THE RELATIONSHIPS AMONG NUTRITION, SOIL INGESTION AND ANTHRAX OCCURRENCE IN ZEBRA AND SPRINGBOK IN THE ETOSHA NATIONAL PARK OF NAMIBIA

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ABSTRACT

The relationship among animal nutrition, soil ingestion and the seasonality of anthrax occurrence in zebra and springbok in the Etosha National Park of Namibia was examined. The nutrient content of zebra and springbok faeces was determined in wet and dry seasons for a period of two years. Nutrient content was also determined for a dominant grass species (*Enneapogon desvauxii*) eaten by zebra. Faecal Crude protein (FCP), faecal phosphorus (FP) and faecal crude fibre (FCF) were assessed as indicators of forage quality. Springbok had a significantly higher faecal CP, P and lower CF content compared to zebra in all seasons. Faecal CP and P were significantly higher in wet seasons than dry seasons while faecal CF were lower in the wet seasons than dry season. It appears that the CP and P content of the faeces reflect the CP and P content of the diet. Also CP and P content of diet follows rainfall patterns. Concentration of silicate (soil) in faeces of springbok and zebra was also estimated, to determine why anthrax occurrence is seasonal, since ingestion of soil exposes animals to the anthrax bacterium. Results indicated that zebra had significantly higher silicate (soil) ingestion compared to springbok. Also the faeces of both species had significantly higher crude silicate content in wet seasons compared to dry seasons. Results also indicated that there was a positive correlation between the seasonality of faecal silicate content and anthrax occurrence in both animals. Faecal crude protein (FCP) of springbok ranged between 9.74 and 12.62% while that for zebra was 6.92 to 8.51% which is higher than the recommended nutrient requirement (6%) all the seasons (wet and dry) while faecal phosphorus FP of springbok ranged from 0.21-0.41% while that zebra is 0.26-0.41% which is higher than the critical value of 0.2% all seasons. Results show that these animals are not nutritionally stressed, so nutritional stress is unlikely related to the timing of anthrax outbreaks in Etosha, however their feeding behaviour may affect how and when these herbivores contact the anthrax bacterium in Etosha.

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DECLARATION

I, Peace Imologhome , hereby declare that this study is a true reflection of my own research, and that this work has not been submitted for a degree in any other institution of higher education.

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DEDICATION

I dedicated this thesis to Almighty God; you have been there all through from the beginning to the very end. I extend this dedication to my late mother. Mummy without your moral and financial support during my undergraduate days I would not be at this level.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Anthrax is a bacterial disease caused by the spore–forming *Bacillus anthracis,* a Gram-positive, rod shaped bacterium, the only obligate pathogen in the large genus *Bacillus.* Anthrax is primarily a disease of herbivores. It has long been the traditionally held belief that ingestion of the spores while grazing is a frequent mode of uptake. Hugh-Jones and de Vos. (2002) state that anthrax occurrence in wildlife in southern Africa is associated with overstocking or the concentration of game around dwindling water or forage and with animals suffering from environmental (nutritional) stress. As grazing biomass is depleted through the dry season, animals will graze very close to the ground and thereby may ingest more soil in dry seasons. The quality of forage is also reduced in dry seasons, and if animals ingest insufficient resources, they may experience reductions in immune defences against incoming pathogens or they may forage in riskier ways in an attempt to improve nutrition, thus increasing exposure to pathogens in the environment. The goal of this study is to test if the seasonality of changes in animal nutrition and soil ingestion relate to the seasonal occurrence of anthrax.

Anthrax is a seasonal disease where it occurs. However the season of occurrence can vary between different locations. In southern Africa, anthrax occurs primarily in dry seasons in areas like Kruger National Park in South Africa (Hugh-Jones and de Vos, 2002)) and Malilangwe Private Reserve in Zimbabwe (Clegg *et al.,* 2007) and primarily in wet seasons in Etosha National Park, Namibia (Lindeque and Turnbull, 1994), which is the focal study area for this research project.

Assessment of nutritional status will provide an understanding of environmental requirements of the animals concerned. Faecal nitrogen and phosphorus can be used to assess the nutritional status of free-ranging animals and seasonal changes in forage (Irwin *et al*, 1993). Also Lindeque and Turnbull (1994) found that anthrax occurrence in Etosha National Park is in the rainy season and incidence of anthrax is higher in zebra than springbok. No study has yet discovered why anthrax outbreaks occur during the rainy season in Etosha, unlike other localities which is in the dry season, or why incidence of anthrax is higher in zebra than springbok. I address these issues by evaluating the quantity of soil derived from the faeces of both animals in different seasons (since *B. anthracis* spores are found in soil), correlating faecal silicate with anthrax occurrence and conducting chemical analysis of faecal sample of both species.

Etosha National Park is a protected environment of international significance, providing habitat to different species of plants and animals. Etosha is Namibia's centrepiece for attracting international tourists, the nucleus for tourist-based economic development on adjacent lands and the only realistic source of certain wildlife species for repopulating depleted areas in Namibia. Etosha National Park supports large herds of plains ungulates and their predators, and large mammals like black rhinoceros (*Diceros bicornis*) and elephant (*Loxodonta africana*). Etosha also contains the only significant remnants of Namibia's once widely distributed plains zebra (*Equus quagga*), blue wildebeest (*Connochaetes taurinus*) and springbok (*Antidorcas* *marsupialis*) populations. Wildlife population restored in Etosha is a source of tourist attraction, and wildlife-based cottage industries. Thus, Etosha is critically important to Namibia's economy and biological diversity (Gasaway *et al*., 1996).

Zebra and springbok are the most abundant larger mammalian herbivores in Etosha. Also there is higher incidence of anthrax in zebra than springbok. Most of the carcasses recovered are from zebra, with relatively few springbok carcasses recovered (Lindeque and Turnbull, 1994), despite these two species sharing the plains habitat.

Zebras are odd-toed ungulates of the *Equidae* family (horse family) native to eastern, southern and southwestern Africa. They are best known for their distinctive white and black stripes, which come in different patterns unique to each individual (Waage, 1981). They are generally social animals and can be seen in small harems to large herds. The odd-toed ungulates (animals having an odd number of toes on each hoof) are mammals which belong to the order *Perissodactyla*. They are usually large, have relatively simple stomachs and a large middle toe. Zebras are very adaptable grazers (McClintock, 1976). Their well adapted digestive system allows them to subsist on diets of lower nutritional quality than that necessary for ruminants. They are hindgut fermenters, that is, they digest plant cellulose in their large intestinal appendage (caecum) rather than stomach (Churcher, 1993).

Zebras are primarily grazers and have dental adaptations for feeding on grasses (Moelhman, 2003). The primary grass eaten by zebras in Etosha is *Enneapogon desvauxii* (Havarua, 2011). The chemical analysis of *Enneapogon desvauxii* was carried out to know if there is a relationship between faecal crude protein and dietary crude protein.

The Springbok belongs to family *Bovidae* (sheep, cows, goat family) and is a medium-sized brown and white antelope that stands about 75 cm high. Springbok mostly eat grass, leaves, shoots and other small plants, and shrubs (Waage, 1981). Springbok are ruminants and hence have a four-chambered stomach which allows them to digest foods, such as grasses, that are too low in nutrients for many other animals (Geoffrey, 1974). No higher animal directly digests cellulose, but like kangaroos, bovids rely on micro-organisms living in their stomachs to break down cellulose by fermentation. Because of the size and weight of their complex digestive systems, many bovids have a solid, stocky build. The more gracile species tend to have more selective diets, and be browsers rather than grazers (Churcher, 1993).

