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Habitat and seasonal effects on the nutrient status of selected roan (*Hippotragus equinus)* and sable antelope (*Hippotragus niger*) populations in South Africa

by

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DECLARATION

Herewith I, Jacques van Rooyen, declare that this thesis, submitted in fulfilment of the requirements of the degree M.Sc. (Agric) Animal science (Animal nutrition) at the University of Pretoria, has never been submitted at any other academic institution as part of the requirements of a degree, neither by me nor anybody else.

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J van Rooyen

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For all the animals of the forest are mine,
And I own the cattle on a thousand hills.
I know every bird on the mountains,
And all the animals of the field are mine.

Psalm 50: 10-11, NLT

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I dedicate this thesis to:

My loving wife, Renate,
And the miracle growing within her,
Who has supported me
Through all the joys, trials and tribulations
We experienced and conquered over the course of this project.

May the Lord bless you richly for your
Love
Support
and
Faithfulness!

ABSTRACT

Roan antelope numbers have declined drastically over the last 30 years in the Kruger National Park (KNP). Various hypotheses have been tested and suggested as reasons for their sharp decline. A change in habitat structure and nutrient deficiencies were among the factors considered for their population decline. The aim of this research was to study the influence of habitat structure on habitat selection in the absence of predators by two separate roan populations in predator-proof enclosures on the northern plains of the KNP. Seasonal variations in the nitrogen, macro- and micronutrient concentrations of the preferred grass species in every enclosure were determined. Faecal samples were collected and pooled seasonally in order to link nutrient concentrations in faeces with that in grass. Water and soil samples were also collected and analysed. The nutrient concentrations of grass and faeces in the wet- and dry season were compared with nutrient concentrations collected similarly from a roan antelope population at Vaalbos National Park, Northern Cape Province, South Africa. Where possible, serum samples were also collected and analysed for macro- and micronutrient concentrations.

Results indicated that roan antelope disregard habitat structure in the selection of vegetation type or foraging areas in the absence of predators. This behaviour may jeopardise survival if captive bred roan are released to supplement free roaming roan populations in the KNP since previous studies indicated that free roaming roan antelope in the KNP prefer lightly wooded areas with a fairly high visibility. Results from grass, faecal and serum analyses indicated that some nutrient deficiencies might occur in each of the populations studied during specific times of the year and especially in animals in nutrient challenging production phases. Results suggested a possible induced copper deficiency in roan at N'washitsumbe in the KNP, mainly from a Cu-Mo-S interaction. Wet season faecal nitrogen levels of roan at N'washitsumbe was marginal to deficient and that of the Graspan population at Vaalbos National Park was marginal. Phosphorus levels of all the populations were sufficient in faeces despite low dietary intakes. Results were compared with faecal and serum nutrient levels of supplemented roan and sable antelope at Sable Ranch, Northwest Province, South Africa. In general, supplemented roan and sable antelope had a significantly higher nutrient status than un-supplemented populations. Significant species and gender differences in serum and faecal nutrient concentrations were identified in supplemented animals at Sable Ranch.

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LIST OF ABBREVIATIONS

Al	Aluminium
Ar	Arsenic
Ca	Calcium
Cd	Cadmium
CF	Crude fibre
Co	Cobalt
CP	Crude protein
Cr	Chromium
Cu	Copper
DM	Dry matter
F	Fluoride
Fe	Iron
H	Hydrogen
I	Iodine
K	Potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
OM	Organic matter
P	Phosphorus
Pb	Lead
S	Sulphur
Se	Selenium
TDS	Total dissolved salts
w/w	Wet weight
$\mathbf{Xx_f}$	Xx concentration in faeces
$\mathbf{Xx_g}$	Xx concentration in grass
$\mathbf{Xx_s}$	Xx concentration in serum
Zn	Zink

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CHAPTER 1

INTRODUCTION

1.1 PROJECT THEME

Applied animal nutrition

1.2 PROJECT TITLE

Habitat and seasonal effects on the nutrient status of selected roan (*Hippotragus equinus)* and sable antelope (*Hippotragus niger*) populations in South Africa

1.3 MOTIVATION

Most estimates of the nutrient requirements of wild herbivores are based on domestic livestock and laboratory standards (Robbins, 1993; Schmidt and Snyman, 2002). The nutritional status of free roaming wild herbivores differ significantly from domesticated animals within the same area, suggesting that requirements cannot necessarily be extrapolated (Grant, 1989). The nutritional requirements of an herbivore species are determined by measuring its production or reproduction performance as a function of the nutrient quality and quantity of its diet (Underwood and Suttle, 1999; Van Ryssen, 2001). Should the diet meet the nutrient requirements of the animal's metabolic and physiological processes, it will be reflected in its productive or reproductive performance, assuming all other factors influencing its performance (e.g. disease, climate, age and sex) are non-significant (Van Ryssen, 2000). Under free roaming conditions, a wild herbivore species has specific habitat preferences influenced by its particular nutritional, climatic, social and environmental demands. It can therefore be assumed that the habitat within which a wild herbivore species establishes itself naturally and in which it performs well in terms of population growth, complies with all or most of its requirements.

Dose response trials remain the most reliable method of determining nutrient requirements in grazing ruminants (Underwood and Suttle, 1999; Van Ryssen, 2003) but these trials are time consuming and costly (Van Ryssen, 2001; Schmidt and Snyman, 2002; Van Ryssen, 2003). It also bares a high risk in the case of stress sensitive wild herbivores like roan and sable antelope (Wilson and Hirst, 1977). Determining the actual nutrient status of an animal requires controlled feeding in a metabolic crate in which all factors affecting nutrient status can be measured, monitored and managed. The nutrient status mentioned in the title of this project should thus be seen in the context of the extensive environment and undomesticated nature of the wild ungulates studied. The emphasis of the project was the evaluation of estimates of the nutrient status of populations given the limitations and scope of available non-invasive methods and data with which nutrient status were quantified. The nutrients included in this study to quantify nutrient status are thus limited to N, P, K, Ca, Mg, Na, S, Cu, Fe, Mn, Zn, Mo, Co and Se.

Although no formal guidelines on the optimal nutrient status of any wild ungulates, with which to assess the nutritional status of a population or its habitat, exists at present, previous studies proved that certain nutritional deficiencies could be anticipated by comparing the nutritional status of different populations of a species between different geographical regions (Wilson and Hirst, 1977; Dörgeloh, 1998). The more data available on the nutritional status of different populations of a wild herbivore species, like roan and sable antelope, and its habitat preferences, the more reliable estimates of nutritional requirements can be made (Myburgh, 2003).

Following an extensive study after the initial decline in roan antelope numbers in South Africa was realised, Wilson and Hirst (1977) stated that the reason behind the decline in roan and sable antelope numbers was a complex of factors involving disease, malnutrition and habitat quality of which a general suboptimum nutritional status might have been the primary reason which affected populations the most severely. Harrington *et al.* (1999) and Grant *et al.* (2002) investigated various previous hypotheses for the sharp decline in rare antelope numbers in the Kruger National Park (KNP) and the possible effects of resulting management actions on these populations. They suggested that predation, grazing pressure, rainfall cycles and habitat quality and quantity all contributed to a greater or lesser degree to the drop in roan antelope numbers.

However, at the same time a decline in roan antelope numbers was experienced at various other geographical locations throughout southern Africa and not just in the KNP (Wilson and Hirst, 1977; Martin, 2003). Grant (2004, pers. comm.)[1] and Codron *et al.* (2009) reported that suboptimum faecal nitrogen (N_f) concentrations in the roan antelope populations on the northern basaltic plains of the KNP had been found.

This indicated the probability of a deficient nutritional status in some or all of the roan antelope populations in the northern KNP (Codron *et al.,* 2009) and thus validating further research into the nutritional status of these roan antelope populations. For comparative reasons, the roan antelope population at the Graspan-Holpan section of the Vaalbos National Park, Northern Cape province, South Africa was included as a study area as well as the sable and roan antelope populations of Sable Ranch, a private game reserve in the Northwest province.

1.4 AIMS AND OBJECTIVES

The main objectives of this study were to:

1. Determine whether habitat structure influences habitat selection by roan in the absence of predators
2. Evaluate and compare the forage, soil and water quality of the different study areas in order to identify possible differences between areas
3. Evaluate the nutritional status of the various roan antelope populations by the comparison of faecal nutrient levels in combination with serum nutrient levels between populations, species and sexes
4. Determine whether there are differences in the nutrient status of roan and sable antelope under similar feeding conditions in both faecal and serum nutrient levels

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[1] Dr C.C. Grant, January 2004. Scientific Services, Skukuza, Kruger National Park. Ph. (013) 735 4415

5. Determine whether there are differences between sexes within roan and sable antelope under similar nutritional conditions in both faecal and serum nutrient levels
6. Determine whether correlations between specific nutrients within and between serum and faecal samples influence the interpretation of the nutrient status of a population
7. Assess whether nutrient deficiencies could have contributed to the sudden decline in roan numbers in the KNP

1.5 THESIS STRUCTURE

The outline of this research project was structured in a way that will enable the reader to follow the line of thought as well as data flow from one chapter into the next. Therefore, chapters four, five and six of this thesis essentially consist of the following:

- Literature review
- Materials and methods
- Results and discussion
- Conclusions

Most of the data from one chapter follows onto the protocol and sub-study of the next chapter. The general conclusion of the thesis is presented in the last chapter (Chapter 7).

CHAPTER 2

LITERATURE REVIEW

2.1. POSSIBLE CAUSES OF THE DECLINE IN SIZE OF RARE ANTELOPE POPULATIONS IN SOUTHERN AFRICA

The roan antelope population in the KNP declined between 1986 and 1993 from 450 to 45 animals despite their high management and research priority (Harrington *et al.,* 1999). This drastic change in roan antelope numbers and other rare antelope species such as sable antelope, gave wildlife managers and conservationists reason for concern and various possible hypotheses were tested through different research approaches (Martin, 2003).

Joubert (1970) studied the social behaviour of the roan antelope population situated at the northern plains of the Kruger National Park and found that the park contained limited suitable habitat for roan antelope. According to reports by early park wardens, the former stronghold of roan in the Lowveld region was along the foothills of the Drakensberg mountain range, excluded from the boundary of the Kruger National Park in the early 1900's. Joubert (1970) stated that according to estimations in 1912, the roan population in the southern half of the KNP was double the size of that of the northern roan population. Throughout the previous century the southern populations declined until they eventually disappeared.

The reason for this disappearance could have been that the roan antelope occurring in the southern parts of the KNP only occurred there because they migrated between the lower-laying areas of the Lowveld and the foothills of the Drakensberg as seasons changed. It could also be that they never occurred in one place all year round because of nutritional deficiencies in preferred areas. Since the fencing of the park boundary, these populations could not continue their seasonal movements and diminished over time. There is the possibility as well that roan antelope in the northern half of the KNP could have moved into more favourable Mozambican areas adjacent to the KNP during certain times of the year or during dry cycles (C.C. Grant, 2004. Pers. comm..)[2].

Dorgelöh (1998) stated that, based on the findings of various scientists, the ecological patterns of rare species such as roan antelope may ultimately lead to their extinction in specific areas. He then summarized specific behaviours and characteristics of roan antelope, as documented by various scientists, on whom he based his statement (Joubert, 1970; Joubert, 1976; Wilson and Hirst, 1977; Heitkönig, 1993):

1. Roan is an ecotonal species that is found in nutrient poor savannahs such as miombo woodlands
2. Roan are highly selective of food plants even under stress situations
3. Roan are always at a disadvantage in a competitive situation as other species are normally more dominant causing roan to withdraw into secluded areas
4. Newly born calves are hidden away from the herd for up to 6 weeks, which contribute to high calf mortalities
5. A high proportion of calves are weaned at the end of the dry season when forage protein and phosphorus levels are too low to provide adequate nutrition
6. Intraspecific aggression by a herd bull may impact significantly on the sub-adult male age group within a herd

The major question of the actual suitability of KNP for sustaining large numbers of roan antelope was answered previously when Joubert (1976) stated that roan antelope habitat within the KNP was marginally sufficient. He stated further that due to the limited suitable habitat in the KNP a population greater than 350 animals in the entire park should never be expected.

In a comprehensive report on the population status of rare antelope species (sable antelope, roan antelope and tsessebe *Damaliscus lunatus*) in southern Africa, Martin (2003) considered the influence of veterinary fences on the movement of these antelope species between Namibia and Botswana to be a major constraining factor. Populations may become isolated and diminish over time.

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[2] Dr C.C. Grant, January 2004. Scientific Services, Skukuza, Kruger National Park. Ph. (013) 735 4415

A further important limiting factor, as was pointed out by Martin (2003), was the negative influence of large numbers of elephant *Loxodanta africana* on the habitats required by these specialist feeders. Martin (2003) reported that the roan antelope and tsessebe populations in the Sebungwe region of Zimbabwe declined almost to extinction over a period coinciding with the rapid increase of the elephant population and the subsequent large-scale structural changes in the habitat. According to Martin (2003) the influence of elephant on the habitat is the most severe limiting factor second to below normal rainfall cycles. Elephant and their effect on habitat could therefore be a key factor influencing the KNP roan antelope population.

Joubert (1970) reported that despite the fact that roan antelope are very fertile, populations seem to have an apparent maximum density at comparatively low levels, which indicate a mechanism of self-regulation. This self-regulating mechanism has to do with the major features determining optimal roan habitat and the social organisation of a roan antelope population (Joubert, 1970). Considerable intra-specific aggression by roan antelope is most probably an evolutionary adaptation to ensure sufficient habitat of good quality for each herd (Wilson and Hirst, 1977). Wilson and Hirst (1977) recommended a maximum population density of 4 animals/km^2 with a minimum range size of 1 500 ha for a population of 40-50 animals. Roan antelope therefore seldom exists as a dominant species in any African savannah ecosystem (Martin, 2003).

Following the earliest, most extensive studies involving rare antelope species, covering an array of possible limiting factors, Wilson and Hirst (1977) concluded that the causes of diminishing roan antelope numbers were a complex of correlated factors involving disease, malnutrition and habitat quality. Roan and sable antelope populations studied in most of the South African locations (Transvaal Nature Reserves) were in a sub-optimal nutritional state compared to populations in Zimbabwe. This was primarily due to the insufficient nutrient status of the natural resources of the habitat of South African populations (Wilson and Hirst, 1977).

Harrington *et al.* (1999) used data in the form of observations and standard data recordings of climatic, animal and vegetation surveys recorded over years of KNP management. Various possibilities for the decline in roan antelope population numbers in the KNP were investigated:

- Competition from zebra and wildebeest following the introduction of water points
- Increased predation following the influx of zebra and wildebeest
- Habitat deterioration driven by prolonged sub-normal rainfall
- Anthrax outbreak
- Stress associated with annual immunization practises

Results from the study indicated that four potential causes of the roan decline seemed prominent and related. The lower rainfall trends through the late 1980's terminating in the 1991/2 droughts degraded favourable grassland structure for all ungulate species preferring taller grasslands. The influx of zebra and wildebeest because of park management's decision to increase the number of waterholes in and around the region where roan antelope populations occurred, aggravated the situation. Zebra and wildebeest kept the grass structure short throughout the dry cycle and drastically increased the herbivore density in the region. The high numbers of zebra and wildebeest increased the lion population in the area and resulted in a sudden higher predation pressure on the roan antelope population, exacerbated by the higher inter-competition pressure for food resources from zebra and wildebeest. The higher predator pressure was most probably one of the major reasons for the increase in adult mortalities during the roan population's period of rapid decline (Harrington *et al.,* 1999).

According to Harrington *et al.* (1999) the fact that it was not lower calf and juvenile survival rates that coincided with the period of declining numbers, suggested that nutritional factors were not the prime cause of diminishing numbers. However, there were some limitations in the data in terms of routine ungulate censuses, limited samples and incomplete animal number data (Harrington *et al.,* 1999).

According to Martin (2003) the primary limiting factor of roan antelope and other rare antelope species in southern Africa is the fact that many areas in which roan antelope are conserved falls within a marginal rainfall area for the species (300–400 mm/annum).

Martin (2003) further stated that the performances of rare antelope species are linked to long term cumulative rainfall surpluses and deficits. His statements supported the findings of Harrington *et al.* (1999), who found that the majority of ungulate population numbers preferring tall grass habitat in the KNP were strongly correlated to rainfall patterns. This was true for roan antelope as well. However, the sudden sharp decrease in roan numbers could not have been due to a single below average rainfall period only (Harrington *et al.,* 1999).

In summary, various factors seemed to have contributed, either jointly or individually, to the sudden decrease in roan numbers.

2.2. CONCLUSIONS

Considerable evidence indicate that an increase in predation as well as grazing pressure contributed to the initiation of the sharp decrease in the roan numbers on the northern plains of the KNP. However, various other populations throughout southern Africa showed the same tendency under different environmental and ecological circumstances. It is therefore perhaps more important to know what to monitor within a specific rare antelope population in order to be able to identify and even anticipate possible threats on a particular population. However, what is of greater concern is the roan population's apparent inability to recover from their population crash. Waterholes have been closed again, rainfall cycles have recovered and grazing/predator pressure have decreased yet the roan population kept decreasing or at least remained more or less stable.

It was consequently decided to embark on a comprehensive evaluation of the nutritional status of the habitat and the populations of the KNP and compare it to that of the Graspan population in order to attempt to quantify the specific habitat and dietary needs of the species. A sub-optimal nutrient status exacerbated by inter- or intra-specific competition in any herbivore population could contribute significantly to its vulnerability under pressures.

Should it be concluded that the nutritional status of the habitat in which roan exist on the northern plains of the KNP is sufficient in supplying the required nutrients and minerals for optimum survival and reproduction, it can be concluded that nutritional deficiencies were not a major contributing reason for their population crash in the 1980's. More so, it will not be the reason for their inability to increase in their population numbers. If proven otherwise, nutritional deficiencies might have contributed extensively to the roan antelope's vulnerability during specific periods of the year, to both competition as well as predation. Too many pressures on the population at one specific point in time might have pushed the population over a stress-threshold from which it could not recover under natural conditions or in the presence of acceptable, natural pressures.

CHAPTER 3

STUDY AREAS

3.1. KRUGER NATIONAL PARK

The remnant free roaming populations of roan antelope within the boundaries of the KNP are scattered across the north-eastern basaltic plains to the north and south of the Shingwedzi River. This region forms part of the Letaba Formation (Lebombo Group, Karoo Supergroup) and is characterized by heavy black clay soils. Soil depth varies considerably and may exceed one metre. The underlying formation is mostly comprised of a deep impermeable layer of calcareous rock (Joubert, 1976; Mucina and Rutherford, 2006). The main vegetation type is *Colophospermum mopane* shrub veld. Within this region two roan populations exist within two separate enclosures. The N'washitsumbe enclosure is situated approximately 50 km to the north of the Shingwedzi River (Shingwedzi Rest Camp) and the Capricorn enclosure is situated approximately 50 km to the south of the Shingwedzi River, close to the Mopani Rest Camp. Both these enclosures are situated in the Mopane Basalt Shrubland Veldtype (Mucina and Rutherford, 2006).

3.1.1. N'WASHITSUMBE

The N'watshitsumbe enclosure was built in 1967 for the purpose of more intensive research on the ecology of roan antelope (Joubert, 1970). The enclosure was erected in the middle of preferred roan habitat on the north-eastern basaltic plains covered primarily by mopani shrubland. The enclosure itself consists mainly of mopani shrubland savannah, but also contains a small piece of grassland savannah in a vlei area and a *Sclerocarya birrea – Acacia nigresescens* tree savannah area (Joubert, 1970). The enclosure was 254 ha in size and properly fenced in order to keep all predators and herbivores out.

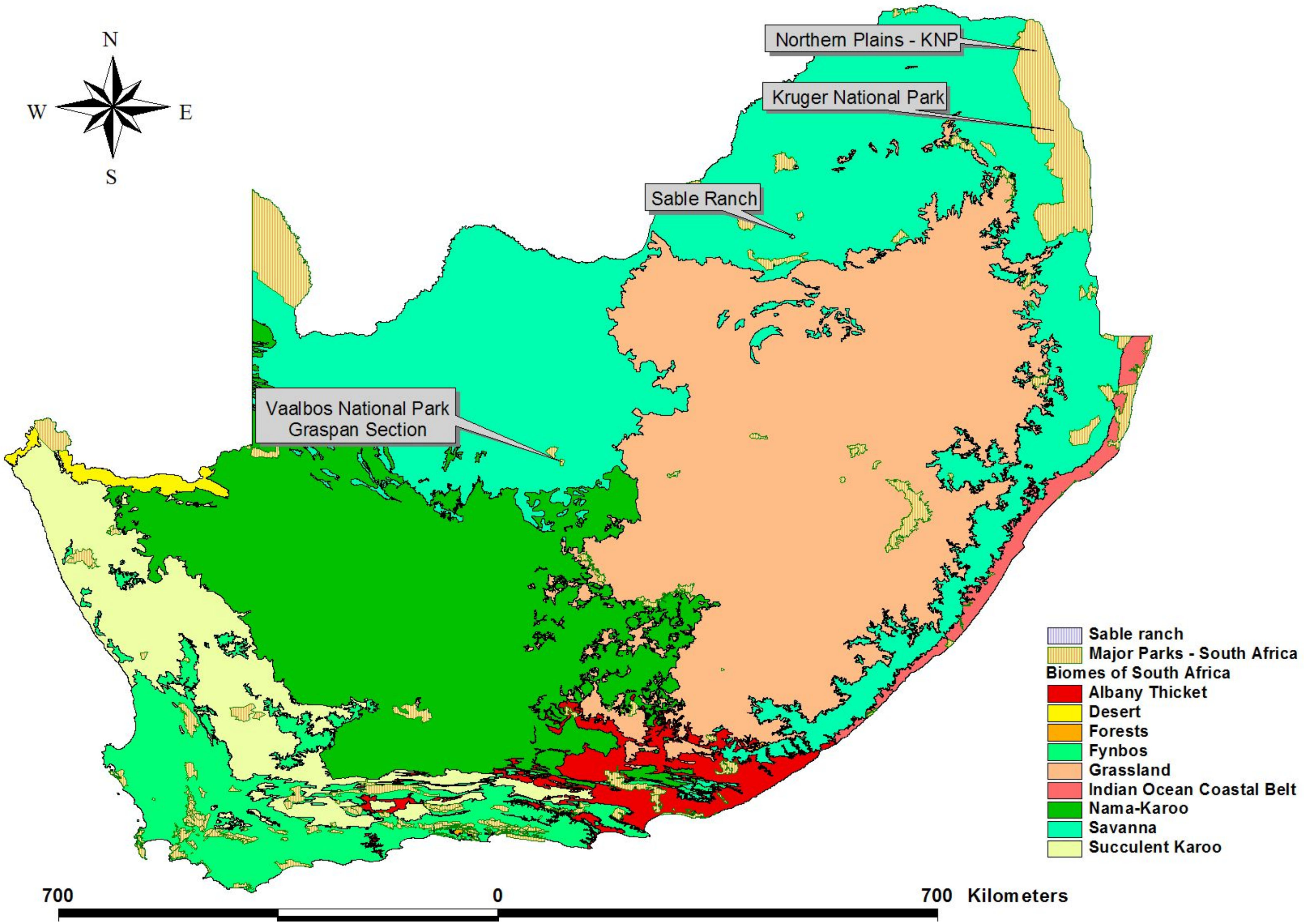


Figure 3.1 Biomes of South Africa indicating all the study areas within the Savannah Biome

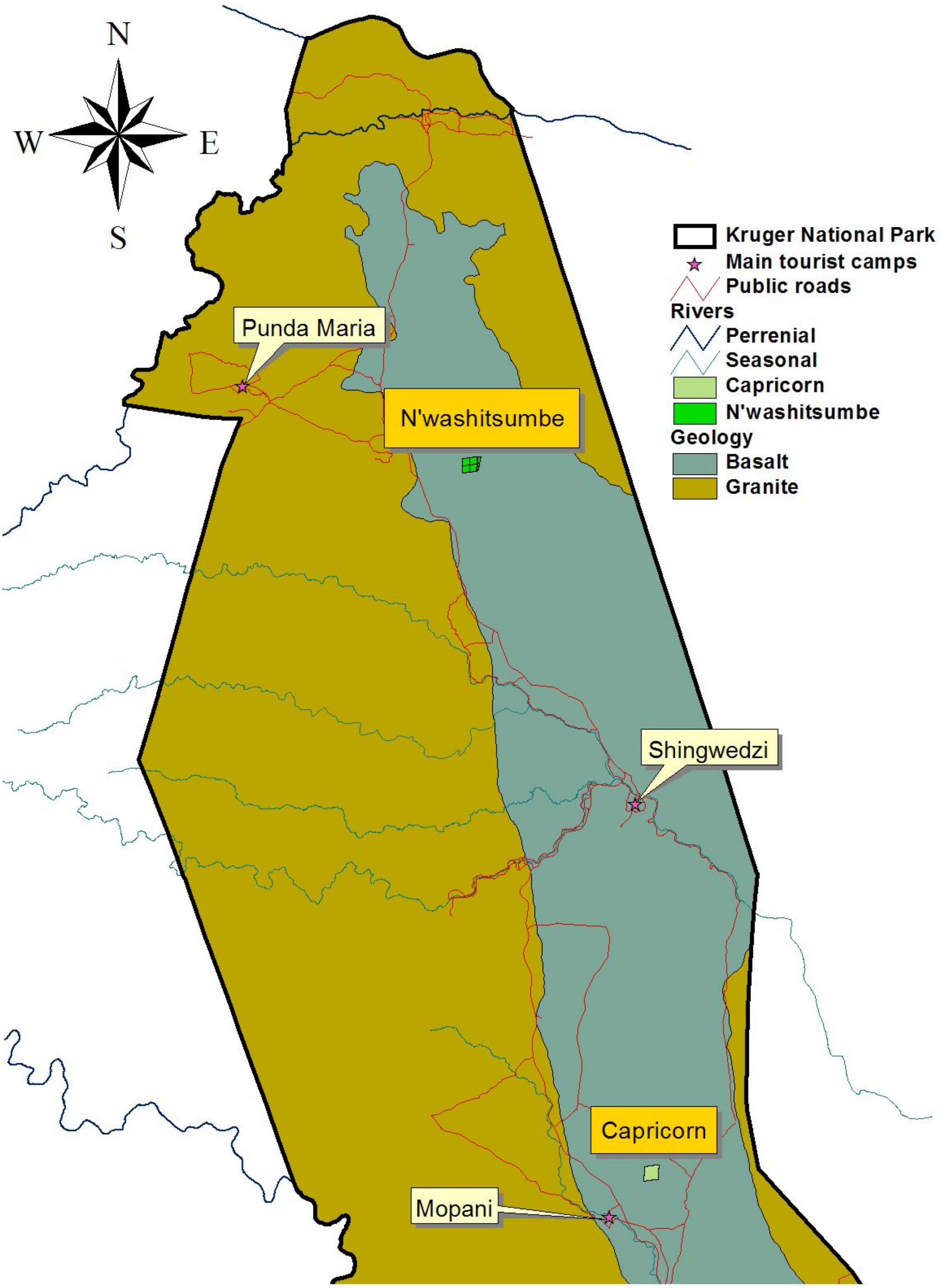


Figure 3.2 Geology of the northern plains of the Kruger National Park, indicating the two roan enclosures, N'washitsumbe and Capricorn, situated on the basalts

In 1984 another 48 ha were added to the eastern boundary of the enclosure to include a part of the adjacent watercourse and the associated ecotonal wetland vegetation (Grant, 2003).

3.1.2. CAPRICORN

Roan antelope were fairly recently introduced to the Capricorn or Mooiplaas Rare Antelope Enclosure. In March 2003, 19 animals were removed from N'washitsumbe and relocated to the Capricorn enclosure. The enclosure is 500 ha in size and is also situated on the northern basaltic plains of the mopani shrub savannah region (Grant, 2003). Three vegetation types have been identified within this enclosure: mopani shrub savannah, mixed savannah and open grassland (Grant and De Buys, 2004).

3.1.3. FREE ROAMING POPULATION

At present, very little is known about the free roaming roan population on the northern basaltic plains of the KNP. During the course of the study, at least one sighting of roan antelope outside the enclosures was made within the vicinity of the N'washitsumbe enclosure, which suggested that they still mainly occurred within the proximity of the enclosures. According to the annual species survey data of 2003, a free-roaming population of approximately 33 roan still existed within the entire KNP.

Figure 3.3: Lush vegetation of N'washitsumbe in the wet season– March 2006

Figure 3.4: Very low biomass and poor grazing quality in N'washitsumbe in the late dry season before the first summer rains – October/November 2005

3.2. VAALBOS NATIONAL PARK

Vaalbos National Park is situated in the Northern Cape Province of South Africa. It consists of two separate sections - the Than-Droogeveld section (18 129 ha) and the Graspan-Holpan (GH) section (4 576 ha) of which the latter is situated 25 km west of Kimberley (Bezuidenhout, 1995). The GH section is subdivided into several camps of which the largest (north-western section) was occupied by a herd of roan antelope (approximately 55 animals). This section is part of the Kalahari Thornveld veld type (Mucina and Rutherford, 2006) even though the savannah was dominated by *Tarchonanthus camphoratus* shrubs.

The GH section is mainly underlain by three geological systems that vary from andesitic to basaltic lava outcrops as well as tillite and shale outcrops in the south, to red fresh-coloured aeolian sand with surfacing limestone patches in the north (Bezuidenhout, 1995). The soil consisted mainly of deep sandy to loamy soils of the Hutton soil form and formed slightly undulating sandy plains (Mucina and Rutherford, 2006). Small pans often formed in the low laying areas where some *Acacia erioloba* trees often occurred. This part was mainly covered by the *Hermannia tomentosa – Schmidtia pappophoriodes,* short closed grassland and the *Tarchonanthus camphoratus – Rhus ciliata* high closed shrubland vegetation types (Bezuidenhout, 1995). *Schmidtia pappophoroides, Eragrostis lehmanniana* and *Stipagrostis uniplumis* were among the dominant grass species which occurred in both vegetation types (Bezuidenhout, 1995). Scattered stands of *Themeda triandra* also occurred within these vegetation types.

3.3. SABLE RANCH

Sable Ranch is a private game reserve approximately 25 km north of the town Brits in the Northwest Province of South Africa. The reserve is approximately 3000 ha in size and contains one of the largest privately owned sable antelope populations in South Africa. Roan and sable antelope are bred semi-intensively in large enclosures varying between 50 -150 ha in size. Sable Ranch has a supplemental feeding program as well as a comprehensive record keeping system for their rare antelope breeding programme.

The habitat of Sable Ranch mostly consist of the Mixed Bushveld veld type (Acocks, 1988) or Central Sandy Bushveld veld type (Mucina and Rutherford, 2006). Most of the property is situated on sandy granite soils. The annual rainfall of the area is approximately 620 mm per year and the geology is predominately red granite of the Lebowa Granite Suite and some granophyres of the Rashoop Granophyre Suite of the Bushveld and Vaalian Complexes (Dörgeloh, 1998; Mucina and Rutherford, 2006).

The soil consists mostly of well-drained, deep Hutton and Clovelly soils often with a catenary sequence due to all the granite outcrops in the largest part of the central reserve area (Mucina and Rutherford, 2006).

Figure 3.5: Dry season landscape of the Graspan/Holpan section of Vaalbos National Park – July 2005

CHAPTER 4

ROAN ANTELOPE HABITAT AND FEEDING PREFERENCES

4.1 LITERATURE REVIEW

In this chapter preference was given to habitat and dietary selection by roan antelope above that of sable antelope since the primary habitat and nutritional evaluation was that of the roan antelope populations in the two KNP enclosures and the Graspan population. The habitat selection and preference of sable antelope was included since the nutritional status of roan and sable was compared later in the study.

The roan antelope is the second largest antelope species in Africa with adult bulls weighing between 250-275kg and adult cows between 225-245kg (Skinner and Chimimba, 2005; Furstenburg, 2006). The shoulder height of adult bulls varies between 130-145cm and that of females range between 125-135cm. Under optimal conditions roan are very fertile and can breed prolifically (Joubert, 1976). Females can conceive at two years already and have an average gestation period of 270-290 days. Their inter-calving period can be as short as 317 days (10-11 months) (Joubert, 1976; Furstenburg, 2006).

Sable antelope are closely related to roan antelope but are smaller in body size and shoulder height. The live weight of mature sable bulls range between 200-250kg and that of females between 180-220kg (Skinner and Chimimba, 2005; Furstenburg, 2006). The average shoulder height of bulls range between 130-140cm and the shoulder height of females range between 115-130cm (Skinner and Chimimba, 2005; Furstenburg, 2006). Age at first conception of sable females (2.5 years) is generally later than that of roan females. However, their age at first conception could be decreased to below two years under optimal, intensive production systems (Skinner and Chimimba, 2005).

Sable have a similar gestation period as roan of approximately 266 days (Skinner and Chimimba, 2005; Furstenburg, 2006) with an average inter-calving period of 12 months (Furstenburg, 2006).

The habitat and grazing ecology of the roan antelope have been thoroughly researched since the 1970's in the KNP (Joubert, 1970; Joubert, 1976; Harrington *et al.,* 1999; Grant *et al.,* 2002) and across southern Africa (Joubert, 1976; Erb, 1993; Heitkönig and Owen-Smith, 1998; Martin, 2003). Roan antelope are widely distributed throughout the central and western parts of Africa, occupying all savannah areas and peripheral semi-arid areas south of the Sahara (Skinner and Smithers, 1990; Toms and Joubert, 2005).

South Africa hosts the most southern roan antelope populations implying that the habitat occupied by roan antelope in South Africa is marginally sufficient (Joubert, 1976; Wilson and Hirst, 1977). Wilson and Hirst (1977) stated that: "The whole complex of habitat use and the relevant feeding strategies of roan and sable antelope on Transvaal nature reserves are of major significance in their maintenance and successful conservation."

Various factors affecting the preference of roan antelope for a specific habitat have been studied (Joubert, 1970; Joubert, 1976; Wilson and Hirst, 1977; Dörgeloh, 1998; Harrington, *et al.,* 1999). These studies investigated factors such as soil type, grass species composition, greenness, plant structure, forage quantity and quality, browse availability, canopy cover, tree density and visibility. Joubert (1976) found that the condition of the grass stratum (grass height) is as critical as the density of the shrub strata in determining the quality of roan habitats.

4.1.1 HABITAT STRUCTURE

In general, the habitat preferred by roan antelope is described as a lightly wooded savannah with open areas (wetlands) of medium to tall grass (Joubert, 1976; Apps, 1996; Dörgeloh, 1998c; Martin, 2003). Roan antelope are tolerant to low bush growth of up to 1.5 m tall, provided it is open and patchy. Roan antelope are well adapted to tall grass of heights up to 1.5m and consequently avoid short grass areas, for instance the green flush after burns (Martin 2003).

