changes in liver mitochondria and cell membranes to reductions in the activity of MnSOD. Mitochondria are responsible for the majority of O_2 consumption and may be particularly vulnerable to free radical damage (Leach & Harris, 1997).

Paynter & Caple, (1984) noted that the bulk of the Mn in ovine heart is present as MnSOD, and that this is the predominant dismutase in this tissue and also in muscle. A significant reduction of MnSOD in heart and lung tissue was noted by Masters, Paynter, Briegel, Baker & Purser (1988), in Mn depleted animals. This led them to speculate that in circumstances of oxidant stress (e.g. from PUFA) or depletion of other dietary antioxidants (for example, Cu, Se and vitamin E), Mn deficiency may lead to dysfunction. The effects on MnSOD, following the administration of tumour necrosis factor α (Wong & Goeddel, 1988),

suggest a need for added protection against oxidative stress associated with the inflammatory responses to some infections.

3.3.4 Se

A dietary Se-deficiency in ruminants can cause white muscle disease, a decrease in selenoproteins, forfeiture of glutathione peroxidase activity and suppression of the immune system. Se-supplementation is often necessary as many feeds are produced in areas of the world where there is a Se-deficiency (Pherson, Knutsson & Gyllensward, 1989).

Various tissue concentrations have been proposed as indicative of the Se-status of the animal and to monitor the effects of Se supplementation. The measurement of Se-dependant functions, like glutathion peroxidase activity in serum or isolated blood cells, can also relay valuable information. Glutathione peroxidase activity in the erythrocytes is useful but the analytical technique is difficult to standardise among laboratories (Van Ryssen, 1997). The activity of Se supplements can also be determined by investigating biological and clinical effects (Nève, 1994).

Selenoproteins are present in every cell type. There exists at least 20-30 selenoproteins of which a number were partially or fully described (McKenzie, Rafferty & Beckett, 1998). Deiodinase enzymes and glutathione peroxidase are among these selenoproteins.

3.3.4.1 Cytosolic peroxidase

The first peroxidase to be recognised and investigated in detail is known as cytosolic or GPX1. It is the predominant GPX and source of Se in erythrocytes and liver, and all the Se responsive diseases are accompanied by reduction in blood and tissue GPX1 activities. Se is present in GPX1 in stoichiometric amounts, with 4g atoms Se mol^{-1}. The tetrameric enzyme catalyses the reduction of hydrogen peroxide (H_2O_2) and of hydroperoxides formed from fatty acids and other substances, according to the general reaction:

ROOH + 2GSH ∧ R-OH + HOH + GSSG

It is questionable whether GPX1 ever becomes a rate-limiting factor in protecting erythrocytes or tissues from peroxidative damage during Se-deficiency, as its activity in erythrocytes and liver can decrease to levels, not far from undetectable, without apparent pathological or clinical changes (Arthur & Beckett, 1994b). Sunde, (1994) proposed therefore that GPX1 is essentially a storage selenoprotein. Seleno-amino acids can, however be incorporated directly and non-specifically into body proteins (Kincaid, Rock, & Awadeh, 1999). Furthermore, its suitability as a vehicle for transferring Se to sites of synthesis of other selenoproteins is questionable (Kincaid *et al.*, 1999). It is more probable to make up a depot for excess Se, while providing a reserve of antioxidant potential, which may be beneficial at times of oxidant stress (Kincaid *et al.*, 1999). Animals can survive without the gene for GPX1 (Cheng, Ho, Ross, Valentine, Combs, & Lei, 1997) suggesting that the enzyme itself is non-essential.

3.3.4.2 Other peroxidases

The plasma or extracellular peroxidase, GPX3, is also tetrameric and synthesised principally in the lung and kidney. Its main functions may possibly be the protection of the renal proximal tubule from peroxidative damage. GPX3 does not have a major extracellular protective function, as glutathione (GSH) concentrations in plasma are too low to support such a role. The gastrointestinal peroxidase (GPX2) may also act locally to protect the intestinal mucosa from dietary hydroperoxides expression characterisation (Chu, Doroshow, & Esworhy, 1993). The peroxidases influencing the immune system are completed by the phospholipid hydroperoxidase form (GPX4), a monomer associated with intracellular membranes. GPX4 is conserved during Se-deficiency, suggesting a rate-limiting importance, which may be accountable for the synergistic relationship between Se and vitamin E (Arthur & Beckett, 1994b).