Principally, dietary silica can occur as characteristic crystals in plant cell walls (phytoliths), or can be ingested as dust or contaminations of soil (Kaiser and Schulz, 2006) and it is known to pass through the digestive tract without any significant degradation or absorption (Jones and Handreck, 1965). Anthrax was hypothesized to be caused by ingestion of soil containing the spores. Spores can persist in soil for decades, wait to be taken up by another host, where germination and multiplication can again take place upon infection. The cycle of infection is influenced by factors related to the season, such as available grazing, and of the health of the host. It is a long-held belief that animals generally acquire anthrax by ingestion of spores while grazing (WHO, 2008).

First signs of an anthrax outbreak in the more susceptible livestock species are one or two sudden deaths within the herd or flock with retrospective recall of preceding mild illness. In more resistant species, local signs such as swellings of the oral and pharyngeal region are seen. In wildlife, sudden death is the invariable sign, often (but not always) with bloody discharges from natural orifices, bloating, incomplete *rigor mortis* and then absence of clotting of the blood (WHO, 2008).

Many methods have been used in the past to determine the quality of herbivore's diet but they suffer a variety of problems. Direct field observations are tedious and it is also difficult to assess the exact proportions of each plant species consumed because of variation in bite size. Fistulation is more direct but invasive, costly and ethically questionable technique for assessing diet quality (Wrench *et al.*, 1997) and oesophageal fistulation is impractical for free-ranging animals. Faecal indices of diet quality, such as those using crude protein, crude fiber and phosphorus, have shown to be useful tools.

1.2 Statement of the problem.

Anthrax occurrence is in the wet season in Etosha National Park, when animals are expected to have access to high quality forage. This is unlike other localities, for example Kruger National Park in South Africa, where anthrax tends to occur in the dry seasons. In these other systems, anthrax seasonality has been attributed to 1) foraging closer to the soil as available forage biomass is reduced in dry seasons and 2) nutritional stress during dry seasons (Hugh-Jones and Vos, 2002). The quality of forage is reduced in dry seasons and it has been hypothesized that animals might not have access to sufficient resources during dry seasons to mount an immune response against *B. anthracis*. This study examines firstly how nutrition changes seasonally and to what extent nutrition may play a role in the seasonal timing of anthrax outbreaks. This will enable us to know if these animals meet their nutrient requirements in both seasons or not. Secondly animals are expected to ingest more soil in the dry seasons, as a result of dwindling food and water resources. This study will also examine if there is seasonal difference in how animals may contact *B. anthracis*, since ingestion of soil may expose animals to *B. anthracis* spores (WHO, 2008).

This study therefore is aimed at assessing the seasonal relationships among nutrition, soil ingestion and anthrax occurrence in zebra and springbok from Etosha National Park, Namibia.

1.3 Purpose of the study

The purpose of this study was to evaluate how zebra and springbok nutrition and soil ingestion change seasonally and to relate these patterns to the seasonal occurrence of anthrax for each species. This study also contrasted the nutrition and soil ingestion between zebra and springbok. The methods were based mainly on chemical analysis of faecal samples from zebra and springbok to determine indirectly the quality of the forage and quantity of soil material ingested. The study examined how the quality of an important grass species varied monthly throughout a year. The results of these studies were then related to the seasonal difference in zebra and springbok anthrax incidence.

1.4 Objectives of the study

(i) To determine and compare the seasonal changes in nutritional factors for zebra and springbok.

(ii) To determine the quantity of soil ingested by both species and the relationship between faecal silica content and season.

(iii) To determine how the nutritional composition of the grass *Enneapogon desvauxii,* one of the main grass on the Etosha plains, changes monthly over a year.

(iv) To determine if the seasonal patterns in animal nutrition or soil ingestion relate to the seasonality of anthrax occurrence.

1.5 Hypothesis

1. Seasonality has effect on the nutritional status of zebra and springbok
2. Soil ingestion vary seasonally for zebra and springbok
3. Faecal silica content differ between zebra and springbok
4. Seasonal patterns of anthrax occurrence observed in Etosha National Park can be explained by seasonal changes in animal nutrition or soil contact

1.6 Significance of the study

The result of this study will provide us with an understanding of the relationships among nutrition, anthrax and soil ingestion. Sound knowledge of changes over time in nutrient and health of animals in the rangeland can help to prevent stock losses. This will give us an idea if anthrax occurrence reflects nutrition and/or soil ingestion in this system. Also this study will provide an understanding of environmental requirements of animals concerned. Comparing the nutrient quality of faecal samples from zebra and springbok may give a real reflection on the quality of the forage available to or selected by these species. This will enable us to know if the animals are nutritionally stressed or not. Determining the level of soil derived in the faeces of zebra and springbok could give useful information on why incidence of anthrax differs between seasons and species. This study could be used as a basis for making decisions in rangeland management. If faecal profiling is used regularly, ranch managers can estimate whether forage in the rangeland is meeting the threshold requirement.

1.7 Definitions

Nutrition: science that deals with food and their effect on health.

Forage sample: grasses, leaves and shrubs eaten by animals.

Faecal sample: the undigested material excreted from animals

Anthrax: disease caused by bacterium *Bacillus anthracis*

CP: crude protein

CF: crude fibre

P: phosphorus

CHAPTER TWO

LITERATURE REVIEW

2.1 Feeding

The natural diet of zebra and springbok has effect on the growth and the successful production of young zebra and springbok and their population. A thorough knowledge of the feeding ecology of these wild ungulates is therefore an important component of game ranch management.

2.1.1 Protein

For optimum growth National Research Council, (1997) gives dietary crude protein recommendation of 16-18% for springbok and 9-14% for zebra. The dietary crude protein requirements for maintenance of adult mammals range from 5.5-9% for wild ruminants (Van Soest, 1982). Protein requirements, for gestation and hair growth, would be intermediate to the requirements for rapid neonatal growth and adult maintenance. Protein concentrations of 10% for maintenance, 11-12% for lactation and 14-15% for growing animals respectively, are required.

It is generally accepted that a crude protein content of 8%, and above in vegetation is necessary for young, growing herbivores, whereas a crude protein content of 5% is required for African ungulates (Van Hoven, 1983). It appears that wild herbivores cope better than domestic ones under poorer rangeland conditions. Ruminants, including wild ungulates, require a minimum of 5% crude protein (0.8% N) in their food to maintain body weight (Liversidge and Berry, 1995). Also, proper rumen fermentation is disrupted if faecal nitrogen concentrations are not at least 1.10-1.20% or 6-8% crude protein (Grant *et al*., 1995).

Mbatha, (2008) observed that there was a significant difference in faecal CP among species from the study performed in South Africa. Mbatha (2008) also observed that springbok had a higher faecal CP of 11.66% than zebra CP 8%, the explanation was that springbok being a mixed-feeder usually has small mouth and can easily select forage with higher nutritive value compared to zebra, a grazer which is not as selective. Mbatha (2008) also observed that faecal CP content of animals tended to increase as the rainfall increased and the lowest CP was in the dry season. There is variation in the nutritive value of the vegetation of the African savannas; its chemical composition varies from season to season (Meissner *et al.*, 1999). Orr and Holmes (1984) and Mbatha and David (2007) observed that faecal CP content was positively correlated to annual rainfall.

From a study performed in South Africa, specifically looking into chemical content of forage selected by zebra and blue wildebeest, it was observed that crude protein (CP) concentrations of forage fluctuated between 2.93% to 7.01% for zebra and 2.7% to 7.7% for wildebeest. The CP content was significantly higher in the wet season when there was more regrowth available than during the drier months (Bodenstein, *et al*., 2000). Wrench *et al.,* (1997) observed that there is a positive correlation between the protein content of the feed and that of faeces. They also observed that diet crude protein content follows rainfall patterns.