However, according to observations made by Dörgeloh (1998c) over a two year period in the Nylsvlei Nature Reserve, South Africa, the new short grass of burnt areas was utilised by roan antelope. Joubert (1970) described different habitat types frequented by roan antelope populations in Angola and the KNP. Although very different in botanical composition, there were three major similarities:

1. Open grass plains or grass patches of variable sizes
2. Easily accessible cover in the form of a shrubby, wooded fringe
3. Open surface water

According to Joubert (1970) these habitat characteristics could be typical of any optimum habitat for roan antelope. Dörgeloh (1998c) found that the roan breeding herd in Nylsvlei Nature Reserve preferred areas with grass taller than other available plant communities during the summer and winter seasons. Of various habitat aspects grass height, biomass, tree density and canopy cover were found to be the most important factors influencing roan habitat selection. Specific sites selected within specific plant communities preferred by roan antelope showed that of all the different habitat facets measured, vegetation structure differed significantly. Roan antelope selected sites with significantly higher total grass height, above-ground biomass and grass tuft height and lower canopy and tree densities in comparison to areas not preferred. Similarly, Heitkönig (1993) found that roan in Nylsvlei showed no significant preference for a particular soil type but rather for the physiognomy of the habitat. As reported by Joubert (1976), Heitkönig (1993) also found that roan had a significant preference for grasslands over savannahs. These findings proved that habitat structure is one of the most important factors influencing habitat preference by roan antelope (Dörgeloh, 1998).

4.1.2 DIET PREFERENCE

In a comprehensive study within a newly built roan antelope enclosure, N'washitsumbe, in the northern plains of the KNP, Joubert (1976) found that the diet of roan antelope consisted 75 % of grasses and 25 % of browse. The main grass species selected by roan antelope in the N'washitsumbe enclosure was well represented in the preferred roan habitat.

The selected grass species consisted of the following (ranked according to a preference rating):
Themeda triandra, Eragrostis superba, Heteropogon contortus, Schmidtia pappophoroides, Digitaria pentzii, Setaria woodii and *Cenchrus ciliaris.*

Other important species also preferred by roan but not as readily available at that stage were:
Urochloa mozambicenses and *Enneapogon cenchroides.* Of the three main plant communities available in the enclosure (*Schmidtia pappophoroides – Pannicum coloratum* (72 %), *Schmidtia pappophoroides – Digitaria pentzii* (20 %) and *Setaria woodii – Panicum coloratum* (8 %)) the most preferred plant community on utilisation per unit area basis was found to be the *Setaria woodii – Panicum coloratum* community. This “vlei” community comprised only 8% of the total surface area of the enclosure but was preferred twice as much as the other communities according to a preference rating.
This was especially the case in the dry season. Joubert (1976) also observed a sharp increase in the utilisation of *Schmidtia pappophoroides, Cenchrus ciliaris, Digitaria pentzii* and *Heteropogon contortus* during the late dry season.

The woody species utilised by roan antelope in the N’washitsumbe enclosure were the following: *Dalbergia melanoxylon, Lonchocarpus capassa, Indigofera sp., Albizia harveyi* and *Dichrostachys cinerea.* Most of these woody plants were browsed during the dry period of the year but also in some instances in the wet season when the plants produced green shoots (Joubert, 1976).

In Percy Fyfe Nature Reserve in the Limpopo Province, South Africa, Wilson and Hirst (1977) studied the feeding preferences of the roan and sable antelope. Two roan antelope bulls were placed in a 0.5ha enclosure erected within a habitat area preferred by roan antelope. During an eleven-day period roan showed a strong preference for the following grass species: *Heteropogon contortus, Rhynchelytrum repens, Eragrostis superba, Schizachyrium sanguineum.* Other species also preferred were *Hyparrhenia hirta, Trichoneura grandiglumis* and *Themeda triandra.* An interesting observation by Wilson and Hirst (1977) was that the diet of the two roan bulls consisted only of approximately 15% of the available grass species in the enclosure. Most of the dominant grass species (e.g. *Digitaria pentzii*) were not selected by the roan bulls. This proved the extent of the highly selective feeding habits of roan antelope (Wilson and Hirst, 1977). Observations from sable antelope in the Rustenburg Nature Reserve, Loskop Dam Nature Reserve, Hans Merensky Nature Reserve and Matetsi area (Zimbabwe) showed that *Themeda triandra, Heteropogon contortus* and *Eragrostis* sp. were grass species preferred by all the populations (Wilson and Hirst, 1977).

In 1972 roan antelope in Percy Fyfe Nature Reserve occurred in a 430ha enclosure (Wilson and Hirst, 1977). Roan antelope maintained their body condition much better and had a lower disease incidence than sable antelope during the dry months in Percy Fyfe. This was attributed to the fact that roan antelope increased their browsing frequency up to 59% of their total feeding time during the dry season. On the contrary, sable antelope were never observed browsing during the course of the study in any of the then Transvaal reserves (currently Limpopo and Mpumalanga Provinces) (Wilson and Hirst, 1977). The woody species mostly selected by roan antelope in Percy Fyfe were *Acacia karroo* and *Rhus lancea.* Other preferred browsed species included *Olea africana, Acacia caffra,* and *Faurea saligna.* However, in 1973 the roan antelope population gained access to a much larger area (2 061 ha) and their browsing incidence dropped significantly to approximately 11%, suggesting that roan antelope prefer grazing but could readily switch to browsing should preferred grass species decline and feeding competition increase.

In a study in the Waterberg Plateau Park, Namibia, Erb (1993) found that roan antelope selected *Brachiaria nigropedata, Panicum kalahariense, Eragrostis jeffreysii, Andropogon schirensis, Triraphis schinzii* and *Digitaria seriata.* All these species with the exception of *E. jeffreysii* were climax grass species. The feeding height ranged between 25.6cm and 8.1cm. Woody plants browsed included *Grewia reticulate, Lonchocarpus nelsii, Acacia ataxacantha* and *Ochna pulcra.* Most of the browsing was observed in the drier months between April and November.

In Nylsvlei Nature Reserve, Limpopo Province, Heitkönig (1993) found that roan antelope preferred grass swards with a favourable green:old leaf ratio on alluvial soils and a favourable leaf:stem ratio on felsitic and sandstone soils. Areas with grass consisting of very high stem densities were avoided. During the dry season roan preferred areas that offered higher densities of green leaves.

In Nylsvlei the diet of roan consisted 84% of grass and 8.2% of browse with no significant increase in browse during a specific season (Heitkönig, 1993). Grass species making up the diet consisted of *Cenchrus ciliaris, Eragrostis lehmanniana, Schmidtia pappophoroides, Hyperthelia dissoluta, Digitaria eriantha, Heteropogon contortus* and *Eragrostis pallens.* There was no consistent preference for or against any of these grass species. However, *E. lehmanniana* was the dominant grass in the *Acacia*-site but was generally avoided accept during August/September. *S. pappophoroides, P. maximum and C. cilliaris* encompassed 29% of the available tufts and were generally preferred all year round. *Hyperthelia dissolute* and *Panicum maximum* were preferred over *Eragrostis pallens* which was mostly avoided (Heitkönig, 1993). Woody plants selected were *Combretum molle, Grewia flavescens* and *Burkeya africana*, all year round.

Dörgeloh (1998) found that during the summer months roan antelope breeding herds preferred the open, lower floodplain of the Nylsvlei Nature Reserve, Limpopo Province, South Africa. The floodplain's mean grass height was higher than other plant communities. In the winter months a reduction in green leaf density (Heitkönig and Owen-Smith 1998) and a reduced forage quality most probably forced roan antelope out of the floodplain (Dörgeloh, 1998). These observations corresponded with the observations of Heitkönig and Owen-Smith (1998) on the same roan antelope population in Nylsvlei. According to Heitkönig and Owen-Smith (1998) roan antelope in Nylsvlei Nature Reserve preferred open grassland to wooded savannah areas. Contrasts in the physiognomy of the landscape primarily influenced the habitat preferred by roan. The particular soil type was of secondary importance in the selection of the landscape (Heitkönig and Owen-Smith, 1998).

Knoop and Owen-Smith (2006) found a significant seasonal difference in plant species as well as grazing area selection by roan antelope in the N'washitsumbe enclosure. However, the extent of plant species and grazing area preference was largely influenced by seasonal rainfall patterns. The roan population generally preferred the bottomland vlei area during the winter months and this corresponded with the findings of Joubert (1976). The dominant grass species *Sporobolus pyrimidalis* and *Ischaemum afrum* within the wetland was extensively utilised during the dry winter months. These species were utilised much less during the summer months. *Sporobolus ioclados* was restricted to sodic patches on the ecotone between the bottomland and upland.

S. ioclados was little utilised except in the late summer. *P maximum* and *Panicum coloratum* were extensively utilised in the upland region, mostly in summer but also extensively in the drier than normal 2002 winter months. *T. triandra* was generally utilised to a much lower degree than what would have been expected but made a larger dietary contribution in the very dry winter of the year 2002. These findings did not coincide with the findings in a study by Joubert (1976) where it was found that *P. coloratum* played a less significant role in the diet of roan antelope within N'washitsumbe compared to *T. triandra,* which was the most important food source in all the seasons at that time.

Knoop and Owen-Smith (2006) further observed that *S. pappohoroides* and *U. mosambiscensis* was generally not well utilised except in abnormally dry seasons. Contradictory, Joubert (1976) found *S. pappophoroides* and *Urochloa mosambiscensis* to be a major constituent in the diet selection of roan in N'washitsumbe.

Codron *et al.,* (2009) found through $\delta^{13}C$ stable isotope analysis of herbivore faeces in the northern basaltic plains of the KNP that roan antelope are almost exclusively grazers with a maximum of 7% browse in the late dry season. Due to the relatively small quantities of browse utilised by roan it is feasible to focus on grass nutrient concentrations in combination with faecal nutrient concentrations for dietary nutrient evaluations.

4.1.3 PREDATOR EVASION AND HABITAT SELECTION

Inducible defences in herbivores are, for instance, the changes in the behaviour of a species or population in response to predation (Creel *et al.,* 2007). Inducible defences in herbivores can reduce the predation risk of an individual or population but could come at a cost (Creel *et al.,* 2007). In Yellowstone National Park, USA, Creel *et al.* (2005) found that elk *Cervus elaphus* adapted their grazing patterns and habitat selection after the introduction of wolves into the park. Elk favoured grassland for foraging but readily moved into wooded areas in the presence of wolves. Over time this behaviour not only seemed to have affected the vegetation, but also the reproduction rates of the elk population.

Even though predation pressure was correlated with calf:cow ratios, there were very little evidence of direct wolf predation on young calves. Results therefore suggested that direct predation had a lesser effect on calf survival than, for example, the decreased ability of cows to raise calves successfully when utilising less preferred foraging areas as part of their predation evasion measures (Creel *et al.,* 2005; Creel *et al.,* 2007).

Roan antelope are primarily grazers and only utilise browse extensively when resources are limited (Wilson and Hirst, 1977). Roan has a stronger preference than sable for open savannahs with a low tree density and canopy cover, hence higher visibility (Joubert, 1976; Wilson and Hirst, 1977). This includes patches of open grassland (dambo's) surrounded by a denser woodland fringe (Joubert, 1976). Valeix *et al.* (2009) found that the behaviour of herbivores in an African savannah was influenced by the risk of lion predation. Most herbivore species in the Hwange National Park, Zimbabwe, shifted to open grassland areas in the presence of lions whereas some grazers preferred denser vegetation for short periods with lions in close proximity. Most browsers moved into open grassland areas when lions were in the vicinity.

In a study of the habitat preference and utilisation of roan during two consecutive winter seasons within the N'washitsumbe enclosure, Knoop and Owen-Smith (2006) found that roan utilised a wide variety of grass species throughout the enclosure. However, there were particular preferences to grass species within the wetland of the enclosure during the dry months. The more severe the dry season, the wider was the diet as well as habitat selection throughout N'washitsumbe.

4.2 OBJECTIVES

The objectives of this study were to:

- Evaluate whether or not habitat structure influences habitat selection by roan in the absence of predators
- Identify preferred grass species by roan in the dry and wet season in order to sample a representative diet selection with which to estimate dietary nutrient levels

4.3 MATERIALS AND METHODS

Habitat structure surveys were only conducted within the two KNP study areas and not in the Vaalbos National Park study area because a buffalo herd of over 100 animals was introduced onto the study area at Vaalbos National Park's Graspan/Holpan section a few months after the study commenced. The presence of a large number of bulk grazers, such as buffalo, within a relative small confined area, such as within the enclosure at Graspan, jeopardised the indication of habitat preference by means of signs of forage utilisation and/or trampling by roan antelope. Habitat overlap occurred between buffalo and roan antelope and it was not possible to correlate forage utilised and trampled to a specific herbivore species, unless it was utilised very recently so that fresh tracks were present.

The presence of other herbivores in large numbers within the same range area of the roan antelope in the Graspan section of Vaalbos National Park made it therefore impossible to use the same methods to measure habitat structure and to try and correlate it with habitat preference, as in N'washitsumbe. The Graspan study area was therefore not included in the habitat preference surveys. However, through tracking grazing roan herds within Graspan, many observations of areas and grass species utilised and preferred were recorded and will be discussed where applicable.

In Capricorn a herd of approximately 20 tsessebe shared the 500 ha enclosure with approximately 20 roan antelope. This enclosure was largely under-stocked and it was therefore decided to include the enclosure in habitat preference surveys with the assumption that all utilised areas were that of roan antelope. In many instances sample plots were put where roan antelope have been seen grazing. However, a fair degree of overlap might have occurred.

Habitat selection and preference surveys were conducted during the mid wet season (March) in N'washitsumbe and Capricorn. Due to the tendency of roan to select for plant greenness, especially in winter months, it was argued that habitat structure was of less importance in the dry season when preferred food sources were scarce. During the mid rainy season all grass species are abundantly available, equally green and highly nutritious.

It was therefore assumed that the relevance of habitat structure in habitat selection would be more significant in the wet season when most areas could be utilised and animals are not necessarily forced to utilise areas where the habitat physiognomy is not preferred.

4.3.1 HABITAT STRUCTURE

Since the habitat physiognomy is of significant importance in determining the habitat suitability for roan antelope (Joubert, 1976), a standard method should be used in order to measure the height and density of the grass and woody layers within a specific habitat. For measuring the tree density within the respective vegetation types in habitat preferred by a species, Joubert (1976), Wilson and Hirst (1977) and Ben-Shahar (1995) all used variations of the “Density Board” method (De Vos and Mosby, 1969) as described by Joubert (1976).

The density board measures the density of the vegetation in terms of the degree of visual obstruction. The degree of lateral visibility implies the measure of cover provided by the vegetation at different height levels (Joubert 1976). However, this method is time consuming and laborious since more than one person is needed to conduct the surveys. For the purpose of this study it was therefore decided to use the simpler and quicker Point-Centred Quarter Method to measure tree density and canopy cover (Ben-Shahar, 1995; Arvanitis and Portier, 1997; Mitchell, 2005).

Sampling plots within a study area were randomly allocated within each vegetation type. In some instances a sample plot was allocated in a spot where roan antelope have been seen grazing in order to measure the degree of grazing and/or trampling in an area obviously preferred by roan. This was also done to determine the specific grass species selected by the grazing herd.

At each sampling plot a 100m transect line was set out straight in a specific direction, away from any disturbances like roads and fences. Along the transect line an imaginary line was set perpendicular at every 25m across the transect line, forming four 90 degree quarters.

Within each quarter the nearest tree to the centre point of the four quarters was identified and measured: the distance from trunk to the centre point, its height from the ground to the highest tip of the tree and its canopy diameter at its widest point. A tree was represented by a Tree Equivalent (TE), which is defined as all trees and shrubs 1.5m and higher (Tainton, 1999). The distance to a tree was measured to the trunk of the tree or main stems of a shrub. The distances to trees were measured using a range finder or a measuring tape, depending on the distance - with a cut-of distance set at 100m. Tree heights were measured by means of a four metre measuring rod.

Grass height measurements were conducted every metre of the ten last metres preceding a 25m quadrate point. Grass heights were measured using a range rod and the height of the closest grass tuft/inflorescence at each one of the ten metres was recorded. There was no differentiation between plant parts and merely the highest point of a specific sward was recorded.

All together four Point-Centred Quarter surveys were conducted per sampling plot. Generally, three sampling plots were identified within every plant community of each habitat studied. In some instances where the specific vegetation type was very small only two random sampling points were surveyed and in other instances where the vegetation type was large and more sampling points were necessary, four random sampling plots were surveyed. Consequently, on average, twelve Point-Centred Quadrate surveys were conducted per vegetation type (three sampling plots of four quadrates each).

Absolute tree densities were calculated at every quadrate in order to correlate the degree of foraging and trampling to the tree density, canopy cover as well as tree height on the spot. The tree densities were calculated according to the method stipulated by Mitchell (2005) where:

1

Absolute Tree Density / hectare $= \lambda = \acute{r}^2 \times 10'000$

Where ŕ = the mean distance to a tree at that point

A total of 56 quadrates was sampled in N'washitsumbe along 14 randomly placed transects and 40 quadrates in Capricorn along ten randomly placed quadrates. Broad vegetation types were used in each enclosure in order to ensure that all the major veld types were sampled. N’washitsumbe had higher visible vegetation diversity and the enclosure was divided into four broad vegetation types – Mopani shrubland, Marula savannah, sodic ecotone and the wetland.

Capricorn is a larger enclosure than N’washitsumbe with less distinct vegetation types. Its vegetation was broadly classified into three veld types – Mopani-veld, Combretum-veld and a wetland area. These areas were classified based on the dominant or prominent tree species within the specific veld type unless it was a wetland area.

4.3.2 DIET PREFERENCE

The identification of utilised grass species from fibre fragments in the faeces of roan antelope can be used to determine the specific grass species utilised.
This method, as described by Wilson and Hirst (1977) and Erasmus *et.al.* (1978), could give valuable information about the grass species utilised by roan in the different study areas. However, the method is very time consuming and for the preparation of reference slides, extra funds and some knowledge of selected grass species are needed. The identification of preferred grass species was very important for determining the grasses comprising the main diet component of roan. These preferred grass species were then sampled in order to determine their respective nutrient and mineral concentrations.

Observations of grass species selected are best presented as a fraction of the relative availability of each grass species. In other words the frequency of occurrence of each grass species within the specific habitat linked to the degree of selection gives the best indication of the species preference of a herbivore (Joubert 1976). This was only partially done in this study and complete species frequency surveys were not done in all enclosures. Information on the utilisation of key grass species based on previous studies (Joubert 1976; Wilson and Hirst, 1977; Knoop and Owen-Smith, 2006) was mainly used to identify key grass species that could serve as an indication of habitat nutrient status.

This data was further expanded and supplemented through observations made by tracking herds, walking through all areas of the study locations in search of faeces as well as from many observations made during the Point-Centred Quadrate surveys in summer.

In N'washitsumbe, roan antelope was the dominant animal species with a small group of Lichtenstein's hartebeest *Sigmoceros lichtensteinii* present. A herd of 20 Tsessebe were present in Capricorn. No other large herbivores occurred within the enclosures. Nevertheless, the assumption that every utilised tussock was from foraging roan was made in instances where no signs of a specific species were present. In Vaalbos National Park a herd of approximately 100 buffalo was introduced into the Graspan section within the roan enclosure and therefore jeopardised accurate utilisation surveys. Animals were nonetheless followed closely in both seasons in order to determine the grass species preferred.

Habitat utilisation surveys were conducted jointly with habitat structure surveys in the wet season in the KNP enclosures. At every Point-Centred Quadrate survey point in N'washitsumbe and Capricorn, all utilised grass species were noted within a five metre radius from the centred point. Utilised species were identified and an utilisation score was allocated to the point according to the degree of selection as a function of availability on a scale of 0, 2, 3.5 and 5. The scores were allocated accordingly:

0	No utilisation
2	Very little utilisation (at least one tussock utilised but less than a third of what was available)
3.5	Fair amount of utilisation (at least one third of what was available but less than two thirds)
5	High degree of utilisation (two thirds and more of what was available)

The same was done in terms of the degree of trampling at every Point Centred Quadrate survey point. Trampling was defined as all signs indicating the presence of roan antelope in the form of laying spots, fresh faeces and tracks.

A differentiation was made between utilisation and trampling in order to differentiate between areas where animals actually prefer to graze as opposed to areas where they merely spent time or pass through in order to get to a preferred grazing area.

In Graspan, observations were made while tracking roan herds and moving around within the study areas. The presence of other herbivores in Graspan jeopardised the use of alternative quantitative surveys. Grass species sampled on Sable Ranch were based on grass species generally preferred by roan and sable antelope as well as species occurring in relative abundance in the area.

In many instances a wider variety of grass species was utilised than that which was sampled. However, the species sampled formed the bulk of what was available and what was consistently selected within each study area in a particular season. Budget constraints prevented the sampling and analysis of more grass species.

One-way ANOVA analyses were used to determine means and standard deviations of the data (SAS JMP, 2001). Significant differences between means were determined using All pairs Tukey-Kramer HSD comparisons of means at a 95 % confidence level (SAS JMP, 2001). Multivariate correlations were conducted using Spearman’s Rho correlations (SAS JMP, 2001). All statistical analysis was conducted using SAS JMP 4 (2001) software.

4.4 RESULTS AND DISCUSSION

4.4.1 DESCRIPTIVE STATISTICS

In N’washitsumbe grass height in the wetland (vlei) was significantly ($P<0.05$) higher than in all the other veld types (Table 4.1). The dominance of the very tall *Sporobolus pyramidalis* in N’washitsumbe’s vlei area contributed mostly to the significant difference in grass height in this enclosure. No significant ($P=0.77$) differences between the grass heights of the various veld types in Capricorn were observed.

Table 4.1: Mean and standard deviation (SD) of the grass height, nearest tree distance, tree height, crown diameter, degree of trampling and foraging as well as tree density in N'washitsumbe and Capricorn

Veld Type	**n**	**Grass Height (Meter)**		**Tree Distance (Meter)**		**Tree Height (Meter)**		**Crown Diameter (Meter)**		**Degree of Trampling**		**Degree of Foraging**		**Tree Density (Trees / ha)**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
N'washitsumbe															
Ecotone	16	1.07^{a}	0.29	12.56^{a}	5.58	3.80^{a}	0.69	3.66	1.06	2.00^{a}	0.00	0.25^{a}	0.68	245.90	609.87
Marula	16	1.16^{a}	0.17	5.87^{b}	2.02	4.07^{a}	1.41	3.22^{a}	1.11	3.22^{b}	1.13	1.50^{b}	0.89	404.08	262.16
Mopani	16	1.09^{a}	0.18	4.61^{b}	1.02	2.60^{b}	0.70	3.16^{a}	0.63	2.56	0.75	2.19^{b}	1.50	539.73^{a}	250.12
Wetland	8	1.61^{b}	0.15	59.94^{c}	11.12	4.32^{a}	0.43	4.53^{b}	0.64	2.19^{a}	0.53	0.00^{a}	0.00	3.12^{b}	1.50
Capricorn															
Combretum	16	1.01	0.23	11.00^{a}	5.22	3.43	0.87	3.14	0.72	3.13^{a}	1.02	2.78	1.46	151.70	159.67
Mopani	12	0.97	0.09	8.45^{a}	6.10	3.13	0.82	3.54	0.43	3.63^{a}	1.00	3.75^{a}	1.25	482.44^{a}	705.83
Wetland	12	1.02	0.17	29.23^{b}	8.60	3.24	0.65	3.16	0.55	1.75^{b}	1.20	1.54^{b}	1.61	16.00^{b}	12.86

$^{a/b/c}$ Means with different superscripts differ significantly ($P<0.05$)

The plant species distribution in Capricorn was very homogenous in general and the vlei area did not have grass species of considerable height as in N'washitsumbe. The mean distance to the nearest tree of the wetland areas in both N'washitsumbe and Capricorn were significantly further (P<0.0001) than in the rest of the veld types emphasizing the significant difference in vegetation physiognomy between the vlei and the bushveld/savannah veld types in both enclosures. The ecotonal tree distance in N'washitsumbe was significantly less than in the wetland and significantly further than in the Marula and Mopani veld types (P<0.05). The intermediate structure of the tree layer of the ecotone is consistent with its transitional position between the vlei with few trees and the savannah area with many trees. The distance to the nearest tree in the Mopani and Marula veld types in N'washitsumbe was significantly shorter than in all the other veld types (P<0.05).

The tree height in the Mopani veld type in N'washitsumbe was significantly shorter (P<0.0001) than in the rest of the veld types. The dominance of the *Colophospermum mopane* shrubs in this veld type contributed largely to the significant height difference compared to other veld types in N'washitsumbe.

Few other larger trees existed within the Mopani shrublands and the tree layer was mostly restricted to other shrubs, like *Grewia* species. In Capricorn, tree height (P=0.59) and crown diameter (P=0.17) did not differ significantly between the veld types and were therefore consistent with the little variation in habitat structure and species composition within the enclosure. The crown diameter of trees in the wetland of N'washitsumbe was significantly larger (P=0.006) than that of trees in the Mopani and Marula veld types. The presence of many shrubs and smaller trees among some large *Sclerocarya birrea* and *Combretum imberbe* trees within the Marula veld type reduced the overall average crown diameter within that veld type to resemble that of the Mopani veld type. The tree layer in the wetland area of N'washitsumbe consisted mainly of a few scattered tall trees, mostly *Combretum imberbe*, and almost no small shrubs. This explained the larger average crown diameter within the wetland of N'washitsumbe compared to that of the Marula veld type that had larger tree specimens than in the wetland.

The degree of trampling in the wetland of N'washitsumbe was significantly lower (P= 0.0002) than in the Marula veld type. Similarly, degree of trampling in the wetland of Capricorn was also significantly lower (P=0.0003) than in both the other veld types within that enclosure. The degree of foraging in N'washitsumbe's wetland and ecotone was significantly lower (P<0.0001) than in the Mopani and Marula veld types. In the same way, the degree of foraging in the wetland of Capricorn was significantly lower (P=0.003) than in its Mopani veld type.

The mean tree density differed significantly in both N'washitsumbe (P=0.01) and Capricorn (P=0.02) between the wetland areas and the Mopani veld types. However, there were large standard deviations (larger than half the value of the mean) in the tree density of the Ecotone and Marula veld types of N'washitsumbe as well as in all the veld types of Capricorn.

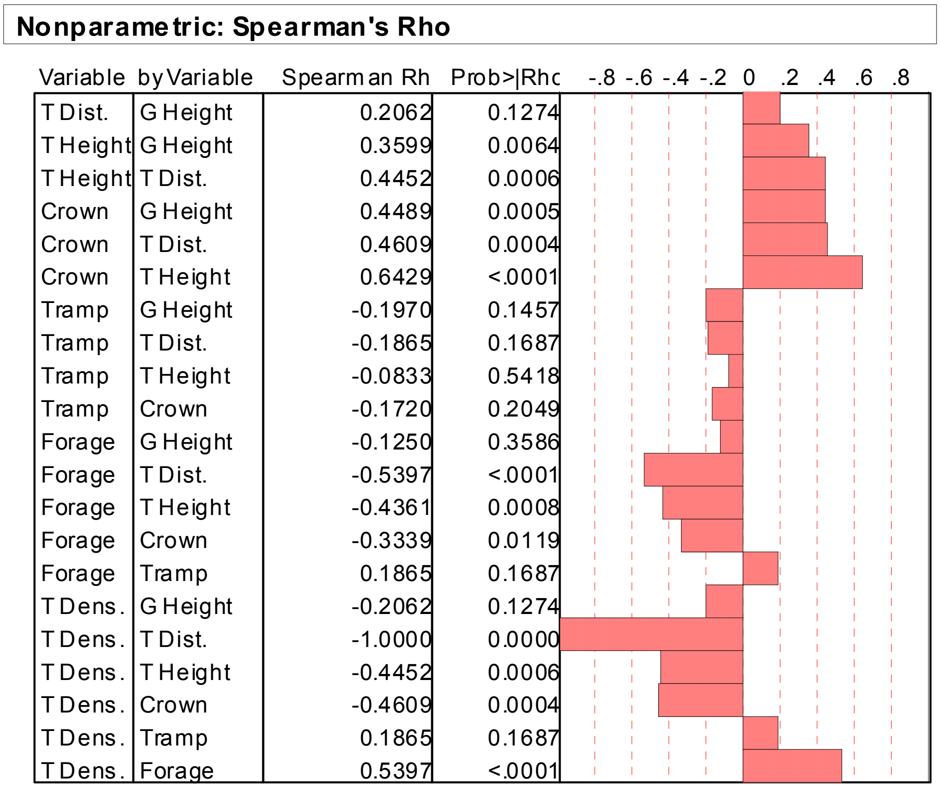
4.4.2 CORRELATIONS

Multivariate correlations between all the variables in both N'washitsumbe and Capricorn are summarized in Tables 4.2 and 4.3.

In N'washitsumbe, there were significant positive correlations (P<0.05) between tree height and grass height, crown diameter and grass height as well as distance to the nearest tree and tree height. Tree distance and grass height were not significantly correlated (P=0.13). The degree of foraging and the distance to the nearest tree were significantly negatively correlated (P<0.0001), as was the correlations between the degree of foraging and tree height as well as crown diameter (P<0.05).

There was a significant positive correlation (P<0.0001) between tree density and the degree of foraging in N'washitsumbe but tree density was significantly negatively correlated with tree distance, tree height as well as crown diameter (P<0.05).

Table 4.2: Spearman's Rho non parametric, multivariate correlations between degree of foraging and trampling and tree height, grass height, tree density, crown diameter and the distance to the nearest tree in N'washitsumbe

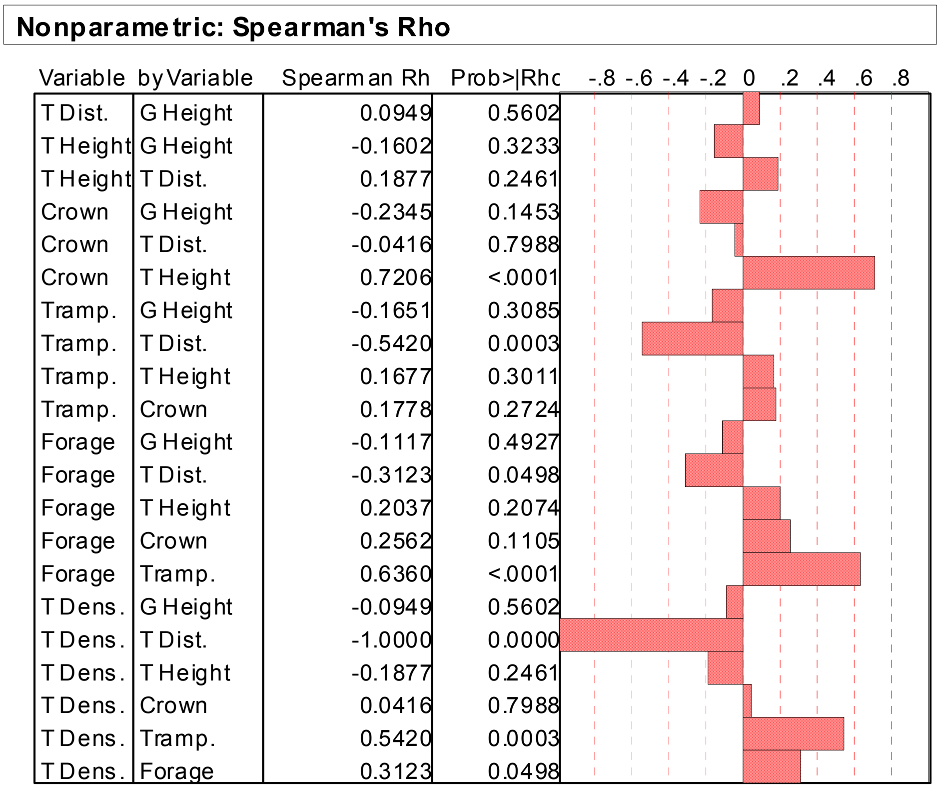


Nonparametric: Spearman's Rho

Variable	by Variable	Spearman Rh	Prob>|Rhc
T Dist.	G Height	0.2062	0.1274
T Height	G Height	0.3599	0.0064
T Height	T Dist.	0.4452	0.0006
Crown	G Height	0.4489	0.0005
Crown	T Dist.	0.4609	0.0004
Crown	T Height	0.6429	<.0001
Tramp	G Height	-0.1970	0.1457
Tramp	T Dist.	-0.1865	0.1687
Tramp	T Height	-0.0833	0.5418
Tramp	Crown	-0.1720	0.2049
Forage	G Height	-0.1250	0.3586
Forage	T Dist.	-0.5397	<.0001
Forage	T Height	-0.4361	0.0008
Forage	Crown	-0.3339	0.0119
Forage	Tramp	0.1865	0.1687
T Dens.	G Height	-0.2062	0.1274
T Dens.	T Dist.	-1.0000	0.0000
T Dens.	T Height	-0.4452	0.0006
T Dens.	Crown	-0.4609	0.0004
T Dens.	Tramp	0.1865	0.1687
T Dens.	Forage	0.5397	<.0001

Forage - Degree of foraging
Tramp - Degree of trampling
T Height - Tree height
G Height - Grass height
T Density - Tree Density
Crown - Crown diameter
T. Dist - Distance to the nearest tree

Table 4.3: Spearman's Rho non parametric, multivariate correlations between degree of foraging and trampling and tree height, grass height, tree density, crown diameter and the distance to the nearest tree in Capricorn



Nonparametric: Spearman's Rho

Variable	by Variable	Spearman Rh	Prob>|Rhc
T Dist.	G Height	0.0949	0.5602
T Height	G Height	-0.1602	0.3233
T Height	T Dist.	0.1877	0.2461
Crown	G Height	-0.2345	0.1453
Crown	T Dist.	-0.0416	0.7988
Crown	T Height	0.7206	<.0001
Tramp.	G Height	-0.1651	0.3085
Tramp.	T Dist.	-0.5420	0.0003
Tramp.	T Height	0.1677	0.3011
Tramp.	Crown	0.1778	0.2724
Forage	G Height	-0.1117	0.4927
Forage	T Dist.	-0.3123	0.0498
Forage	T Height	0.2037	0.2074
Forage	Crown	0.2562	0.1105
Forage	Tramp.	0.6360	<.0001
T Dens.	G Height	-0.0949	0.5602
T Dens.	T Dist.	-1.0000	0.0000
T Dens.	T Height	-0.1877	0.2461
T Dens.	Crown	0.0416	0.7988
T Dens.	Tramp.	0.5420	0.0003
T Dens.	Forage	0.3123	0.0498

Forage - Degree of foraging
Tramp - Degree of trampling
T Height - Tree height
G Height - Grass height
T Density - Tree Density
Crown - Crown diameter
T. Dist - Distance to the nearest tree

In Capricorn there was a significant positive correlation between crown diameter and tree height (P<0.0001) as well as between the degree of foraging and trampling (P<0.0001). There was a significant positive correlation between tree density and both the degree of trampling (P=0.0003) and the degree of foraging (P=0.05).

Significant negative correlations existed between the distance to the nearest tree and both the degree of foraging (P=0.05) and trampling (P=0.0003). There was also an expected significant positive correlation between tree density and tree distance (P=0.000).

4.5 CONCLUSIONS

Results from this study indicate a tendency for roan antelope in especially N'washitsumbe but also Capricorn, to select grazing areas associated with habitat with a higher tree density in the wet season. The stronger correlation between degree of trampling and tree density compared to between degree of foraging and tree density in Capricorn, suggested that roan in Capricorn only moved through very dense areas in order to forage in less dense areas. There was no significant correlation between tree density and trampling in N'washitsumbe. The fact that there was a significant negative correlation between tree density and tree height as well as between tree density and crown diameter in N'washitsumbe, emphasized the stronger tendency for roan to forage in the dense mopani shrublands during the study period. This was the opposite of what was found by Knoop and Owen-Smith (2006) in the dry season using another method.