Activity of GPX1 and also the distribution of the enzyme in the body are dramatically influences by Se intake (Ganther, Hafeman, Lawrence, Serfass & Ganther, 1975; Lei, Dann, Ross, Cheng, Combs, & Roneker, 1998). Over an

extended period, erythrocyte GPX1 activities increase logarithmically in relation to Se intake, eventually reaching a plateau. Incorporation of selenocysteine into erythrocyte GPX1 occurs at erythropoiesis (Wright, 1965). In the shorter term, there is therefore a lag before the newly 'packaged' enzyme is released into the bloodstream and a further lag before it disappears, when the erythrocyte reaches the end of its normal lifespan. Other GPXs also show contrasting tissue responses to supplementation, earlier plateaux being reached for GPX4 in the thyroid and pituitary than in the liver or heart of young pigs (Lei *et al.*, 1998).

3.3.4.3 Deiodinases

Deiodinases are necessary in the metabolism of thyroxine. Se-deficiency may thus indirectly influence basic metabolic rate and a wide range of physiological processes, with adverse effects on production (Underwood *et al.*, 1999).

3.3.4.4 Glutathione peroxidase activity

An experiment where the efficiency of organic and inorganic forms of Se in grower finishing diets of pigs, was compared, showed that the levels of dietary Se required to maintain maximum serum glutathione peroxidase activity were in the region of 0.05 – 0.10 ppm Se. Although the inorganic form of Se usually leads to higher glutathione peroxidase levels at lower dietary levels, both forms clearly reached maximum glutathione peroxidase activity levels at lower dietary concentrations than which are usually fed to pigs. This study showed that higher dietary levels of Se does not further stimulate production of glutathione peroxidase, and that the activity of the enzyme increases with increase in age of the pig (Mahan & Kim, 1996). Pherson *et al.* (1989) found that fermented Se or selenomethionine was twice as active as selenite in increasing glutathione peroxidase activity in erythrocytes of Se-deficient heifers. This discovery suggests that glutathione peroxidase activity cannot reliably be used as a gauge for Se bioactivity.

Thus the activity of glutathione peroxidase decreases in animals with a Se-deficiency and recovers with Se supplementation. Enzyme activity shows saturation at levels that agree with Se requirements for this important enzymatic function (Nève, 1994).

3.3.4.5 Effect of chemical form of Se on immunity

Experimental data (Stabel *et al.*, 1991) indicates that addition of organic and inorganic forms of Se increased *in vitro* IgM production in peripheral blood mononuclear cells of mitogen-stimulated cattle. The cells were isolated from animals receiving normal levels of Se in their feed. These results indicate that both inorganic and organic forms of Se increase *in vitro* B cell function.

3.3.4.6 Se as an antioxidant

Peroxynitrite, a product of superoxide and sulphureoxide "salpeteroksied", is produced in the skin during exposure to ultraviolet radiation and causes breakage of single strand DNA. Studies with ebselene, a synthetic Se compound which functions as glutathione peroxidase, showed that the enzyme can prevent this breakage. Ebselene also inhibits the pro inflammatory enzymes sulphur oxide synthase and protein kinase C. Furthermore, glutathione peroxidase plays an important role in the synthesis of arachidonic acid metabolites (McKenzie *et al.*, 1998).

3.3.4.7 Se and its influence on the immune system

Se has, besides its inclusion in the glutathione peroxidase as a selenocystein residue, various effects on the immune system, *inter alia*, the expression of specific and non-specific humoral and cell mediated reactions. The anti oxidative role of GSHPx in the elimination of inorganic and organic hyperoxides plays an important role in cells of the immune system, as well as in various tissues. A principal function of Se in immune cells, especially the phagocytic cells, is to curb excessive production of peroxidative substrates like H_2O_2 (Spallholtz, 1990).

According to McKenzie *et al.*, (1998) there are three mechanisms through which Se-supplementation promotes cellular immunity:

1. Se stimulates the expression of the high affinity IL-2 receptors of T-lymphocytes and increases reactions of T-lymphocytes. Considering that the T-lymphocytes aid the B-lymphocytes in the production of antibodies, this may account for the effect of Se on antibody production (Nicholson, Bush & Allen, 1993).
2. Se prevents damage to immune cells, which may be caused by oxidative stress. The most familiar function of Se is its role as an antioxidant in the GSHPx system. Endogenous production of $O_2^{\bullet-}$ and H_2O_2, together with other free radical species during the respiratory burst of phagocytic cells is obligatory for the cytotoxic destruction of invading species. Vitamin E, vitamin C, superoxside dismutase and GSHPx protects the phagocytic cells against self-destruction by free radical peroxidation. If the GSHPx activity in macrophages decreases, there will be a concomitant increase in the release of H_2O_2. Spallholz & Boylan (1989) have found that macrophages of mice, which have a Se and GSHPx deficiency, indeed produce more H_2O_2 during the respiratory burst. Furthermore it was established that the production of $O_2^{\bullet-}$ in these cells also was increased. In addition to the antioxidant function of GSHPx in cells, Se and/or GSHPx may also fulfil other functions in the immune system, directly or indirectly related to the increased endogenous release of H_2O_2 (Spallholtz, 1990).
3. Se changes blood platelet aggregation by decreasing the ratio of thromboxin to leucotrine production.