2.1.2 Phosphorus

Faecal phosphorus below 0.2% is considered to be indicative of phosphorus limitation (Dorgeloh *et al.* 1998). NRC (1997) gave dietary phosphorus recommendation of 0.10-0.44% for springbok, and 0.15-0.34% for zebra.

Mbatha (2008) observed that there was a significant difference in faecal P among species, from the study performed in South Africa. Mbatha (2008) also observed that springbok had higher faecal phosphorus (0.49%) than zebra (0.21% phosphorus), the explanation was that springbok are a more selective feeder than zebra.

Rainfall can affect the mineral composition of grass (McDonald *et al.*, 1996). For example, phosphorus appears to be present in higher levels when rainfall is high (Bothman, 1989 and McDonald *et al.*, 1996). In other studies, wet conditions resulted in low P concentration (Wilson 1982). Jessen, *et al.*, (2004) looking into chemicals of diet selected by eland in northern Namibian, observed that phosphorus content of forage was significantly higher in the wet season when there was more regrowth available than during the drier months. Faecal P reflects dietary P (Karn, 1997).

2.1.3 Fibre

Buys (1990) reported crude fibre content in the diet of eland as fluctuating between 22 and 44%. He also observed that crude fibre in the diet of eland is higher in the dry season than in the wet season. From the studies performed in Namibia, specifically looking at the quality of diet selected by eland, it was observed that crude fibre fluctuated between 19-39% (Jessen *et al.*, 2004).

Erasmus and Penzhorn (1978) reported that faecal fibre (68%) in zebra is higher than faecal fibre (46%) in springbok; the explanation was that it could be due to that fact that zebra ate less selectively than springbok and therefore consumed more grasses that are high in fibre. Erasmus and Penzhorn (1978) observed that faecal fibre is significantly higher in dry seasons than wet seasons.

2.2 Forage intake of grazing animals

Forage intake of grazing animals varies with body weight as well as forage quantity and availability. Presently, intake for grazing animals is most commonly expressed as weight of forage consumed as a percentage of the animal's body weight. Review of literature below shows that dry matter consumption by most range ruminants is about 2% of their body weight per day when values for different seasons are average across the year. However, considerable seasonal variation occurs, with values as low as 1% during periods when forage is low in quality and availability, to over 2.5% when quality and availability is high. Horses (same family as zebra), which have an enlarged caecum rather than a rumen; consume about 60% to 70% more forage than ruminants of similar body size when quality is comparable (Johnson *et al*., 1982).

Bodenstein *et al*., (2000) reported that daily dry matter intake of zebra was 7.8kg and that of wildebeest 3.7kg. Boutouba *et al.* (1990) reported daily dry matter intake of 1.9-2.2% in goat. Rice *et al.* (1974) reported daily dry matter intake of 1.9% in sheep.

2.3 Digestibility

Digestibility is estimated as the difference between the amount of feed ingested and the amount excreted (Neil, 2000). Digestibility is usually positively related to the concentration of nutrients in the forage and their intake (Neil, 2000). This is because the larger the quantity of nutrients in forage, the more easily they can be digested (Neil, 2000).

Generally forbs and foliage from shrubs have higher *in vitro* digestibility values than grasses at comparable stage of maturity (Wofford and Holechek, 1998). Digestibility varies among forages, although leaves and fruits are consistently higher in digestibility than are stems and twigs (Huston *et al.*, 1981). Leaves from forbs and shrubs reach their potential extent of digestion much more quickly than those from grasses (Wofford and Holechek, 1998). This appears to be partly explained by higher level of cells soluble and quick microbial access to cell soluble in forbs and shrub leaves and stems.

In vitro digestibility of forage is evaluated by incubating forage in buffers and live rumen fluid, at body temperature, under anaerobic (no air) conditions. Bodenstein *et al.*, (2000) reported *in-vitro* digestibility of organic matter of forage consumed by zebra and wildebeest to be 42.2% and 47.9%, respectively.

2.4 Forage eaten by springbok and zebra

Some of the even-toed ungulates evolved an extra stomach (the rumen) which partially break down the food before passing it back to the mouth for further chewing before being sent down to the true stomach for final digestion. This, of course, is the proverbial "chewing of the cud." These are known as the ruminants. The success of these more advanced ungulates can be shown by the number of existing species.

There are 194 species of even-toed ungulates and only 16 odd-toed ungulates, of the horse and zebra are the only exclusive grazer among them (Milton *et al.,* 1992).

This cud chewing allows for longer time for food to be processed (70 - 100 hours through the gut) than, say, for a horse (30 -45 hours). The central difference, then, is that the digestive system of the horse is less efficient than that of the ruminant, but in compensation the horse eats greater quantities of food; the emphasis in ruminants is on highly efficient digestion and on more selective feeding, but not on high rates of food intake. Given food in short supply, the ruminant will probably survive after the horse has starved to death (Van Hoven, 1983). Horse and zebra are the same family; they also have the same digestive system.

The springbok is a common plains antelope in the arid lands of southern Africa, occupying a range of landscapes from savannas to the Namib Desert and elevations from the South African rangelands (2,000 m) to sea level (Skinner *et al.,* 1984). Springbok avoid mountains, rocky hills, woodlands, and other areas where vegetation restricts movement and visibility (Bigalke, 1972; Smithers, 1983). Springbok typically prefer grasslands or savannas with short-growing grasses that are associated with edges of dry lake beds (Bigalke, 1972; Milton *et al*., 1992; Skinner and Smithers, 1990).

Springboks are seasonal grazers and browsers that shift from grasses in hot, rainy seasons to shrubs in cold, dry seasons (Bigalke, 1972; Van Zyl, 1965). Springboks feed on a variety of karooid vegetation found along riverbeds and in dry lake beds. Springboks also forage on tall shrubs by standing on their hind legs or eating fallen leaves from the ground, and they occasionally dig for roots (Bigalke, 1972; Van Zyl, 1965). Springboks in the Lombard Reserve, Western Transvaal, feed on 68 plant species, 20 of which are eaten 3 months of the year and are considered primary food plants.

These includes nine grasses (*Cynodon dactylon*, *Eragrostis bicolor*, *E. curvula*, *E. lehmanniana*, *E. obtuse*, *Panicum coloratum*, *Sporobolus fimbriatus*, *S. ioclados*, and *Themeda triandra*) and eleven shrubs (*Aster muricatus*, *Cyperus usitatus*, *Gazania oxyloba*, *Lycium oxycladum*, *Nenax microphylla*, *Nolletia ciliaris*, *Osteospermum leptolobum*, *Pentzia calcarea*, *P. virdis*, *Salsola rabieana*, and *Ziziphus mucronata* (Van Zyl, 1965). Important items in the winter diet of springbok include shrubs (*Chrysocoma tenuifolia*, *Lycium*, *Pentzia calcarea*, *P. globosa*, *P. incana*, *P. lanata*, *Rhus ciliata*) and trees (*Acacia tortilis*, *Zizyphus mucronata*) Bigalke (1972). Important items in the summer diet of springboks include *Cymbopogon plurinodis*, *Cynodon dactylon*, *Cynodon hirsutus*, *Schmidtia bulbosa*, *Tragus berteronianus*, and *Urochloa stolonifera*.

Zebras are equids, which means that they are primarily grazers and have dental adaptations for feeding on grasses (Moelhman, 2003). The primary forage eaten by zebras in South Africa is *Themeda triandra*, other grasses consumed include:*Cymbopogon plurinodis, Heteropogon contortus, Setaria neglecta*, and *Enneapogon scoprius* (Grobler, 1983; Nowak, 1999).

According to (Bodenstein *et al.*, 2000), the grasses that were mostly consumed by zebra were *Panicum maximum*, *Themeda triandra*, *Heteropogon contortus* and *Urochloa mosambicensis*.