With food availability and presumably diet quality not being a limiting factor in the wet season, roan in both the KNP enclosures could in the absence of predators, select areas mostly preferred in terms of diet availability and/or habitat structure. In the dry season preferred food sources become limited as plant quality and quantity decrease. This result in shifts in the preference of diet and habitat by most grazers. N'washitsumbe in particular, but also Capricorn to some extent, represents relatively small confined areas compared to natural home ranges of roan antelope (Wilson and Hirst, 1977; Skinner and Chimmimba, 2005). Previous studies have even indicated that home ranges of sable antelope in high quality habitats are smaller compared to home ranges in areas of lower dietary quality (Wilson and Hirst, 1977).

Skinner and Chimimba (2005) stated that the home ranges of roan may increase in the dry season.

The KNP enclosures restrict the movement of the residing roan populations to other grazing areas. Therefore, the drier a season becomes the animals must adapt their foraging preferences more in order to acquire the necessary nutrients to survive within the available space. In the absence of predators, the animals do not have to consider any risk of predation when foraging or selecting feeding sites. The animals are most probably driven by diet availability and diet quality – with habitat structure, hence visibility, probably playing a less significant role in habitat selection.

Nevertheless, studies in Nylsvlei Nature Reserve indicated that, if given a choice, roan may still prefer areas of lower tree densities and canopy covers in the absence of large predators (Wilson and Hirst, 1977; Heitkönig, 1993; Dörgeloh, 1998). Ben-Shahar (1986) found to the contrary that roan in the Waterberg of the Limpopo Province did not seem to be influenced by habitat structure in their habitat selection.

While elk preferred open grassland in the absence of predators and only adapted their foraging preference towards woodland areas after the threat of predation (Creel *et al.* 2005), results indicate that habitat selection by roan antelope in the absence of predators might be more related to diet quantity and quality. The roan antelope population in N'washitsumbe have been without direct contact with any large predators for many generations. The roan population introduced into the larger Capricorn enclosure originate from the captive bred roan population in N'washitsumbe and has therefore never been predated upon either. There even had been reports of roan antelope within the enclosures staring through the fence at lions on the outside, apparently not much startled (C.C. Grant, 2004; pers. comm.)[3].

The strong tendency of foraging roan antelope in N'washitsumbe and, to an extent in Capricorn, to disregard habitat structure is most probably a combination of two factors: limited access to more grazing land as well as ignorance towards instinctive predator evasion tactics due to the absence of predators for many years.

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[3] [3] Dr C.C. Grant, January 2004. Scientific Services, Skukuza, Kruger National Park. Ph. (013) 735 4415

The fact that free roaming roan antelope in the KNP naturally selected areas with higher visibilities, lower canopy covers and tree densities than for instance sable antelope (Joubert, 1976), suggested that an enclosure such as N'washitsumbe might not be suitable to breed roan antelope for the purpose of releasing the animals into the wild. If captive bred roan antelope that are not adapted to predation risk were to be released back into the KNP in small groups, their tendency to select for specific grass species or forage quality without considering habitat structure might increase their risk of falling prey to predators such as lions.

Results of the present study suggested that roan kept in the enclosures in the absence of predators readily utilised grazing within dense shrub and tree areas in both the wet season and late dry season (Knoop and Owen-Smith, 2006). These populations even seemed to avoid more open wetland areas during the wet season.

If free roaming roan populations prefer more open areas throughout the year in the presence of predators, as was found by Joubert (1976), it could at least in part be due to antipredator behaviour. In such an instance the cost of selecting for habitat types based more on structure than on diet quality could limit animal production, as was the case with elk in Yellowstone National Park (Creel *et al.,* 2007).

As far as is known, the combination of an utilisation/trampling factor with the Point-Centred Quadrate survey has not been used in order to describe the behaviour and grazing preferences of wild herbivores relative to habitat structure. The method used proved to have potential in this application but the categories and degrees of utilisation/trampling should be better defined in order to reduce possible bias. The number of sampling plots should also be increased to get an even greater representation of the overall utilisation patterns by the grazing herbivore. Other methods describing the structure of vegetation, for instance the BECVOL-method, could prove to be more applicable since the estimation and quantification of visibility at different height classes and quantification of tree density would then be possible.

The critical evaluation of the diet and nutrient status of animals within the KNP enclosures, in comparison to roan from other parts of South Africa in the following chapters, indicated to what extent the confined enclosures are suitable for breeding roan. Possible reasons for habitat selection described in this chapter will be identified and possible limitations in the nutrient status of selected habitats will be quantified.

CHAPTER 5

EVALUATION OF THE NUTRIENT STATUS OF THE HABITAT

5.1. LITERATURE REVIEW

5.1.1. INTRODUCTION

The nutrient status of the habitat in which a species of herbivore occurs, largely determines the nutrient status of the animal living in the habitat. The nutrient intake of an animal is determined by the nutrient content of everything ingested and the nutrient absorption is a function of the availability and requirement of a particular ingested nutrient. The nutrient sources of a herbivore consist mainly of plant material, water and to an extent, soil (McDowell, 1977; Maskall and Thornton, 1996; Judson and McFarlane, 1998; McDowell, 2003). Should the available diet of an herbivore be insufficient in terms of the nutrient requirements of a specific animal, it could induce a deficiency in the nutrient status of the animal itself (Underwood and Suttle, 1999). The nutrient deficiency in the habitat could therefore be reflected in the production and/or survival of the animal if not altered by possible mineral interactions within the animal's body.

Under free ranging conditions malnutrition can cause a population decline in a herbivore species through secondary factors such as predation (Harrington *et al.,* 1999; Creel *et al.,* 2007). Determining the nutrient requirements of a herbivore implies a controlled feeding trial in which different planes of nutrients are fed to producing animals and the response in production indicates whether the nutrient intake is sufficient for the level of production (Maynard *et al.,* 1975; Underwood and Suttle, 1999). In wild ungulates the option of a controlled feeding trial in order to determine the nutrient requirements of a species is possible, but especially with very expensive animals such as roan and sable the costs involved limits the possibility. The impact of stress on wild herbivores in a small captive environment might further influence results. Alternative measures therefore need to be taken.

It has been suggested that wild herbivores do have the ability to select a habitat that serves in their specific nutrient requirements. It has been shown that the nutrient status of a diet available in a specific habitat influences largely the migration of wild herbivores (Ben-Shahar and Coe, 1992).

However, factors like social behaviour (Joubert, 1976), production status (Dörgeloh, 1998), grazing and animal density pressure (Joubert, 1976, Harrington *et al.,* 1999), habitat structure (Ben-Shahar, 1995; Dörgeloh, 1998) and water availability (Joubert, 1976; Holecheck *et al.,* 2001) all play a very important role in the selection of a specific habitat. In the commercial game farming industry and small nature reserves, game proof fences also inhibit free roaming and therefore, selection of a preferred habitat or food source.

5.1.2. NUTRIENT CONTENT OF FORAGE

As in animals, plants also have specific nutrient requirements in order to aid essential physiological functions within the plant (Blasi, 2001). Similarly to animals, plants have four basic primary physiological purposes for which certain nutrient elements are essential:

1. Basic structure
2. Energy storage and transfer
3. Ionic charge balance
4. Activation of enzymes and electron transport

Plants are composed of approximately 94% carbohydrates. Of the remaining 6% of the plant's constituents, 1.5% is N and the remaining 4.5% is what is left for utilisation by the animal's digestive system. Hence, this is what an animal uses to meet its nutritional requirements (Blasi, 2001).

The wide variations in nutrient and mineral concentrations in plants have been thoroughly documented (Du Toit *et al.,* 1940; Boyazoglu, 1976; Whitehead, 2000; Blasi, 2001).

Kabata-Pendias (2000) reported that big differences in element uptake between plant species and also genotypes of a species have been demonstrated in many studies. There are various reasons for the vast differences in nutrient concentrations in and between plants.

Early in the 20th century Du Toit *et al.* (1940) reported that most of South Africa's natural pasture was deficient in phosphorus for most of the year, especially during the dormant three to four months of the year. Du Toit *et al.* (1940) stated that deficiencies would be more pronounced during winter times in the summer rainfall areas or in times of drought. Mineral deficiencies could disappear completely in times when abundant new growth is available. McDowell (1996) reported that in most cases Cu, Co, Fe, Se, Zn and Mo levels declined as the plant matures. Blasi (2001) also reported that most forage species decline in nutritive value over the course of the growing season. The plant's priority for allocating nutrients for specific physiological functions changes as it matures. When the plant is in its early growth stage, it supplies nutrients for enhanced leaf growth area to encourage photosynthetic activity for maximum uptake of energy and nutrients. During the reproductive stage, the nutrient flow is directed towards the development of a rigid stem needed to support the inflorescence and the eventual distribution of seeds. During the dormant seasons, nutrients are redistributed to the root system where they remain (stored) until the next growing season (McDowell, 1996, Meissner, 1999). The mineral levels in a plant usually also follow the digestibility pattern in the plant (Blasi, 2001).

It could therefore be concluded that the mineral levels found over the growing season in plants and, more specifically the plant parts, corresponds with the element's specific function. For example, trace elements that are components of various enzyme systems would be expected to be located in active metabolic tissue such as the leaf. Consequently, with advancing maturity and a resulting decline in the leaf:stem ratio and digestibility, there will be a decline in Co, Cu and Zn levels (Whitehead, 2000; Blasi, 2001). Blasi (2001) further reported that the internal movements of absorbed nutrients might be characterized as mobile, variably mobile or immobile. From the basis of diagnosing the sufficiency of an element and for determining if and when a supplemental mineral programme is necessary, the extent of a mineral's mobility has significant consequences.

Mobile nutrients like N, P and K cycle to the sites of metabolic activity in the plant while immobile elements such as Ca, B, Mn and Mo move to growing plant tissues and remain there until the plant part dies and falls off (Blasi, 2001).

Plant species and varieties also have variations in nutrient concentrations (Underwood and Suttle, 1999; Whitehead, 2000). In a study in Kenya's Lake Nakuru National Park, Maskall and Thornton (1996) found that there are not just great variations in nutrient content between tropical grass species but even greater differences between grass species and woody species. They also determined that grasses sampled in the park tended to contain higher concentrations of Cu and Co and lower concentrations of Se than the woody plants.

Other factors influencing the nutrient concentrations in plants are various environmental factors such as rainfall patterns, animal excreta and soil type (Underwood and Suttle, 1999; Whitehead, 2000).

5.1.2.1. Nutrient content of forage preferred by roan and sable antelope

In their study of roan and sable antelope in the old Transvaal reserves of South Africa (present day Limpopo and Mpumalanga Provinces), Wilson and Hirst (1977) sampled the most palatable and most preferred grass species in the dry season for nutrient analyses. These samples were collected in all of the Transvaal's nature reserves where roan- and sable antelope occurred at that time. The different areas sampled varied in soil and veld type as well as in plant species composition. The Ca content of grasses differed considerably from area to area due to its reflection of cell wall construction. The Ca:P ratio was considerably higher compared to the optimal of 2:1. For *Eustachys mutica* the ratio went as high as 13:1. These were attributed to the soil deficiency of P rather than an excess in Ca, although Lukhele and Van Ryssen (2003) found similar high Ca:P ratios in the leaves of several *Combretum* species. Most grasses had P levels between 0.02 and 0.05% (wet weight) throughout the sour regions of Transvaal. The highest P level was in *Panicum maximum* (0.09%) in the Hans Merensky Nature Reserve situated within a sweetveld region.

Values were compared with nutrient concentrations of grasses in Matetsi, Rhodesia (now Zimbabwe) where sable and roan antelope seemed to be doing very well. The average P levels in Matetsi were between 0.08-0.36%. Mg and K were also substantially lower in the Transvaal reserves than in Zimbabwe.

Of the trace minerals, grasses had much higher Fe and Cu concentrations in Matetsi than in the Transvaal except for the Rustenburg Nature Reserve. The protein content of the forages was generally low in all the regions, especially in the sour veld areas during the dry months.

Heitkonig (1993) observed that roan antelope at Nylsvlei Nature Reserve preferred the lower floodplain in summer and the *Eragrostis pallens – Burkea africana* Savannah in the winter. The nutrient status of the most preferred grass species in these plant communities were determined and compared by Dörgeloh (1998).

Plant species preferred by roan in the lower floodplain included *Oryza longistaminata, Panicum schinzii, Paspalum scrobiculatum* and *Leersia hexandra.* Species selected in the *Eragrostis pallens – Burkea africana* Savannah plant community included *Cenchrus cilliaris, Digitaria eriantha, Eragrostis lehmanniana, Eragrostis pallens, Heteropogon contortus, Hyperthelia dissoluta, Panicum maximum* and *Schmidtia pappophoroides.*

Significant differences in nutrient content between plant communities in summer and winter months were reported (Dorgelöh, 1998). Inter-plant community differences in terms of moisture%, P%, K% in winter and Ca% in late summer was not significant. In general, few significant differences were found between the different plant communities in the same season. In winter the sampled grasses of the lower floodplain contained significantly lower levels neutral detergent fibre (NDF) and organic matter (OM) than those in the *Eragrostis pallens – Burkeya africana* savannah. The lower floodplain still had a higher forage quality in winter compared to the *Eragrostis pallens – Burkeya africana* savannah. In late summer there was a significant difference in % OM and % K between the lower floodplain and the *Eragrostis pallens – Burkeya africana* Savannah. Generally, the forage quality in terms of % N and macro mineral content were higher during early summer than during late summer and winter in all the veld types.

The Ca and P% in preferred grass species in the *Eragrostis pallens – Burkeya africana* savannah were higher in the winter and late summer compared to the grasses of the lower floodplain. The Na% was more than double the concentration in grasses in the floodplain than in the *Eragrostis pallens – Burkeya africana* savannah. Dorgelöh (1998) concluded that Ca, P and Na played an important role in the selection of a habitat by roan antelope, especially breeding herds. The Ca:P ratio of preferred grass species in the *Eragrostis pallens – Burkeya african* savannah was, similar to the K:Na ratios, more favourable than in the lower floodplain.

The nutrient status of preferred grass species within a specific plant community could also vary. Dörgeloh (1998) found that the nutrient status of preferred grass species correlated well with a specific site preferred by roan antelope within the same plant community. The grasses in the preferred site in the floodplain of Nylsvlei Nature Reserve had significantly lower moisture and Na levels than the rest of the floodplain. N, Ca and OM, amongst others, were higher at the preferred site. P%, K%, NDF, acid detergent fibre (ADF) and grass height did not differ significantly. This again correlated significantly with structural facets of the habitat within these preferred areas.

Jooste (2000) found that sable antelope in the Sandveld Nature Reserve utilised a few woody species mainly in the winter months. These included *Acacia erioloba, Grewia flava, Lycium cinereum and Lycium hirsitum.* Grass species that made up the bulk of the sable's diet were *Cynodon dactylon, Anthphora pubescens, Eragrostis lehmanniana, Urelytrum agropyroides, Themeda triandra, Setaria sphacelata, Brachiaria nigropedata, Panicum stapfianum* and *Heteropogon contortus.* There were considerable variations between the diet composition and species selection between seasons. Various other grass species were also utilised to a limited extend.

Furthermore, Jooste (2000) found that the crude protein content of nine of the most preferred grass species in the late winter were 4.77% whereas the CP content of leaves of selected woody species were much higher at 17.36%. The significantly higher protein content of woody plant leaves compared to that of grass was most probably why sable supplemented their diet with browse. Skinner and Chimimba (2005) reported that the diet of sable generally contained up to 15% browse.

Data on the species selection in consecutive dry seasons by roan antelope in the N'washitsumbe enclosure were documented by Knoop and Owen-Smith (2006). Unfortunately the actual nutrient content of grass species selected were not determined to establish to what extent the nutrient content of grass species influenced seasonal selection. However, the study did aid in the sampling of the correct grass species as a representative sample of the animals' dietary composition. The nutrient content of selected grass species in the other study areas were included to establish spatial differences between the overall forage quality as well as that of specific key grass species.

5.1.3. NUTRIENT CONTENT OF SOIL

The nature of the soil parent material, other than N, is the primary influence on the amounts of the nutrient elements present in the soil (Maskall and Thornton, 1996; Judson and McFarlane, 1998; Whitehead, 2000). For example, the nutrient levels in soil of basaltic parent material are substantially higher than those of granitic parent material (Kabata-Pendias, 2000). The findings correlate with the evaluations of various researchers regarding grazing patterns in KNP where basaltic and granitic plains lies adjacent to one another. Observations that wild herbivores strongly select for the more nutritious grazing on the basaltic plains compared to that of the granitic plains were made (C.C. Grant, 2003. pers. comm.)[4]. Whitehead (2000) stated that generally, the concentrations of most of the nutrient elements are higher in clay soils than in sandy soils, with loam and sodic soils being intermediate. Almost all soil N is present in the organic matter and its concentration in the soil is therefore not related to parent material. Other elements, particularly P and SO_4, are also present in soil organic matter, influencing their total concentrations in soil (Whitehead, 2000).

Maskall and Thornton (1996) and McDowell (1996) stated that most naturally occurring mineral deficiencies in herbivores were associated with specific regions and were directly related to soil characteristics and soil parent material.

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[4] Dr C.C. Grant. Scientific Services, SANParks, Skukuza, Kruger National Park. South Africa. Tel. (013) 735 4415. Personal communication. August 2003

Soil nutrient concentrations from geochemical surveys can therefore be used as an indicator to possible nutrient deficiencies in forage and the subsequent animal (Maynard *et al.* 1979; Maskall and Thornton, 1996; Underwood and Suttle, 1999). Kabata-Pendias (2000) stated that although trace minerals are minor components of soils in the solid phase, they play an important role in the fertility of soil. According to Holecheck *et al.* (1982) N and P are the most deficient nutrients in soils available for plant growth. An average annual rainfall of above 500 mm exacerbates nutrient deficient soils because of leaching.

For ecologists and wildlife managers, however, soil is seen as a growth medium for plants, therefore determining the actual quality of forage for wild herbivores remains essential. Holecheck *et al.* (2001) stated that, in general, soils low in nutrient concentrations produces nutrient deficient forage. Unfortunately, looking only at the soil in determining the nutrient status or potential for forage quality is not recommended due to complex interactions between soil and plants (Maynard *et al.* 1979; Holecheck *et al*. 2001).

The nutrient availability in the soil is therefore not necessarily reflected in the nutrient status of the forage (Holecheck *et al.,* 1982) due to various factors in the soil that influences the nutrient availability for forage uptake (Maskall and Thornton 1996; Judson and McFarlane 1998; Whitehead 2000; Holecheck *et al.* 2001). These factors are as follows:

- **The geochemical composition of parent material** from which some soils are derived can influence directly or indirectly, both the balance of elements within those soils and the trace element content of herbage growing on them. Regional differences of Cr, Cu, I, Fe, Se and Zn and excesses in Ar, Cd, F, Pb and Se arise from such causes. McDowell (1996) stated that most trace elements are more abundant in young, alkaline geological formations than in the older, more acid, coarse, sandy formations.
- **Differences in soil moisture and soil pH** can markedly affect the availability and consequent uptake of specific elements into vegetation. High pH soils or irrigation water will greatly increase Se and Mo availability. Iron-rich irrigation water restricts the uptake of Se. Extensive water leaching increase I, P, Cu, Co, Mg, Zn and Na

losses and reduce the intrinsic availability of Se and Zn. Low pH conditions increase the availability of Al, Fe and Mn.

- **Cation exchange capacity of the soil:** During the weathering process some extremely small, negatively charged particles referred to as colloids are formed. Consequently, colloids are able to attract the positively charged ions (cations) such as K, Na, H, Ca and Mg and repel the negatively charged ions (anions) such as chlorides, nitrates, sulphates and phosphates. As a result, the more colloids the soil has the better it can attract cations.
- **Soil organic matter** has a strong influence on the concentration and availability of certain nutrients in the soil. Almost all of the N and much of the P and S in grassland soil occur in the soil organic matter and are being released in plant available forms by the soil micro-organisms. Soil organic matter has only a small effect on the availability of macronutrients K, Na, Ca and Mg. It does however have a much greater influence on the availability of micronutrients Fe, Mn, Zn, Cu and Co.
- **Soil redox conditions** affect the availability of many of the nutrient elements such as N, sulphate, Fe, Mn, Zn, Cu and Co. Some of the effects are purely chemical while others are due to changes brought about by microbial activity.

Wilson and Hirst (1977) found that the soils of all the then Transvaal nature reserves where roan and sable antelope occurred were P deficient. Rustenburg Nature Reserve had a low Ca content and most reserves were low in Mg. When compared to the reference area (Matetsi) in Zimbabwe, Matetsi generally had much higher trace element concentrations, especially Fe, Cu, Zn, Co and Mn. It was nonetheless stated that total nutrient concentrations of the soil were analysed and not plant available nutrients, implying that the forage would not necessarily show the same concentrations (Wilson and Hirst, 1977)

5.1.4. NUTRIENT CONTENT OF WATER

Water is one of the major components of most herbivores' diets. However, water is not considered as one of the major sources of minerals to grazing animals (Wilson and Hirst, 1977; Judson and McFarlane, 1998; Simyu *et.al.,* 1999) and consequently received very little attention in wildlife studies thus far (Casey and Meyer, 2001). Nevertheless, water quality has serious wildlife management consequences through its effect on reproductive health and immune responsive disorders (Bothma, 2005). Data from wildlife studies that included water quality was usually inadequate and superficial (Bothma, 2005). Studies including water quality mostly focused only on macro minerals. However, the potentially hazardous constituents of water are the trace minerals which have a lesser effect on water palatability but can have a major impact on animal physiology (Casey and Meyer, 2001). The palatability of a water source is determined by the quality of the water, mainly by the level of total dissolved salts (saline or brackish) (Bothma, 2005). Judson and McFarlane (1998) reported that considerable levels of especially salts like Na, Ca and Mg could be found in sub-surface water pumped for drinking supply to grazing animals. On the other hand, water palatability is not necessarily a measure of the potentially hazardous constituents of a water source (Casey and Meyer, 2001).

Determining the water quality or the quality of the water taken in by wildlife, pose a number of challenges (Casey and Meyer, 2001). Animals differ in physiology, water turnover, territory and feeding spectrums and can further use numerous water sources within a given territory (Casey and Meyer, 2001). Underground water is one of the most common water sources in semi-arid countries. Underground water quality is largely affected by soil geochemistry. The mineral concentrations in a water trough differ significantly from the water reservoir supplying the trough due to evaporation, resulting in a concentrating effect of elements in the trough (Judson and McFarlane, 1998).

Water quality may further have various ecological and management implications for any ecosystem. Water contains organic and inorganic elements that can either be a valuable supplement or potential health risk for wild animals (Casey and Meyer, 2001). Sulphates, Mg, Na, Ca and total dissolved salt (TDS) concentrations, especially the ratio in which they occur, are key elements affecting water palatability. Water palatability can have a big influence on the habitat selection of specific wildlife species (Casey and Meyer 2001).

After analysing the nutrient content of all the preferred water sources by roan and sable in the then Transvaal nature reserves, Wilson and Hirst (1977) concluded that the nutrient status of water had a very limited contribution to the nutrient intake of the animals.

However, since water quality can play a significant role in the nutrient intake of an animal, standard water analysis on the water sources within the study areas were conducted.

5.1.5. RELATIONSHIPS BETWEEN THE NUTRIENT CONTENT OF FORAGE, SOIL, AND WATER

The utilized ecosystem is characterised by dynamic, complex interactions of soil, plants and animals (Coleman *et.al.,* 1989; Holecheck *et al.,* 2001). The primary reason for the existence of areas of mineral deficiencies in grazing animals, such as those of P, Na, Co and Se, is that the soils of the areas are inherently low in plant available supplies of these minerals (Underwood and Suttle, 1999). This has led to the surveys of the occurrence of possible nutrient deficient areas that could be correlated to nutrient deficiencies in soils occurring in such an area (Du Toit *et al.,* 1940; Maynard *et al.,* 1979; Mtimuni, 1982; Maskall and Thornton, 1996). P is a good example of a mineral that reflects a linear relationship between the concentrations in the soil, forage and animal (Maynard *et al.,* 1975).

To determine the nutrient status of the habitat and therefore possible deficiencies in animals, the first step usually was to sample and determine the nutritive quality of the soil and forage during various seasons (Wilson and Hirst, 1977; Reid and Horvath, 1980; Schmidt and Snyman, 2002). However, various researchers have claimed that using soil and vegetation analysis alone in predicting possible nutrient and/or mineral deficiencies in herbivores is insufficient (Suttle, 1986; Underwood and Suttle, 1999; Blasi, 2001; Webb *et al.,* 2001; Schmidt and Snyman, 2002). The reasons for this insufficiency are that the associations between soil and herbage composition and marginal or functional deficiency in the grazing ruminant have not been well defined (Suttle, 1986). Very complex interactions and relationships between various factors within the soil and plants further complicate assessments (Blasi, 2001).

Whitehead (2000) stated that usually only a small proportion of the soil content of any nutrient element is available for uptake by plants at a specific time.

Using herbage and soil samples are even more difficult in wild herbivores since their diets are too selective and consist of a wide variety of grasses, shrubs and trees (Schmidt and Snyman, 2002).

By evaluating as many possible different facets in the habitat, Grant *et.al.* (1996) found that specific correlations between the various facets could give valuable information regarding possible nutrient deficiencies in animals. By using Spearman rank correlations between the nutrient status of sable antelope and the nutrient status of soil and forage, Wilson and Hirst (1977) found significant relationships. P, Ca, Se and Na concentrations in the habitat were significantly correlated to that within the animals. There were also positive relationships between Cu and Fe and negative relationships between P and Zn, and Fe and Cu. Contrary to the forage P content, soil P levels correlated significantly with total blood protein but not with the kidney fat index. Forage P levels correlated significantly with live weight of sable antelope (Wilson and Hirst, 1977).

An additional problem with soil and plant samples is the many external sources of minerals to animals, which cannot be predicted by soil and plant samples alone. These external sources of minerals include geophagia (eating of soil), dust on plants and water mineral content. The actual nutrient availability in the digestive tract of the animal is unpredictable and can play a major role in the animal's nutrient status (Maskall and Thornton 1996; Underwood and Suttle 1999). Maskall and Thornton (1996) reported that little was known about the effects of geophagia in wild animals and the effect it can have on the nutrient status of wild herbivores. Wilson and Hirst (1977) and Apps (1996) reported many examples of geophagia and pica among various wild herbivore species, for example in sable antelope and giraffe *Giraffa camelopardalis.*

According to Maskall and Thornton (1996), earlier studies in national parks and reserves in Kenya have shown that broad variations of the trace element content of soils were largely attributable to differences in soil parent material. Pedogenic processes however influenced the distribution in soil profiles of trace and major elements, which have an effect on soil properties and nutrient availability to plants. Underwood and Suttle (1999) consequently stated that geochemistry could be used to map broad areas of possible nutrient deficiencies/toxicities.

Nonetheless, Krauskopf (1972) stated that soil-forming processes vary widely from one climatic region to another and that a general soil composition for a specific area can have little significance. This is to a large extent true for plants and water. However, Sutton *et al.* (2002) were able to make certain predictions of possible mineral deficiencies in the diet of sable antelope in a game park based on parent rock compositions, such as the high possibility of a P deficiency.

5.2. AIMS AND OBJECTIVES

The purpose of this study was to evaluate the habitat in which a roan antelope population occur based on the assumption that the specific habitat was preferred above other available habitats and should therefore supply most of its nutrient requirements needed to sustain a viable population. In order to determine the requirements of a wild ungulate the assessment of the nutrient status of the habitat could reveal the nutritional suitability of the habitat and possible nutrient requirements of the animals (Maynard *et al.,* 1975; Underwood and Suttle, 1999). Van Ryssen (2000) stated that despite the knowledge of the mineral status of an animal, as much as possible information on the quantity of every mineral and its antagonists that are supplied through the animal's feed and water should be obtained.

The aim of this study was therefore to take a holistic approach in collecting as much data as possible on nutrient and mineral concentrations within the habitat of a roan antelope population. Consequently, valuable assessments of the nutrient status of the habitat could be made in order to assess the nutritional suitability of the habitat in meeting the animals' nutritional demands.

The objectives of assessing the nutrient status of the habitat in which various roan antelope populations occurred were to:

1. Evaluate the nutrient status of forages in the wet as well as the dry season in every study area
2. Determine possible nutrient or mineral deficiencies within the habitat based on the assumptions that:

   a. the nutrient concentrations within the grasses analysed reflect that of the diet of the roan antelope
   b. the nutrient requirements of roan antelope resemble that of beef cattle
3. Evaluate the water and soil nutrient and mineral concentrations of the different study areas to identify further possible anomalies which could significantly affect the nutrient status of a roan population

5.3. MATERIALS AND METHODS

Soil, water and forage samples of the two KNP enclosures, N'washitsumbe and Capricorn, as well as that of Graspan were collected in both the dry and wet season. Sable Ranch only became part of the original study at a later stage and only dry season herbage samples were collected.

5.3.1. GRASS SAMPLING AND ANALYSES

The selection of specific grass species for sampling and analysis was based on the criteria used by Ben-Shahar and Coe (1992). The species, which were firstly one of the dominant grass species in terms of biomass production and secondly, also formed a large part of the diet of the studied animal, were selected and sampled. Roan and sable antelope have the ability to select only specific preferred grass species, which are not necessarily the dominant species (Joubert, 1976; Wilson and Hirst, 1977; Jooste, 2000) and it is therefore very important to determine the grass species selected or avoided during the respective seasons in different habitats. Previous studies suggested that roan and sable antelope do tend to utilise different grass species during different times of the year (Dörgeloh, 1998; Harrington *et al.,* 1999; Jooste, 2000; Knoop and Owen-Smith, 2006).

During habitat evaluations, herd monitoring and faecal sampling in every study area, all utilised grass species were noted. These utilised grass species together with other dominant grass species were then sampled. All preferred grass species were sampled more or less at the height at which it were utilised at that time by roan antelope within the specific study area. Specific areas where a herd had just grazed were visited and tufts of utilised grass species were sampled according to the manner in which the specific grass species were grazed.

Dörgeloh (1998) reported that there were some similarities between the nutrient concentrations in forage sampled in winter and early summer and therefore late winter and late summer samples were used for nutrient content comparisons in his study. Grass species sampled varied to an extent between seasons in each study location due to a shift in the selection preferences by roan antelope between seasons (Knoop and Owen-Smith, 2006). Only the most abundant and most utilised species in every study location were sampled for analysis. Grass species sampled in N'washitsumbe were considerably more than in the other enclosures mainly due to the larger botanical species composition as a result of a wider habitat variety within this enclosure. In certain instances, species utilised in one season and not in the other were sampled in both seasons in order to determine whether the seasonal change in plant quality might have affected selection preferences by roan. Data from Sable Ranch were only collected during the winter months of 2006 and not during the summer months as well.

At least ten individual plants of a species were sampled per study area in order to represent most regions of the study area where the particular species occurred. All the sampled specimens per species were then pooled to one sample per species per study area. These pooled samples were dried either in the sun for at least a month or in a dry-oven for at least 12 hours at approximately 55 ˚C. The dried samples were then milled in a miller with a 1 mm sieve which produced one pooled sample per species per study area for a specific season.
A grab sample from each pooled sample was used for the analysis. CAL[5] analysed the grass samples for N, P, Ca, K, Na, Mg, Cu, Zn, Mo, Co, Fe, Mn, S and Se. Analyses were done by means of NIR (Near-Infrared Reflectance Spectroscopy) according to the official AOAC (Association of Analytical Communities) (1995) method Nr. 989.03. Wet chemistry analyses were conducted on CP, Ca, P, moisture, CF, fat, ash and NDF according to the following AgriLASA methods:

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[5] Central Analytical Laboratories. Pelindaba, P.O.Box 812, Ifafi, 0260. E-mail: cal@calsa.co.za.

Analysis	**Method**	**Reference**
CP	6.3.1	N reagents (1977). *Technicon Industrial Method.* Method 329-74W
Ca	6.3.1	Ca reagents (1978). *Technicon Industrial Method.* Method 606-77A
P	6.3.1	P reagents (1972). *Technicon Industrial Method.* Method 144-71A
Moisture	2.1	AOAC (1995) Methods 934.01 and 920.36
Crude Fat	2.3	AOAC (1995) Method 920.39
CF	2.4	AOAC (1995) Method 962.09
Ash	2.5.1	AOAC (1995). Method 942.015
NDF	3.2.1	Robertson and Van Soest (1981)

It must be noted that the use of NIRS technology for the analyses of micronutrients in plant and faecal material is not generally promoted since wet chemistry techniques are more accurate and reliable. However, in this study laboratory analyses were limited to techniques used by CAL laboratories. Concentrations of some minerals vary considerably and some values differ substantially from known norms and values of especially micronutrients should therefore be evaluated in this context. Care was taken to use the same laboratory and analytical methods for all samples in order to make results comparable within the scope of this study.

The mean nutrient content of every mineral within all the sampled grass species per study was used to compare between study areas as well as seasons (Appendix 1). Since the sample sizes were relatively small, non-parametric statistical methods were used to compare means. One-way ANOVA-analyses were used to determine means and standard deviations of the data (SAS JMP, 2001). Significant differences between means were determined using all pairs Tukey-Kramer HSD comparisons at a 95 % confidence level (SAS JMP, 2001). All statistical analyses were conducted using SAS JMP 4 (2001) software.

Dry season grass samples in N'washitsumbe and Capricorn were collected in very late dry season at the end of October beginning of November 2005. It was a very long dry season and samples were collected just before the first spring rain fell at the beginning of November. In general, the grass layer was very poor and very dry due to the long dry season. The veld condition in N'washitsumbe was particularly poor compared to the other enclosures. *Urochloa mosambicensis* samples were collected from plants grazed virtually to the ground in N'washitsumbe at the late dry season.

However, this species already showed some greenness in some swards other than the other grass species sampled in the same area. The chemical analysis of *U. mosambicensis* resembled the sampling of green, young leaves (Appendix 1) with significantly higher N and other mineral levels. *U. mosambicensis* was therefore omitted from the statistical analysis in N'washitsumbe in order to obtain results that were more representative of a normal peak dry season diet composition.

5.3.2. SOIL SAMPLING AND ANALYSES

Soil samples were collected at the end of the wet season (late summer) in all the different study areas. In his study, Mtimuni (1982) did not find any significant differences between both upland and lowland soil pH and extractable Ca, K, Mg, Na, Fe, Co, Cu, Mg, Zn levels between the different seasons. Only extractable Al (aluminium) showed a significant difference. Soil samples in this study were therefore not collected during the dry and the wet season but only in late summer at the end of the active growth period of the vegetation. Similarly, Wilson and Hirst (1977) collected soil samples within one month of the dry season in order to provide background information on the availability of nutrients to forage during the wet season.