3.3.4.8 The effect of Se on antibody production

The primary antibody reaction and total IgM concentration in both monogastric and ruminant animals are influenced by Se-deficiency. Se-supplementation can also increase serum IgG and the production of secondary antibodies in response to an antigen, which indicates that the helper T-lymphocyte dependent class is also affected by a Se-deficiency (Nicholson *et al.*,

1993). The immunological effects of Se-supplementation on antibody reactions is dependent on both age and type of antigen (Spallholz, Martin, Gerlach, & Heinzerling, 1973; Martin & Spallholz, 1976; Baalsrud & Øvernes, 1986; Jelinek, Ellis, Wroth, Sutherland, Masters & Petterson, 1988; Larsen, Moksnes & Øvernes, 1988a; Reffett, Spears & Brown, 1988a; Reffett, Spears & Brown, 1988b; Droke & Loerch, 1989; Hayek, Mitchell, Harmon, Stahly, Cromwell, Tucker & Barker, 1989; Stabel, Spears, Brown & Brake, 1989; Swecker, Eversole, Thatcher, Blodgett, Schurig & Meldrum, 1989; Ellis, Masters, Hustas, Sutherland, & Evans, 1990; Knight & Tyznik, 1990; Larsen, 1993). According to Baalsrud & Øvernes (1986) and Droke & Loerch (1989) the supplementation of both Se and vitamin E is necessary in horses and cattle to achieve an increased antibody reaction. In lambs, however, no additive effect was attained with supplementation of Se and vitamin E (Larsen *et al.*, 1988a; Reffett *et al.*, 1988b).

Spallholz *et al.* (1973) found that sodium selenite administered by intra peritoneal injection increased the primary immune reaction to ovine erythrocytes. These authors also found that the increase in primary immune reaction in response to ovine erythrocytes was largest when the Se was administered just prior to or simultaneously with the antigen.

Se-deficiency reduced glutathionene peroxidase and immuno-competence. Elevated Se of 0,7 – 2,8 ppm as sodium selenite increased antibody titres seven to 30 times respectively when challenged. The supplementation of Se in calves stressed by weaning, transport and Pasteurella haemolytic inoculation resulted in higher serum IgM concentrations and serum antibody titers (Boyazoglu, 1997).

Nemec, Hidiroglou, Nielsen & Proulx (1990), found no effect of Se or vitamin E supplementation on IgG_1, IgG_2, IgA or IgM antibody levels in response to *Brucella abortus* as antigen. Results of Finch & Turner (1986) also showed that lambs with a marginal Se-deficiency had strong antibody titers in response to a bacterial antigen (*Salmonella dublin*) and that supplementation with Se only marginally increased the immune reaction.

In a study by Reffett *et al.* (1988a) calves receiving two different levels of Se-supplementation received a primary and secondary inoculation with IBR (infectious bovine rhinotracheitis virus), to evaluate the effects of Se-deficiency on the primary and secondary humoral immune response. Serum antibody titres did not differ between treatments 14 days after the challenge but were greater in Se-adequate calves than in Se-deficient calves 14 days after the second IBR challenge.

Nicolson *et al.* (1993) also did not observe large differences between antibody titres of Se supplemented and not supplemented cattle after challenge with ovine erythrocytes and ovalbumine. The results of this experiment support the proposal by Swecker *et al.* (1989) that blood Se levels of more than 100:g L^{-1} are necessary for optimum functioning of the immune system.

In the experiment by Swecker *et al.* (1989) calves received diets containing 20, 80, 120, 160 en 200 mg kg^{-1} Se supplements. Increases in body mass was not affected by Se level of the diet, which suggests that 20 mg kg^{-1} is sufficient for. IgG titres, in response to chicken egg lysosime, was higher in all groups receiving Se-supplementation greater than 20 mg kg^{-1}. This data indicates that the immune system has a greater requirement for Se than growth.