2.5 Link between anthrax, seasonal trends in nutrition and feeding behaviour.

Much has been written and hypothesized about the effects of season, rainfall, soil, vegetation, and host condition and population density on the epidemiology of anthrax. The primary conditions affecting the seasonality of anthrax in any one place would appear to be temperature and rains or drought (climatic factors).

Climate probably affects the animal directly, by influencing the way in which it makes contact with the spores through the soil, for example, grazing closer to the soil in dry periods when the grass is sparse, or enforced grazing at restricted sites when water becomes scarce (WHO, 2008). (Sussman & Halvorson, 1966) suggests that the rate and yield of germination may be influenced by temperature in a manner that varies with pH and that spores will not germinate at a pH of < 5, a temperature of < 8 °C and relative humidity of < 96%.

It has long been the traditionally held belief that ingestion of the soil exposes animals to anthrax spores while grazing is a frequent mode of uptake (WHO, 2008). However, among angiosperms, grasses are best known to be silica accumulators, while dicots are generally characterized by lower silica contents (Jürgen *et al*., 2010). Silica contents have been quantified to be 4.95 per cent dry matter (DM) in grasses compared with only 0.56–1.46% DM in browse (Dougall *et al*., 1964)

Wong *et al*., (1988) stated that geophagy (soil, dirt or clay eating) may be quantitatively assessed on the basis of stool silica excretion rate: the rate of silica excretion is proportional to the rate of ingestion, and normal dietary silica levels are sufficiently low to be differentiated from stool silica levels due to geophagy.

The hypothesis that was put forward by Canadian researchers is that persisting anthrax outbreaks occur when the animals become immune-compromised from the stress that results when meteorological conditions lead to diminished food and water sources (Dragon *et al.*, 1999). The invariable occurrence of natural outbreaks in hot dry weather, as opposed to feed-related winter outbreaks, supports the hypothesis that innate immunity is depressed under such climatic stress, thereby reducing the necessary minimal infective dose sufficient for some exposed grazing animals to become infected and diseased. Stress-inducing factors of these types are, in fact, thought to be important in the seasonality of anthrax in any anthrax-enzootic environment worldwide (WHO, 2008).

Lindeque and Turnbull (1994) noted that in the Etosha National Park in Namibia the over all peak anthrax activity is in the rainy season, in contrast to most other enzootic localities, where it is in the dry season. They also observed that there is higher incidence of anthrax in zebra than springbok.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area and vegetation

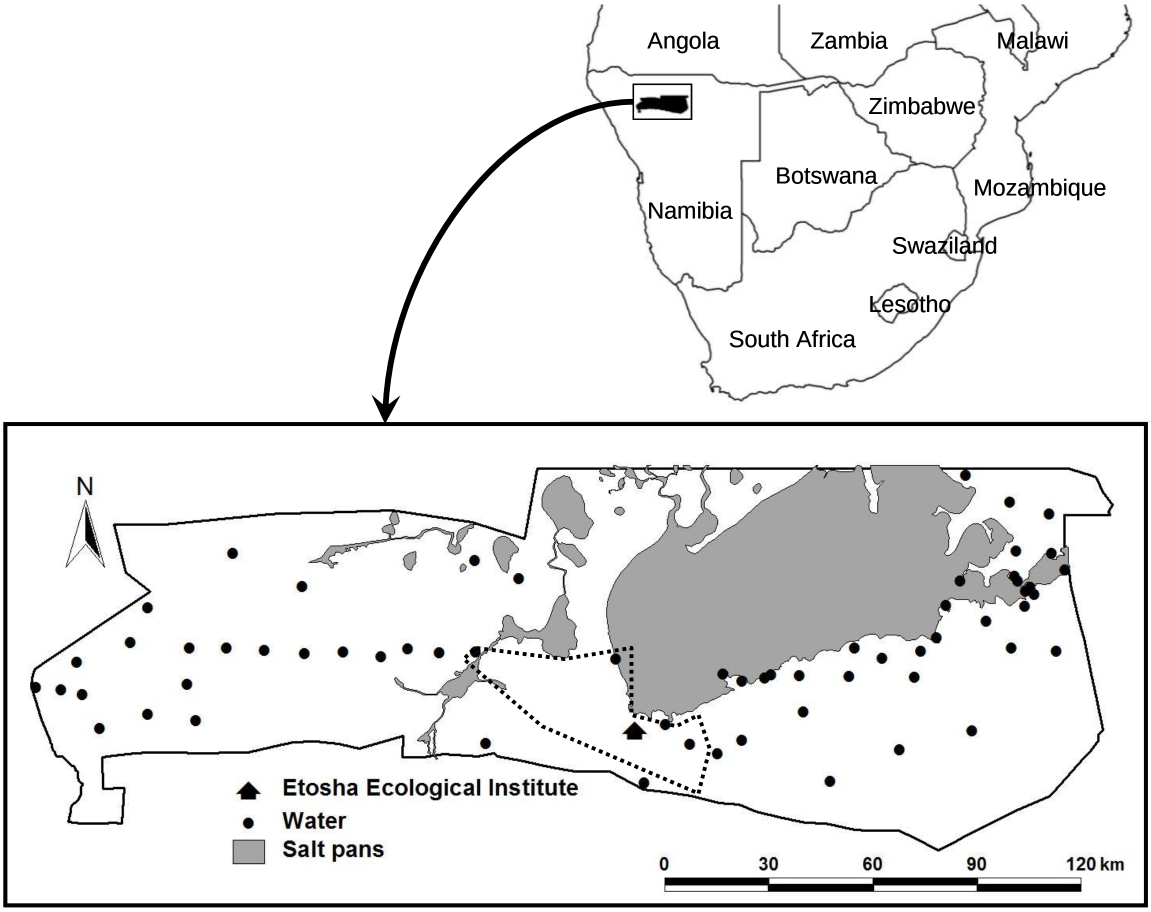

Figure 3.1 Etosha National Park in northern Namibia, showing the pans (saline depressions).

This study was conducted in Etosha National Park (22,915km²) situated in northern Namibia located at, 18º30'-19º30'S and 14º15'-17º10'E. Etosha is approximately 295 km east-west and 65-110 km north-south at of elevations 1000-1250m. The study area was at the centre of the park, which is the grassland/dwarf shrub savanna habitat around Okaukuejo. This area is easily accessible from the research facilities and it is a preferred habitat of springbok and zebra animal.

Vegetation is classified as arid savanna (Huntley 1982) and consists of plains and savanna shrub lands and wood-lands. The sweet grass plains extend southeast, south, and south-west of the Etosha Pan and are dominated by short–medium grasses*:* *Enneapogon desvauxii*, *Eragrostis nindensis*, *Eragrostis cenchroides*, and *Aristida adscensionis* (le Roux *et al.,* 1988). Also Etosha is dominated by mopane woodland (*colophospermum mopane*), and grassland plains that surround the barren salt pan (le Roux *et al.*, 1988).

Rainfall varies from mean annual rainfall of 445mm in the east, to 309mm in the west of the park. Rainfall occurs mainly between January and April, with highest precipitation recorded in February (Le Roux 1988).

Temperature at Okaukuejo, in the centre of the park ranges from a maximum of 34ºC and minimum of 17 ºC in November to a maximum of 25.0ºC and a minimum of 6.0ºC in July.

3.2 Methods for sample collection

The samples that were used for this research are:

- Faecal samples
- Forage samples
- Blood samples

3.2.1 Faecal sample collection

This study was blended into a bigger project that has been running from 2005. As a result faecal samples previously collected in 2006 was analysed together with those collected in 2009 so as to get results spanning for two years. In both cases, the method of collection and timing within season was the same.