A soil sample of the top 10-15 cm of the soil was collected at random sites within each vegetation unit in the different study areas. Samples were either air dried or dried in an oven at 60°C for at least 12 hours. Different soil samples within the same vegetation type were pooled together and a sample from each pool was analysed. Samples were analysed by CAL for soil pH and P, K, Ca, Mg, Na, Cu, Zn, Mn, Fe, Se concentrations. All soil analyses were conducted according to the methods as described by the Handbook of Standard Soil Testing Methods for Advisory Purposes (1990) as developed and printed by the Agri Laboratory Association of Southern Africa (AgriLASA). The following methods were used to determine the element exchangeable levels:

pH - method No 2
P Bray 1 - method No 20
Na, K, Mg, Ca (Ammonium Acetate) - method 8
Fe, Mn, Cu, Zn (0.1M HCl) - method 14

5.3.3. WATER SAMPLING AND ANALYSES

Water samples were collected from the one available water trough in Capricorn and the two available water troughs in N'washitsumbe. The three water troughs most preferred or in the vicinity of the habitat preferred by roan antelope in Graspan were sampled. Tracks and signs at waterholes were also used to determine which waterholes were visited by roan antelope in Graspan. Each of the sampled water troughs was supplied by a wind mill which pumped underground water to the surface and into a reservoir. From the reservoir the water was pushed by gravity and/or pump-pressure to the water trough. The water level in the troughs was regulated by standard ball-valve systems.

Since the nutrient concentrations in a drinking trough could differ considerably from the water source or reservoir (Judson and McFarlane 1998), water was sampled from the trough (Point-of-use). The water in the trough is what was available to the animal. Water samples were collected in 0.5 L clean, clear plastic bottles and were kept cool until delivered to the laboratory (Simiyu *et al.* 1999). The water samples were delivered to CAL for analysis as quickly as possible after sampling in order to prevent possible changes in the chemical properties of the water (Wilson and Hirst 1977).

Water samples were collected and analysed for pH, EG, TDS (Total Dissolved Salts), Ca, Mg, K, Na, Cl, SO_4, CO_3, HCO_3, NAV/SAR, B, NH_4, NO_3, Mn, Cu, F and Co. Standard Inductively Coupled Plasma (ICP) analyses were used to determine mineral levels and standard Ph and EC meters were used to measure the Ph and EC of the water[6].

5.4. RESULTS AND DISCUSSION

5.4.1. GRASS ANALYSES

The specific grass species sampled at every study area per season as well as their respective nutrient and mineral concentrations were summarized in Tables 1 – 4 of Appendix 1.

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Appendix 1 presents the means and standard deviations of the mineral and nutrient levels of all the grass species sampled and analysed in each season in every study area.

5.4.1.1. Nitrogen and macrominerals

The N content of forages did not differ significantly between the study areas (P=0.74) in the wet season (Table 5.1). The average N concentration in the wet season was above the minimum recommended N concentration in wild herbivores diets of 0.8% (5% crude protein divided by 6.25) (McDonald *et al.,* 1998; Schmidt and Snyman, 2002). The N content of grass at N'washitsumbe was the highest (1.11%) in the wet season with the N concentration in grass at Graspan and Capricorn equal at 0.95% (Table 5.1).

There was a significant difference (P=0.09 at 90% confidence level) in dry season N concentrations in grass between the study areas and only grasses at Sable Ranch (0.87%) contained more than the minimum required N level. The N levels in grasses at Graspan and the KNP enclosures ranged between 40-31% lower than the minimum required dietary level for beef cattle. The grasses at Capricorn had the lowest winter N level of 0.48%. At N'washitsumbe a few *U. mosambicensis* tussocks in the wetland area started sprouting at the end of the dry season and was already utilised, as opposed to those growing in the upland area showing neither signs of greenness nor signs of utilisation. Only *U. mosambicensis* growing in the wetland of N'washitsumbe had a very high N content (Appendix 1 - Table 3) compared to the same species at the same time in Capricorn. However, contrary to not being grazed in the upland of N'washitsumbe, *U. mosambicensis* at Capricorn was utilised in the late dry season without showing any signs of greenness. The only other grass species in all the enclosures that showed some greenness was *Diplachne fusca* in the wetland at N'washitsumbe and to a very limited degree, *S. ioclados* on the sodic patch in the ecotone at N'washitsumbe. Of these two grass species at N'washitsumbe, only *D. fusca* was mostly utilised at the time of sampling in the late dry season even though only *S. ioclados* had an elevated N content. Although still utilised, the N content of *D. fusca* was similar to other grass species not showing any greenness at the time of sampling.

Table 5.4: Nitrogen and macromineral concentrations of wet season grasses in every study area on Dry Matter basis

Place	**n***	**% N**		**% P**		**% K**		**% Ca**		**% Mg**		**% Na**		**% S**	
		ū	**SD**	**ū**	**SD**	**ū**	**SD**	**Ū**	**SD**	**ū**	**SD**	**ū**	**SD**	**ū**	**SD**
Graspan	5	0.95	0.30	0.07^{a}	0.04	0.71	0.40	0.40	0.11	0.13^{a}	0.05	0.0049	0.0004	0.14	0.04
N'washitsumbe	10	1.11	0.56	0.21^{b}	0.03	1.31	0.57	0.28	0.17	0.20	0.07	0.38	0.56	0.16	0.08
Capricorn	5	0.95	0.39	0.27^{b}	0.04	1.22	0.41	0.42	0.20	0.25^{b}	0.09	0.19	0.24	0.14	0.03
Minimum Req. (NRC 1996).		**0.8**		**0.17-0.59**		**0.6-0.7**		**0.26**		**0.1-0.2**		**0.06-0.08**		**0.15**	

$^{a/b}$ = P<0.05

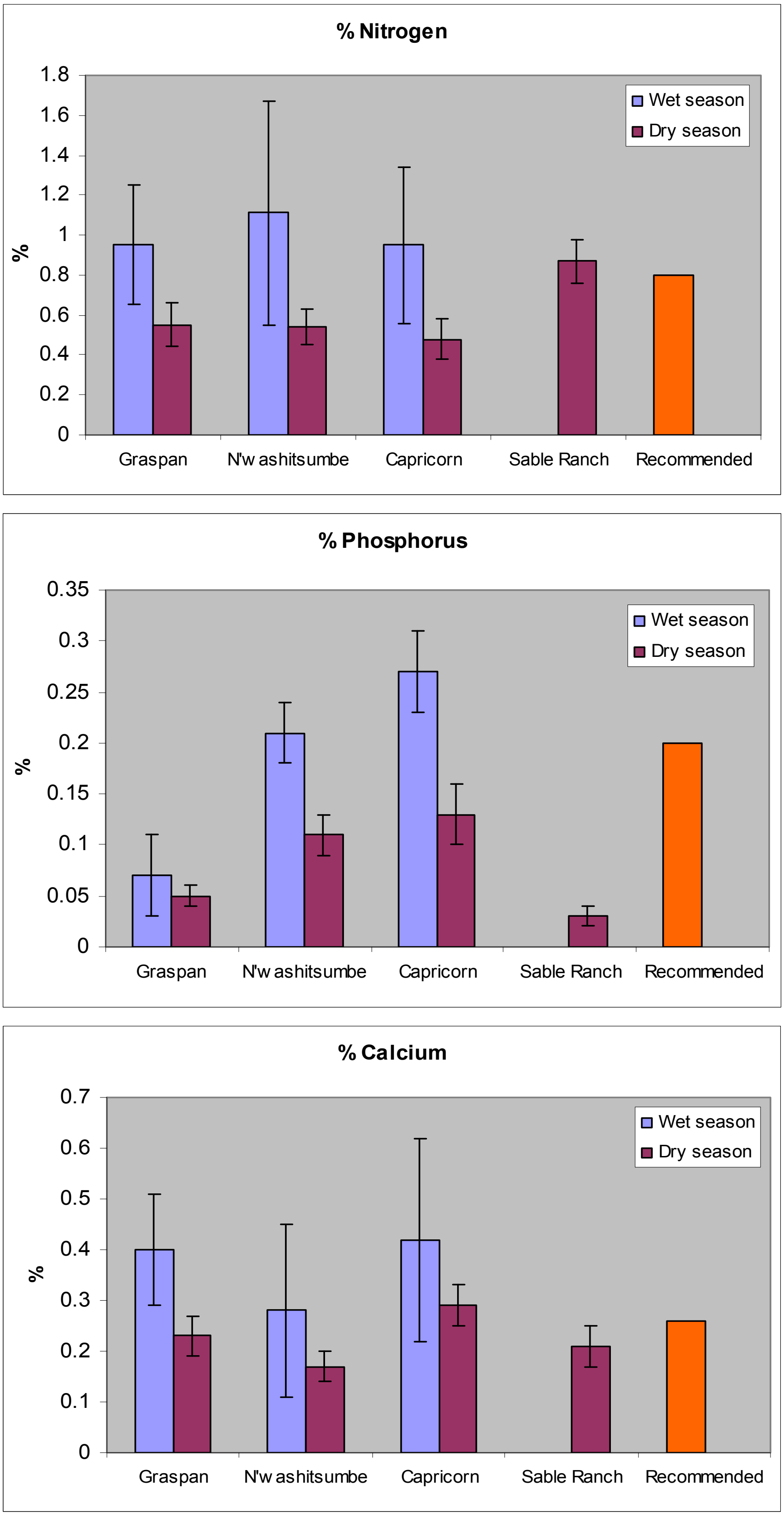
* n = number of pooled species per study area per season (Appendix 1)

Table 5.5: Nitrogen and macromineral concentrations of dry season grasses in every study area on Dry Matter basis

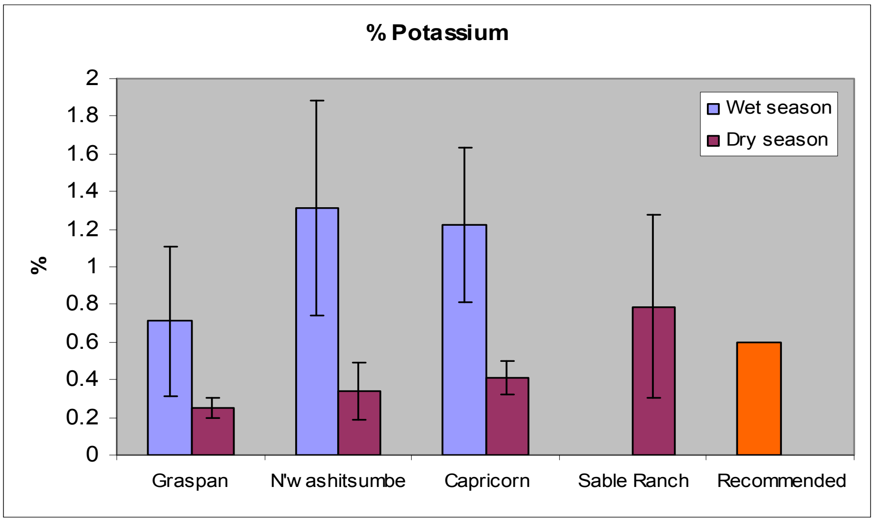
Place	**n***	**% N**		**% P**		**% K**		**% Ca**		**% Mg**		**% Na**		**% S**	
		ū	**SD**	**ū**	**SD**	**ū**	**SD**	**Ū**	**SD**	**ū**	**SD**	**ū**	**SD**	**ū**	**SD**
Graspan	4	0.55	0.11	0.05^{a}	0.01	0.25^{a}	0.05	0.23	0.04	0.06	0.02	0.0049	0.0007	0.12	0.02
N'washitsumbe	7	0.54	0.09	0.11^{b}	0.02	0.34^{a}	0.15	0.17	0.03	0.12	0.06	0.150	0.207	0.19	0.12
Capricorn	5	0.48	0.10	0.13^{b}	0.03	0.41	0.09	0.29	0.04	0.11	0.07	0.070	0.096	0.13	0.03
Sable Ranch	4	0.87	0.11	0.03^{a}	0.01	0.79^{b}	0.49	0.21	0.04	0.11	0.05	0.006	0.003	0.14	0.03
Minimum Req. (NRC 1996)		**0.8**		**0.17-0.59**		**0.6-0.7**		**0.16-1.53**		**0.1-0.2**		**0.06-0.08**		**0.15**	

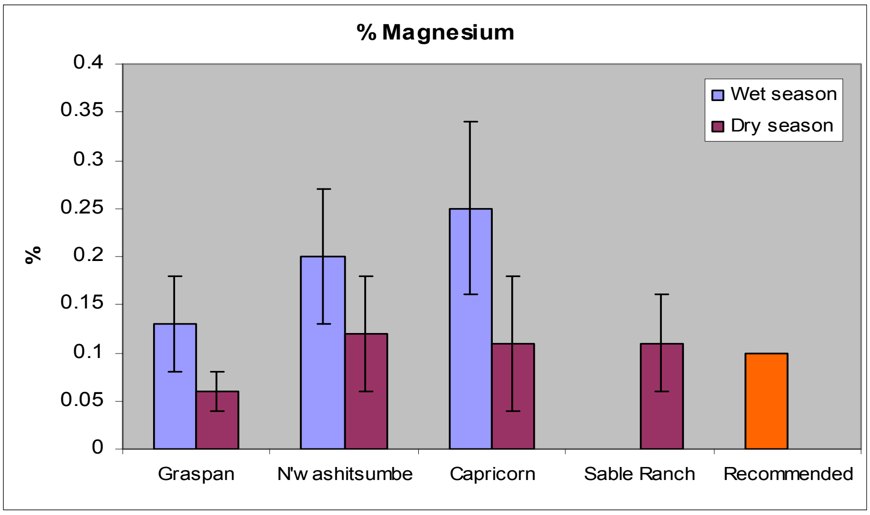
$^{a/b}$ = P<0.05

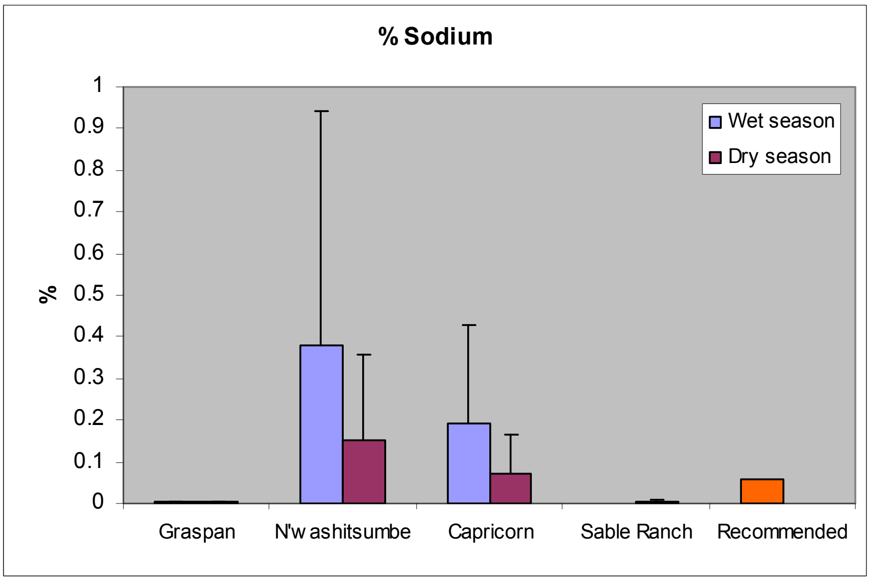
* n = number of pooled species per study area per season (Appendix 1)



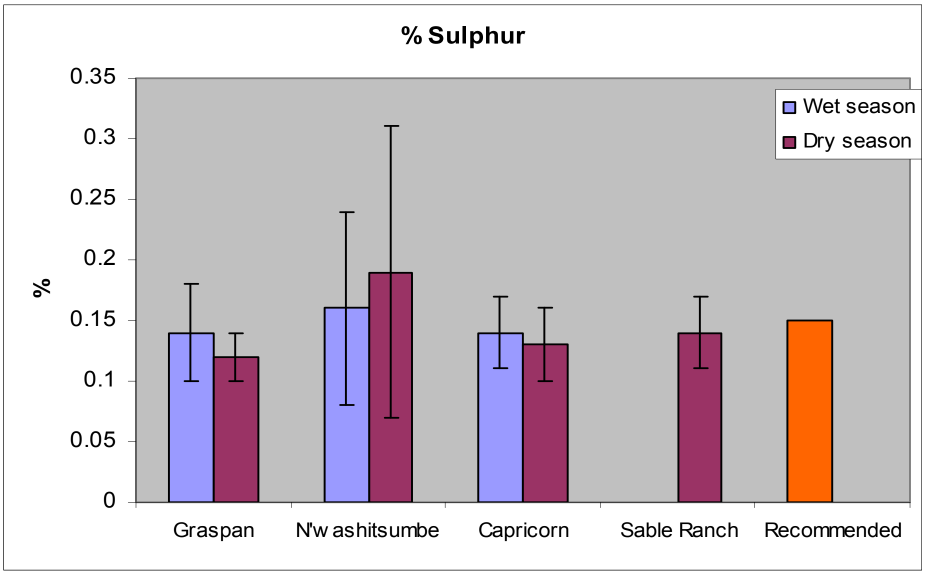
Graph 5.1: N, P and Ca concentrations (DM) of grasses in the wet and dry season

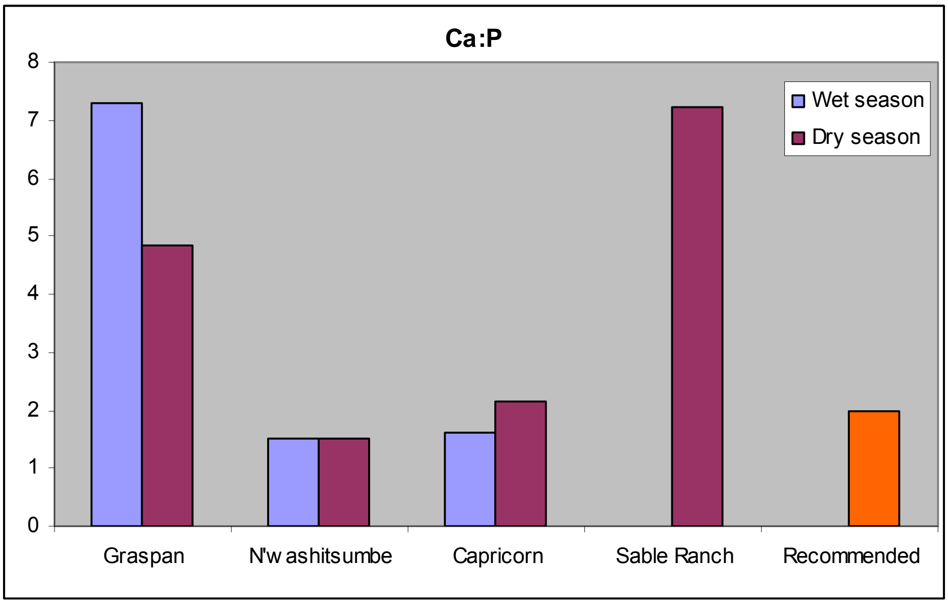






Graph 5.2: K, Mg and Na concentrations (DM) of grasses in the wet and dry season





Graph 5.3: S concentrations (DM) and Ca:P ratio's of grasses in the wet and dry season

The requirement for P in beef cattle diets range between 0.17-0.59%, depending on the level of production as well as the age and sex of the particular animals (NRC, 1996; McDowell, 2003). The lowest margin (0.17%) is closer to maintenance requirements for beef cattle (McDowell, 2003). The daily P requirement for pregnant or lactating cattle is much higher than general production requirements (Underwood and Suttle, 1999; NRC, 2005) and therefore it can be assumed that the daily dietary P requirement for pregnant and lactating roan females will be higher than the maintenance requirement of beef cattle - most probably at least 0.2-0.3%. It is highly unlikely that roan antelope females will require as high P levels as high producing beef cows, mainly due to their much lower daily milk production and smaller body size.

The P content of grasses at Graspan was significantly lower (P=0.01) than grasses at KNP enclosures in summer and 67% lower than the presumable minimum required level of 0.2%. P levels in wet season grasses at both the KNP enclosures were above 0.2 % with grasses at Capricorn the highest at 0.27%. In the dry season the P content of grasses at Graspan and Sable Ranch were significantly lower than in grasses at the KNP enclosures (P<0.05). The P levels in grasses at Sable Ranch and Graspan were respectively 84% and 76% lower than the required minimum level of 0.2%. The P concentration in grass at N'washitsumbe was 47% lower and at Capricorn 34% lower than the recommended 0.2% for beef cattle diets (NRC, 1996).

The wet season K content in grasses did not differ significantly (P=0.11) between the study areas but in the dry season the K content in grasses at Sable Ranch were significantly higher (P=0.02) than in grasses at N'washitsumbe and Graspan.

The variance between the K content of grass species at Sable Ranch was high with a standard deviation of 0.49% above or below the mean of 0.79%.

In summer, the K content in grasses at all the study areas was sufficient. However, the K content of dry season grasses at all the study areas except at Sable Ranch (0.77%) were below the minimum required level of 0.6% (NRC, 1996). The K content in grasses at Graspan was 58% lower, at N'washitsumbe 43% lower and at Capricorn 32% lower than the recommended level.

The Ca levels in grasses in the wet season were within acceptable ranges at all the study areas but fell to marginal content of 0.17% in winter at N'washitsumbe. The required range for Ca in beef cattle diets is 0.16-1.53% (NRC, 1996) of which the exact level is depended on the production level of the animal, particularly in the case of lactation. Maintenance diets in adult cattle typically contain 0.45-0.6% Ca whereas diets for lactating cattle contain at least 0.7-0.9% Ca (NRC, 2005). Diets that high in Ca as is recommended for lactating cows could have negative effects on mature bulls (NRC, 2005). The actual required Ca level for producing wild ruminants is most probably much lower than for producing beef cattle and could probably be met by the maintenance requirements of beef cattle. The content of 0.17% in the grasses at N'washitsumbe therefore just met the assumed minimum requirement level for wild ruminants but could be still insufficient for lactating roan females. The Ca levels in grasses at all the other study areas were within the acceptable range in the dry season with grass at Capricorn having the highest content of 0.29%. However, these levels could still be insufficient for lactating roan cows. Ca levels did not differ significantly between study areas in any season (P=0.16 dry season; P=0.24 wet season).

The Ca:P ratio in the diet of ruminants is very important when evaluating the nutritional value of a diet. Research indicated that a dietary Ca:P ratio of below 1:1 and above 7:1 had significant negative effects on the growth and feed efficiency of beef cattle (McDowell, 2003). A dietary Ca:P ratio of between 1:1-2:1 is considered to be the ideal dietary ratio for most producing livestock (McDowell, 2003). The dietary Ca:P ratio's in grasses at the different study areas in the dry season varied from an average of 1.5:1 in Capricorn to 7.2:1 in Sable Ranch. The ratio in grasses at N'washitsumbe was 2.2:1 and at Graspan 4.8:1. The very high ratios in grasses at Graspan and Sable Ranch were due to the very low P levels in the grasses at that study areas whereas the higher P levels in grasses at the KNP enclosures caused the more optimal Ca:P ratios.

The Ca:P ratio in grasses in the wet season at the different study areas ranged from a good 1.5:1 and 1.6:1 at N'washitsumbe and Capricorn respectively, to 7.3:1 at Graspan. The high ratio in Graspan was again due to the still insufficiently low P levels compared to the adequate Ca levels in the grasses. The lower Ca levels and higher P levels in grasses at the two KNP enclosures resulted in average optimal Ca:P ratios in these grasses.

However, there were considerable species differences in the Ca:P ratios in grasses at each study area (Appendix 1). The average values of all the grass species analysed per study area were used to calculate the ratios based on the assumption that a range of the sampled grass species were well represented in the diets of the particular roan population at that time.

The Mg levels in grasses in both seasons were within the required range of 0.1 – 0.2% (NRC 1996) at all the study areas accept at Graspan. Dry season Mg levels in grasses at Graspan were 40% lower than the recommended minimum level of 0.1%. In the wet season, the Mg levels in grasses at Graspan were significantly lower (P=0.05) than in grasses at Capricorn. No significant differences (P=0.43) were found between the Mg content of grasses at all the study areas in the dry season.

The Na concentrations in grasses did not vary significantly (P=0.30) between places in the summer nor in winter (P=0.28). The variance between grass species in summer at N'washitsumbe (Appendix 1) were very high mainly due to very high concentrations in three grass species, *Diphlance fusca, Sporobolus ioclados*, and *S. nitens* which had much higher Na concentrations than the other species sampled in summer. *S. ioclados* and *D. fusca* retained their higher Na content through the dry season as well. *S. ioclados* and *S. nitens* both occured only on the sodic site of the ecotone between the upland and wetland area at N'washitsumbe. Both *S. ioclados and S. nitens* are strongly associated with disturbed saline areas (Van Wyk and Van Outshoorn, 1999). *D. fusca* only occurred within the wetland at N'washitsumbe. *D. fusca* is a palatable grass species which is well utilised by livestock and game and is strongly associated with saline soils (Van Wyk and Van Outshoorn, 1999). Ram (2006) reported that *D. fusca* is one of the salt-tolerant grass species that could be used to improve the soil in salt-affected arable lands in India.

Figure 5.1: Heavy utilisation of *D. fusca* in the wetland at N'washitsumbe in the late dry season of the year 2005. Note the green shoots still present whilst other grasses are completely dry

Figure 5.2: Selection of *T. triandra* by roan at Graspan in the late dry season of 2005

Tsao and Scheper (2003) reported that halophytes are plants that can tolerate or accumulate salts up to 3-7 times normal concentrations. However, some plants can merely tolerate high saline conditions while accumulators actually remove salt from the subsurface and move it into terrestrial tissues (Tsao and Scheper, 2003). The data therefore suggested that *D. fusca, S. ioclados* and *S. nitens* are Na accumulators, which explain their high Na contents. *Ishaemum afrum* occurred in the same wetland habitat than *D. fusca* but had ‘normal’ Na levels, which suggested that it most probably is a salt tolerant species rather than an accumulator.

To some extent the very high variance in the Na content in grasses at Capricorn was mainly due to *Urochloa mosambicensis* in both seasons, which had a Na content much higher than that of other grasses sampled (Appendix 1). In winter, the substantial difference (99%) in the Na concentrations between *U. mosambicensis* and *Themeda triandra* at Capricorn contributed further to the very high species difference in the enclosure. The Na concentration of grass both in summer and winter at Graspan was far below (92%) the required level of 0.06-0.08% in beef cattle diets (NRC, 1996). Sable Ranch was the only other study area with insufficient Na levels in grass and had an average winter grass content 90% lower than what is recommended for beef cattle. Both N’washitsumbe and Capricorn had sufficient Na levels in grasses in both seasons.

Summer S concentrations in grasses at all the study areas were within the required range of 0.15% (NRC, 1996) and did not differ significantly between the areas (P=0.78). Winter S concentrations in grasses did not differ significantly between the study areas (P=0.39) but a high variance in the S concentrations between grass species occurred at N’washitsumbe. The high variance was again caused by *D. fusca* and *S. ioclados* which had S concentrations much higher than the rest of the grasses. The S concentrations in these grasses were the highest in the dry season when it increased to 0.4% and 0.3% respectively. High intakes of these grass species at such high levels of sulphur could have a negative effect on Cu absorption within the animal (McDowell, 2003). Some plant species are known to accumulate S through the biosynthesis of high quantities of sulphur-rich glucosinolates (Parsons, 1976; Dixon, 2006). Graspan had the lowest dry season S concentration in grasses of 20% below the recommended concentration of 0.15%.

Dry season S levels at Sable Ranch and Capricorn were approximately 13-14% lower than the recommended level. The S content in grasses at N'washitsumbe was within the recommended range in the dry season.

5.4.1.2. Microminerals

The Cu concentrations in grass did not differ significantly (P=0.33) between the study areas in summer and were much lower (64-73%) than the recommended level of 10 mg/kg (NRC, 1996) in all study areas. The lowest Cu levels were detected in grasses at Graspan (Table 5.3). The Cu content of Sable Ranch grasses were significantly lower (P=0.01) than that of N'washitsumbe in the dry season. All study areas had considerably lower Cu levels in the dry season in selected grasses than the required level for beef cattle diets (NRC, 1996). The Cu concentrations in dry season grasses were the lowest at Sable Ranch which were 68% lower than the required level. Cu levels in dry season grass at N'washitsumbe were the highest of all the study areas but were still 54% lower than what is required for beef cattle. Summer Cu concentrations were lower than winter Cu levels in grass at all the study areas. Nonetheless, Cu deficiencies as a result of deficient dietary Cu levels are of less concern to animals than induced Cu deficiencies due to high dietary levels of antagonists such as Mo, S and Fe (McDowell, 2003).

The availability of Cu in forages to the animal is generally very low (5-10% in adult animals) and is often as low as 1-3% in ruminants (McDowell, 2003). This is due to the chemical form of Cu in plants but largely also the strong antagonistic interactions between dietary Cu and S, Mo, Fe and to a degree Zn. The assessment of the Cu content of forages is therefore only of value in conjunction with the assessment of the content of its antagonists (NRC, 1996; McDowell, 2003). The primary site of interactions, especially Cu-Mo-S interactions is in the animal's gut (McDowell, 2003).

The Fe concentrations in grasses in the wet season from the KNP enclosures were significantly lower (P=0.006) than in grasses at Graspan, whereas the Fe concentration of grass at Sable Ranch were significantly lower (P=0.04) than in grasses at N'washitsumbe in winter.

Table 5.3: Micromineral concentrations (mg/kg) of wet season grass in each study area on Dry Matter basis

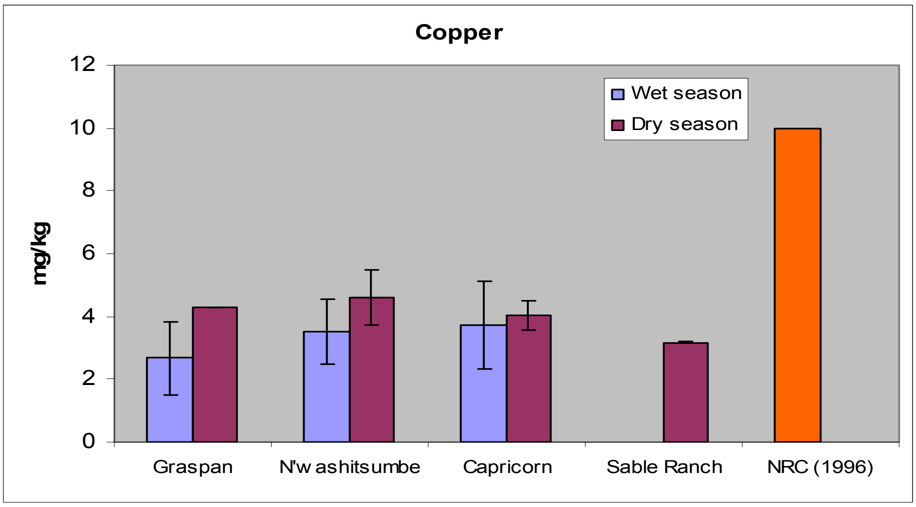
Place	**n**	**Cu**		**Fe**		**Mn**		**Zn**		**Mo**		**Se (µg/kg)**	
		ū	**SD**	**ū**	**SD**	**ū**	**SD**	**ū**	**SD**	**ū**	**SD**	**ū**	**SD**
Graspan	5	2.68	1.17	312.88^{a}	110.11	56.54^{a}	24.67	30.28	10.03	1.04	0.57	10.35	-
N'washit-sumbe	10	3.53	1.04	123.53^{b}	109.47	30.31^{b}	12.13	27.57	118.19	0.86	0.39	96.76	143.69
Capricorn	5	3.72	1.40	111.38^{b}	54.47	32.27	17.53	33.01	6.44	0.60	0.33	39.61	-
Min. Req (NRC 1996)		**10**		**50**		**50**		**30**		**0.5-3.5**		**100**	

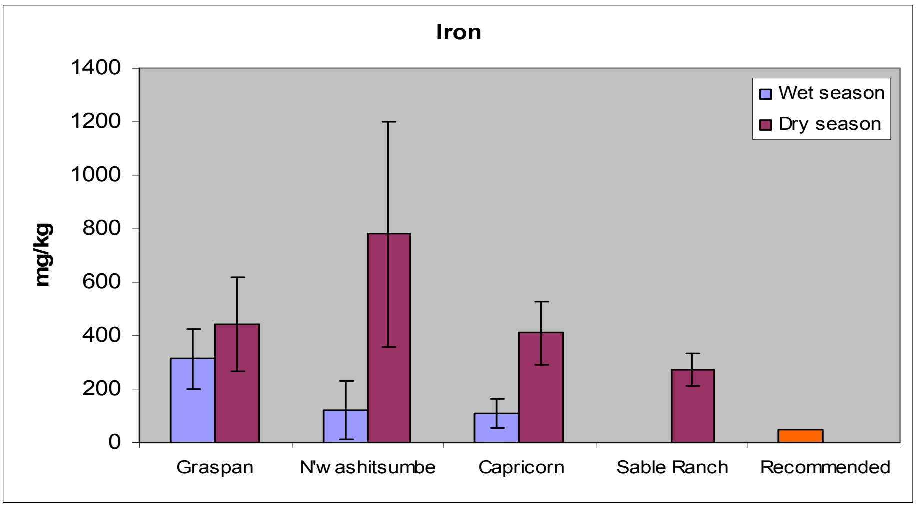
$^{a/b/c}$ = P<0.05

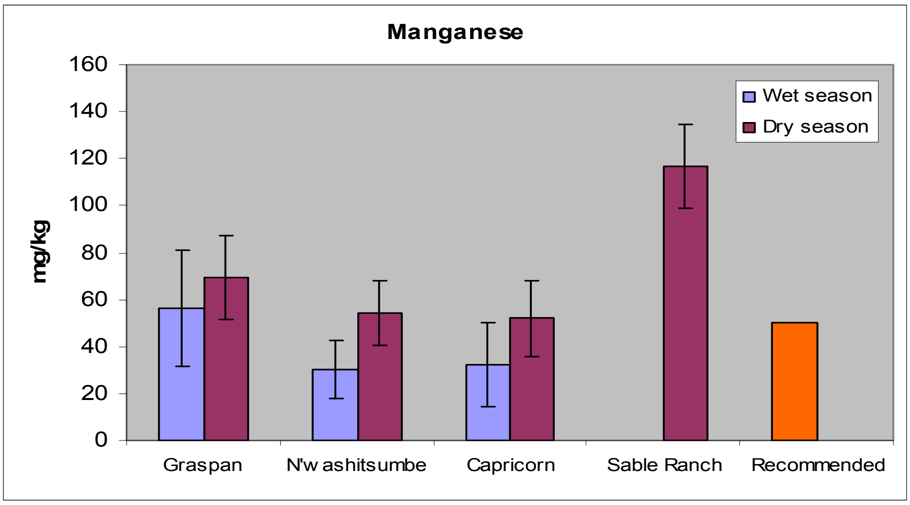
Table 5.4: Micromineral concentrations (mg/kg) of dry season grasses in each study area on Dry Matter basis