Wright, Corah, Stokka & Blecha (1997) used 80 Hereford X Angus calves to evaluate Se and vitamin E combinations. The treatments were as follows: 1) basal diet only; 2) basal + 3 mg of Se/kg; 3) basal + .3 mg of Se/kg + 500 IU of vitamin E; 4) basal + .3 Se/kg + 1000 IU vitamin E; and 5) basal + .3 mg Se/kg + 1500 IU vitamin E. The basal diet was 60% rolled corn, 25% rolled oats, 10% soybean meal, and 5% molasses (as fed basis). Calves were temporarily separated from their dams before weaning and fed their assigned dietary treatments. All calves were vaccinated 17 d before weaning. At weaning, calves were revaccinated and shipped to a commercial feedlot. Antibody titres to IBR and BVD were not affected by treatment, and treatments did not affect serum haptoglobin concentrations. Moreover, treatments did not affect pre- or postweaning gain or transit shrink. The authors concluded that preweaning vitamin E and/or Se-supplementation did not influence postweaning

performance, stress responses, or vaccination responses in beef calves with adequate vitamin E status.

The effect of supplementation on the production of a specific antibody is not necessarily parallel to a change in the total immunoglobulin levels of that same isotype. Different isotypes (e.g. IgM and IgG) are also not necessarily affected in the same manner by supplementation (Reddy, Morrill, Minocha, Morrill, Dayton & Frey, 1986; Larsen *et al* 1988a; Swecker, Thatcher, Eversole, Blodgett & Schurig, 1995). It must thus be accepted that a proposed protocol where supplements are granted, has varying effects on different antibody reactions with different types of antigens (fore example, T-dependant in comparison to T-independent reactions). Different effects can also be observed during different stages of an immune reaction (fore example colostral, as opposed to serum antibodies) (Finch & Turner, 1996). The chemical form of the supplement may also be important: *in vitro* studies on cattle cells have shown that organic Se is more effective than inorganic Se in the stimulation of IgM synthesis (Stabel *et al.*, 1991). A further source of variation may be that animals without real deficiencies are used or that blood Se levels attained are not high enough to show large differences (Nicolson *et al.*, 1993).

3.5.4.9 Se and Lymphocyte Production

Dietary deficiency of antioxidant micronutrients may influence T-lymphocytes to a greater extent than B-lymphocytes. T-cell membranes are more fluid than the membranes of B-cells and T-cell lipids are more sensitive to peroxidation than B-cell lipids. Se deficiencies in isolation or in combination with vitamin E, decreases lymphocyte reaction in response to mitogens (Larsen & Tollersrud, 1981; Turner, Wheatly & Beck, 1985; Larsen, Moksnes & Øvernes, 1988b; Lessard, Yang, Elliott, Rebar, Van Vleet, Deslauriers, Brisson & Schultz, 1991). Adult ruminants are apparently more resistant to the immunological effects of a Se-deficiency on lymphocyte proliferation than are the young. This can be explained in terms of increased availability of rumen microbial Se to lymphocytes (Turner & Finch, 1991).

The effective portion of the T-cell reaction in response to antigens is composed of both stimulation of lymphocytes and activation of phagocytes. In a study by Aziz & Klesius (1985) the mitogen induced production of leukocyte migration inhibiting factor was suppressed in lymphocytes of goats with a Se-deficiency, compared to goats receiving adequate Se. The interleucine-2 production and lymphocyte reaction in response to the mitogen, concavalin A, was however not affected. A decreased production of migration inhibiting factor may also have an effect in circumstances of chronic inflammation, which is associated with macrophage infiltration and activation.

Se-supplementation does not influence the production of IL-1 and IL-2 by macrophages and lymphocytes significantly. This observation suggests that the mechanism(s) responsible for the observed effects of Se on lymphocyte proliferation is independent of the levels of IL-1 or IL-2 (Kiremidjian-Schumacher, Roy, Wishe, Cohen, & Stotzky, 1990). The possible decrease in cell-mediated immunity with a Se-deficiency may be caused by a decrease in interleucine-2 (IL-2) receptor expression, which consequently limits IL-2 mediated cellular immune reactions (Kiremidjian-Schumacher *et al.*, 1990). It is suggested that Se modifies the expression of IL-2 receptors on the cellular surface, which may explain the decreased ability of lymphocytes to react to mitogens and antigens in Se-deficient animals (Larsen, 1993). Studies by Eskew, Scholz, Reddy, Todhunter, & Zarkower (1985) and Koller, Exon, Talcott, Osborne & Henningsen (1986) also showed that changes in the prolific ability of lymphocytes according to Se status, was not as a result of a change in the ability of macrophages to produce IL-1 or to destroy antigens.