In 2006 and 2009, faecal samples were randomly collected from zebra (non ruminant) and faecal samples from springbok (ruminant) in the central Okaukuejo section of Etosha National Park. During sample collection, animals were followed by a vehicle to waterholes using a pair of binoculars to watch individuals animal defecate and the faecal samples were immediately collected within 10 minutes of deposition. Collection from the ground was by hand and it was ensured that faecal sample contaminated was avoided. The samples were then deposited in a zip-top plastic bag. Soil contamination was avoided as much as possible because one of the analyses was to determine the amount of soil in the faecal sample.

Sample sizes for nutrition studies consist of ten pooled faecal samples were used from each species for each season. A pooled sample was composed of five individual samples collected from adults. Sampling took place in February/March (the wet season) and July/August (the dry season), for a total of two dry seasons (twenty samples from zebra and twenty samples from springbok) and two wet seasons (twenty samples from zebra and 20 samples from springbok). Sampling was conducted in 2006 and 2009. 2006 and 2009 were both wetter years than the annual average rainfall recorded at Okaukuejo (2006 had 582.2mm, 2009 had 535.7mm). Sample size for soil ingestion consist of five pooled faecal samples was used from each species for each wet (i.e five sample for zebra and five samples for springbok) and dry (i.e five sample for zebra and five samples for springbok). Sampling took place in February/March (the wet season) and July/August (the dry season) of 2009.

The population of zebra and springbok in Etosha is estimated to be about 13,200 zebra and 15,600 springbok. Since they are wild animals, it was not possible to take samples from all of the animals, so faecal samples were collected from opportunistically selected individuals.

3.2.2 Blood samples

Anthrax occurrence was diagnosed from blood swabs collected from fresh carcass of animals at the Central Veterinary Laboratory in Windhoek.

3.2.3 Vegetation sample collection

Samples of *Enneapogon desvauxii* utilized by zebra were collected by hand-clipping in a manner imitating the observed selection pattern of zebra. Samples were collected every month from October 2008 to November 2009. These samples were placed in a paper bag and air dried to prevent rotting and then taken to the laboratory for drying, grinding and analysis.

3.3 Laboratory Analysis

The unit of analysis consisted of:

- Nitrogen and crude protein
- Crude phosphorus
- Crude fibre
- Soil content in faecal samples

Laboratory analysis was carried out in the Ministry of Agriculture, Water and Forestry laboratory. Faecal and forage samples were oven-dried at 80ºC for 48 hours and grinding was done using a Knifetch Mill to a grain size of 1mm.

3.3.1 Nitrogen and Crude protein determination.

Grass and faecal samples were analysed for nitrogen and crude protein using the Kjeldahl method (Association of Official Analytical Chemists, 1990) Samples were digested with concentrated sulphuric acid in the presence of catalyst (selenium) that increases the boiling point of sulphuric acid. In the process, nitrogen present in the grass or faeces is converted to ammonia, which reacts with excess of acid to form Ammonium sulphate. During distillation, ammonia was liberated by adding sodium hydroxide to the digested sample, distilled off and collected in excess boric acid as ammonium borate. Then titrating the ammonium borate formed with 1M hydrochloric acid (Association of Official Analytical Chemists, 1990).

Crude protein in the samples was calculated from nitrogen present in the protein, according to the Kjeldahl procedure (Association of Official Analytical Chemists, 1990). Two assumptions are made in calculating the protein content from the nitrogen: firstly, that all the nitrogen of the samples is present as protein and secondly that all protein samples contain 16% nitrogen. Hence nitrogen content was multiplied by 6.25(100/16) to give the crude protein %.

3.3.2 Analysis of Phosphorus.

Phosphorus concentration in solution of digested sample of faeces and forage was determined spectrophotometrically by using a phosphovanado-molybdate complex (i.e. addition of phosphorus, HCL, and ammonium) (Association of Official Analytical Chemists, 1990).

3.3.3 Analysis of crude fibre

Fibre is the part of cell-wall of a plant that can not be digested by the enzymes in the intestine. Crude fibre was determined gravimetrically, after chemical digestion and solubilisation of other compounds present (i.e. protein starch and other digestible/soluble carbohydrates) with diluted sulphuric acid and sodium hydroxide (Goering and Van Soest 1970). The fibre mass was corrected for ash content after ignition.

3.3.4 Method for estimating the concentration of silicates in faeces

This method is adopted from the incineration acid wash (ICW) procedure for determining the crude silica content of soil (Hesse, 1971) based on the removal of organic material by high temperature incineration and dissolving inorganic materials in concentrated acid, leaving a residue of acid-insoluble inert silicates.

This procedure has been developed and standardized to ensure optimal efficiency (Wong, *et al.,* 1988). One gram of faeces was dried to constant weight at 105ºC, and then incinerated in a muffle furnace at 800°C for 1 hour. The ash was cooled in a desiccator, covered with 6M HCI for 12 hours, then filtered under vacuum on to Whatman 541 filter pads in a Millipore apparatus. The residue of inert silicates was then dried to constant weight under desiccation at 50°C.

3.4 Statistical Analysis.

Data were analysed using analysis of variance (ANOVA) and were handled with statistical analytical system software (SAS, 2004). Two ways ANOVA was used to examine the effect of seasonality on the nutritional status of zebra and springbok and effect of seasonality on soil ingestion of springbok and zebra. Where significant difference was noted means were separated using Duncan's multiple range test (SAS, 2004). Correlation analysis was also carried out to describe the relationship between soil ingestion and anthrax occurrence (SAS, 2004).

Statistical analysis was not carried out on chemical composition of *Enneapogon desvauxii* and rainfall data their relationship was done using graphs.

CHAPTER FOUR

RESULTS

4.1 Effect of seasonality on the nutritional status of zebra and springbok.

4.1.1 Faecal Crude Protein (CP)

There was significant difference in faecal CP between year 2006 and 2009 (F=14.2, N=40, P<0.0001). Faecal CP was significantly higher in 2006 (9.96±0.15) compared to 2009 (8.91±0.15). There was significant difference in faecal CP between seasons (F=113.22, N=60, P<0.0001). Faecal CP is significantly higher (P<0.05) in wet seasons (10.23±0.39) compared to dry seasons (8.45±0.39). Faecal CP was significantly higher in springbok compared to zebra (F=480.32, N=60, P<0.0001, springbok=11.17±0.39, zebra=7.51±0.39). There was significant difference (F=14.97, P<0.0002) in faecal CP between year and species. Faecal CP in springbok and zebra were significantly higher in 2006 compared to 2009 (Figure 4.1).

Faecal CP differed significantly between springbok and zebra in both wet and dry season of 2006 (wet; F=64.89, N=10, P= <0.0001 dry; F=104.56, N=10, P<0.0001). Springbok had significantly higher CP than zebra. Similarly faecal CP was significantly higher in springbok than zebra in both seasons of 2009 (wet; F=82.10, N=10, P<0.0001, dry; F=119.40, P<0.0001). Springbok had higher concentration of CP than zebra in all seasons (Appendix 1 and figure 4.2).