Place	**N**	**Cu**		**Fe**		**Mn**		**Zn**		**Mo**		**Se (µg/kg)**		**Co**	
		ū	**SD**	**ū**	**SD**	**ū**	**SD**	**ū**	**SD**	**ū**	**SD**	**ū**	**SD**	**ū**	**SD**
Graspan	4	4.30	0.009	440.83	175.80	69.29	17.98	59.92	6.93	0.72	0.33	16.65	3.75	0.28	0.07
N'washit-sumbe	7	4.60^{a}	0.87	780.43^{a}	420.49	54.39	13.58	52.02	5.24	0.32	0.27	52.88	57.06	0.66	0.40
Capricorn	5	4.04	0.47	409.34	115.92	51.99	16.07	51.62	6.20	0.59	0.35	55.47	59.95	0.52	0.15
Sable Ranch	4	3.18^{b}	0.05	274.28^{b}	61.07	116.76	17.97	46.12	6.93	0.91	1.32	-	-	-	-
Min. Req. (NRC)		**10**		**50**		**50**		**30**		**0.5-3.5**		**100**		**0.05**	

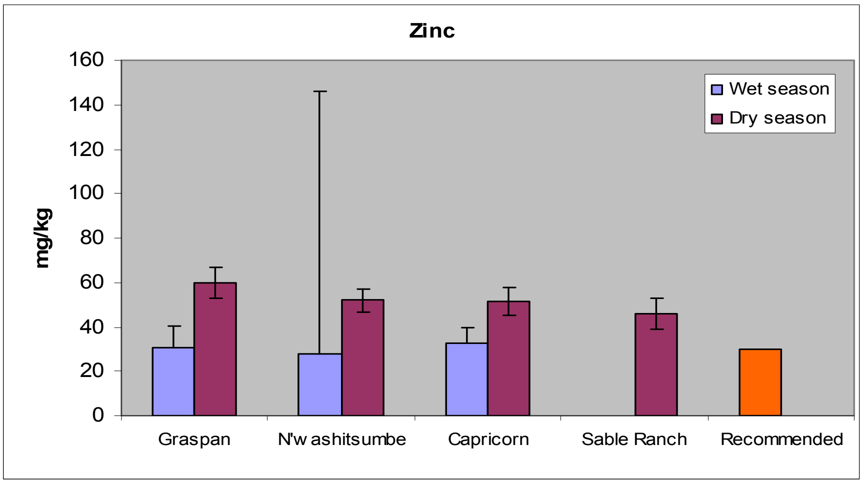
$^{a/b/c}$ = P<0.05

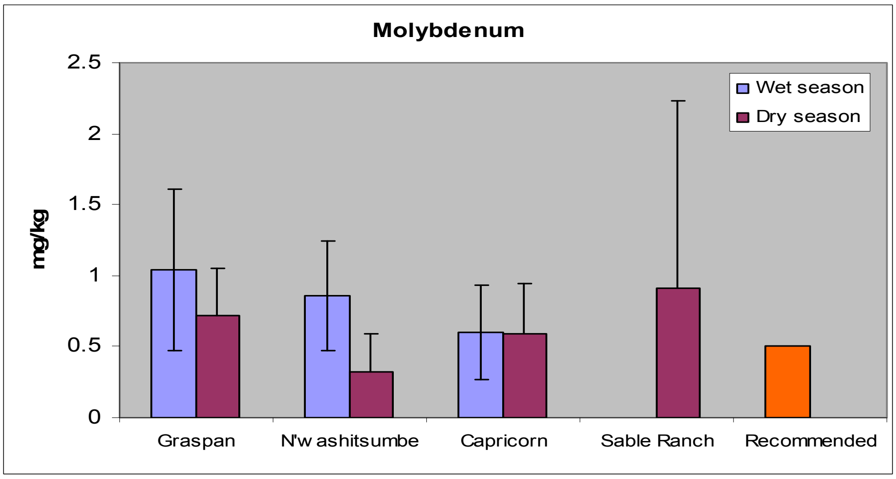


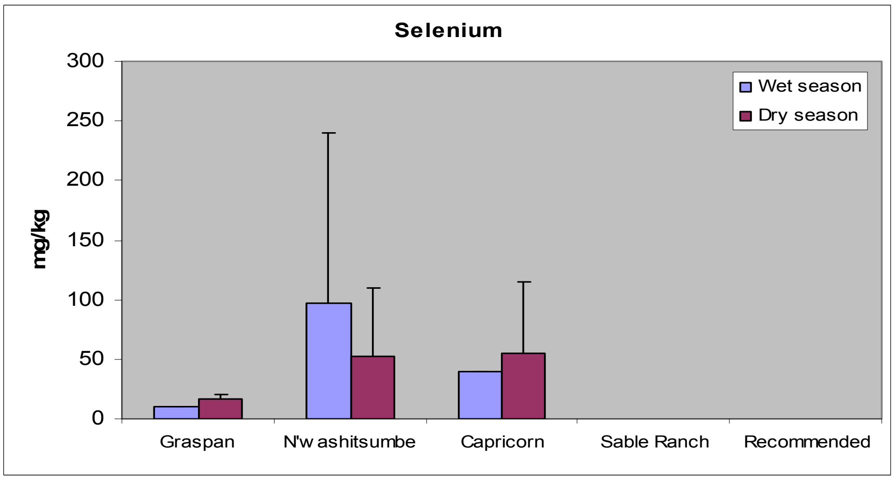




Graph 5.4: Cu, Fe and Mn concentrations (DM) of grasses in the wet and dry season







Graph 5.5: Zn, Mo and Se concentrations (DM) of grasses in the wet and dry season

In both the wet and the dry season the Fe levels in grasses of all the study areas were well above the minimum recommended level of 50 mg/kg (NRC, 1996) but still below the critical dietary level of 1000 mg/kg for cattle and 500 mg/kg for sheep (McDowell, 2003; NRC, 2005). The form of dietary Fe is the main factor affecting critical intake levels. Fe from natural herbage is generally not highly absorbable and can therefore be consumed in very high amounts without causing negative effects or interactions with other minerals.

The very high variation in the Fe content of grasses at most study areas in every season was most probably due to soil contamination of the grass samples. McDowell (2003) stated that Fe deficiencies in grazing animals are rare due to the generally high level of soil contamination on plants. The grass species at N'washitsumbe that contributed most to the high variance and Fe levels were grass species sampled very close to the ground due to the availability of limited plant material as a result of the poor veld condition in the late dry season at N'washitsumbe. The high concentrations and variance could be attributed mainly to *U. mosambicensis, S. ioclados* and to an extent *Schmidtia pappophoroides* (Appendix 1) which all contained very high Fe concentrations. The general veld condition in N'washitsumbe was much poorer in the late dry season than at any other study area at the same time. Grasses at N'washitsumbe were exposed to higher dust contamination than at other enclosures, especially in comparison to Capricorn. It appeared as if the veld condition and herbaceous biomass at Capricorn was retained much better throughout the dry season than at N'washitsumbe.

The Mn concentrations in grass at Graspan were significantly higher (P=0.03) than in grass at N'washitsumbe in summer. The wet season Mn concentrations in grass at N'washitsumbe and Capricorn were respectively 40% and 36% below the minimum required dietary level of 50 mg/kg for beef cattle (NRC, 1996). Dry season Mn concentrations in grass at Sable Ranch were significantly higher (P=0.056) than in grasses at N'washitsumbe even though the Mn levels at all the study areas were above the required level of 50 mg/kg.

Neither summer (P=0.79) nor winter (P=0.58) Zn concentrations in grasses varied significantly between the study areas. Nonetheless there was a strong tendency towards a significant difference in Zn levels in grasses between study areas in winter.

The very high variation in Zn levels between grass species at N'washitsumbe in the wet season (mean 27.57 mg/kg; SD ± 118 mg/kg) probably contributed to the lower probability of a significant difference between the areas. Summer Zn concentrations in grasses at all the study areas were above or very close to the minimum required level of 30 mg/kg (NRC, 1996). Winter Zn concentrations in grasses at all the study areas were higher than in summer and above the minimum required level of 30 mg/kg for beef cattle (NRC, 1996). The Zn concentration in grasses at Graspan was the highest in the dry season at 55.75 mg/kg and was the lowest at Sable Ranch with an average concentration of 46 mg/kg.

The Mo levels in grasses did not vary significantly between the study areas in summer (P=0.29) nor in winter (P=0.51). Only the Mo content of grasses at N'washitsumbe was lower (40%) during the dry season than the recommended minimum level of 0.5% (Puls, 1994). Puls (1994) recommend a dietary Mo level of 0.5-3.5 mg/kg but McDowell (2003) stated that the requirement of Mo by ruminants cannot be quantified due to the strong metabolic interactions of Mo with S and Cu. Summer Mo concentrations ranged between 0.6-1.04 mg/kg at all the study areas with the highest concentrations in grass at Graspan. Winter Mo levels in grasses at all the study areas were on average lower than in summer and ranged between 0.32-0.91 mg/kg with the highest levels at Sable Ranch. In contrast, the Cu levels in grasses at all the study areas were lower in summer than in winter.

The Mo content in grasses at every study area varied highly between grass species. In Sable Ranch the standard deviation was almost 1.5 times the overall mean. McDowell (2003) stated that the Mo content of natural forages is often lower than 0.35 mg/kg without causing a deficiency in grazing ruminants. McDowell (2003) further stated that Mo levels are more favourable for Cu absorption at such low concentrations and that th Cu:Mo ratio within the diet is more important. The critical Cu:Mo ratio in ruminant diets necessary to secure sufficient Cu availability vary between 2:1 and 5:1 (McDowell, 2003). The Cu:Mo ratio in grasses sampled at each study area ranged between 3.4:1 (Graspan) and 8.4:1 (Capricorn) in the wet season and between 7.2:1 (Graspan) and 79.8:1 (N'washitsumbe) in the dry season. It therefore seemed that the risk of a Cu deficiency based on the Cu:Mo ratios in grasses was higher in the wet season than in the dry season at all the study areas.

Nevertheless, Cunha (1973) stated that a Cu deficiency usually occur when forage Cu levels are below 5 mg/kg and Mo levels are higher than 3 mg/kg. The highest Mo levels in grasses at all the study areas were measured in dry season *Digitaria eriantha* at Sable Ranch which had a Mo content of 2.84 mg/kg and a Cu content of 3.26 mg/kg. The rest of the grasses had much lower Mo levels.

The Se concentrations in most of the grasses at Graspan and Capricorn were below 4-5 µ/kg in summer and winter and a specific value could therefore not be determined by means of the analytical method used by CAL laboratories (Appendix 1). Such values were consequently not included in the statistical analysis. Only *Schmidtia. pappophoroides* at Graspan and *Urochloa. mosambicensis* at Capricorn had values of 10.35 µg/kg and 39.61 µg/kg respectively in summer. *Sporobolus. ioclados* and *S. nitens* at N'washitsumbe had higher Se values compared to the Se content in the rest of the grass species and ranged between 279.04-405.01µg/kg respectively (Appendix 1). In general, grasses at N'washitsumbe had much higher Se levels in summer than grasses at Capricorn and Graspan and was the only study area that contained the minimum required level for beef cattle diets of 100 µg/kg (NRC, 1996). However, McDowell (2003) stated that the Se requirement for livestock in general probably range between 50-300 µg/kg. The average wet season Se content in grasses at both Graspan and Capricorn were therefore below the recommended level (Table 5.3).

Winter Se concentrations were not determined in grasses at Sable Ranch and the three grass species at N'washitsumbe and two at Capricorn that did not have absolute values were omitted in the statistical analysis. The Se levels in grasses at the three study areas analysed for Se concentrations in the dry season did not differ significantly (P=0.47). Winter Se levels in grasses at Capricorn and N'washitsumbe were on average between 52-56 µg/kg. The variation of Se concentrations between grass species were very high at Capricorn and N'washitsumbe in winter and the SD ranged between 57 µg/kg at N'washitsumbe and 60 µg/kg at Capricorn. The Se concentration of grasses in winter at Graspan was much lower than at the KNP enclosures and substantially lower than the minimum required level for beef cattle diets of 100 µg/kg (NRC, 1996). Graspan therefore contained very deficient Se levels in grass in both seasons whereas the Se levels in grasses at the KNP enclosures were marginally deficient in both seasons.

Dry season Co concentrations were determined only in grasses at Capricorn, N'washitsumbe and Graspan. The Co concentrations in gasses did not differ significantly between the study areas (P=0.14). The dry season Co concentrations in grass at all the study areas were above the critical minimum level of 0.05 mg/kg for beef cattle diets (NRC, 1996).

5.4.2. SOIL ANALYSES

Results from soil analysis at each study area did not reveal any abnormal levels of soil mineral concentrations that could possibly have significant effects on plant mineral concentrations at any of the study areas (Table 5.5) (Prof AS Claassens, Pers. comm.)[7]. Both N'washitsumbe and Capricorn are situated on the basaltic plains of the Lebombo flats in northern KNP and similar soil mineral concentrations between these two enclosures were anticipated. However, substantial differences between the sandy Graspan soils and the clayey basaltic soils of the KNP enclosures were evident from the results.

The P concentration in soil at Graspan was approximately 50% lower than at the KNP enclosures. The Ca, Mg and K concentrations in soil were the highest at Capricorn with that at N'washitsumbe respectively 56%, 17% and 30% lower. The Ca, Mg and K concentrations in soil at Graspan was respectively 87%, 83% and 57% lower than at Capricorn. The Na concentration in soil at N'washitsumbe was on average much higher (83-95%) than at Capricorn and Graspan.

The higher average Na content in soils at N'washitsumbe were largely attributed to the high Na concentrations in the sodic ecotonal area (466 mg/kg) as well as the adjacent wetland (414 mg/kg). The Na content in soil from these areas were substantially higher than the Na concentrations in the upland areas at N'washitsumbe and explained the very high Na concentrations in most of the grasses that grew in the sodic site as well as the wetland. Only *Ishaemum afrum* was Na tolerant and did not accumulate higher Na levels (Appendix 1).

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Table 6.5: Soil analyses of the three study areas with special reference to the different veld types in the KNP enclosures

Place	Veld type	pH (KCl)	P BRAY1 mg/kg	Ca mg/kg	Mg mg/kg	K mg/kg	Na mg/kg	Ca:Mg 1.5-4.5	Ca+Mg)/K 10.0-20.0	Mg:K 3.0-4.0	Zn mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Se ppb
A	Mopani	5.66	10.00	4696.0	997.0	589.0	42.0	2.87	21.01	5.42	1.40	0.60	29.00	1.00	<5
	Combretum	6.03	8.00	4393.0	1003.0	1184.0	39.0	2.67	9.97	2.71	1.20	0.40	27.00	1.00	<5
		5.85	**9.00**	**4544.5**	**1000.0**	**886.5**	**40.5**	**2.77**	**15.49**	**4.07**	**1.30**	**0.50**	**28.00**	**1.00**	**<5**
B	Wetland	5.65	5.00	1614.0	1055.0	630.0	414.0	0.93	10.38	5.37	6.10	0.70	53.00	2.00	<5
	Mopani	5.65	10.00	2873.0	1061.0	674.0	38.0	1.65	13.38	5.05	1.60	1.70	36.00	2.00	<5
	Marula	5.72	11.00	6050.0	988.0	772.0	34.0	3.74	19.42	4.10	1.00	0.40	22.00	1.00	<5
	Ecotone	5.70	12.00	1278.0	196.0	398.0	466.0	3.98	7.86	1.58	1.00	0.60	38.00	12.00	<5
		5.68	**9.50**	**2953.8**	**825.0**	**618.5**	**238.0**	**2.58**	**12.76**	**4.03**	**2.43**	**0.85**	**37.25**	**4.25**	**<5**
C	-	5.28	4.00	415.0	112.0	117.0	9.0	2.26	10.00	3.07	0.30	0.90	7.00	8.00	33.00
	-	5.50	5.00	741.0	232.0	643.0	13.0	1.95	3.41	1.16	3.60	0.80	8.00	2.00	28.00
		5.39	**4.50**	**578.0**	**172.0**	**380.0**	**11.0**	**2.11**	**6.71**	**2.12**	**1.95**	**0.85**	**7.50**	**5.00**	**30.50**

A – Capricorn **B –** N'washitsumbe **C –** Graspan

The Zn concentration in soil at N'washitsumbe was 47% higher than in soil at Capricorn and 20% higher than at Graspan. Cu concentrations were the same in soils at Graspan and N'washitsumbe (0.85 mg/kg) but was 41% higher than Cu in soils at Capricorn. The Mn concentrations in soils at Graspan were the lowest (7.50 mg/kg) and were 73% lower than in soils at Capricorn and 80% lower than in soils at N'washitsumbe. The Fe concentrations in soils at Capricorn were on average 76% lower than in soils at N'washitsumbe and 80% lower than in soil at Graspan. Most soils at N'washitsumbe resembled the Fe content in soils at Capricorn but were on average approximately 84% lower than the Fe content in soil from the sodic ecotone at N'washitsumbe. The Fe content in the soils in general at all the different study areas were very low (Prof AS Claassens, pers. comm..).

In general, the Se concentration in soil at Graspan was by far the highest (30.50 ppb) compared to soils at the KNP enclosures which all had a Se concentration of below 5 ppb. The Se content in the soils at the study areas therefore exhibited the opposite concentrations of Se in grasses at the study areas. The Se content in grasses at Graspan was the lowest compared to the Se content in grasses at the other study areas.

5.4.3. WATER ANALYSES

Water samples were collected and analysed merely to see whether severe mineral/nutrient toxicities occurred within a particular water source. Excessive intakes of specific minerals and nutrients through water can have adverse effects on the nutrient/mineral status of animals and over time could cause substantial animal losses (Dr JA Meyer, pers. comm.)[8]. Standard water analyses from CAL laboratories were used but since neither the complete trace mineral profile nor bacterial content were analysed, the analysis was insufficient for the assessment of fitness for animal use (Dr JA Meyer, pers. comm.).

None of the mineral levels in water were above normal levels at any study area except Cu concentrations in two of the Graspan water troughs (Table 5.6).

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Table 5.6: Mineral and nutrient content of water from water troughs (point-of-use) at the different study locations

Trough	Place	pH	TDS mg/l	Ca me/l	Mg me/l	K me/l	Na me/l	Cl me/l	SO4 me/l	CO3 me/l
1	**A**	**9.18**	**652.00**	**0.16**	**2.45**	**2.19**	**8.74**	**3.78**	**0.07**	**2.80**
1	**B**	9.69	1538.00	0.19	4.45	3.67	16.35	12.89	0.08	4.00
2		9.21	860.00	0.73	7.41	2.46	7.26	5.89	0.36	4.80
		9.45	**1199.00**	**0.46**	**5.93**	**3.07**	**11.81**	**9.39**	**0.22**	**4.40**
1	**C**	8.35	366.00	3.15	2.59	0.14	1.16	0.47	0.22	0.40
2		8.49	292.00	1.76	2.38	0.13	1.20	0.48	0.20	0.40
3		8.45	516.00	3.87	3.83	0.16	1.94	1.16	0.68	0.80
		8.43	**391.33**	**2.93**	**2.93**	**0.14**	**1.43**	**0.70**	**0.37**	**0.53**

Ref. #	Place	HCO3 me/l	NH4-N mg/l	NO3-N mg/l	Mn mg/l	Cu mg/l	F mg/l	Co µg/l
1	**A**	**5.40**	**0.00**	**0.00**	**0.00**	**0.04**	**0.50**	**<0.01**
1	**B**	7.20	0.80	0.00	0.00	0.00	0.50	<0.01
2		5.40	0.30	0.00	0.00	0.00	0.50	<0.01
		6.30	**0.55**	**0.00**	**0.00**	**0.00**	**0.50**	**<0.01**
1	**C**	4.90	0.90	4.60	0.01	0.02	<0.5	<0.01
2		3.50	0.00	5.60	0.00	4.96	<0.5	<0.01
3		6.20	0.00	2.40	<0.01	6.09	<0.5	<0.01
		4.87	**0.30**	**4.20**	**0.01**	**3.69**	**<0.5**	**<0.01**

A – Capricorn **B –** N'washitsumbe **C –** Graspan

Cu concentrations above 1 mg/L can have a negative effect on the nutrient status of an animal mainly due to antagonistic interactions with other minerals. The high Cu in the two water troughs at Graspan could stem from contamination within the water troughs (dissolved Cu from ball-valve parts) or from the supply pipeline via the windmill and water reservoir (Dr JA Meyer, pers. comm.).

The water pH at Graspan was slightly lower (8-11%) than at the KNP enclosures. The difference of more than two units between the water and the soil pH within each enclosure was questionable and it could be related to laboratory analytical errors (Prof AS Claassens, Pers. comm.). The Total Dissolved Solids (TDS) levels in water at Graspan and Capricorn were in general respectively 67% and 46% lower than in water at N'washitsumbe. The Ca content in water at Graspan was much higher than in the water at Capricorn and N'washitsumbe, which had respective water Ca concentrations 95% and 84% lower than at Graspan. Mg in water at Capricorn and Graspan was on average approximately 50% lower than at N'washitsumbe. The K content in water at Graspan was on average considerably lower (94-95%) than in water at the KNP enclosures. Water at Graspan had also much lower Na (84-88%) and Cl (81-93%) levels than water at the KNP enclosures.

The sulphate (SO_4) level in water at Capricorn and N'washitsumbe was respectively 81% and 41% lower than at Graspan. Water at N'washitsumbe had on average the highest carbonate (CO_3) level with water at Capricorn and Graspan respectively 36% and 88% lower. Hydrogencarbonate (HCO_3) levels in water varied only 14-23% between study areas. Ammonia-nitrogen (NH_4-N) in water was lower (45%) at Graspan than at N'washitsumbe with water at Capricorn having no value. Nitrate-nitrogen (NO_3-N) had values only at Graspan with an average of 4.20 mg/L in all three water troughs sampled. Very low Mn levels were found in water with only one trough at Graspan having a value of less than 0.01 mg/L. The F concentrations in water at Capricorn and N'washitsumbe were the same (0.5 mg/L) but were even less than 0.5 mg/L in water at Graspan. The Co concentrations in water were below 0.01 mg/L at all the study areas and below the detectable levels for the analytical methods used by CAL.

At N'washitsumbe there were substantial differences in some mineral levels in water between the two water troughs available to roan as could be seen in the 44% lower TDS level in water from trough no. 2 than from trough no. 1.

The two water troughs were less than a kilometre from one another but were supplied from two separate boreholes. Trough no. 1 was the northern trough and no.2 the southern trough close to the enclosure's entrance gate. Water from trough no. 2 had a 55% lower Na and Cl concentration than water from trough no 1. The Ca and Mg level in trough no. 1 was respectively 74% and 40% lower than in trough no. 2. The SO_4 level in trough no 1 was 78% lower than in trough no. 2. These differences could influence the preference for drinking water by the resident roan antelope at N'washitsumbe.

Differences in mineral levels between water from various water troughs at Graspan did not vary substantially with only large differences seen in the Cu levels. Trough no. 1 had a 99.7% lower Cu concentration than trough no. 3 and a 99.6% lower level than trough no. 2. Only water in trough no. 1 at Graspan had a NH_4-N value (0.90 mg/L). The NO_3-N level in water from trough no 3 was 57% lower than in water from trough no. 2 and 18% lower than in water from trough no. 1.

5.5. CONCLUSIONS

Animals in a production phase have higher dietary nutrient demands than animals only maintaining their particular body weight or condition. Growth, pregnancy and lactation are all production phases that require specific amounts of nutrients for the specific physiological and metabolic processes involved to function and produce according to the genetic performance level of the species or individual. Lactating females in general has some of the highest nutrient demands than most other production phases. Joubert (1970; 1976) found that both free roaming and enclosed roan on the basaltic plains of the KNP calved throughout the year with an almost 50/50% division between the dry and wet seasons.

This implies that in general, a roan herd will have at least a few females in production at any given time in the KNP. The dietary nutrient levels should therefore be able to support the required production levels throughout the year to prevent deficiencies that could result in limited production or survival levels. The animal can only produce to the level that the most limiting nutrient allows it to (NRC, 1996). If the supply of protein is limiting performance more than P, protein is the most limiting nutrient and the supply of P or any other nutrients will not affect performance significantly unless the protein supply is increased according to the requirements.

The priority of this chapter was therefore firstly to identify the nutrients in the diets of roan at all the study areas that most probably limited the production and/or survival in each season and secondly to identify other possible nutrient deficiencies during specific times of the year within each study area.

The most deficient nutrients in the grasses of the KNP enclosures in the wet season appeared to be mostly Cu but possibly also Mn. The availability of dietary Cu largely depends on the dietary Mo and S levels (McDowell, 2003). Roan preferred bottomland areas in N'washitsumbe during the dry season (Knoop and Owen-Smith, 2006) and the high S levels in favoured bottomland grasses (mainly *Diphlachne fusca*) may limit the availability of Cu during the peak dry season at N'washitsumbe. Both the dry and wet season Cu-Mo-S ratio's were acceptable and within the recommended ranges. The possible intake of excessive Fe due to most probably geophagia, could reduce Cu availability even more, especially at N'washitsumbe. Very high dietary Fe concentrations, as in grasses at N'washitsumbe in the dry season could exacerbate the antagonistic effect of the high S levels on Cu availability for absorption in the animal. The low Cu level is most probably not the most limiting nutrient in the dry season in the forages of the KNP enclosures but could have a severe subclinical effect on roan reproduction and calf survival.

Both N and P are very important for animal production – especially reproduction and lactation (NRC, 1996; Underwood and Suttle, 1999; NRC, 2005). P is generally the most limiting nutrient in South African pastures and is widely supplemented to both livestock and wildlife. However, the Ca:P ratio in the KNP enclosures are sufficient despite the deficient P levels in grasses which could limit the effect of the P deficiency. The protein content of grasses might therefore be even more limiting in the dry season at the most of the study areas. A dietary crude protein content of at least 5% is necessary to maintain rumen microbes for optimal forage utilisation. The dry season crude protein content of grasses at the KNP enclosures dropped to approximately 3% and will most probably not be sufficient in sustaining especially lactating roan females. However, it is neither impossible nor uncommon for roan to supplement their N intake in the late dry season with browse. The K levels in grasses are deficient in dry season grasses at N'washitsumbe and Capricorn, as well but generally ruminants have greater problems dealing with dietary K excesses than deficiencies.

The P levels in wet and dry season grasses were most likely one of the most limiting nutrients at Graspan, even though the Na levels were well below the recommended levels as well. A Na deficiency in ruminants is often caused by a dietary deficiency and is mostly more prominent in lactating females due to the Na content of milk (McDowell, 2003). The fact that the Ca:P ratio at Graspan was much higher than the recommended minimum in both seasons was mainly due to the very low P levels in grass relative to the Ca levels. The crude protein of forages in the dry season was also below the minimum requirement at Graspan and could limit production even further. Of the micro minerals in grasses at Graspan, only Cu was deficient in the both in the wet and dry seasons. S levels in grass at Graspan were adequate but the Mo levels were slightly elevated, especially in the wet season. This might reduce the Cu availability in grass for roan even further during the summer months at Graspan. However, two of the three water troughs sampled at Graspan had very high Cu concentrations. Should a continuous high level of Cu be supplied through contaminated water at Graspan, the constant higher Cu in the water intake could supplement the low Cu levels in the grass. The K levels in the water at Graspan were very low compared to that of water sources at the KNP enclosures.

Similarly to the P levels in grass at Graspan, the P concentrations of dry season grasses were most likely the most limiting nutrient at Sable Ranch. The very low levels of P in dry season grass will most certainly affect production levels of particularly lactating females in winter. The adequate N concentration of grasses at Sable Ranch supported the probability that P was the most limiting nutrient. The K levels were also deficient in grasses at Sable Ranch and as with P, the Cu concentrations in grasses were the lowest of all study areas.

The soil P and K concentrations at Graspan were much lower than at the KNP enclosures and were most probably responsible for the deficient P levels in the grasses. The low Mg soil concentrations at Graspan were not reflected in the grasses. The very low Fe levels in all the soils sampled and analysed were generally not reflected in the grasses.

All the possible nutrient limitations in the assumed diets of roan antelope identified and described at all the study areas within this chapter will be tested further in the next chapter. The response of roan at each study area to the specific dietary nutrient levels described in this chapter will be measured in serum and faecal samples and evaluated further in the next chapter.

CHAPTER 6

EVALUATION OF THE NUTRITIONAL STATUS OF ROAN AND SABLE ANTELOPE

6.1. INTRODUCTION

The nutritional requirements of a wild animal must be thoroughly understood to ensure its survival and at the same time maximise its production (Holecheck *et al.,* 2001). Unfortunately mineral nutrition in ruminants is rather complex and requires a solid appreciation and knowledge of various interactions (Blasi, 2001). Both synergistic and antagonistic interactions between minerals are the most important factors influencing the bioavailability of these elements in the diet to animals (Van Ryssen, 2000). Estimating the requirements of a specific nutrient for a ruminant species grazing natural pastures is therefore complicated. Predicting the dietary Cu availability, for example, very complex because of its interactions with Mo and S. Above a specific level, Fe also inhibits Cu and Zn availability whereas Cd may play a role as well (Judson and McFarlane, 1998).

Even though the nutrient content of soil and especially forage could be a good indicator of possible deficiencies in animals (Myburgh, 2003), the determination of dietary nutrient status alone is insufficient in predicting the nutrient status of an animal (Suttle, 1986; Irwin *et al.,* 1993; Kincaid, 1999; Myburgh, 2003). Diet analyses provide useful supporting data should a representative sample be obtained (Kincaid, 1999). The chemical analysis of the diet should include all those elements with important interactions (Kincaid, 1999). Nonetheless, no diagnostic decisions should be made upon the results of forage and soil analysis alone, but results should rather be used in conjunction with data obtained from animal samples as well (Suttle, 1986; Kincaid, 1999; Underwood and Suttle, 1999; Myburgh, 2003). Blood, liver and various other animal tissue samples are excellent and widely accepted indicators of the nutrient status of an animal (Grant, 1989; Herdt *et al.,* 2000; Van Ryssen, 2003). Although animal tissue and fluid samples are generally considered the most reliable means of determining the nutrient status of an animal or population (Herdt *et al.,* 2000), it is difficult in its application to wild herbivores.

In order to collect samples, animals must be either dead or immobilized and in animals like roan and sable antelope this could be a very expensive and risky procedure. Fresh faecal samples could be used successfully as a non-invasive and affordable tool to estimate the nutrient status of the diet and the animal (Erasmus, *et al.,* 1978; Holechek *et.al.,* 1985; Howery and Pfiser, 1990; Irwin *et al.,* 1993; Grant *et.al.,* 1995; Grant *et.al.,* 1996; Wrench *et.al.,* 1996, Dörgeloh *et al.,* 1998). The best results will however be obtained should faecal analysis be used in conjunction with an actual animal sample like liver or blood, as well as dietary data - especially in estimating the nutrient status of a specific population of animal species (Myburgh, 2003).

In order to assess the nutritional status of the last viable population of sable antelope in Shimba Hills National Park, Kenya, Sutton *et al.* (2002) did a geochemistry reconnaissance survey in the park. As part of the study, vegetation was randomly sampled and analysed. Results indicated that estimates of mineral imbalances in sable antelope were limited by the absence of element levels in animal tissues and the lack of knowledge of the nutrient requirements of sable antelope. These results emphasized that a good understanding and scientific knowledge of the nutrient requirements of rare species, like roan and sable antelope, will increase the success of conservation and breeding efforts, especially since millions of Rand are currently invested in such efforts, both privately and federally within southern Africa alone.

6.2. LITERATURE REVIEW

6.2.1. CORRELATIONS BETWEEN NUTRIENT CONCENTRATIONS IN ANIMALS AND THE HABITAT

To identify and demarcate specific areas in which anticipated nutrient imbalances could be monitored over the long-term, Grant *et.al.* (1996) used soil, vegetation and geology as natural classification systems to define cattle ranching areas in central and northern Namibia. The nutrient concentrations in these systems were then compared to the nutrient concentrations in tissue, milk and faecal samples from cattle within those specific areas. Cu, Fe, Zn, Mn and Co concentrations were determined in liver samples from abattoirs, while P, Ca samples were collected from faecal grab samples and I from milk samples.

Results indicated that areas of low P could be identified by all classification systems. The sandy Kalahari farms showed the lowest P concentrations. Animals with I deficiencies were detected in the north by means of milk samples. Areas that supported animals with marginal Cu deficiencies could best be identified by detailed soil-classification systems of irrigation potential. However, the authors found that most areas low in mineral levels were best indicated by using geological classification systems, even though significant monthly variations for all minerals were detected within the classification system.

Holecheck *et al.* (1985) found that dietary P content of grazing cattle may be accurately predicted from faecal P concentrations using simple regression equations. The P intake of cattle was strongly associated (r^2=0.94) with daily faecal P output.

In a study in the Hell's Gate National Park, Kenya, Simiyu *et.al.* (1999) assessed the impact of geothermal activities on the chemical quality of the habitat and its health implications for grazing wild animals. Water, soil, grass and tissue samples from Burchell's zebra *Eguus burchelli* were used to determine the influence from possible toxic Zn, Cu, Cd, Pb and Mo concentrations in the habitat on wild animals occupying the area. The study revealed that a linear relationship existed between Zn, Cu, Cd and Mo. A roughly linear relationship between the trace mineral concentrations for water, soil, grass, plasma and liver samples were found in Zn, Cu and Cd, except for Mo. Grass and water Mo concentrations increased while soil, plasma and liver Mo concentrations, decreased.

Hintze *et.al.* (2001) found in a study on Se concentrations in soil, forage and beef cattle that the geographic region in which the beef cattle occurred was the greatest source of variation of bovine skeletal muscle Se content. Management practises in the form of supplemental feed Se did not increase the Se concentrations in skeletal muscles significantly. The Se concentrations in soil ($r = 0.53$; $p>0.01$) and grass ($r = 0.63$; $p>0.01$) were strongly correlated to Se content of skeletal muscle (Hintze *et al.,* 2001). Using linear regression models Hintze *et al.* (2001) further found that the best predictors of Se concentrations of skeletal muscle that could be obtained in the field were whole blood, hair and soil Se solubles (grass was not used in the model even though stronger correlated than soil). Whole blood Se concentrations were stronger correlated to skeletal muscle Se content than Se concentrations in hair.

Se concentrations in hair could also be influenced by factors like hair colour making it less attractive as a predictor of skeletal muscle Se content (Hintze *et al.,* 2001).

Wilson and Hirst (1977) compared the concentrations of Ca, P, Mg, Na, K, N, Fe, Cu, Zn, Co, Mn and Se in milk, liver, blood, grass and soil samples from sable antelope populations in different geographic regions in the Transvaal (now referred to as the lowveld of the Mpumalanga and Limpopo Provinces, South Africa). Changes in the concentration of any one mineral between one area in the environment and the body tissues of an animal were constant. The results therefore suggested that the initial concentrations in soil and vegetation were primarily responsible for setting the tissue levels within the animals grazing in the same habitat. - unless the specific mineral was controlled by corticoid hormones or significantly influenced by changes in gut absorption by for instance mineral interactions (Wilson and Hirst, 1977). Ca concentrations in soil and forage in most of the areas showed linear correlations. The blood Ca content was very similar in all areas due to corticoid control but high dietary Ca concentrations were reflected in high liver and milk Ca concentrations. For P, high soil and vegetation levels led to high blood P concentrations but relatively constant milk P content. Mg was similar in its passage from soil to forage but the grass showed a wide variation in availability to the animal. Blood and milk P and Mg levels were very constant implicating that P and Mg were supplied at a constant level to milk from the female body despite a wide range of availability in the diet. This implied that P and Mg deficient diets induced a negative P and Mg balance in lactating females within P and Mg deficient areas (Wilson and Hirst, 1977). Na concentrations were linearly related to soil, forage and animal tissue and fluids.