Although the production of IL-1 is not influenced by the Se-status, modified secretion or arachidonic acid metabolites by macrophages may have important regulatory implications. GSHPx, and thus Se is necessary in the arachidonic acid cascade for the production of prostaglandines, thromboxanes, leucotries and other mediators of inflammatory and immune reactions. Cells with a deficiency of GSHPx and Se have a decreased conversion of the arachidonic acid cyclo oxygenase product, prostaglandin G_2 (PGG_2), to the alcohol PGH_2

(Spallholtz, 1990). Maddox, Reddy, Eberhart & Scholz (1991), have shown that there are definite effects of dietary Se on milk eicosanoid concentrations in reaction to *Eschericha coli* infection. Eicosanoids are a family of compounds derived from arachidonic acid and are the principle mediators of inflammation. Increased concentrations of thromboxsane B_2, prostaglandin $F_{1\forall}$, prostaglandin E_2 and leucotrin B_4 were associated with significantly larger numbers of bacteria in the milk, possibly as a result of decreased destruction of *E. coli* by neutrophils of animals with a Se-deficiency. Increased inflammation and tissue damage, caused by endo-toxins and inflammatory mediators, will induce a higher tissue hydroperoxide concentration. During a Se-deficiency, this may lead to increased activity of cyclo-oxygenase and lipoxygenase enzymes, with consequent higher eicosanoid levels.

Turner *et al.* (1985), Finch & Turner (1989) and Stabel *et al.* (1991) found that the addition of sodium selenite to cell culture of ruminants increased the lymphocyte reaction in response to mitogens. The mitogen induced immunoglobulin production is also increased when serum or pooled plasma of animals with a high Se-status is added to cell cultures. This discovery indicates that the effects of Se transpire in the microenvironment of the cell rather than in the cell itself. Finch & Turner (1989) furthermore also observed that the amount of Se required *in vitro* to increase ovine lymphocyte reaction to the phitohaemagglutenin mitogen, was in the region of 1-10 ng Se/ml. Toxic effects were obtained above 1 g/ml. Similar results were obtained by Larsen *et al* (1988b). Sodium selenite or seleno-methionine at a concentration of 0.1 of 0.5: g/g added to the diet, increased the lymphocyte reaction of lambs in response to mitogens, while 1: g/g seleno-methionine had an inhibitory effect.

Pollock, McNair, Kennedy, Kennedy, Walsh, Goodall, Mackie & Crockard (1994) provided calves with a diet with insufficient vitamin E and Se and supplemented the animals with vitamin E and/or Se to test the possible interacting effects of these micronutrients on cellular immune reactions. *In vitro* lymphocyte proliferation was used to determine the effect of diet on cell-mediated immunity. It was found that this effect was dependent on the type of serum used

to cultivate the cells. These results indicate that the micro nutrient status of both the donor animal and of the cultivating medium must be considered when results of *in vitro* studies on lymphocyte function are interpreted.

3.5.4.10 Se and Phagocyte Function

The production of free radicals is a prerequisite for the destruction of bacterial pathogens by phagocytes. The destructive process is usually limited to intracellular vacuoles, which contain the phagocitised pathogen. The reactive molecules, however, leak from the phagolysosome into the surrounding cytoplasm and intracellular space. These products may damage the cell if they are not detoxified. Neutrophils contain superoxide dismutase, catalase and glutathione / glutathione peroxidase to protect the cell against auto oxidation. During conditions of antioxidant deficiency, the cells may, however, be damaged by the reactive oxygen species (Larsen, 1993).

Se and/or glutathione peroxidase is found in phagocytic and other lymphoid cells. High specific activity of GSHPx was found in bone marrow, spleen and lymph nodes of sheep (Paynter, 1979). Scholz & Hutchinson (1979) found that leukocytes of dairy cows contained 4 times more GSHPx than erythrocytes. This and other examples of Se and GSHPx concentrations in immune tissue and related lymphoid cells demonstrate that Se and GSHPx serve important functions in these cells. The Se-requirement of these cells can be seen when animals are provided with Se-deficient diets. Organs like the liver, heart, kidneys and muscles lose Se relatively quickly, while other tissues, like lymphoid cells retain Se for relatively longer (Spallholz, 1990).

The ability of phagocytes to migrate towards a region of infection or inflammation may also be influenced by an animals Se / vitamin E status. Se-deficiency reduced the random migration and chemotactic reactions of goat neutrophils; either supplementing the animal itself or supplementing the isolated neutrophil in vitro (Aziz, Frandsen & Klesius, 1984) could reverse both these reactions. Aziz & Klesius (1986) also found that a Se-deficiency had the ability to limit the production of chemotactic factors, which play a key role in the

recruitment of cells to a region of infection. This decrease in chemotactic factors could be reversed by supplementation with Se.