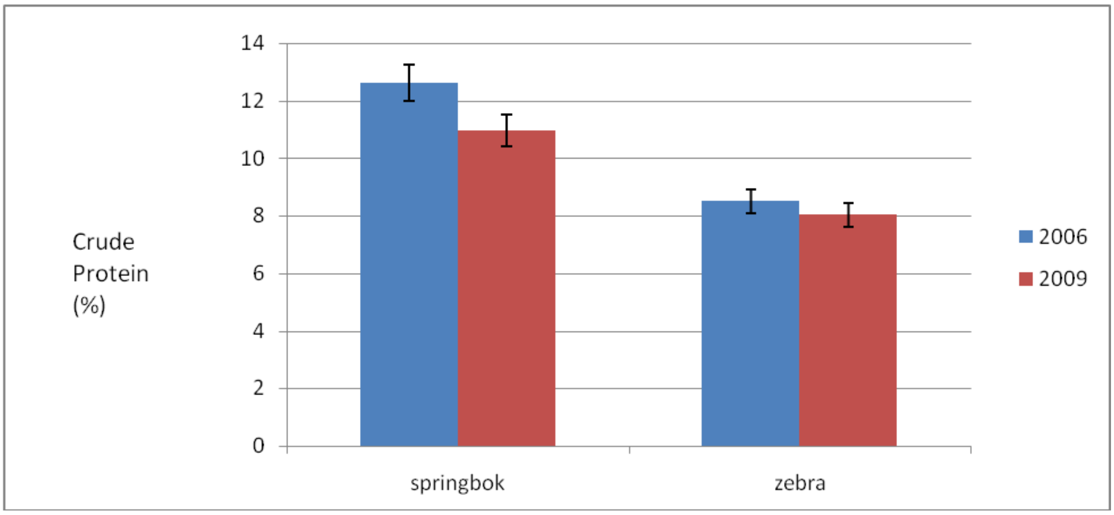

Figure 4.1 Comparison of mean difference of faecal CP between year and species. Bars indicate standard errors (SE).

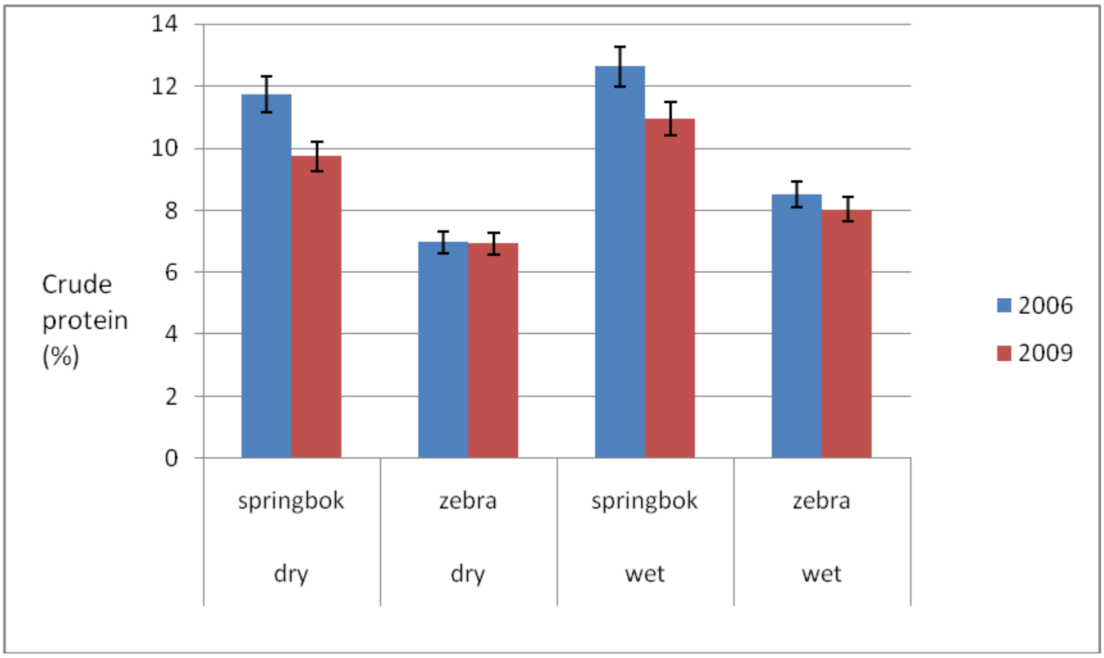

Figure 4.2 Comparison of mean difference of faecal CP between year, species and season. Bars indicate standard errors(SE).

4.1.2 Faecal Crude fibre (CF)

There was a significant difference (P<0.05) in faecal CF between years (F=25.77, N=40, P<0.0001). Faecal CF was significantly higher in 2009 (30.56±0.46) compared to 2006 (28.10±0.46). There was significant difference (F=74.45, N=60, P<0.0001) in faecal CF between seasons: the dry season (30.51±1.21) was significantly higher compared to the wet season (25.93±1.21). Faecal CF was significantly higher in zebra compared to springbok (F=106.69, N=60, P<0.0001, zebra=30.96±1.21 and springbok=25.47±1.21). There was no significant difference in faecal CF between year and season (F=0.18 and P=0.67). There was no significant difference (F=2.31 and P=0.13) in faecal CF between year and species. Faecal CF in springbok and zebra were significantly lower in 2006 compared to 2009 (Figure 4.3).

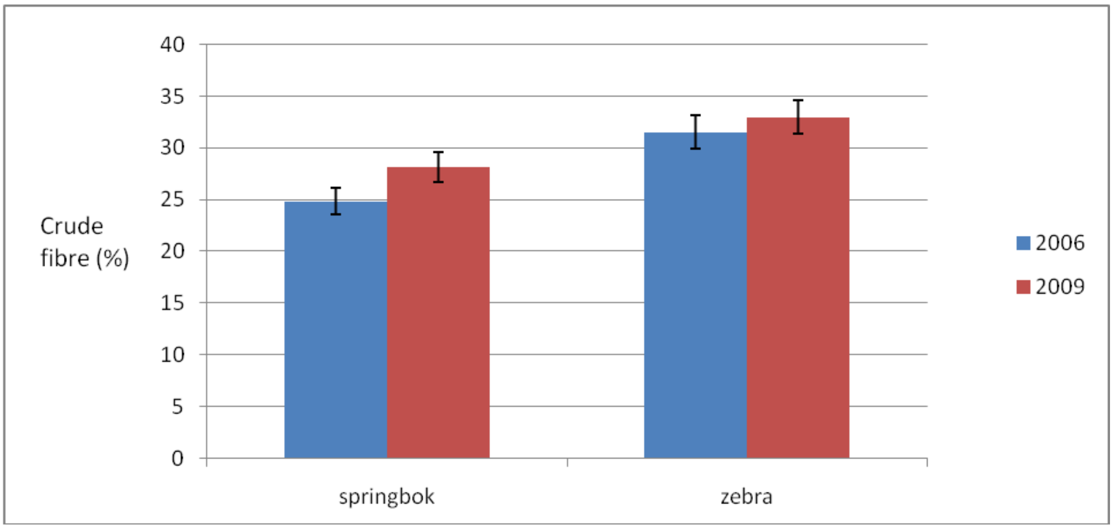

Figure 4.3 Comparison of mean difference of faecal CF between year and species. Bars indicate standard errors (SE).

Faecal CF was significantly higher in zebra than springbok in both wet and dry seasons of 2006 (wet; F=26.74, N=10, P<.0001 dry; F=104.56, N=10, P<0.0001). Feacal CF was significantly higher in zebra than springbok in wet season of 2009 (wet; F=32.01, N=10, P<0.0001). A non significant difference in Faecal CF between springbok and zebra in dry season of 2009 (dry; F=3.90, P=0.06) was observed (Appendix 1 and figure 4.4).