The nutrient availability in water was also determined by Wilson and Hirst (1977) through samples taken in the dry season from water sources frequented by roan and sable antelope in the different regions. The trace element content of all water samples was very low. Wilson and Hirst (1977) found therefore that the water intake of sable and roan antelope contributed very little to total mineral nutrient intake in their study areas.

6.2.2. NUTRIENT AND MINERAL CONCENTRATIONS IN FAECES AND BLOOD SERUM

6.2.2.1. Faecal nutrient concentrations

The use of faecal analysis of animals as an indicator of a population or an individual's nutrient and mineral status have been properly researched in both domestic- and wild herbivores (Erasmus *et.al.*, 1978; Holechek *et al,.* 1985; Grant *et al.,* 1995; Grant *et al.,* 1996; Wrench *et al.,* 1996, Dörgeloh *et al.,* 1998). Generally, the faecal concentrations of N and P are accepted as an indication of the sufficiency of these nutrients in an animal's diet.

Wilson and Hirst (1977) estimated P intakes of sable antelope and found that the intakes of populations in the then Transvaal region of South Africa averaged between 2-3 g/d compared to the 9 g/d of Zimbabwean populations and the 10 g/d recommended for cattle. Irwin *et al.* (1993) found that a linear relationship existed between N_f (faecal N) and N_d (dietary N) and a further linear relationship existed between N_f and dry matter digestibility in bighorn sheep. It was also found that winter diets ≤ 7% crude protein was considered sub-maintenance for ungulates and that N_f values < 1.3% may indicate a potential nutrient deficiency in bighorn sheep (Irwin *et al.* 1993).

During a severe drought in the KNP between 1992-1993, N_f and P_f concentrations and their relationship proved a useful indicator of forage quality (Grant *et al.* 1995). The N_f and P_f levels were significantly affected by species and feeding category, season and landscape (Grant *et al.* 1995). It was found that the N_f and P_f concentrations between different species within the same feeding category did not differ significantly from each other. This implied that a possibility existed of categorising wild herbivores in terms of their nutrient requirements. Grant *et al.,* (1995) found that the dry winter months showed the lowest N_f and P_f concentrations in wild herbivores. The highest N_f concentrations were recorded during January – March and the highest P_f were recorded during October – December. The highest P_f concentrations were recorded from animals occurring on the basaltic landscapes and the lowest concentrations were recorded from animals on the granitic landscapes. However, the highest N_f concentrations were related to granitic landscapes and the lowest were related to the basaltic landscapes.

Furthermore it was confirmed by Grant *et al.,* (1995) that from the three different feeding categories (browsers, mixed feeders and grazers), the grazers had the lowest N_f, the mixed feeders second lowest and the browsers the highest N_f concentrations. The P_f concentrations were the highest in the mixed feeders and the lowest in the browsers. The P_f was also significantly related to N_f but not conversely. N_f concentrations were lower at P_f concentrations less than 2.2 g/kg OM than at concentrations higher than 2.2 g/kg OM (Grant *et al.,* 1995). Body condition score of animals correlated significantly with N_f concentrations of the different species.

The findings of Grant *et al.* (1995) correlated strongly with findings from a more recent study by Codron *et al.,* (2009) using N isotopic composition analysis of the faeces of herbivores from different feeding categories on the northern basaltic plains of the KNP. It was found that the trends of the N isotopic composition of faeces ($\delta^{15}N$) and the N_f concentrations correlated significantly. The trends in mean N% values in the faeces of animals from the different feeding categories were as follow:

Giraffe and Kudu	Impala	Elephant	Zebra and Buffalo	Wildebeest and Roan
2.5% N	1.9% N	1.3% N	1.2% N	1.0% N
Browsers	**Mixed feeders**		**Grazers**	

Roan antelope had the lowest N_f values for both variables compared to sympatric herbivores, including representative grazers like tsessebe, mixed feeders and browsers. This finding implied either that the diet of roan antelope have a lower crude protein content than other herbivores utilizing the same habitat or that roan have the ability to extract relatively greater proportions of protein from total food ingested and therefore excretes lower amounts of N. However, the low $\delta^{15}N$ level in the faeces of roan indicated that the source for the low faecal N was likely to be a diet low in crude protein because the $\delta^{15}N$ level in faeces has been shown to be correlated to low dietary N (Codron *et.al.,* 2009). The lowest $\delta^{15}N$ levels were found in November whilst the lowest %N levels were observed in July/August. These findings strongly suggested that the roan antelope populations in the KNP were at the time of the study subjected to nutritional stress, which could be linked to the populations' inability to recover from its populations collapse (Condron *et al.,* 2009).

Irwin *et al.* (1993) stated that bighorn sheep with an N_f level of ≤ 1.3% could be suffering from a nutrient deficiency. The comparison of the nutrient status of the KNP roan populations with other roan populations in a different geographic area could reveal more insight into what the actual N level in roan faeces should be under different habitat conditions.

The results of Grant *et al.* (1995) and Codron *et al.* (2009) indicated the strong possibility that the nutrient requirements of various different wild herbivores could be narrowed down to their different feeding categories. Should this be true for the broader nutrient requirement spectrum, more species specific and efficient supplementation would be possible. However, a direct comparison of the nutrient status of roan and sable antelope in similar habitat and feeding regimes has not been studied before and could reveal whether related animals of the same feeding category could in fact have similar nutrient requirements.

Animals utilising browse species containing high levels of tannins could impair N_f values since tannins bind to protein making it unavailable to the animal (Howery and Pfister, 1990; Irwin *et al.,* 1993; Van der Waal *et.al.*, 2003). In the case of roan antelope it has been shown that very limited amounts of browse is utilised throughout the year (7-8%) (Joubert, 1976; Codron *et al.,* 2009) but the diet of roan could consist of up to 50% browse should the available grass be limited (Wilson and Hirst, 1977). This factor will have to be considered when evaluating results from faecal analysis.

The faecal nutrient levels of roan antelope have been studied in order to prove the relevance of the use of faecal analysis in a comparative study as well as to evaluate possible nutrient variations between different habitat types and seasons (Dörgeloh *et al.*, 1998). Some of the populations compared received supplementary feeding making the basis of comparison difficult. Nonetheless, this study did indicate that the analysis of faecal samples could be a relevant indicator of the nutrient status of a roan antelope population under free ranging, natural conditions. Such an evaluation is especially valuable in comparing the response in nutrient levels of faeces of ruminants to available nutrients in different habitat types.

A further study by Van der Waal *et al.* (2003) on kudu *Tragelaphus strepsiceros* in the Limpopo Province of South Africa proved that the N_f of kudu was significantly affected by the month in which the samples were taken and the study area in which samples were collected.

The N_f was therefore strongly correlated to the N content of forage. Fluctuations in N_f followed to a large extent the fluctuations in N content of preferred forage species due to seasonal rainfall and climatic changes. Population density was another factor that influenced N_f concentrations of the species. Van der Waal *et al.* (2003) found that N_f was negatively correlated to kudu density and therefore served as an indicator of the nutritive value of forage and forage quantity as well as decreased forage quality as feeding competition increased. The N_f of populations that received supplementary feeding during certain seasons were found to be significantly higher than those of populations that did not receive supplements during the same periods. This was similar to the findings of Dörgeloh *et al.* (1998).

The conclusions of the study suggested that the faecal N (N_f) content was a useful indicator of the nutritional status of kudu since:

- Dry season N_f was negatively related to kudu densities
- Low dry season N_f levels were associated with poor physical condition and mortalities
- The dry-hot season N_f value of 1.5 g/kg was similar to the nutritional threshold value proposed for southern African herbivores of 1.4 g/kg

Van der Waal *et al.* (2003) concluded furthermore that the values from the study could be used to assess and determine the appropriate stocking densities for kudu in the study area. It could also be used to base management decisions such as supplementary feeding on.

Dörgeloh (1998) reported seasonal variation in N_f and fibre with the highest quality in the summer and the lowest quality in the winter. The faecal macromineral concentrations did not change significantly between seasons in the same area but did vary significantly between areas. Faecal macromineral concentrations can therefore be used to compare nutrient status of animals between areas. N_f and P_f could be indicators of seasonal forage quality. The N_f and P_f concentrations of the NNR were significantly lower than other populations implying possible nutrient deficiencies.

Wrench *et al.* (1996) found that 20 mm of rain on faecal samples did not affect the N levels in faeces but as little as 5 mm of rain influenced the P levels significantly. Exposure of faeces to direct sunlight for two days decreased the P and N levels dramatically in comparison to the control. Faecal samples that had been processed by dung beetles had significantly higher N levels, while P levels were not affected (Wrench *et al.,* 1996). Wrench *et al.* (1996) found that it was possible to use faecal samples as an indicator of, for example the N or protein status of a specific herd of wild herbivores if the recommended sample sizes were sampled and a range of other guidelines on the various environmental factors, collection and storing of the samples were followed.

Knoop and Owen-Smith (2004) found that the N_f of roan at the N'washitsumbe enclosure in the KNP was the lowest (approximately 0.85%) in July/August and P_f was the lowest (approximately 0.3%) in August/September.

6.2.2.2. Blood serum/plasma nutrient and mineral concentrations

Analysis of animal tissue and fluid samples (liver, blood, serum, hair, bone) from living or dead animals remain the most reliable method for determining the nutrient status of a specific animal. Webb *et al.* (2001) used liver samples from African buffalo *Syncerus caffer* in the KNP as an indicator species of possible mineral deficiencies or toxicities due to pollution. The collection of samples was possible because, as part of the KNP bovine tuberculosis monitoring programme, 660 African buffalo were culled in areas representing all regions of the park.

The problem with using tissue samples in wild herbivores is to obtain a statistically significant representative sample of a population on a game farm or reserve, especially in the case of rare antelope species like roan and sable antelope. To be able to get tissue samples, animals must be either tranquillised or dead, making it an expensive, risky and time-consuming operation (Wrench *et al.,* 1996).

Blood serum is considered one of the most preferred indicators of animal nutrient status for most nutrients since it is highly correlated to the animal nutrient status and it is less invasive than tissue samples (Kincaid, 1999; Herdt *et al.,* 2000). Puls (1994) indicated that serum samples could be used the most extensively for mineral deficiency testing in animals, although it was not the most suitable indicator for some specific nutrients. Serum levels of Ca, Co, Cu, Fe, Mg, P, K, Se, Na and Zn are adequate indicators of deficiencies in animals but could be influenced by many factors. Feed mineral levels are suggested for the testing of Ca, Cu, Mn, P, K, Se, Na, S and Zn while liver mineral levels should rather be used for Cu, Mn, Se and Zn levels in an animal (Puls, 1994). However, care must be taken not to contaminate blood samples and it must be kept in mind that the nutrient levels in blood may react slowly to dietary levels due to homeostatic control mechanisms (Kincaid, 1999). Herdt *et al.* (2000) emphasized that all sources of variation must be considered when interpreting results from blood analysis. In the case of analysis for nutritional status, the dietary nutritional status is very important for relevant interpretation (Herdt *et al.,* 2000).

Van Ryssen (2003) explained that most macro- and micromineral levels within an animal could be accurately determined with blood or blood plasma analyses. Nevertheless, there is a degree of variation regarding the suitability of blood samples for some nutrients. The P concentration in blood is only indicative of the dietary P level and shouldn’t be used to determine a deficiency. Underwood and Suttle (1999) stated that the only way of accurately determining the P status or requirement of an animal is through a dose–response trial.

In the interpretation of the Cu level in serum/plasma, it is of utmost importance to know the dietary levels of all the Cu antagonists (Mo, S, Fe and Zn) (Van Ryssen, 2003). In Se analysis whole blood concentrations is linearly correlated to the dietary intake of Se. The Se serum concentration is further considered as an indication of short-term Se intake while whole blood reflects long-term Se intake (Van Ryssen, 2003). The diagnosis of Co in the animal is difficult and should therefore be analysed in the diet. Mn levels in blood are responsive to intake but are present in very low concentrations making it difficult to measure. Serum K levels from the jugular vein is very dependent on the salivary gland activity. Na concentrations in blood are considered of little value because the Na level in blood decreases only in the terminal stages of a deficiency. Blood Zn levels should be used in conjunction with dietary Zn levels in order to interpret results meaningfully (Underwood and Suttle, 1999; Van Ryssen, 2003).

6.3. OBJECTIVES

The nutrient concentrations in faeces resemble both the endogenous loss of minerals during metabolic processes as well as minerals excreted due to an excess in the supply of a particular mineral (Underwood and Suttle, 1999). The use of faecal concentrations of minerals as an indicator of the mineral status of an animal should therefore be done with great caution, but could serve as a valuable link between the intake and status of an animal when the sufficiency of a diet is evaluated. This link becomes even more important when evaluating and monitoring the nutritional status of a wild herbivore of which the mineral requirements are unknown. Studies on the nutrient status of wild herbivores seldom include complete mineral profiles and generally only focus on a few macrominerals by means of evaluating one or two of the acceptable measurable nutritional parameters – dietary, faecal or animal tissue/fluid analyses.

In this study the response of a wild herbivore to the nutrient concentrations in the presumed diet was evaluated using all three nutritional indicators together. Through the use of the nutrient levels in the habitat (soil, water and grasses) together with faecal and blood serum nutrient concentrations the nutritional interactions between animal and diet as well as interactions between minerals within the animal should be described the best. The correlations between blood nutrient concentrations and faecal nutrient concentrations were used to identify interactions and relationships between different minerals.

Where possible and feasible, the comparison of the nutrient status of the different roan antelope populations included in the present study was used to better quantify the nutrient requirements of roan antelope. This was mainly done through the validation of expected dietary deficiencies identified in the previous chapter, with the actual nutrient status of animals' utilising those habitats.

The nutrient requirements of many minerals and nutrients are influenced, among various factors, by gender and species (Underwood and Suttle, 1999; McDowell, 2003; NRC, 2005). Inter-species and inter-gender comparisons of faecal and serum mineral levels were used in this chapter to identify significant differences between the nutrient levels in roan and sable antelope faeces and serum under similar habitat and dietary conditions. These differences were then extended to identify important variation between male and female animals within each species as well.

The comparison of nutrient levels within both faeces and serum of the same roan bulls were used to identify possible significant correlations between faecal nutrient levels and the measured serum nutrient status of the animal at a specific point in time.

Animal specific serum and faecal analysis of the tested mineral spectrum were correlated with one another to describe the occurrence of antagonistic and synergistic interactions between minerals in the animal body of Graspan male roan. This extended the information on the use of faecal analysis as a tool to determine possible nutrient deficiencies and interactions in wild ungulates.

It must be stated that since faecal excretion is not the main excretion route of many minerals and nutrients (Underwood and Suttle, 1999) and the fact that urine excretion levels were not measured in this study, the scope and decisiveness with which these comparisons were made, were limited.

6.4. MATERIALS AND METHODS

6.4.1. BACKGROUND

The method of habitat- and animal sampling was aimed at the roan antelope habitats of the KNP and the Graspan section of Vaalbos National Park (study period 2005–2006). These were conservation areas with very little animal and population specific data. Extensive management regimes at these study areas unfortunately did not provide enough opportunities to sample specific animals and the focus therefore shifted mainly to the habitat within which these populations occurred. Although faecal samples were collected from these areas, the samples were largely not animal or sex specific and could only provide general information on the populations. In July 2005 SANParks (South African National Parks) immobilised and removed approximately 15 mature roan antelope bulls from the Graspan population in an attempt to rectify skewed sex ratios. Several sub-adult bulls were seen either dead or badly injured due to harsh skirmishes with breeding bulls. It was then established that the population consisted of too many bulls for the size of the camp which resulted in a negative effect on the population growth and increased inter-bull aggression. During the capture operations animal specific faecal and blood samples were collected from most of the bulls immobilised.

The need arose to obtain more species, animal and sex specific data to establish particular mineral differences between animal species, populations and sexes. During the winter of 2006, the roan antelope and sable antelope populations of Sable Ranch were included into the study as an opportunity to obtain more in-depth animal- and species-specific data within the same management system, habitat conditions and feeding regime. Many male and female sable and roan antelope were annually immobilised as part of the ranch's management operations which created the ideal sampling opportunity. Both sexes and species were kept apart in separate enclosures of approximately 60-100 ha each. Even though the habitat was similar in all the enclosures, the veld conditions did vary. The same feed supplements were provided in each enclosure in similar amounts (average of 1 kg antelope cubes per animal). However, the exact intake of both vegetation and supplements by each individual animal could not be determined under such semi-intensive conditions and some variation in the amount of supplements provided to each enclosure was later identified.

6.4.2. FAECAL SAMPLES

Generally, faecal samples were collected on as many occasions as possible per season at every study area. It was attempted to collect samples at least every two to three months but due to the distance between the study areas, it was not always possible – especially in the rainy season on the northern plains of the KNP. It was difficult to obtain fresh faecal samples not affected by rain or already infested by insects. Special care was taken to avoid the collection of soil or insect contaminated faeces, although it wasn't always possible. In order to limit the influence of the climate and rainfall, no samples collected were older than 24 hours - in other words, still soft and damp inside the pellets (Wrench *et al.,* 1996). At least five to ten individual dung pellets were collected from each individual dung heap. Where necessary any obvious or excessive plant and soil material was carefully removed from pellets to reduce contamination. Samples were collected in brown paper bags and either oven dried at approximately 60°C for approximately 24hours or air-dried for approximately one month (Irwin *et al.,* 1993). Due to limited funds for analysis and often limited samples collected, the samples collected within each season from each study area were pooled for analysis.

To secure an even representation when pooling samples, the samples were first ground individually. A grab sample of exactly the same weight was taken from each individual sample and pooled together into a container. The pooled sample was thoroughly mixed prior to a grab sample collected for chemical analysis. Several pooled samples were often analysed per season per study area in order to use an even more representative value.

On Sable Ranch faecal samples were collected rectally from immobilised animals. Latex gloves were used to retrieve enough pellets from each animal's rectum and were collected in brown paper bags with the specific animal's tag number on it. Blood samples were collected at the same time from each animal and numbered in correspondence to the animal's unique tag number. This was done in order to be able to correlate the faecal sample of an individual animal with the serum sample from the same animal. Generally, faecal samples collected rectally were clean from soil or plant contamination. Only hair contamination had to be cleaned as and when detected.

Faecal samples were milled and sieved prior to analysis. Faecal samples were analysed for N, P, Ca, K, Na, Mg, S, Cu, Zn, Mo, Fe, and Mn concentrations. Analyses were done by means of NIRS (Near-Infrared Reflectance Spectroscopy) according to the official AOAC (Association of Analytical Communities) method Nr. 989.03. Wet chemistry analysis for N, Ca and P were conducted according to AgriLASA method 6.3.1, as was described for the application in grass samples in Chapter 5.

- Nitrogen reagents, 1977. *Technicon Industrial Method,* method 329-74W
- Calcium reagents, 1978. *Technicon Industrial Method,* method 506-77A
- Phosphorus reagents, 1972. *Technicon Industrial Method,* method 144-71A

Statistical analyses of the differences in the faecal nutrient concentrations between the different study areas in every season were not executed due to inadequate number of samples from each area per season. Averages were used for comparisons in this evaluation.

Figure 6.1: Schematic layout of collected and pooled faecal samples in the dry season on various study areas

Figure 6.2: Schematic layout of collected and pooled faecal samples in the wet season on various study areas

6.4.3. BLOOD SERUM SAMPLES

Blood samples were collected from immobilised roan and sable antelope whenever the opportunity emerged. Blood samples were collected during management operations in Graspan and Sable Ranch in the dry seasons of 2005 and 2006 respectively. Samples were collected either from the jugular vein (Graspan) or from one of the major arteries in the ear (Sable Ranch) of the immobilised antelope. Blood samples from two mature roan females from N'washitsumbe were obtained from the blood bank of the SANP veterinary unit in Skukuza, KNP. The samples were collected during routine management operations in the dry season of 2005. Unfortunately, these were the only serum samples available from the KNP roan populations.

Blood was mainly collected during the course of the morning before the day temperature increased too much for safe darting and immobilisation of antelope. The collected blood was centrifuged in the afternoon of the same day. Serum was separated from whole blood samples by centrifugation at 2'500 rpm for 15 minutes on a Beckman Coulter Allegra X-22 centrifuge. The collected serum was then frozen at -30˚C until analysed.

Preparation of samples for ICP analyses were done by means of standard micro wave digestion processes (Nadkarni, 1984; Kingston and Jassie, 1986; Pougnet and Wandt, 1986). Approximately 2 g of each sample was weighed and mixed with 6 ml 65% HNO_3 which was added to 1 ml of 30% H_2O_2. The solution was then left over night and digested in a microwave oven. After digestion, the sample was rinsed into a 50 ml volumetric flask containing deionised water and consequently analysed.

Blood serum samples were analysed as follows:

ICP-MS (Inductively Coupled Plasma Mass Spectroscopy):

- Se

ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy):

- Co, Cu, Mn, Mo, Zn, S, Ca, K, Mg, Na, B, Fe, P

One-way ANOVA-analyses were used to determine means and standard deviations of the data (SAS JMP, 2001).

Significant differences between means were determined at a 95% confidence level using the non-parametric Wilcoxon/Kruskal-Wallice as well as the All Means Tukey-Kramer HSD tests (SAS JMP, 2001). Multivariate correlations were conducted using the Spearman Rho model (SAS JMP, 2001). All statistical analyses were conducted using SAS JMP 4 (2001) software.

6.5. RESULTS AND DISCUSSION

6.5.1. CONCENTRATIONS OF NUTRIENTS IN FAECES AND BLOOD SERUM IN ROAN

6.5.1.1. Macronutrients

All mineral and nutrient concentrations in faeces collected in every season at every study area were analysed and summarised in Table 6.1. The mineral concentrations from serum samples collected in every season at every study area are presented in Table 6.2. The relationship between the mineral concentrations in the forage, faeces and serum at every study area sampled are graphically presented in Graphs 6.1-6.4.

Similar to the N content of grass in Chapter 5, wet season N levels in roan faeces at Graspan were higher than that of the dry season levels. By contrast, dry season N_f levels of roan at N'washitsumbe were higher than in the wet season as was explained previously. This could be ascribed to the fact that some grass swards in and on the edge of the wetland area at N'washitsumbe already produced a few green shoots at the end of the dry season when the faeces were collected. The roan clearly selected for such green shoots (visual observations) which appeared in the late dry months. The selection of these green shoots could have caused the higher N_f levels. The N_f level of roan at N'washitsumbe (wet season) dropped below the recommended threshold value of 1.3% (Irwin *et al.,* 1993) or 1.4% (Van der Waal *et al.,* 2003) but was sufficient in most instances according to Grant *et al.* (1995) who recommended N_f thresholds of 1.1-1.2% for wild herbivores. Only wet season N_f levels of roan at N'washitsumbe seemed slightly deficient despite sufficient N_g concentrations. This value could be questioned since the dry season N_f level of roan at N'washitsumbe was within the threshold value and dry season N_f concentrations have been found to be higher in the wet season than in the dry season in non-supplemented roan populations (Dörgeloh, 1998).

Table 6.1: Seasonal differences in the nutrient content of roan antelope faeces collected within every study area

MACRONUTRIENTS								
Study area	**n***	**% N**	**% P**	**% K**	**% Ca**	**% Mg**	**% Na**	**% S**
Wet season								
Graspan	8 (28)	1.45	0.28	0.93	0.87	0.23	0.019	0.21
N'washitsumbe	1 (8)	0.96	0.77	1.32	0.88	0.38	0.159	0.16
Dry season								
Graspan	2 (22)	1.29	0.21	0.79	0.97	0.18	0.02	0.24
N'washitsumbe	2 (8)	1.39	0.22	0.59	0.59	0.34	0.20	0.17
Capricorn	2 (5)	1.60	0.27	0.62	0.70	0.24	0.18	0.18
Sable Ranch	11	2.17	0.37	0.84	1.18	0.32	0.09	0.24

MICRONUTRIENTS						
Study area	**n***	**Cu (mg/kg)**	**Fe (mg/kg)**	**Mn (mg/kg)**	**Zn (mg/kg)**	**Mo (mg/kg)**
Wet season						
Graspan	8 (28)	12.39	1757.20	150.05	69.03	0.52
N'washitsumbe	1 (8)	11.73	1737.74	116.20	58.64	0.48
Dry season						
Graspan	2 (22)	8.12	1355.09	151.96	52.65	0.77
N'washitsumbe	2 (8)	12.26	4365.67	117.80	72.49	1.89
Capricorn	2 (5)	8.00	1711.62	90.09	34.65	3.83
Sable Ranch	11	13.76	1147.90	214.87	112.81	1.28

n* - The number of pooled samples analysed is given with the total number of samples collected given in brackets. All samples from Sable Ranch were analysed individually.

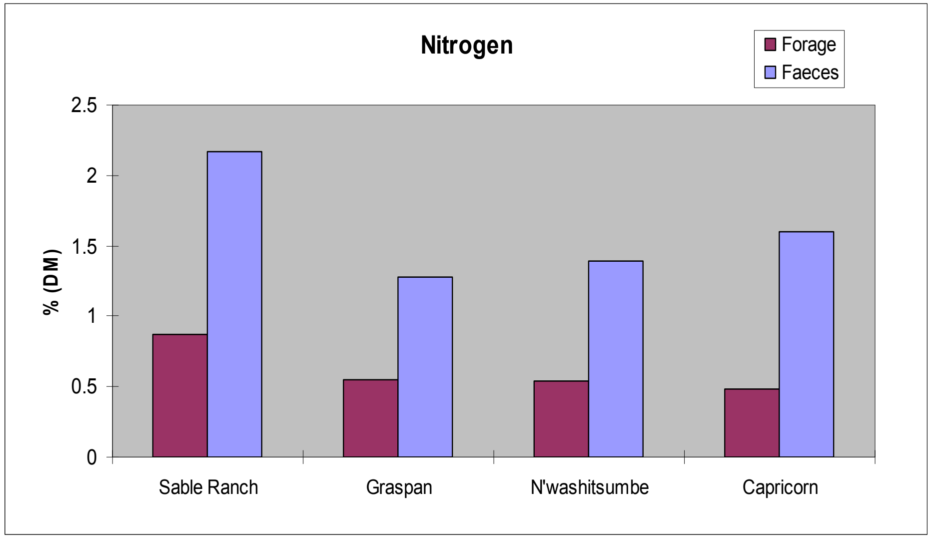
Table 6.2: Dry season macro- and micromineral concentrations in the serum of roan antelope at Sable Ranch, Graspan and N'washitsumbe

Study area	n	P (mg/L)		K (mg/L)		Ca (mg/L)		Mg (mg/L)		Na (mg/L)		S (mg/L)	
		Ū	SD	ū	SD	ū	SD	ū	SD	ū	SD	ū	SD
Sable Ranch	10	110.83^{a}	11.71	181.96^{a}	20.22	72.31^{a}	4.31	14.84	1.90	2872.12	116.60	-	-
Graspan	11	74.10^{b}	15.83	165.69^{b}	11.42	95.18^{b}	10.52	11.63	2.24	2945.60	107.40	916.61	79.98

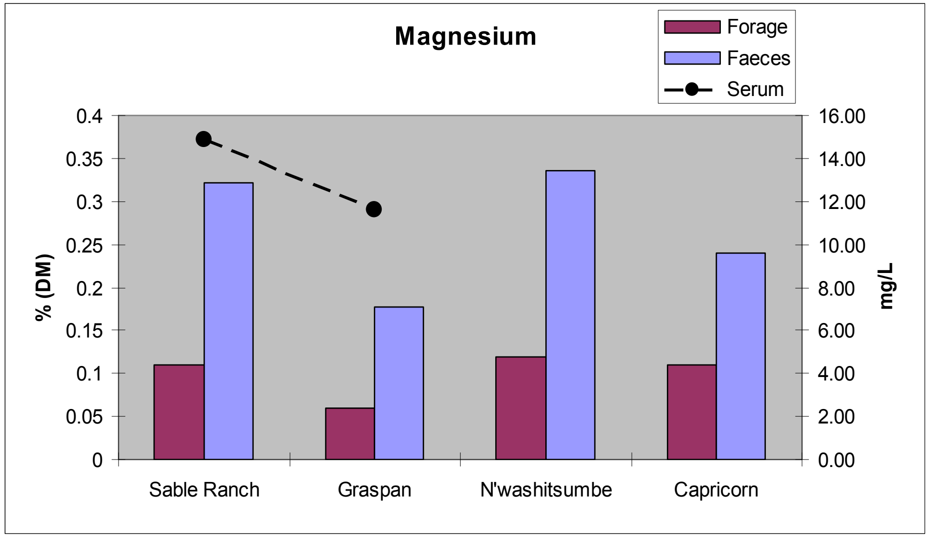
$^{a/b/c}$ = P≤0.05

Study area	n	Cu (mg/L)		Fe (mg/L)		Mn (mg/L)		Zn (mg/L)		Mo (mg/L)		Co (mg/L)		Se (µg/L)	
		ū	SD	ū	SD	ū	SD	ū	SD	ū	SD	ū	SD	ū	SD
Sable Ranch	10	3.63^{a}	1.03	5.90	2.72	0.35^{a}	0.26	7.74	12.87	0.04^{a}	0.04	0.022^{a}	0.013	16.55^{a}	2.06
Graspan	13	1.31^{b}	0.36	63.97	198.61	0.02^{b}	0.00	2.45	2.21	0.01^{b}	0.00	0.002^{b}	0.002	6.41^{b}	1.03
N'washitsumbe	2	0.55^{b}	0.03	1.77	0.70	0.01	0.00	0.80	0.16	0.01^{b}	0.00	0.003	0.0003	0.63^{c}	0.12

$^{a/b/c}$ = P≤0.05



Graph 6.1: Dry season N, P, K and Ca concentrations in grass, faeces and serum collected at every study area



Graph 6.2: Dry season Mg, Na and S concentrations in grass, faeces and serum collected at every study area

A very high nutritional demand by roan during the wet season could have pushed the N_f concentration down despite an apparent adequate N intake. There was only a slight drop in N_f values of roan between Graspan's wet and dry seasons. The dry season N_f was marginal at 1.29%. The dry season N_f of roan at Capricorn and Sable Ranch was above the threshold value with the N_f at Sable Ranch being substantially higher at 2.17%. The nutritional supplements provided to the Sable Ranch animals clearly increased their N_f concentrations, implying that they received adequate dietary protein.

Dry season P levels in faeces of roan varied between study areas as the P concentrations in the forage varied (Graph 6.1). The dry season P_f and P_g in grass and faeces at Capricorn were the highest of all the reserves with the P levels at Graspan the lowest in both faeces and grass. The P_f of roan at Sable Ranch was the highest despite of its lowest P_g levels. The high level of P excretion could be attributed to the supplementary feed supplied to the Sable Ranch animals.

There was a 72% drop in P_f levels from the wet season to dry season at N'washitsumbe (Table 6.1). The drop in P_f concentration of roan at N'washitsumbe and Capricorn coincided with the substantial seasonal drop in P_g at those study areas (Chapter 5). The seasonal difference in P_f of roan at Graspan was much less, probably due to the low seasonal variance in P_g. Nonetheless, despite the deficient P levels in the presumed diets of roan at Graspan, N'washitsumbe and Capricorn (Chapter 5), their faeces contained P levels sufficiently above 0.2% (Grant *et al.,* 1995). This implied either a misrepresentation in terms of the diet sampled due to roan's known ability of diet selection, or roan have a physiological ability to utilise P more efficiently. The other probability is that roan might have a much lower P requirement compared to other wild ruminants.

The dry season inorganic phosphorus (Pi) levels in serum of roan sampled at Graspan was significantly lower (P=0.0007) than that of supplemented roan at Sable Ranch. Puls (1994) reported a Pi concentration in serum of adult cattle between 45-60 mg/L in cattle with adequate levels of serum whereas adequate levels in juvenile cattle ranged between 60-90 mg/L. High levels of Pi in adult cattle ranged between 80-120 mg/L. NRC (2005) reported that the P_s of weaned angus heifers with a P intake of between 0.12-0.2%, ranged between 45-47 mg/L.

Despite the apparent very low P intake of Graspan roan, roan bulls had a mean P_s of 74.1 mg/L compared to the very high P_s concentrations in roan at Sable Ranch (110.83 mg/L). The NRC (2005) reported that Holstein cows on a diet containing 0.69% P had a P_s of 71.8 mg/L. The P_s of roan at Graspan confirmed the sufficient P_f concentrations in roan bulls which had sufficient faecal and serum P levels despite apparent insufficient dietary P levels. However, serum from female roan was not sampled at Graspan and it could be anticipated that their P status would be lower, especially when lactating and reproducing. This probability was emphasized by reports of roan farmers close to Graspan which claimed up to 40% losses in reproduction rates in roan herds not supplemented with a P lick (Dr J. Kriek, 2008. Unpublished data)[9].

Wet season faecal K levels of roan at both Graspan and N'washitsumbe were higher than the dry season levels. Dry season K_f values of roan ranged from 0.59% at N'washitsumbe to 0.84% at Sable Ranch. Despite the comparatively low K_g concentrations at Graspan, the K_f of the roan population at Graspan was almost as high as that of the supplemented roan population at Sable Ranch. However, the K_s level of roan at Sable Ranch was significantly higher ($P<0.05$) than in roan bulls at Graspan. The data therefore suggested a much higher K excretion level in roan at Graspan. According to Puls (1994), adequate K_s concentrations in cattle range between 160 mg/L-215 mg/L. Therefore, K_s concentrations in serum of roan sampled at both Sable Ranch and Graspan seemed adequate. The high excretion rate of K in roan faeces at Graspan was most probably due to a lower K demand by roan bulls compared to that of lactating females and therefore caused a higher excess, hence excretion. Up to 12% of K is taken up in milk production in cows while only approximately 13% is excreted in the faeces (Preston, 1985; McDowell, 2003).

The wet season Ca_f concentration of roan at Graspan was lower than the dry season faecal concentration. At Sable Ranch however, the Ca_f concentration of roan was higher in the wet season than in the dry season (Table 6.1). The dry season Ca_f concentration of roan at N'washitsumbe was the lowest (0.59%) of all the study areas, as was the Ca_g concentration at N'washitsumbe. The Ca_f of roan at Sable Ranch was the highest (1.18%) with that of roan at Graspan second highest at 0.97%. However, despite the fact that the dry season estimated Ca intake of roan at Graspan was below what is required for beef cattle, the Ca_s concentration in roan at Graspan was significantly higher ($P=0.0001$) than that of the supplemented roan population at Sable

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[9] Dr Johan Kriek, private veterinarian and roan and sable antelope breeder. Presentation of private observations. Annual workshop of Wildlife Veterinary Group, 22 November 2008, Mattanu Game Reserve, Barkley West, South Africa. Ph: 082 820 8305

Ranch (Table 6.2). Puls (1994) reported adequate Ca_s concentrations in serum of cattle to range between 80-110 mg/L. Both the roan populations at Sable Ranch and Graspan therefore absorbed adequate levels of Ca despite their higher excretion levels, which was most probably due to excessive intakes.