Dietary deficiency of Se and/or vitamin E impedes macrophage and neutrophil activity. The Se-deficiency however is not associated with a decrease in phagocytosis (Grasso, Scholz, Erskine & Eberhart, 1990), although phagocytosis can be decreased where there is a deficiency of both Se and vitamin E (Turner & Finch, 1990). The intra cellular destruction of yeast and bacteria by macrophages and neutrophils of animals with a Se-deficiency was reduced (Grasso *et al.*, 1990). Grasso *et al* (1990) found that a Se-deficiency decreased the *in vitro* ability of neutrophils to destroy *E. coli* by 30%, and the ability to destroy *Staphylococcus aureus* by 50%. In a study by Serfass & Ganther (1975) neutrophils of rats with a Se-deficiency could not destroy *Candiba albicans* after phagocytosis had taken place. Boyne & Arthur (1979) confirmed the fact that Se-deficiency in steers did not affect the ability of neutrophils to phagocitise *C. albicans,* but did affect the ability to destroy the ingested organism. The possible cause, being a decrease in GSHPx activity in the neutrophil, which would allow peroxide and lipid hydroperoxide to accumulate to toxic levels. It was also found that Se-deficient mice, infected with *C. albicans* died faster than mice receiving sufficient Se in their diet (Spallholtz, 1990). The oxidative ability of neutrophils isolated from goats (Aziz & Klesius, 1986) and cattle (Arthur & Boyne, 1985; Grasso *et al.*, 1990) receiving vitamin E sufficient but Se-deficient diets, was decreased. This limitation in function could be repaired by *in vitro* incubation with sodium selenite (Aziz *et al.*, 1984; Aziz & Klesius, 1986) or supplementation of the animal with Se and/or vitamin E (Aziz *et al.*, 1984; Politis, Hidiroglou, Batra, Gilmore, Gorewit & Scherf, 1995).

The finding that Se-deficiency reduces the microbicidal activity of neutrophils (Serfass & Ganther, 1975; Boyne & Arthur, 1979) suggested that low Se-status might be relevant to infectious diseases. Evidence from survey data (Arthur *et al.*, 1979) however suggests that less than 5% of herds will have a sufficiently low Se-status (<0.01 mg Se/l) for microbicidal activity to be decreased.

Considering that Se itself may be toxic and that it produces H_2O_2 and $O_2^{\bullet-}$ with oxidation of glutathion by selenite, it may be that dietary Se, i*n vivo*, is cytotoxic in immune cells (Spallholtz, 1990).

CHAPTER 4

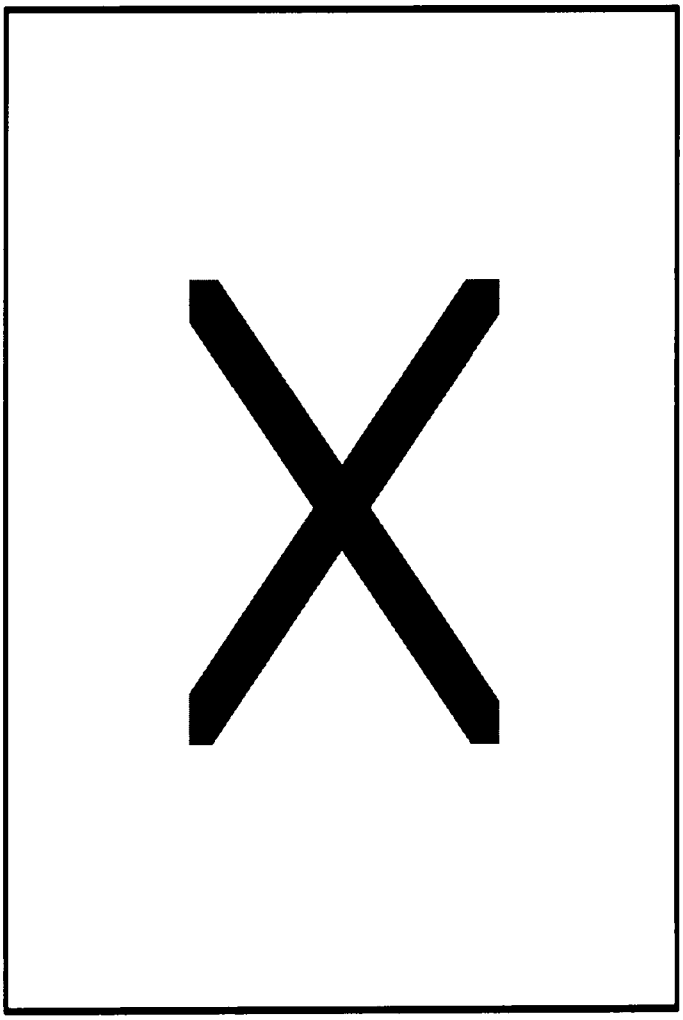
MATERIALS AND METHODS

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^2 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 22º19' and 25º32' South and longitudes 30º52' and 32º03' 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.