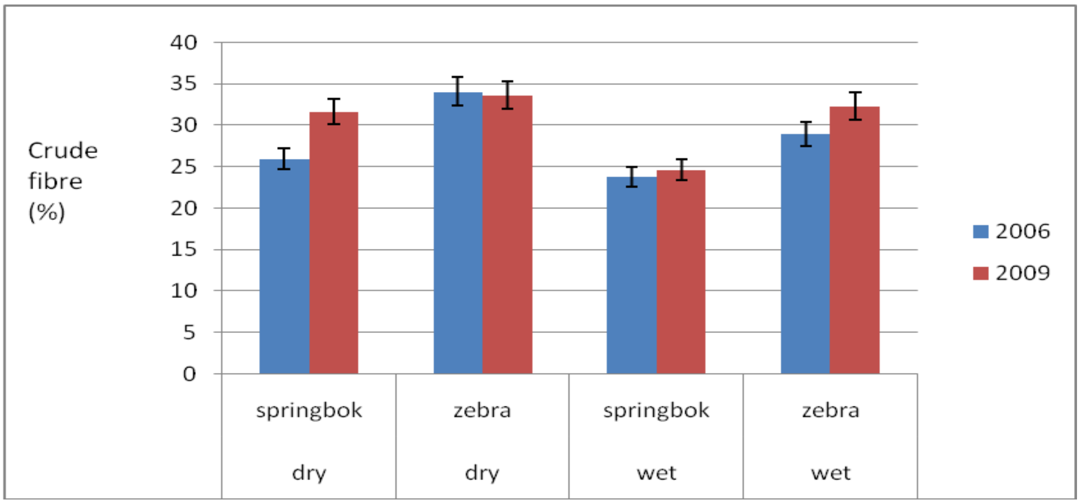

Figure 4.4 Comparison of mean difference of faecal CF between year, species and season. Bars indicate standard errors (SE).

4.1.3 Faecal Phosphorus (P)

There was significant difference in faecal P between years (F=2.16, N=40, P=0.006). Faecal P was significantly higher in 2006 (0.36±0.030) compared to 2009 (0.32±0.030). There was significant difference in faecal P between seasons (F=93.94, N=60, P<0.0001). Faecal P is significantly higher in the wet season (0.42±0.023) compared to dry season (0.25±0.023). Faecal CF significantly higher (F=20.33, N=60, P<0.0001, springbok=0.38±0.023, zebra=0.29±0.023) in springbok compared to zebra There was no significant difference in faecal P between year and species (F=2.21 and P=0.14). In both animals faecal P was significantly higher in 2006 compared to 2009 (Figure 4.5).

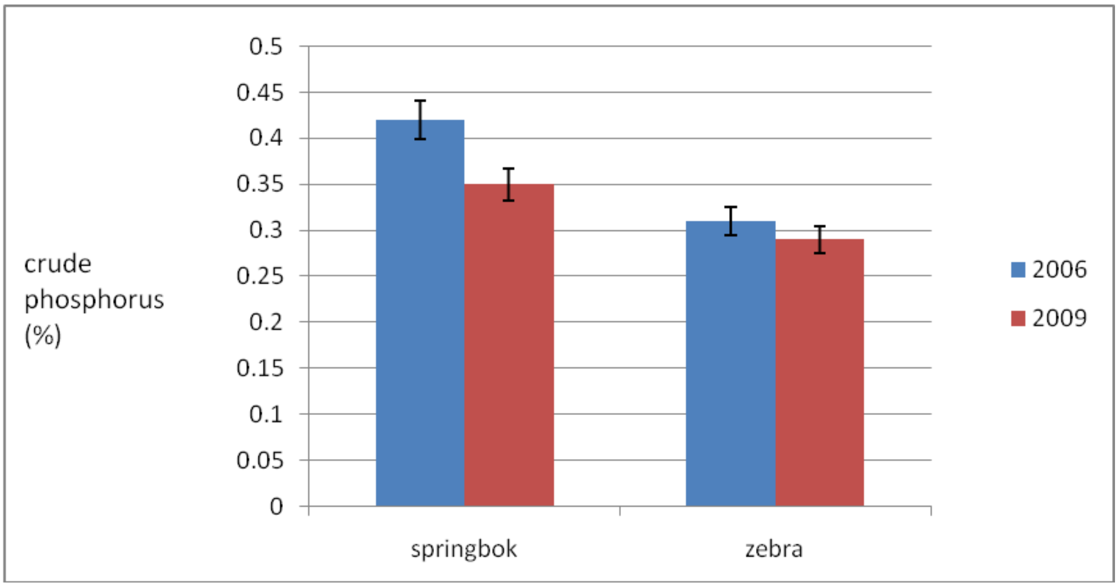

Figure 4.5 Comparison of mean difference of faecal CP between year and species. Bars indicate standard errors (SE).

Faecal P was significantly higher in springbok than zebra in 2006 (wet; F=4.60, N=10, P= <0.046 dry; F=20.45, N=10, P=0.0003) and 2009 (wet; F=32.01, N=10, P=0.03, dry; F=3.90, P=0.03) in both seasons. (Appendix 1 and figure 4.6).

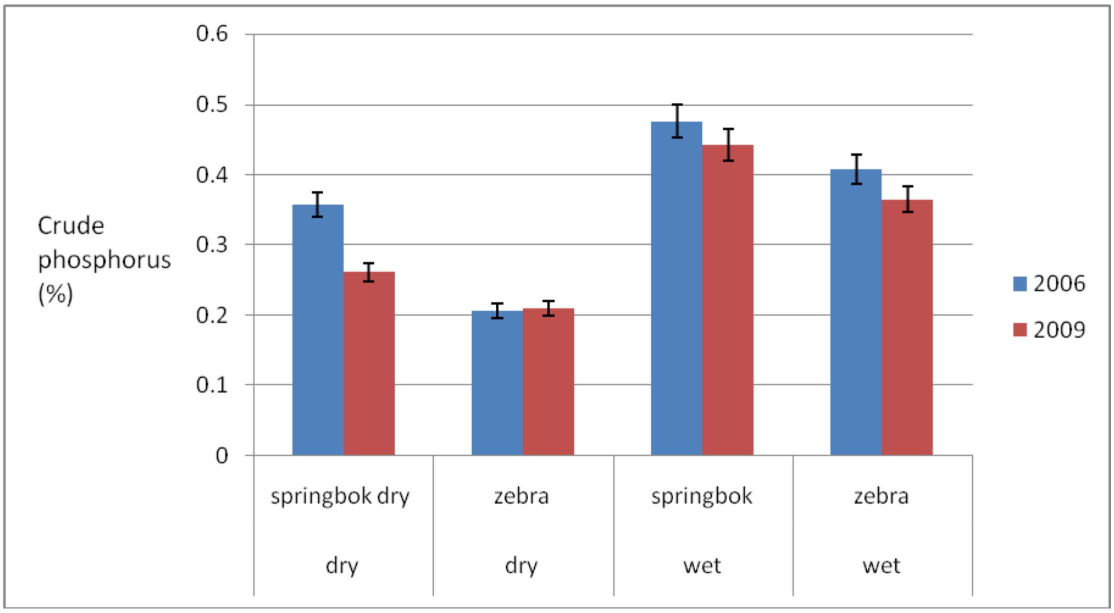

Figure 4.6 Comparison of mean difference of faecal P between year, species and season. Bars indicate standard error (SEs).

4.2. Effect of seasonality on soil ingestion of zebra and springbok and it relationship with anthrax occurrence.

Crude silicate was significantly higher in zebra than springbok (F=39.05, P=<.0001 and N=10) in both seasons (Figure 4.7). Crude silicate was significantly higher in wet than dry season (F=87.35, N=10, P= <.0001 in both species (Figure 4.7). There is a positive correlation (r=0.33, P<0.05) between seasonal faecal silicate content and anthrax occurrence (Appendix 2 and 3).