The Mg_g levels of roan at both Sable Ranch and Graspan were slightly higher in the wet season than in the dry season (Table 6.1). This was similar to their seasonal fluctuations in forage concentrations and coincided with results from previous studies (Wilson and Hirst, 1977). The Mg concentration in grass and roan faeces at Graspan was the lowest of all the study areas in the dry season (Graph 6.2). The Mg concentrations in grass and roan faeces at N'washitsumbe were the highest despite the fact that the roan population at Sable Ranch was supplemented and had a lower faecal concentration than roan at N'washitsumbe. The Mg_f of roan at Capricorn was 30% lower than at N'washitsumbe despite similar concentrations in dry season grasses.

Underwood and Suttle (1999) reported that high K intakes or a dietary Na deficiency that causes higher ruminal K levels through increased K levels in saliva could inhibit Mg availability for absorption in the rumen. However, the apparent K intake of roan at Capricorn was higher than at N'washitsumbe - yet they excreted lower Mg_f levels. Roan at both N'washitsumbe and Capricorn had sufficient dietary Na levels. Roan at Graspan had deficient Na_g concentrations and exhibited marginal Na_s concentrations in the roan bulls. This could explain the high levels of K excreted in faeces of roan at Graspan despite their low K intake and acceptable K_s concentrations (Graph 6.1). The Mg_s content in roan at Sable Ranch was not significantly higher (P=0.12) than in roan at Graspan. According to Puls (1994) adequate Mg_s concentrations in cattle range between 18-30 mg/L and marginal levels are between 12-18 mg/L. The Mg_s concentration in roan at Graspan might be marginal but should still be sufficient for adult male roan antelope. The Mg_s levels in roan at Sable Ranch were sufficient.

The wet season Na_f concentrations of roan at Graspan were similar in both the wet and dry season, although the wet season Na_f concentrations of roan at N'washitsumbe were 25% lower than in the dry season (Table 6.1). Grass in the wetland area of N'washitsumbe had much higher Na levels than the upland area and the higher utilisation of the wetland area by roan in the dry season compared to in the wet season could have caused the higher dry season Na_f levels of roan at N'washitsumbe.

The Na_g content at Graspan was well below recommended levels (Chapter 5) in both seasons compared to the very high levels of Na_g at N'washitsumbe and Capricorn. Roan at Sable Ranch had very high Na_f concentrations compared to the low Na_g concentrations at Sable Ranch, most probably due to the high supplementation levels at Sable Ranch.

An animal's Na status is homeostatically well maintained at fairly constant levels (McDowell, 2003). This was evident from the fact that the Na_s concentrations in roan at Sable Ranch and Graspan did not differ significantly ($P>0.05$) even though the animals at Sable Ranch received supplementary feed and the dietary Na level in grasses at Graspan appeared to be deficient. Puls (1994) reported deficient Na_s concentrations in cattle to be between 2'300-2852 mg/L with marginal levels between 2989-3220 mg/L. The roan bulls at Graspan were therefore marginally sufficient in Na despite their very low Na intake. McDowell (2003) reported that the animal's kidney will excrete very little Na when intakes are deficient in order to secure enough Na within the body. The faeces are not the primary excretion route for Na. The Na_s concentration in roan at Sable Ranch was slightly deficient irrespective of being supplemented.

The very high intake and excretion of K in roan at Sable Ranch may be indicative of an excessive K intake, which could inhibit Na absorption in the rumen. Such a scenario could explain the lower Na_s content in roan at Sable Ranch compared to in roan at Graspan (Underwood and Suttle, 1999; McDowell, 2003).

The S levels in faeces collected in both the wet and dry season at Graspan and N'washitsumbe were similar in both seasons with the dry season concentrations slightly higher in both study areas than in the wet season. The S content in faeces of roan collected at Graspan and Sable Ranch in the dry season was similar and faeces collected at Capricorn and N'washitsumbe had similar S concentrations (Table 6.1). The apparent dietary intake of S by roan at N'washitsumbe was much higher relative to their faecal excretion in comparison to other populations (Graph 6.2). However, the estimated level of dietary S at N'washitsumbe remained just within the level of prescribed sufficiency (Chapter 5) and could therefore be that rumen microbial utilisation of S was able to utilise the comparatively higher S intakes and therefore similar S levels was excreted in faeces of roan at N'washitsumbe compared to roan at Capricorn.

The mean total blood S concentration in male roan in the dry season at Graspan were 1’714 mg/L. Puls (1994) reported adequate total blood S concentrations in cattle to range between 1’500-1’800 mg/L. McDowell (2003) reported deficient S_s concentrations in cattle to be below 10 mg/L. The blood and serum S status of roan at Graspan seemed therefore adequate and in accordance to their estimated intake levels (Chapter 5).

6.5.1.2. Micronutrients

The concentration of Cu in the faeces of roan at Graspan was higher in the wet season than in the dry season (Table 6.1). The Cu_f of roan at N’washitsumbe was slightly lower in the wet season than in the dry season. In the dry season, the Cu_f concentration of roan at Sable Ranch was the highest (13.76 mg/kg) despite the most deficient Cu concentrations in grass at Sable Ranch (Graph 6.3). Even though the Cu_g content at N’washitsumbe was as deficient as at Graspan and Capricorn, the Cu_f level of roan at N’washitsumbe was much higher than of roan at other reserves and resembled the excretion level of the supplemented population at Sable Ranch. Despite the high Cu excretion levels in faeces of roan at N’washitsumbe, the Cu_s level of the two female roan sampled in the dry season at N’washitsumbe had significantly lower serum levels than roan at Sable Ranch (P=0.0004). The Cu_s in roan at N’washitsumbe was 58% lower than in non-supplemented roan bulls at Graspan (Table 6.2).

Puls (1994) reported marginal Cu_s levels in deer to range between 0.55-0.70 mg/L and marginal levels in cattle not receiving Se supplements to range between 0.50-1.20 mg/L. Roan cows at N’washitsumbe had a mean Cu_s concentration of 0.55 mg/L in the dry season, which was marginal according to Puls (1994). Roan bulls at Graspan had a mean Cu_s concentration of 1.31 mg/L despite similar Cu concentrations in grass at N’washitsumbe. However, Cu_f levels of roan at Graspan were 8.12% lower than that of roan at N’washitsumbe. The data suggested that there was a lower level of absorption and higher level of faecal excretion of Cu in the roan population at N’washitsumbe in the dry season in comparison to roan at the other study areas.

Graph 6.3: Dry season Cu, Fe and Mn concentrations in grass, faeces and serum collected at every study area

Graph 6.4: Dry season Zn and Mo concentrations in grass, faeces and serum collected at every study area

The possible difference in Cu requirements between male roan at Graspan and female roan at N'washitsumbe must be considered since it could affect Cu absorption, storage and mobilisation significantly. Nonetheless, the possible significant effect of the high S concentrations in wetland grasses preferred by roan at N'washitsumbe in the dry season, exacerbated by the high Fe levels in dry season grasses (Chapter 5) cannot be ignored. For instance, *Diphlanche fusca* had a dry season S concentration of 0.4% and *Urochloa mosambicensis* had an S concentration of 0.29%. At such a high intake level of S in grass and a very low estimated dry season Mo intake by roan at N'washitsumbe (Chapter 5), S could have formed insoluble Cu-Mo-S complexes with Cu (CuS_2 or $CuMoS_4$), which reduces the Cu absorption into the animal's body and increase its level of excretion, especially in combination with low Mo and Cu intakes and high Fe intakes (Underwood and Suttle, 1999; McDowell, 2003). However, the comparatively similar level of S_f excretion of roan at N'washitsumbe and Capricorn suggested that the level of S intake of roan at N'washitsumbe was probably lower than estimated, yet high enough to interact with Cu in the formation of complexes.

McDowell (2003) stated that the most severe impact of Cu deficiencies is mostly caused by sub-clinical Cu deficiencies in free ranging ruminants. Unpublished information of a roan antelope farmer in similar environment to that of Graspan has identified a sub-clinical Cu deficiency as the reason for the sporadic outbreak of pneumonia in roan females (D. van Zyl, 2007. Pers. comm.)[10] The supplementation of that particular herd with extra Cu eradicated the sub-clinical deficiency in improved the production of the entire population. These findings emphasize the importance of monitoring the Cu status of male and female roan antelope in both Graspan and N'washitsumbe in order to identify critical periods during which calf survival rates and animals production rates could be limited by a sub-clinical Cu deficiency.

The wet season Fe_f content of roan at N'washitsumbe and Graspan was very similar in comparison (Table 6.1). The dry season Fe_f of roan at Graspan was lower than that of the wet season faecal levels, while the dry season Fe_f content of the roan at N'washitsumbe was more than twice the concentration of the wet season faecal content. The concentrations reflected the dry season Fe_g levels within the grass at these study areas. Despite this, the dry season Fe_s concentration of roan females at N'washitsumbe was the lowest of roan at both Graspan and Sable Ranch (Table 6.2) but still within the adequate range of 1.3-2.5 mg/L for serum in cattle (Puls, 1994). McDowell (2003) reported deficient Fe_s levels in cattle to be below 1.1 mg/L.

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[10] Mr Dreyer van Zyl, experienced roan and sable antelope farmer. Personal interview, July 2007. Hartenbosch, Western Cape Province. Ph: 082 571 6279.

The high dry season Fe concentrations in both the grass at N'washitsumbe and the faeces of roan at N'washitsumbe could most probably be attributed to soil and dust contamination of samples collected in very dry and dusty conditions. The Fe on grass was seemingly not absorbed by the roan population in the area which caused the high grass and faecal concentrations. The Fe_s in roan at Graspan was excessively high (63.97 mg/L) with an even higher standard deviation of 198.61 mg/L (Table 6.2). This extremely high value and standard deviation implied contamination during sampling or the handling of the samples. The Fe_f levels of roan at Graspan were similar to that of roan at Sable Ranch and Capricorn (Graph 6.3), further emphasizing the likelihood of contamination of the serum samples at Graspan. Roan at Sable Ranch received feed supplements and had a high mean Fe_s concentration of 5.90 mg/L (Table 6.2) (Puls, 1994). However, most domestic animals have a high resistance to Fe toxicities and Fe toxicities are therefore not a common occurrence (NRC, 2005).

The Mn_f level in both the dry season and wet season faeces of roan at both Graspan and N'washitsumbe was very similar (Table 6.1). Roan at Capricorn had the lowest Mn_f level in the dry season (90 mg/kg) and roan at Sable Ranch the highest (214.87 mg/kg). The dry season faecal excretion levels of roan at all the study areas seemed to be correlated to that of the level of Mn in the grasses (intake) at the various study areas (Graph 6.3). This is in accordance with previous research which found that the level of Mn excretion is linked to Mn intake and that excess dietary Mn resulted in lower Mn absorption with an increase in excretion (McDowell, 2003). The mean Mn_s level in roan at Sable Ranch was significantly higher ($P=0.0004$) than in roan at Graspan. The Mn_s in roan at N'washitsumbe was the lowest of all the roan populations at 0.01 mg/L.

According to Puls (1994) adequate Mn_s levels in cattle ranged between 0.006-0.070 mg/L, which suggested that the Mn_s in roan at Graspan and N'washitsumbe were within the normal range. The Mn_s of roan at Sable Ranch however, was considered toxic if compared to the prescribed level in serum of cattle (0.08-1.45 mg/L) but adequate if compared to the Mn_s levels in sheep (0.2-0.35 mg/L) (Puls, 1994). Nevertheless, McDowell (2003) and NRC (2005) reported that Mn is regarded as one of the essential elements that is most unlikely to cause a toxicity.

The Zn_f concentration of roan at Graspan was higher in the wet season than in the dry season (Table 6.1). On the other hand, the Zn_f concentration of roan at N'washitsumbe was higher in the dry season than in the wet season (Table 6.1) as was the Zn concentrations in grasses at N'washitsumbe (Chapter 5).

In the dry season the Zn_f level of roan at Sable Ranch was the highest (112.81 mg/kg) of all the study areas with that of roan at Capricorn the lowest (34.65 mg/kg). The estimated Zn intake of roan at N'washitsumbe was similar to roan at Capricorn but the faecal excretion of roan at N'washitsumbe was much higher (Graph 6.3). The estimated Zn intake of roan at Graspan was higher than in roan at N'washitsumbe even though roan at N'washitsumbe still excreted higher levels of Zn. Nonetheless, roan at N'washitsumbe had the lowest Zn_s levels compared to roan at both Sable Ranch and Graspan (Table 6.2). This indicated either contamination of the faecal samples at N'washitsumbe or an interaction between Zn and an antagonistic mineral which prevented Zn absorption and an increased in Zn excretion. The analyses of grass at N'washitsumbe revealed very high variation in the Zn content between grass species in the wet season but not in the dry season (Chapter 5). Dry season Zn_g levels at all the study areas were above the dietary limit of beef cattle.

Puls (1994) reported deficient Zn_s levels in cattle of between 0.2-0.4 mg/L, adequate levels between 0.8-1.4 mg/L and toxic levels between 3.0-12.0 mg/L. Despite the high level of excretion of Zn by roan at N'washitsumbe, the serum levels indicated a sufficient Zn status while the Zn_s concentrations in roan at Sable Ranch were very high despite the high excretion level which could indicate an over supplementation of Zn to roan at Sable Ranch. Animals can tolerate very high Zn intakes (NRC, 2005). However, high Zn intakes (>750 mg/kg) could exacerbate a marginal Cu deficiency in a ruminant population (McDowell, 2003) but most probably not at the estimated Zn intake levels of roan at Graspan and N'washitsumbe of between 52-59 mg/kg (Chapter 5). The lower level of absorption of Zn in roan at N'washitsumbe might indicate a lower requirement level and therefore the high excretion level.

Mo_f concentrations of roan at Graspan and N'washitsumbe were lower (32% and 75% respectively) in the wet season than in the dry season (Table 6.1) despite the higher estimated Mo intakes in the wet season at both study areas (Chapter 5).Faecal samples collected at Capricorn had the highest Mo_f levels (3.83 mg/kg) with that of Graspan roan the lowest (0.77 mg/kg). Despite the lowest dry season Mo_g levels at N'washitsumbe and Capricorn compared to other study areas, the Mo_f concentrations in faeces of roan at the KNP enclosure in the dry season were higher than of roan at both Sable Ranch and Graspan (Graph 6.4). The higher Mo intake in supplemented roan at Sable Ranch seemed to cause a higher Mo absorption compared to other study areas where higher levels of Mo was excreted despite lower estimated intake levels (Chapter 5).

The higher Mo faecal excretion of roan at N'washitsumbe despite their low estimated Cu and Mo intakes may be linked to their apparent high S intake in the dry season. A high S intake suppresses Mo absorption through the binding with disulfide (MoS_2) which increases Mo excretion, mainly through urine (McDowell, 2003). Mo_s levels above 0.1 mg/L are considered potentially inhibiting to Cu absorption (Puls, 1994) and therefore the Mo_s concentrations of roan at all study areas were considered sufficient.

Co_s levels usually reflect dietary Co intake levels (Puls, 1994). This fact supported the significantly higher ($P<0.05$) Co_s levels observed in supplemented roan at Sable Ranch in comparison to non-supplemented roan at Graspan (Table 6.2).
The Co_s concentrations of roan females at N'washitsumbe were slightly higher than that of roan males at Graspan. Puls (1994) reported normal Co_s levels for cattle to be between 0.9-15 μg/L. The NRC (2005) reported that Co toxicity in ruminants is highly unlikely under natural grazing conditions since the toxicity level is 100 times the normal intake level. The Co_s levels in roan at all the study areas given in Table 6.2 could therefore be regarded as sufficient.

The Se_s concentration of supplemented roan at Sable Ranch was significantly higher ($P<0.05$) than in roan at both Graspan and N'washitsumbe (Table 6.2). The Se_s levels in roan at Graspan were significantly higher ($P<0.05$) than in roan at N'washitsumbe despite the higher Se levels in grasses at N'washitsumbe (Chapter 5). The two *Sporobolus* species which occurred in the sodic area at N'washitsumbe were mainly responsible for the very high average Se concentration in the grasses at N'washitsumbe. These grass species are most probably also Se accumulators. Unfortunately the Se levels in faeces of roan at N'washitsumbe were not analysed.

The faecal excretion levels might have indicated whether substantial levels of Se were indeed taken in by roan without being available for absorption. Puls (1994) reported that deficient serum Se levels in cattle occurred below 0.002 mg/kg in the diet. The Se_s concentration in roan at Graspan was 0.00063 mg/kg which was substantially lower than the recommended level. The low serum Se concentrations in roan at N'washitsumbe therefore supported observations of capture myopathy in tsessebe capture operations in the vicinity of the roan enclosures at the KNP (Dr Rina Grant, 2009. Pers. Comm.)[11].

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[11] Dr Rina Grant, 2009. Personal communication. Scientific Services, South African National Parks, Skukuza, South Africa. Ph. 013-7354415.

This possible deficiency should be further investigated and suggested that roan at N'washitsumbe utilised limited amounts of dry season *Sporobolus* species which contained high concentrations of Se.

6.5.2. COMPARISONS OF NUTIENTS IN FAECES AND SERUM BETWEEN SEXES AND SPECIES

6.5.2.1. Comparisons between roan and sable within the same sexes

In general, the faecal concentrations of eight of the 13 nutrients analysed were higher in sable females than in roan females of which K, S and B was significantly higher ($P<0.1$). Only the Mn level was significantly higher ($P<0.05$) in the faeces of female roan compared to that of sable females (Table 6.3).

Similarly, the concentrations of 11 of the 13 minerals analysed in serum of roan and sable females were higher in sable females than in roan females of which P, Cu, Mo and Se was significantly higher ($P<0.1$). Only the Mg level in the serum of roan females were significantly higher ($P<0.05$) compared to that in sable females (Table 6.5). K, Ca, Na, Cu, B and Mo levels were higher in both faeces and serum of sable females but none was significantly higher in both faeces and serum. Both Mg and Zn were lower in the faeces as well as the serum of sable females compared to that in roan females.

Similar to what was found in the comparison between roan and sable females, sable males had higher nutrient levels in most of the nutrients analysed in both faeces and serum compared to roan males. Sable antelope males had higher nutrient concentrations compared to roan males in nine of the 13 nutrients analysed, of which N and S was significantly higher ($P<0.05$). Only the K_f level was significantly higher ($P<0.05$) in roan males in comparison to that in faeces of sable males (Table 6.6). Similarly, the mineral levels in the serum of sable males were higher in eight of the 13 minerals analysed, of which the Se level was significantly higher ($P<0.05$) in serum of sable males. Only the Pi concentration in the serum of roan males was significantly higher ($P<0.05$) than in the serum of sable males (Table 6.6).

Table 6.3: Nutrient levels in faeces of roan and sable antelope females sampled at Sable Ranch in the dry season

Nutrient	Roan (n=5)		Sable (n=10)		P-factor
	Mean	SD	Mean	SD	
N%	1.72	0.26	1.89	0.19	0.17
P%	0.30	0.17	0.28	0.06	0.33
K%	0.55^{a}	0.15	0.74^{b}	0.20	0.07
Ca%	0.96	0.28	1.05	0.15	0.27
Mg%	0.30	0.07	0.26	0.05	0.17
Na mg/kg	751.00	358.39	798.50	732.41	0.46
S%	0.21^{a}	0.03	0.28^{b}	0.04	0.00
Cu mg/kg	11.00	4.06	12.00	2.71	0.21
Fe mg/kg	972.80	220.74	950.90	270.11	0.85
Mn mg/kg	188.60^{a}	52.77	140.50^{b}	31.64	0.05
Zn mg/kg	85.40	45.92	83.90	20.82	0.46
B mg/kg	31.40^{a}	3.51	35.40^{b}	1.58	0.02
Mo mg/kg	1.73	1.13	2.25	1.11	0.33

^{a,b} = P≤0.01

Table 6.4: Nutrient levels in faeces of roan and sable antelope males sampled at Sable Ranch in the dry season

Nutrient	Roan (n=5)		Sable (n=10)		P-factor
	Mean	SD	Mean	SD	
N%	2.30^{a}	0.17	2.74^{b}	0.44	0.03
P%	0.38	0.18	0.46	0.14	0.21
K%	0.99^{a}	0.23	0.62^{b}	0.24	0.01
Ca%	1.23	0.28	1.29	0.54	0.83
Mg%	0.30	0.09	0.32	0.10	0.91
Na mg/kg	882.50	528.85	713.60	430.97	0.75
S%	0.25^{a}	0.02	0.38^{b}	0.08	0.00
Cu mg/kg	14.50	2.07	14.90	2.42	0.78
Fe mg/kg	1163.33^{a}	408.13	788.70^{b}	209.81	0.08
Mn mg/kg	212.33	26.27	213.00	112.42	1.00
Zn mg/kg	122.83	16.40	106.60	27.49	0.19
B mg/kg	36.50	4.09	41.70	11.82	0.70
Mo mg/kg	0.76	0.77	1.53	1.05	0.13

^{a,b} = P≤0.01

Table 6.5: Nutrient levels in serum of roan and sable antelope females sampled at Sable Ranch in the dry season

Nutrient	Roan (n=5)		Sable (n=10)		P-factor
	Mean	SD	Mean	SD	
P mg/L	116.81^{a}	12.78	146.51^{b}	36.17	0.03
K mg/L	178.41	13.70	182.90	23.24	0.90
Ca mg/L	74.93	4.48	77.94	3.83	0.11
Mg mg/L	14.95^{a}	2.36	12.41^{b}	1.93	0.04
Na mg/L	2850.22	106.75	2917.90	100.21	0.33
Cu mg/L	3.53^{a}	0.71	5.83^{b}	1.40	0.01
Fe mg/L	4.81	1.93	8.54	5.73	0.18
Mn mg/L	0.34	0.09	0.42	0.29	0.67
Zn mg/L	12.14	17.95	6.36	5.27	0.90
B mg/L	0.49	0.31	0.54	0.30	0.71
Mo mg/L	0.05^{a}	0.01	0.07^{b}	0.03	0.06
Co mg/L	0.01	0.01	0.02	0.02	0.58
Se µg/L	16.15^{a}	2.42	21.22^{b}	4.51	0.02

^{a,b} = P≤0.01

Table 6.6: Nutrient levels in serum of roan and sable antelope males sampled at Sable Ranch in the dry season

Nutrient	Roan (n=5)		Sable (n=10)		P-factor
	Mean	SD	Mean	SD	
P mg/L	104.85^{a}	7.47	80.25^{b}	18.02	0.02
K mg/L	185.51	26.47	179.27	14.84	0.90
Ca mg/L	69.69	2.14	72.63	5.00	0.27
Mg mg/L	14.72	1.58	15.83	2.04	0.27
Na mg/L	2894.02	134.15	2937.75	53.22	0.46
Cu mg/L	3.74	1.36	4.28	1.24	0.33
Fe mg/L	7.00	3.15	4.57	2.88	0.11
Mn mg/L	0.35	0.38	0.18	0.05	0.36
Zn mg/L	3.34	1.42	3.50	0.75	0.71
B mg/L	0.83	0.98	0.43	0.30	1.00
Mo mg/L	0.04	0.01	0.04	0.02	0.31
Co mg/L	0.03	0.02	0.11	0.12	0.42
Se µg/L	16.95^{a}	1.81	27.26^{b}	6.94	0.00

^{a,b} = P≤0.01

The significant differences found between the nutrient levels in faeces of sable and roan males and females, did not correspond with the significant differences found in the minerals in serum of the same animals. Only the S_f level was significantly higher in both sable females and males compared to roan faecal levels.

The K_f level was significantly higher in sable females but significantly lower in sable males in comparison to the faecal levels of roan females and males. When serum mineral levels were compared, only the Se level was significantly higher ($P<0.05$) in both sable males and females. The Pi concentration in the serum of sable females was significantly higher ($P<0.05$) compared to that in roan females but the Pi concentration in serum of sable males was significantly lower ($P<0.05$) than in the serum of roan males.

6.5.2.2. Comparisons between male and female animals within species

In general, the concentrations of ten of the 13 nutrients analysed in sable faeces were higher in sable bulls than in sable cows, of which the concentration of N, P, S, Cu, Zn and Mo was significantly higher ($P<0.01$) in sable bulls (Table 6.7). However, the mineral levels in sable serum samples were mostly higher in sable cows.

The concentrations of only four of the 13 minerals analysed in sable serum were higher in sable bulls than in cows, while nine mineral concentrations were higher in serum of sable cows compared to that in bulls (Table 6.9). The concentration of Na was higher and that of Mg, Co and Se was significantly higher ($P\leq0.05$) in the serum of sable bulls compared to sable cows. The concentration of P, Ca, Cu, Fe, Mn, Zn and Mo in serum was significantly higher ($P\leq0.05$) in sable cows than in sable bulls. Even though there were significant sexual differences in the concentrations of P, Cu, Zn and Mo in both sable faeces and serum, the higher values in the faeces were in sable bulls while the higher values in serum were in sable cows in all four these minerals. This meant that sable bulls absorbed lower levels of these minerals compared to sable cows and could possibly be indicative of a sexual difference in the requirements for these minerals. Females in production (3rd trimester in pregnancy as well as lactation) generally have higher nutrient demands than mature bulls. The level of absorption of most minerals is influenced by the metabolic requirement at that moment (Underwood and Suttle, 1999). Animals with a lower requirement will absorb less and excrete more at high levels of mineral intake, which was most probably the situation within the sable bulls.

Table 6.7: Nutrient levels in faeces of male and female sable antelope at Sable Ranch in the dry season

Nutrient	**Male (n=10)**		**Female (n=10)**		**P-factor**
	Mean	**SD**	**Mean**	**SD**	
N%	2.74^a	0.44	1.89^b	0.19	0.00
P%	0.46^a	0.14	0.28^b	0.06	0.00
K%	0.62	0.24	0.74	0.20	0.27
Ca%	1.29	0.54	1.05	0.15	0.94
Mg%	0.32	0.10	0.26	0.05	0.22
Na mg/kg	713.60	430.97	798.50	732.41	0.94
S%	0.38^a	0.83	0.28^b	0.04	0.00
Cu mg/kg	14.90^a	2.42	12.00^b	2.71	0.03
Fe mg/kg	788.70^a	209.81	950.90^b	270.11	0.14
Mn mg/kg	213.00	112.42	140.50	31.64	0.55
Zn mg/kg	106.60^a	27.49	83.90^b	20.82	0.06
B mg/kg	41.70	11.82	35.40	1.58	0.85
Mo mg/kg	1.53^a	1.05	2.25^b	1.11	0.07

^{a,b} = P≤0.01

Table 6.8: Nutrient levels in faeces of male and female roan antelope at Sable Ranch in the dry season

Nutrient	**Male (n=6)**		**Female (n=5)**		P-factor
	Mean	**SD**	**Mean**	**SD**	
N%	2.30^a	0.17	1.72^b	0.26	0.01
P%	0.38	0.18	0.30	0.17	0.12
K%	0.99^a	0.23	0.55^b	0.15	0.01
Ca%	1.23	0.28	0.96	0.28	0.10
Mg%	0.30	0.09	0.30	0.07	0.71
Na mg/kg	882.50	528.85	751.00	358.39	0.86
S%	0.25^a	0.02	0.21^b	0.03	0.02
Cu mg/kg	14.50^a	2.07	11.00^b	4.06	0.08
Fe mg/kg	1163.33	408.13	972.80	220.74	0.58
Mn mg/kg	212.33	26.27	188.60	52.77	0.20
Zn mg/kg	122.83	16.40	85.40	45.92	0.14
B mg/kg	36.50^a	4.09	31.40^b	3.51	0.08
Mo mg/kg	0.76	0.77	1.73	1.13	0.20

^{a,b} = P≤0.01

Table 6.9: Nutrient levels in serum of male and female sable antelope at Sable Ranch in the dry season

Nutrient	**Male (n=10)**		**Female (n=10)**		**P-factor**
	Mean	**SD**	**Mean**	**SD**	
P mg/L	80.23^{a}	18.02	146.51^{b}	36.17	0.00
K mg/L	179.27	14.84	182.90	23.24	1.00
Ca mg/L	72.63^{a}	5.00	77.94^{b}	3.83	0.02
Mg mg/L	15.83^{a}	2.04	12.41^{b}	1.93	0.00
Na mg/L	2937.75	53.22	2917.90	100.21	0.41
Cu mg/L	4.28^{a}	1.24	5.83^{b}	1.40	0.03
Fe mg/L	4.47^{a}	2.88	8.54^{b}	5.73	0.02
Mn mg/L	0.18^{a}	0.05	0.42^{b}	0.29	0.04
Zn mg/L	3.50^{a}	0.75	6.36^{b}	5.29	0.03
B mg/L	0.43	0.30	0.54	0.30	0.47
Mo mg/L	0.04^{a}	0.02	0.07^{b}	0.03	0.01
Co mg/L	0.11^{a}	0.12	0.02^{b}	0.02	0.05
Se µg/L	27.26^{a}	6.94	21.22^{b}	4.51	0.01

^{a,b} = P≤0.05

Table 6.10: Nutrient levels in serum of male and female roan antelope at Sable Ranch in the dry season

Nutrient	**Male (n=5)**		**Female (n=5)**		**P-factor**
	Mean	**SD**	**Mean**	**SD**	
P mg/L	104.85^{a}	7.47	116.81^{b}	12.78	0.05
K mg/L	185.51	26.47	178.41	13.70	0.60
Ca mg/L	69.69^{a}	2.14	74.93^{b}	4.48	0.08
Mg mg/L	14.72	1.58	14.95	2.36	0.92
Na mg/L	2894.02	134.15	2850.22	106.75	0.75
Cu mg/L	3.74	1.36	3.53	0.71	0.92
Fe mg/L	7.00	3.15	4.81	1.93	0.18
Mn mg/L	0.35	0.38	0.34	0.09	0.25
Zn mg/L	3.34	1.42	12.14	17.95	0.25
B mg/L	0.83	0.98	0.49	0.31	0.92
Mo mg/L	0.04	0.01	0.05	0.01	0.10
Co mg/L	0.03^{a}	0.02	0.01^{b}	0.01	0.08
Se µg/L	16.95	1.81	16.15	2.42	0.92

^{a,b} = P<0.01

In roan, however, the results of faecal analysis corresponded with that of sable antelope but not in serum concentrations. The mineral concentration in the faeces of roan bulls was higher than in female faeces in 11 of the 13 minerals analysed. Of these, the level of N, K, S, Cu and B was significantly higher (P<0.1) than in roan females (Table 6.8). Unlike sable, roan males had higher serum mineral levels than females in eight of the 13 minerals analysed of which only Co was significantly higher (P<0.1). The serum level of P and Ca was significantly higher (P<0.1) in roan females than in roan males (Table 6.10). No mineral had significant sexual differences in both roan faeces and serum. Overall, it seemed as if sexual differences in roan were less significant compared to sable. This might be due to the fact that the sample sizes were half that of sable and there was a lower degree of conformity in the roan herds compared to the sable herds. The sable bulls were all the same age and the cows were all mature cows in production (mostly lactation as well as pregnancy). Some of the roan females were young heifers, not yet producing while most of the bulls were young actively growing bulls. This would have decreased the degree of difference in mineral requirements between the sexes within the roan antelope population.

Nevertheless, both roan and sable females had significantly lower (P<0.1) faecal N, S and Cu concentrations compared to bulls (Tables 6.7 and 6.8). The level of P and Ca in serum was significantly higher (P<0.1) in both sable and roan females compared to male serum levels, while the level of Co was significantly higher (P<0.1) in the serum of both sable and roan males compared to female serum (Tables 6.9 and 6.10).

6.5.3. CORRELATIONS BETWEEN MINERAL LEVELS IN FAECES AND SERUM

Various significant positive and negative correlations occurred between a number of minerals in the serum and faeces sampled simultaneously within the same roan bulls. However, since samples were limited in both number and repetition, the focus will only be the discussion on evidence of known interactions – mainly between Cu, Mo and S.

Correlations between mineral levels in faeces and serum sampled in the same animal at the same time revealed that Cu and S levels in faeces had the most significant positive correlation (P=0.012) while the Cu and S levels in serum had the most significant negative correlation (P=0.017). This implied that the level of Cu excretion was strongly and significantly linked to the excretion level of S.

On the other hand, the level of absorption of Cu into the bloodstream increased significantly as the level of S absorbed decreased, and *vice versa*. Underwood and Suttle (1999) reported that the formation of insoluble copper sulphide CuS_2 in the rumen reduces Cu absorption and will therefore increase Cu and S levels in faeces. The higher the level of S in the diet, the more S will be absorbed into the bloodstream or could bind to Cu prior to absorption and be excreted as an insoluble compound. The lower the level of inorganic S in the diet, the less Cu is bound to S and the more Cu gets absorbed into the bloodstream, which could therefore substantiate the significant negative correlation between concentrations of Cu and S in roan serum (Table 6.12). High dietary Fe can further reduce Cu availability for absorption in the animal (Sutton and Underwood, 1999). The strong positive correlation (P=0.018) between Cu and Fe concentrations in the faeces (Table 6.11) might indicate that Fe further reduced Cu availability in roan bulls at Graspan. Underwood and Suttle (1999) reported that soil Fe can reduce Cu absorption by either the formation of ironsulfide (FeS) in the rumen or by the adsorption of Cu by insoluble Fe compounds.

Since no significant interaction between Fe and S was found, the possibility of the adsorption of Cu to insoluble Fe, which will then be excreted via the faeces, was highly likely. Puls (1994) further reported that insufficient Cu levels adversely affected Fe absorption in cattle.

Mo is readily absorbed in the ruminant’s gut and it is as readily excreted, mainly by the kidneys (McDowell, 2003). Depending on the form of Mo and the level in the diet, up to 30% of dietary Mo can be absorbed (McDowell, 2003). The rest will be excreted. Therefore, the higher the Mo in the diet, the higher the absorption as well as faecal excretion of unabsorbed Mo. This explained the significantly positive correlation (P=0.0237) between the Mo concentration in faeces and serum (Table 6.11 and Table 6.13). The rate of Mo absorption is furthermore inversely correlated to the level of dietary S due to sulphate that inhibits the membrane transport of molybdate (Ryan *et al.,* 1987; McDowell, 2003). This interaction at the area of absorption between Mo and S was evident in the significant negative correlation (P=0.0424) between Mo and S concentrations in the serum of roan bulls (Table 6.12). There was also evidence of a possible Mn-Cu interaction with the significant negative correlation (P=0.0347) between the level of Mn excreted in faeces and the level of Cu in the serum.