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 (succinyldicholine 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 ºC. 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)[a]	1.76 (0.07)	8.76 (0.56)[b]	90.889 (5.166)
M	185.71 (5.43)	30.42 (0.33)	57.16 (2.04)	10.80 (0.36)[b]	1.85 (0.08)	9.55 (0.55)[a]	90.442 (7.514)

[a,b]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 ± SD)	% Bone (mean ± SD)	% Muscle (mean ± SD)	% Drip loss (mean ± SD)	% BMF (mean ± SD)
Â	11.66 ± 4.65	30.33 ± 4.21	56.35 ± 6.24	1.81 ± 1.10	90.63 ± 6.67
2	10.53 ± 2.04	33.80 ± 3.51	54.22 ± 3.78	1.35 ± 0.60	84.48 ± 10.85
3	11.26 ± 4.28	30.67 ± 4.11	56.37 ± 6.13	1.69 ± 1.01	90.10 ± 6.53
4	12.27 ± 5.18	29.60 ± 4.28	56.57 ± 6.18	2.06 ± 1.23	91.74 ± 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 ± SD)	% BMF (mean ± SD)	Pearson correlation coefficient (significance)
0	9.494 ± 4.093	90.750 ± 5.675	0.1310 (0.2265)
1-6	11.784 ± 4.262	90.621 ±7.015	0.1069 (0.0971)
7-11	12.946 ± 5.264	90.658 ± 6.455	0.2449 (0.0200) Significant
12+	14.412 ± 5.531	89.701 ± 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)^{a}	61.37 (2.56)	9.91 (0.46)^{c}	1.84 (0.09)	9.65 (0.17)^{a}	92.04 (4.85)^{a}
Central	169.59 (5.48)^{b}	29.59 (0.31)^{b}	55.65 (1.93)	12.49 (0.34)^{a}	1.88 (0.08)	9.31 (1.56)	91.79 (7.03)^{a}
Northern	188.82 (5.61)^{a}	30.17 (0.32)^{b}	57.00 (1.96)	11.45 (0.35)^{b}	1.74 (0.08)	8.50 (0.33)^{b}	87.80 (7.22)^{b}

^{a,b,c}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)[1]	32.11 (0.53)[A1]	64.85 (3.29)[A]	10.18 (0.59)[a]	9.18 (0.23)[B]	1.83 (0.11)
	M	186.35 (7.98)	31.69 (0.65)[A]	56.31 (4.02)	9.79 (0.72)[A]	10.14 (0.26)[A]	1.84 (0.13)
	Â	177.10 (5.26)	31.90 (0.42)[a]	60.58 (2.60)	9.99 (0.46)[1]	9.66 (0.17)[1]	1.83 (0.09)
Central	F	169.40 (6.90)	29.53 (0.39)[B]	54.87 (2.43)[B]	13.38 (0.43)[b]	Non-est	1.78 (0.10)
	M	167.58 (8.79)	29.66 (0.50)[2]	57.11 (3.09)	11.42 (0.55)[B]	9.71 (1.55)	2.02 (0.13)
	Â	168.49 (5.59)[A]	26.59 (0.32)[b]	55.99 (1.96)	12.40 (0.35)[2]	Non-est	1.90 (0.08)
Northern	F	186.38 (7.09)	30.06 (0.40)[B]	56.70 (2.47)[B]	11.96 (0.44)[c]	8.33 (0.41)[B]	1.73 (0.11)
	M	190.50 (8.98)[2]	30.34 (0.51)[B]	57.67 (3.11)	10.99 (0.55)[l]	8.50 (0.55)[B]	1.73 (0.13)
	Â	188.44 (5.72)[B]	30.20 (0.32)[b]	57.19 (1.99)	11.48 (0.35)[2]	8.41 (0.34)[2]	1.73 (0.09)

[A,B,1,2,a,b,c]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 ± SD)
2	N	10.13±2.07
	Y	11.03±2.32
3	N	11.50±0.33
	Y	10.39±0.62[b]
4	N	12.26±0.37[a]
	Y	10.30±1.16

[a, b] 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 ± SD)	% Fat (mean ± SD)	% Fat (mean ± SD)
N	11.30±0.71%	12.84 ± 5.43%	9.90 ± 2.91
Y	11.24±0.89%	11.64 ± 4.36%	10.18 ± 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[a]	6.12±0.06[a]
Southern	5.77±0.02[b]	5.89±0.03[b]