Table 6.11: Significant (P<0.05) positive correlations between mineral concentrations in both faeces and serum of roan antelope bulls (n=11) sampled in winter at Graspan

Variable	by variable	Spearman Rho	Prob>Rho
Mo-serum	Mo-faecal	0.736	0.024
P-faecal	N-faecal	0.725	0.012
Cu-faecal	S-faecal	0.722	0.012
Fe-faecal	Cu-faecal	0.692	0.018
Ca-faecal	K-serum	0.685	0.020
Mo-serum	Cu-serum	0.678	0.045
B-faecal	N-faecal	0.667	0.025
Fe-serum	K-faecal	0.658	0.028
S-faecal	K-serum	0.650	0.031
Cu-faecal	K-serum	0.641	0.033
Na-faecal	P-serum	0.627	0.039
Fe-serum	S-faecal	0.622	0.041

Table 6.12: Significant (P<0.05) negative correlations between mineral concentrations in both faeces and serum of roan antelope bulls (n=11) sampled in winter at Graspan

Variable	by variable	Spearman Rho	Prob>Rho
Zn-faecal	Na-faecal	-0.604	0.049
K-faecal	P-serum	-0.635	0.036
Na-serum	K-serum	-0.636	0.035
B-serum	P-faecal	-0.678	0.045
Mo-serum	S-serum	-0.683	0.042
S-faecal	Na-serum	-0.701	0.016
Mn-faecal	Cu-serum	-0.703	0.035
Mo-serum	P-faecal	-0.720	0.029
B-faecal	Cu-serum	-0.761	0.017
Cu-serum	S-serum	-0.762	0.017
Cu-serum	N-faecal	-0.829	0.006
Mo-serum	N-faecal	-0.855	0.003

The significant positive correlation (P=0.0116) between the faecal excretion of P and N was consistent with the findings of previous authors and substantiated the link between P and N in bacterial protein formation, diet digestibility as well as forage quality (Grant, 1989; Belonje, 1980; Grant *et al.,* 1995). There was a significant negative correlation (P=0.0033 and P=0.0058) between the N_f level and the Mo_s and Cu_s concentrations respectively. The apparent interaction between the excretion of N and absorption of Mo and Cu was most probably linked to the indirect two-way interaction of S. The higher the level of S in the rumen, the higher the microbial formation and output of microbial protein would be, hence an increased output of faecal N, whereas an increased level of S in the rumen will also reduce the level of Mo and Cu absorption through the formation of insoluble Cu-Mo-S complexes (copper thiomolybdate ($CuMoS_4$)) (McDowell, 2003). There was a significant positive correlation (P=0.0251) between faecal B and faecal N concentrations (Table 6.11).

The Ca concentration in faeces had a significant positive correlation (P=0.02) to the K concentration in serum. Both faecal S and Cu concentrations had a significant positive correlation (P<0.05) to serum K concentrations (Table 6.11) whereas the K_f level had a significant positive correlation to the Fe_s concentration.

The K_s level had a significant negative correlation (P=0.0353) to the Na_s concentration whereas the K_f concentration had a significant negative correlation (P=0.0359) to the level of P in serum (Table 6.12). The Na_f concentration had a significant positive correlation (P=0.0388) to the P_s concentration (Table 6.11) but had a significant negative correlation (P=0.049) to the Zn_f concentration (Table 6.12). The level of P in faeces had a further significant negative correlation (P<0.05) with both the B_s and Mo_s concentrations (Table 6.12).

The S_f concentration had a significant negative correlation (P=0.0163) to the Na_s concentration and the B_f concentration had a significant negative correlation (P=0.0173) to the Cu_s concentration as well (Table 6.12).

Table 6.13: Positive and negative correlations for specific mineral levels between faeces and serum of roan bulls (n=11) sampled at Graspan (dry season)

Variable	by variable	Spearman Rho	Prob>Rho
P-faecal	P-serum	-0.112	0.744
K-faecal	K-serum	0.585	0.059*
Ca-faecal	Ca-serum	-0.598	0.052*
Mg-faecal	Mg-serum	0.175	0.607
Na-faecal	Na-serum	0.027	0.937
S-faecal	S-serum	0.176	0.604
Cu-faecal	Cu-serum	-0.129	0.741
Fe-faecal	Fe-serum	-0.246	0.467
Mn-faecal	Mn-serum	-0.285	0.458
Zn-faecal	Zn-serum	0.059	0.881
B-faecal	B-serum	-0.196	0.614
Mo-faecal	Mo-serum	0.736	0.024**

* = P<0.1

** = P<0.05

The concentration of Mo in both serum and faeces within the same roan bulls at Graspan had a significant positive correlation ($P=0.024$) with one another (Table 6.13). The K_f concentration of the same roan bulls had a significant positive correlation ($P<0.1$) with the K concentration in the serum of every bull, while the Ca concentration in the faeces of these bulls had a significant negative correlation ($P=0.052$) with the Ca_s concentrations within every bull. All the other minerals which were analysed showed no significant correlation between faecal and serum concentrations.

These results suggested that the faecal concentrations of Mo, Ca and K of these roan bulls at Graspan could have served as indicators of the Mo, Ca and K status of those animals at that specific point in time. However, the sample size was very small and many variables could influence these results. The findings should therefore be verified.

As a matter of reference, the same statistical analyses were run on faecal and serum concentrations of male and female sable antelope at Sable Ranch. Faeces and serum were also sampled simultaneously in these animals as was done with the roan bulls at Graspan. The sample sizes were similar ($n=10$) to that of the roan bulls at Graspan but the animals were in a different environment and received supplementary feed. The aim was to verify the significant correlations between the Mo, Ca and K concentrations within faeces and serum than what was found in the Graspan bulls.

Results from correlations within the ten male sable antelope at Sable Ranch indicated that K_s and K_f again had a positive correlation ($P=0.0498$) while Mg_s and Mg_f concentrations had a significant negative correlation ($P=0.021$). Correlations within the female sample group of sables at Sable Ranch showed that P_s and P_f concentrations had significant positive correlations ($P=0.044$) while the Ca_s and Ca_f ($P=0.065$) as well as B_s and B_f ($P=0.031$) concentrations showed significant negative correlations.

These results confirmed that the significant correlations between faecal and serum concentrations of the same minerals could vary based on species, area as well as feeding regime and gender. Only the significant negative correlation between Ca in serum and Ca in faeces occurred in both roan bulls and sable females while the significant positive correlation between K_s and K_f was also repeated in sable males. These results will therefore have to be substantiated further with larger sample sizes under more controlled conditions before more significance could be attributed to these correlations.

6.6. CONCLUSIONS

6.6.1. NUTRIENT LEVELS IN FAECES AND SERUM OF ROAN

Results from the evaluation of nutrient levels in faeces as well as serum of different roan antelope populations provided a good basis for comparison of the nutrient response to estimated dietary nutrient levels within the various populations. It provided much needed additional information of the evaluation of the habitat by means of water, soil and forage nutrient analysis (Chapter 5). Results indicate that even though the nutritional evaluation of the diet may be very useful in predicting and identifying possible nutritional anomalies, the testing of a species' nutritional response to the nutrient supply provided by the habitat is much more conclusive and insightful. Not only does it confirm expected deficiencies, but it also highlights important and often significant nutrient interactions which may have important implications on a population's nutrient status and nutritional management.

The combination of dietary, animal fluid and excreted nutrient levels of an individual animal or population is the most appropriate in evaluating the nutritional suitability of the habitat. However, since direct animal samples are often expensive and invasive, the combination of dietary and faecal analyses might be very useful in identifying nutrient interactions which cannot be identified by means of forage samples alone. Nevertheless, the use of faecal samples becomes much more useful when interpreted in combination with data from other animals or populations.

Whether faecal samples alone will ever be meaningful as an indicator of nutritional status is unlikely because of so many physiological processes and interactions influencing the absorption and excretion of minerals via the faeces. However, a database of similar studies in the same species in future may come close to providing baseline data for faecal nutrient profiles and could provide a very useful tool for identifying possible mineral deficiencies or interactions.

The close link to and influence by dietary nutrient levels, will always necessitate the evaluation of faecal nutrient levels in combination with at least that of the diet. Faecal analysis can further substantiate the accuracy of the representation of dietary samples relative to the actual selected diet of a wild herbivore.

For instance, results from this study indicate that the estimated S level in the diet of roan sampled at N'washitsumbe did not necessarily correlate with the level of S in the faeces in comparison to that of other populations with similar estimated intakes. However, the possible reason will be speculation unless animal tissue or fluid samples could be collected in order to confirm possible theories – again emphasizing the important role of animal samples in building a conclusive profile in the evaluation of the response of an animal to a specific mineral supplied by a specific natural environment.

In general, it can be concluded that roan antelope seems to have the ability to either select or extract higher levels of certain nutrients than cattle on similar intake levels. These findings can only be quantified and substantiated in more controlled trials. For instance, results of this study show that the P levels in grass sampled at Graspan were extremely low and insufficient for domestic ruminants, but faecal and serum P levels of roan did not indicate any deficiencies. These findings should however be substantiated by dose-response trials in order to determine whether the preferred level of serum P in roan should not be higher than in cattle. In fact supplemented roan antelope male and female animals at Sable Ranch have very high serum P levels in comparison to beef cattle despite the tendency in animals to reduce the efficiency of P absorption from the moment that the P requirement have been met.

In summary, the following conclusions were made from the evaluation of the nutrient status and occurrence of possible nutritional anomalies in animals at the various study areas:

- Wet season N_f levels of roan sampled at N'washitsumbe appeared marginal to deficient
- Dry season N_f levels of roan sampled at Graspan appeared marginal
- Roan from all the non-supplemented populations appeared to be resistant to very low dietary P levels and did not reveal deficient faecal or serum P levels
- The K and Ca status of all the populations seemed sufficient whereas the intake of K in roan sampled at Sable Ranch seemed excessive and possibly interacted with Na absorption as a result
- The Na status of roan sampled at Graspan and Sable Ranch appeared to be marginally sufficient and may have had specific interactions with K and Mg
- The Mg status of roan sampled at Graspan appeared marginal
- The S intake of roan sampled at N'washitsumbe might have been over-estimated or a mineral interaction caused higher levels of faecal excretion. The

possibility existed that the grass species sampled and the fractions in which it was included into the general dietary analyses did not represent the actual diet selection of roan antelope at the time at N'washitsumbe

- A secondary Cu deficiency is highly likely in roan at N'washitsumbe in the dry season and should be monitored or verified. An interaction with high S and low Mo intakes seemed likely and could be further exacerbated by high Fe intakes. Roan bulls at Graspan had a sufficient Cu status despite similarly low intakes than roan at N'washitsumbe which further highlighted a possible Cu-Mo-S interaction in the roan population at N'washitsumbe. However, pregnant females and young animals are more prone to Cu deficiencies and roan females at Graspan might suffer subclinical Cu deficiencies during certain times of the year. There is a strong possibility that the Cu contamination of water sources at Graspan might in fact supplement deficient dietary Cu levels of roan at Graspan
- There appeared to have been Fe contamination of dry season faecal samples sampled at N'washitsumbe and serum samples of roan at Graspan
- The Mn status of roan at Sable Ranch appeared toxic in comparison to cattle but sufficient when compared to that of sheep, further indicating possible significant species differences in nutrient requirements
- The data suggest a possible over-supplementation of Zn to roan at Sable Ranch or a lower general Zn requirement by roan than in cattle
- Mo intake and excretion levels in roan at N'washitsumbe supported the possibility of a Cu-Mo-S interaction even though Mo levels were within sufficient margins in serum
- The Se_s concentrations of roan at N'washitsumbe indicated a possible dry season Se deficiency and should be investigated further since a very small sample size was used

The abovementioned possible deficiencies and interactions must be investigated further in controlled experiments within each population. The evaluation of the nutritional status of various roan populations in this study was broad and only highlighted possible deficiencies and mineral interactions.

6.6.2. SPECIES AND GENDER COMPARISONS OF NUTRIENT LEVELS

The comparison of male and female animals within each species identified significant gender differences. These differences have implications in the interpretation of results from faecal analyses from wild herbivore populations. Care must be taken to either sample a specific gender or enough samples from a population to dilute possible differences in nutritional status between genders. It would be preferred if the significant differences in nutrient excretion and serum status between sexes within the same species could be verified in more controlled experiments. In this study, exact nutrient intakes could not have been measured and possible variations in nutrient intakes could have accounted for some of the significant differences. Nonetheless, differences in the faecal excretion and serum nutrient levels of sable male and females, indicated a generally higher nutrient demand in producing females compared to mature males.

Results from the comparison of the nutrient status of sable and roan antelope under similar nutritional conditions indicated significant nutritional differences between the species. These results emphasize the danger of categorizing species into similar nutritional requirement clusters based on similar habitat use and genetic relations. However, although the nutrient levels in sable males and females were generally higher than in roan males and females in both serum and faeces, fewer differences were truly statistically significant. The data therefore implies that even though roan seemed to have higher requirements than sable, exact intake levels were not measured and could have contributed substantially to the species differences. In the comparison of females between the two species, the production level and/or phase of the sampled animals could have contributed significantly to the differences in nutritional status as well, especially since approximately half of the sable females sampled were lactating. The significant nutritional differences between the species should therefore be substantiated through further controlled trials.

6.6.3. ANIMAL SPECIFIC NUTRIENT CORRELATIONS BETWEEN LEVELS IN FAECES AND SERUM

Despite very small sample sizes with which these correlations were modelled, the significance of some of the results cannot be ignored. The results substantiate the strong relation between P and N excretion. The results further emphasize significant interactions between minerals which were not necessarily identified in results from serum and faecal analyses alone. The strong correlations between Cu and its antagonists, Mo and S, were evident from the results and these minerals clearly interacted with one another. These interactions are evident despite the apparent sufficient Cu status of the male roan population at Graspan. These interactions may have more dramatic effects on the Cu status of lactating or reproducing roan females and should be investigated further. The results suggest that significantly interactions between minerals can take place in an animal although it doesn't necessarily induce identifiably deficiencies or toxicities within a specific population at the time. The continuous monitoring of the potential nutritional threats identified in this study will prevent negative effects on a roan population due to subclinical or sporadic mineral deficiencies.

The strong positive correlation between faecal Mo levels and serum Mo levels within the same individual animals of a population might suggest that Mo levels in faeces could serve as an indicator of the Mo status in the animal itself. Similarly, K and Ca levels also had significant positive and negative correlations respectively between levels in faeces and levels in serum within the same roan population. However, when these correlations were tested within different species, sexes and areas, only the Ca and K faecal-serum correlation repeated itself. No other minerals repeated a significant correlation under varying conditions and species. These correlations should therefore be repeated under different conditions with larger sample groups before much more value can be attributed to the findings.

As a result, it can be concluded that in the evaluation of the nutrient status of wild herbivores the correlation between minerals in faeces and serum could serve as a valuable tool in identifying important nutrient interactions. The identification of such interactions is very important since it may have serious implications for and animal production and survival rates. Proper data on nutritional anomalies and the extent of mineral interactions within certain animals in specific environment can serve as a valuable tool for responsible nutritional management. It is however very important to note that many of the correlations could be circumstantial without having an essential physiological implication to the animal. The results therefore only highlights known interactions should they occur and must be verified with larger sample sizes before any significant conclusions are made – especially in the instances where unknown nutrient interactions occurred.

CHAPTER 7

GENERAL CONCLUSIONS

There are numerous factors that influence the nutrient status of wild ruminants utilising natural forages. It is virtually impossible to consider all these factors when evaluating the nutrient status of a wild ruminant population. However, some of these factors are relatively well studied and should be central in the assessment of the effect of various environmental factors on animals within specific environments. In the evaluation of the nutrient status of wild herbivores within free roaming, natural environments, non-invasive and low-cost sampling techniques are often used to estimate the nutritional status of such an animal population. Samples are most often restricted to abiotic indicators, for instance samples of water, forage soil/geology or a combination of two or more of these indicators. Lately, the use of faecal samples to estimate the dietary status of wild herbivores, especially the protein and P status of an animal, has gained more popularity.

Faecal samples are fairly cheap, non-invasive and provide an indication of the animal-diet interaction in an animal or population. However, samples of all these indicators are generally only analysed for a few nutrient elements at a time. At the most, the entire macromineral spectrum will be analysed in samples of one or more of these indicators per evaluation. Many of these indicators have been qualified to identify possible nutrient anomalies in specific instances under specific conditions. Unfortunately not one or even combinations of these environmental or faecal indicators seem to provide an accurate estimate of the degree or extent of nutritional anomalies which may or may not occur within a wild ruminant population. One of the major reasons for this limitation is that samples of such abiotic and/or faecal indicators are rarely verified with tissue samples directly from animals utilising the habitat that is used as an indicator. In the few instances where mineral anomalies have been verified with animal specific samples, the mineral profiles analysed were generally limited to only a few minerals.

Data from this study suggest that the most thorough evaluation of a wild ruminant's nutritional response to the nutrient status of its habitat should include a combination of environmental, faecal and animal tissue samples analysed for the same nutrient spectrum. In this study results from water, soil and grass samples alone did indicate possible nutritional anomalies but many of these were omitted after the evaluation of the same minerals in the animals themselves. The use of faecal samples alone indicated possible nutrient deficiencies like N and P for which faecal levels have already been quantified in wild ruminants in previous studies. However, mineral levels in faeces of animals in a specific study area used in combination with nutrient levels from grass samples are much more meaningful than using faecal samples alone. This is especially true if a broad mineral spectrum containing most minerals and their antagonists are used in the analyses. The option of using faecal and environmental samples as indicators of the nutrient status of a wild herbivore population is clearly a more feasible option than using either one of these indicators alone. Such an option will be even more meaningful when values of different populations are compared with one another. However, this study confirmed that results of faecal and grass samples must be verified in the animal in order to provide the most accurate estimate of the animal population's nutrient status.

Results in this study would probably have been more accurate if it was possible to collect a higher number of samples of a wider variety of animal tissues, especially in the case of microminerals, with which anomalies could be identified. The use of serum for this purpose is not ideal for all minerals but representative samples of enough animal organs are rarely available for use in statistical analyses when rare antelope are studied. This emphasizes the importance of using comparative studies in such complex and indirect evaluations. If more comparative studies are undertaken within the same species using similar methods, more accurate estimates of nutrient status of a wild ruminant species can be obtained. It will further aid managers more in identifying nutritional deficiencies or toxicities sooner.

Results indicated that various nutrients and minerals could cause sub-clinical or more serious deficiencies within specific populations included in this study. The roan population at N'washitsumbe is threatened by low N in the wet season even though this finding must be substantiated in further research. Roan antelope at N'washitsumbe and Graspan did not exhibit a P deficiency as was anticipated based on the P content of grasses sampled at the enclosure. However, the possibility of periodic sub-clinical P deficiencies within especially roan females at Graspan is expected and should be monitored. The Na status of roan at Graspan must be monitored as well since the Na status was not verified in female animals as well.

A periodic sub-clinical Cu deficiency in the roan populations of especially N'washitsumbe and Graspan is anticipated. The results did not indicate a severe Cu deficiency within either one population but the significant interactions between the Cu antagonists could lead to deficiencies under certain nutritional conditions. This is especially true for reproducing and lactating females. The survival of juvenile roan antelope could be threatened by sub-clinical Cu deficiencies. Deaths within a free roaming roan population will seldom be linked to a secondary nutrient deficiency and is rather linked to other natural factors such as predation or disease.

Previous findings that adult roan deaths were linked to the initial roan population decline rather than juvenile survival rates in the KNP (Harrington *et al.,* 1999) support the possibility of sub-clinical nutrient deficiencies rather than reject such a possibility. For instance, a chronic sub-clinical Cu deficiency within the roan population on the northern plains of the KNP could have a significant influence on reproduction rates of females as well as male fertility. This could cause a rapid decline in the number of new calves born as well as a drop in the survival rates of juvenile calves. The chances of recording a drop in reproduction rates and/or juvenile deaths are slim in a big open ecosystem such as the KNP. However, adult animals will be more prone to disease and more severe losses in body condition in the presence of a sub-clinical Cu deficiency. These conditions will not necessarily be observed because the animals that do suffer the most will be eliminated the fastest by predators. It is therefore recommended that the Cu status of the environment in which roan occurred previously in other parts of the KNP, be thoroughly evaluated to see whether animals did not move out of more optimum environments due to other stress factors. Should this be true, the cost of moving out of an area because of stress factors such as increased grazing competition or predation pressure could have caused roan to move into areas nutritionally more unsustainable for their survival. A population will then decline slowly without the necessary resilience to recover from periodic stresses. The inability of roan to recover in numbers after the relieving of various competition and survival pressures during the 1990's support the possibility of the presence of sub-clinical nutrient deficiencies within these populations. The Se status of roan at the KNP enclosures should be evaluated further as well since the status seems to be marginally deficient. A Se deficiency could cause further nutritional stress to the roan population in the KNP enclosures as should be further investigated. Various other marginal mineral levels were identified and described in order to assist future research projects as well as conservationists in biodiversity management.

The nutrient content of preferred grass species and the change in nutrient content over seasons in specific grass species preferred by roan antelope were successfully used to clarify certain grazing behaviours of captive roan antelope in the KNP enclosures. Data suggest that habitat structure was of less significance to roan in deciding when and where to graze. The results indicate that for roan in the enclosures, grazing quality was of greater importance in habitat selection than habitat structure, hence visibility. This behaviour is not necessarily conducive to animal survival in the presence of predators and suggests that the cost of adaptation to predation of newly released roan from the enclosures in the greater KNP might be too high for the successful addition to the remnant free roaming roan population.

The objective of the management of the KNP to breed roan antelope within the enclosures for the purpose of reintroducing surplus animals to the remnant free roaming roan population must be questioned. If this is indeed the objective of KNP management, more success will probably be achieved if roan are bred within a miniature reserve scenario with a low predator pressure and/or abundant buffer species. If not, the enclosures should be enlarged in order to build up larger numbers of roan. When released in large groups, the herds will be more resilient to sudden predation pressures and more animals will probably survive by the time the herds have adapted to the presence of predators.

Data indicate that the release of roan from the enclosures in the dry season will benefit the released animals more because of their tendency to select for diet quality over habitat structure within the enclosures. Based on the observations of the present study, newly released roan will move to bottomland areas for better grazing in the dry season. These bottomland areas usually have a lower tree density and therefore a greater visibility which could be to the advantage of animal survival and habituation to predators before the onset of the wet season. In the wet season, roan in the enclosures clearly disregarded habitat structure and utilised grazing within the denser mopani shrublands. This habitat preference will probably result in lower predator evasion success should they be released in the summer months.

A comparative study of the habitat preference over seasons between roan populations inside and outside the enclosures will give more insight into the effect that predator risk has on habitat and diet selection in free roaming roan antelope in the KNP. However, adapted foraging behaviour due to the spatial limitations of the roan enclosures may jeopardize comparative studies.

The lack of enough serum samples from roan in the KNP enclosures, limited the evaluation of nutritional status of those populations. However, specific nutritional anomalies have been highlighted and should be further investigated.

It is suggested that a comparative study in habitat preference over seasons be initiated between both roan populations within the enclosures as well as outside the enclosures on the northern basaltic plains of the KNP in order to determine the driving forces as well as limitations in habitat selection in the free roaming roan antelope populations. A study of the diet quality of preferred habitat types as well as grass species, hence overall diet, within the enclosures as well as preferred habitats over seasons of free roaming roan will probably give much insight into the possible nutritional limitations of the remnant roan populations at the KNP.

It should further be considered that the priority of decision making for or against particular habitats might have changed for free roaming roan antelope in the growing presence of predators on the northern plains of the KNP after the onset of the sudden roan population decline. Joubert (1976) indicated that in most southern African countries, including that of the KNP, roan preferred more open areas than for instance sable antelope. This implied a greater possible sensitivity towards predator risk than sable antelope especially since roan disregarded habitat structure in the absence of predator risk within the enclosures. Therefore, similarly to elk in Yellowstone National Park (Creel *et al.,* 2005), the cost of predator risk evasion in KNP over time, might have contributed to roan opting for less preferred habitat which resulted in a lower overall population resilience against droughts, disease as well as high predator pressure. Data from the present study clearly indicate that the nutrient levels in grasses varied substantially between grass species as well as between veld types in as small an area as 300 ha (N'washitsumbe). This variation also influenced grazing behaviour. If better grazing quality is available for roan in the wet season in the denser mopani shrubs, these areas will probably be avoided due to predator evasion tactics with a potentially limiting nutritional cost. Results indicate that bottomland grasses contain very high levels of nutrients like Na, S and Se in the wet season and might cause antagonistic interactions in the animal that could increase the vulnerability of lactating females and calves. A more detailed study of the seasonal habitat preference by free roaming roan will clarify such possibilities.

CHAPTER 8

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APPENDIX 1

Table 7: Nutrients and macro mineral concentrations in grasses in wet season

Area	Species	% N	% P	% K	% Ca	% Mg	Na (mg/kg)	% S	% CP	% Fat	% CF	% Ash	% NDF
Graspan	*Themeda triandra*	0.82	0.05	0.64	0.25	0.08	49.11	0.08	5.12	1.53	40.80	5.63	79.28
	Heteropogon contortus	0.93	0.06	0.60	0.39	0.15	44.26	0.20	5.79	1.53	37.95	7.32	
	Brachiaria nigropedata	1.46	0.13	1.40	0.43	0.20	50.17	0.14	9.15	1.51	28.91	8.63	
	Schmidtia pappohoroides	0.83	0.06	0.57	0.55	0.11	48.66	0.18	5.18	1.95	39.56	6.03	
	Eragrostis lehmanniana	0.72	0.03	0.36	0.41	0.08	54.20	0.15	4.47	1.00	41.50	6.15	
N'washitsumbe	*Urochloa mosambicensis*	2.26	0.22	1.71	0.32	0.23	406.32	0.08	14.11	1.09	38.87	12.21	73.99
	Digitaria eriantha	1.04	0.30	2.06	0.52	0.25	2373.30	0.12	6.48	2.21	38.47	10.67	70.19
	Sporobolus ioclados	1.03	0.09	0.64	0.14	0.14	13435.30	0.25	6.46	0.79	36.95	5.82	74.87
	Diplachne fusca	0.63	0.13	1.05	0.10	0.13	5257.08	0.30	3.93	0.92	41.35	7.76	82.56
	Themeda triandra	0.52	0.09	0.65	0.22	0.15	260.93	0.10	3.22	1.26	36.16	10.99	73.99
	Ishaemum afrum	1.13	0.24	1.06	0.12	0.25	155.14	0.11	7.06	1.22	35.64	14.38	71.33
	Sporobolus nitens	1.93	0.11	0.90	0.18	0.16	14571.95	0.27	12.05		27.04	8.63	68.83
	Schmidtia pappophoroides	0.83	0.19	1.06	0.33	0.10	297.09	0.14	5.21	1.15	38.44	6.91	74.50
	Panicum maximum	0.92	0.47	1.98	0.27	0.31	672.96	0.13	5.77	0.65	39.13	12.25	74.62
	Cenchrus cilliaris	0.82	0.28	2.00	0.63	0.25	172.61	0.12	5.14	1.24	34.13	16.27	66.08
Capricorn	*Themeda triandra*	0.52	0.13	0.67	0.22	0.10	230.87	0.10	3.24	1.36	34.92	13.36	72.96
	Urochloa mosambicensis	1.46	0.35	1.59	0.51	0.28	6004.38	0.18	9.12	1.50	31.26	14.16	62.92
	Panicum coloratum	0.62	0.34	0.97	0.31	0.34	1056.63	0.12	3.85	0.78	38.59	14.31	73.50
	Digitaria eriantha	1.13	0.24	1.21	0.73	0.30	2137.06	0.14	7.06		34.03	12.04	67.65
	Cenchrus cilliaris	1.03	0.31	1.65	0.36	0.21	73.91	0.15	6.42	1.19	35.62	13.90	69.63

Table 8: Micro mineral concentrations in selected grasses in wet season

Area	Species	Cu mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg	B mg/kg	Mo mg/kg	Se µg/kg
Graspan	*Themeda triandra*	3.07	322.28	53.20	24.55	22.51	0.44	<5
	Heteropogon contortus	4.12	442.57	94.69	22.64	15.44	0.76	<5
	Brachiaria nigropedata	2.09	316.71	54.35	25.09	16.72	1.20	<5
	Schmidtia pappophoroides	3.11	344.76	54.87	32.09	17.60	1.97	10.35
	Eragrostis lehmanniana	1.02	138.05	25.56	47.04	13.29	0.81	<5
N'washitsumbe	*Urochloa mosambicensis*	3.08	63.62	18.47	24.63	20.52	0.85	8.21
	Digitaria eriantha	4.15	112.97	30.05	23.84	19.69	0.99	35.24
	Sporobolus ioclados	4.13	159.16	35.14	6.20	34.11	0.31	279.04
	Diphlachne fusca	2.10	95.49	17.84	20.99	26.23	1.71	64.01
	Themeda triandra	3.09	79.41	49.50	34.03	15.47	0.99	6.19
	Ishaemum afrum	4.11	153.09	38.02	52.40	17.47	0.38	25.69
	Sporobolus nitens	5.36	413.59	47.14	6.43	34.29	0.88	405.01
	Schmidtia pappophoroides	4.17	58.38	14.59	28.15	20.85	1.01	11.47
	Panicum maximum	3.08	53.34	28.72	62.58	16.41	0.80	<5
	Cenchrus cilliaris	2.05	46.23	23.63	16.44	14.38	0.71	35.96
Capricorn	*Themeda triandra*	3.11	76.61	40.38	33.13	10.35	0.90	<5
	Urochloa mosambicensis	5.21	187.64	57.33	33.36	16.68	0.24	39.61
	Panicum coloratum	2.05	45.14	31.80	43.09	11.28	0.44	<5
	Digitaria eriantha	5.14	115.07	16.44	29.80	15.41	0.99	<5
	Cenchrus cilliaris	3.08	132.43	15.40	25.66	12.32	0.43	<5
		3.07	322.28	53.20	24.55	22.51	0.44	<5

Table 9: Nutrient and macro mineral concentrations of selected grasses in the dry season

Area	Species	% N	% P	% K	% Ca	% Mg	Na (mg/kg)	% S	% CP	% Fat	% CF	% Ash	% NDF
Graspan	*Pogonarthia squarosa*	0.61	0.05	0.19	0.18	0.04	59.15	0.11	3.83	0.70	42.73	3.22	83.37
	Eragrostis lehmanniana	0.55	0.04	0.23	0.24	0.05	46.20	0.14	3.42	0.63	40.24	4.24	80.91
	Themeda triandra	0.39	0.04	0.30	0.21	0.09	46.06	0.10	2.41	0.71	40.54	6.35	82.02
	Schmidtia pappophoroides	0.64	0.05	0.27	0.29	0.08	45.22	0.13	3.97	1.27	42.67	3.43	79.95
N'washitsumbe	*Diphlachne fusca*	0.42	0.13	0.50	0.13	0.20	3300.76	0.40	2.65	1.14	37.34	8.01	76.95
	Ishaemum afrum	0.27	0.07	0.23	0.11	0.16	321.64	0.16	1.70	0.66	39.11	11.94	78.92
	Sporobolus ioclados	1.13	0.12	0.34	0.23	0.21	5447.34	0.33	7.08	1.39	32.23	8.21	69.40
	Schmidtia pappophoroides	0.56	0.10	0.23	0.17	0.06	190.73	0.12	3.50	0.83	41.05	7.21	78.45
	Cenchrus cilliaris	0.41	0.13	0.60	0.12	0.06	227.30	0.13	2.55	0.68	41.63	7.40	84.00
	Panicum coloratum	0.52	0.12	0.28	0.23	0.11	796.56	0.13	3.23	0.66	36.67	10.83	76.02
	Themeda triandra	0.46	0.08	0.22	0.18	0.06	192.31	0.09	2.87	1.45	36.73	13.23	75.39
	Urochloa mosambicensis	1.91	0.32	2.06	0.26	0.63	1177.50	0.23	11.92	1.69	24.28	10.32	58.45
Capricorn	*Cenchrus cilliaris*	0.32	0.13	0.45	0.16	0.08	166.22	0.15	2.01	0.76	40.45	7.91	82.88
	Panicum coloratum	0.68	0.16	0.52	0.46	0.20	583.77	0.18	4.27	0.94	37.24	10.73	75.99
	Schmidtia pappophoroides	0.44	0.13	0.29	0.18	0.06	112.88	0.11	2.74	1.02	36.18	11.39	77.25
	Themeda triandra	0.36	0.08	0.36	0.20	0.06	241.38	0.09	2.22	0.73	36.43	11.75	76.38
	Urochloa mosambicensis	0.61	0.16	0.44	0.47	0.18	2391.42	0.13	3.82	0.70	38.26	9.66	75.65
Sable Ranch	*Digitaria eriantha*	0.65	0.03	0.65	0.28	0.14	102.00	0.14	4.07	0.81		8.59	
	Heteropogon contortus	0.73	0.02	0.30	0.21	0.07	24.04	0.11	4.57	0.78		4.73	
	Themeda triandra	0.84	0.03	0.72	0.16	0.05	51.40	0.12	5.24	0.94		7.56	
	Panicum maximum	1.27	0.04	1.47	0.22	0.16	46.51	0.18	7.93	0.22		7.31	
	Antelope Cubes	**3.3**	**0.73**	**1.01**	**0.8**	**0.3**	**4240**	**0.27**	**19.4**	**1.94**	**-**	**6.73**	**-**

Table 10: Micro mineral content of selected grasses in the dry season

Area	Species	Cu mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg	B mg/kg	Mo mg/kg	Co mg/kg	Se µg/kg
Graspan	*Pogonarthia squarosa*	4.30	272.10	65.61	69.91	43.02	0.94	0.22	19.36
	Eragrostis lehmanniana	4.30	338.42	42.97	64.46	39.75	0.35	0.23	13.97
	Themeda triandra	4.28	484.20	92.13	40.71	37.49	1.06	0.31	20.35
	Schmidtia pappophoroides	4.31	668.60	76.44	64.60	39.84	0.54	0.37	12.92
N'washitsumbe	*Diphlachne fusca*	4.34	302.93	24.97	32.57	40.17	0.38	0.28	6.51
	Ishaemum afrum	3.13	327.90	20.89	49.08	31.33	0.02	0.25	<4
	Sporobolus ioclados	5.50	1320.57	102.34	42.92	45.12	0.28	1.39	47.32
	Schmidtia pappophoroides	5.39	1217.67	39.87	71.12	39.87	0.04	0.86	134.70
	Cenchrus cilliaris	4.29	582.18	25.73	53.61	39.67	0.83	0.47	<4
	Panicum coloratum	5.38	1073.20	96.88	67.81	40.90	0.38	0.81	<4
	Themeda triandra	4.18	638.59	70.03	47.03	35.54	0.33	0.56	22.99
	Urochloa mosambicensis	9.81	1940.69	66.51	87.22	50.15	0.03	1.26	39.25
Capricorn	*Cenchrus cilliaris*	3.22	390.35	26.81	45.04	37.53	0.62	0.40	123.32
	Panicum coloratum	4.34	594.62	66.19	66.19	39.06	0.85	0.66	<4
	Schmidtia pappophoroides	4.18	433.74	38.67	43.90	32.40	0.24	0.67	<4
	Themeda triandra	4.18	301.99	56.43	40.75	31.35	1.01	0.33	33.44
	Urochloa mosambicensis	4.29	326.01	71.85	62.20	33.24	0.24	0.53	9.65
Sable Ranch	*Digitaria eriantha*	3.26	303.82	187.72	46.66	32.55	2.84		
	Heteropogon contortus	3.14	340.72	63.75	25.08	26.13	0.63		
	Themeda triandra	3.15	251.76	143.71	45.11	25.18	0.07		
	Panicum maximum	3.17	200.82	71.87	67.65	24.31	0.08		
	Antelope Cubes	**19**	**271**	**80**	**182**	**35**	**1.22**	**-**	**-**