[a,b]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[a]
1-6	5.88±0.02	5.98±0.03[b]
7+	5.84±0.05	5.87±0.08[b]

[a,b]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[b]	5.93±0.03[b]
Y	5.92±0.04[a]	6.07±0.06[a]

[a,b]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_{12} 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) [a]	138.13 (10.63) [a]
Central	0.630 (0.037) [b]	131.54 (9.98) [a]
Southern	0.414 (0.034) [c]	85.26 (9.14) [b]

[a,b,c]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)[a]	146.61 (5.59)[a]	4.04 (0.46)	7.06 (1.38)
Central	0.68 (0.02)[b]	132.39 (6.30)[a]	4.01 (0.44)	7.02 (1.34)
Southern	0.51 (0.01)[c]	83.20 (5.49)[b]	4.32 (0.41)	7.69 (1.56)

[a,b]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) [a]	135.14 (13.66) [a]	3.91 (0.09)	6.75 (0.25) [a]
Southern	0.424(0.026) [b]	64.54 (16.97) [b]	3.82 (0.08)	7.52 (0.24) [b]

[a,b]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)[a]	113.30 (6.69)	4.14 (0.39)	7.29 (1.41)
M	0.82 (0.05)[b]	118.39 (5.95)	4.16 (0.51)	7.50 (1.49)

[a,b]Means within the same column with different superscripts differ significantly (P≤ 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) [a]	180.10 (21.93)	3.96 (0.16)	7.34 (0.53)
M	1.15 (0.13) [b]	165.34 (19.62)	3.99 (0.15)	7.48 (0.48)

[a,b]Means within the same column with different superscripts differ significantly (P≤ 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) [a]	101.40 (15.63)	3.92 (0.11)	7.37 (0.31)
1-6	0.589 (0.015) [b]	103.36 (6.73)	4.08 (0.05)	7.66 (0.14)
7-11	0.611 (0.023) [b]	99.67 (10.12)	3.92 (0.07)	7.13 (0.21)
12+	0.437 (0.082) [a]	94.91 (38.53)	3.54 (0.27)	6.37 (0.77)

[a,b] 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)[a]	7.23 (0.19)
7-11	0.735 (0.036)[a]	127.41 (18.42)	3.82 (0.11)[b]	6.81 (0.29)
12+	0.507 (0.101)[b]	179.08 (52.22)	3.55 (0.32)[b]	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)[a]	3.85 (0.08)	6.99 (0.23)
Y	0.537 (0.028)	86.56 (13.14)[b]	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 regionswas 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_{12} 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.

CHAPTER 6
CONCLUSIONS

The purpose of the present study was to investigate visual body condition scoring (BCS) and percentage bone marrow fat (%BMF) for use on the African buffalo and to correlate these with proximate body fat content, to establish which gives the best estimate of body condition / composition. BCS and %BMF were poor predictors of proximate body composition, although %BMF was more accurate in older animals. It is difficult to obtain a measure of the animals' condition based on a single measurement, as there are numerous factors (nutritional, genetic, pathological, climatic and reproductive), which influence body condition.

The effects of region as well as gender and age, on the carcass composition of African buffalo were also investigated. The BCS did not differ between regions in the KNP, but differences in %BMF were observed ($P=0.0001$). Proximate body composition was influenced by sex, age and ecological region. Fat content was higher ($P<0.05$) and head weights lower ($P<0.05$) in females compared to males. Fat concentration increased with age from 11% in juvenile buffalo to 14% in adult buffalo. Fat content was lower (9.9%) in buffalo from the southern region (high BTB region) compared to those in the central region (12.5%) and northern region (11.5%). Carcass mass was lower ($P<0.05$) in the central region compared to the rest of the KNP. A significant correlation was observed between BTB and body condition of African buffalo in the southern region of the KNP. In this region, BTB was associated with a decrease in the overall body condition of buffalo.

Higher pH values observed in carcasses from BTB positive buffalo were attributed to lower glycogen levels in their carcasses. Regional and age effects on carcass pH were also detected.

From the results obtained for the minerals analysed, it appears that most areas in the KNP have adequate mineral levels when the trace mineral tissue levels of cattle (Puls, 1994) are used as reference. The tissue mineral levels for

Se and Cu indicate that there are significant differences between the different regions of the KNP, with a marginal deficiency in their levels in the southern region. A significant difference in Se status between different sexes was also observed in the KNP, particularly in the northern region. In the central and southern regions of the KNP there were significant differences in the Cu-concentrations between BTB positive and BTB negative buffalo.

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