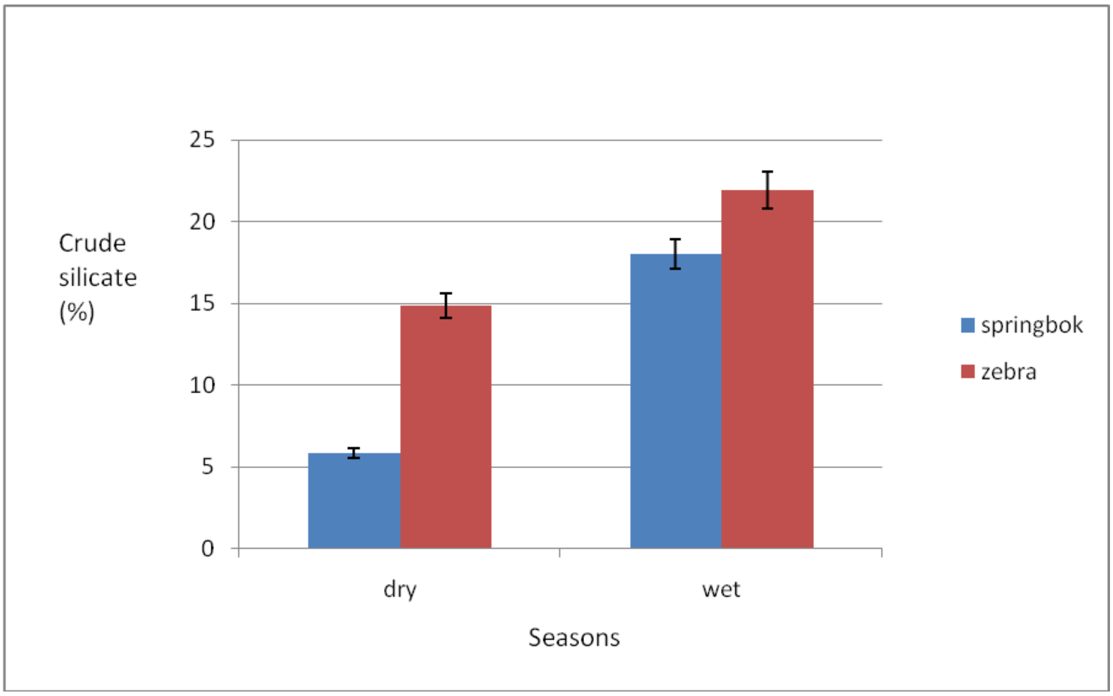

Figure 4.7 Comparison of mean difference of faecal Crude Silicate between species and season. Bars indicate standard errors (SE).

4.3 Chemical composition of *Enneapogon desvauxii* and it relationship with rainfall.

4.3.1. Plant Crude Protein

From figure 4.8., it is evident that crude protein and rainfall were higher in November-March (wet season) while crude protein and rainfall were low in April-October (dry season).

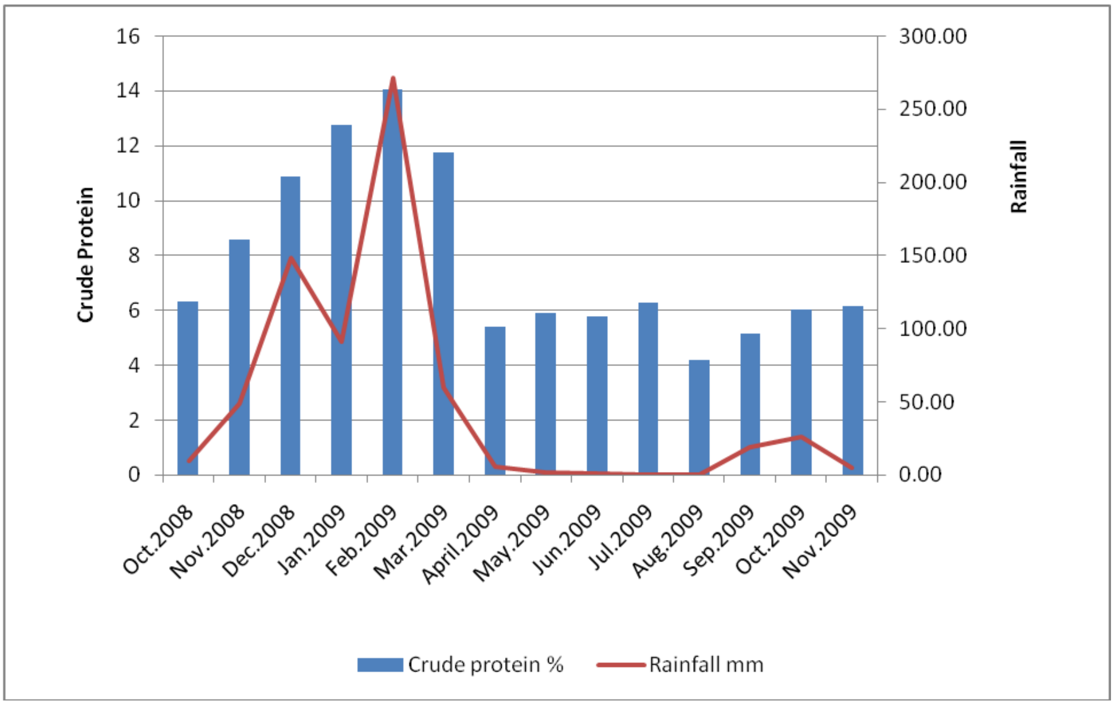

Figure 4.8 Plant crude protein and rainfall of different months in 2008-2009.

4.3.2 Plant Phosphorus

It can be observed from figure 4.9. that phosphorus fluctuated throughout the year. Peaks, however occurred during the months of November-March (wet season). This is the period of highest precipitation in Etosha. Phosphorus concentration becomes low during the months of April, June, July, August and October which is during dry season.

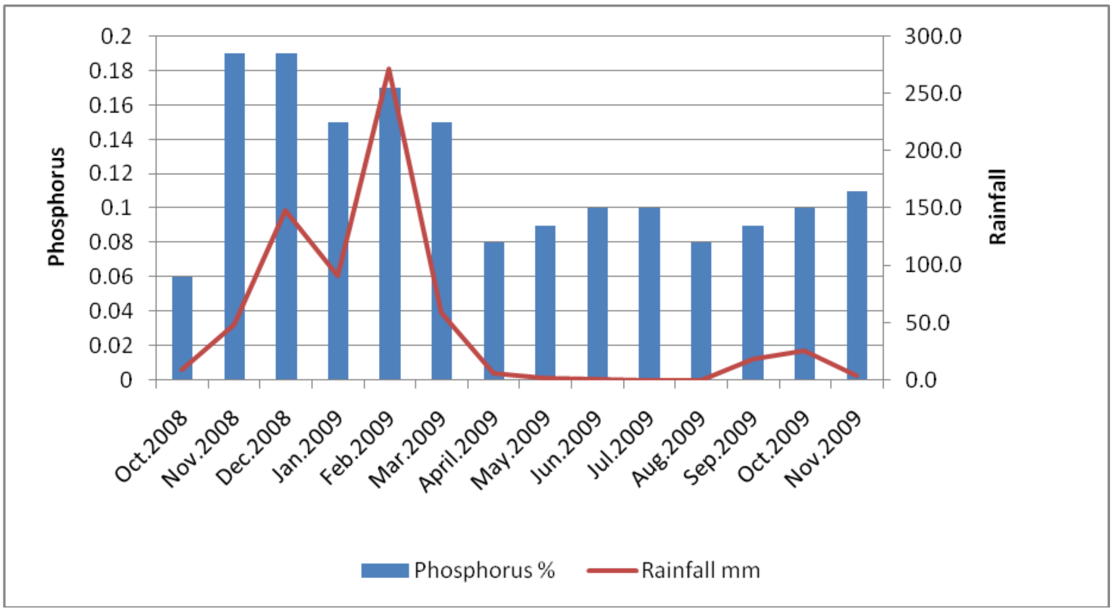

Figure 4.9 Plant crude phosphorus and rainfall of different months in 2008-2009.

4.3.3. Plant crude fibre

Figure 4.10 depicts the crude fibre content on monthly basis throughout the year. It can be observed from the figure that the fibre content of *E. desvauxii* has no strong seasonal pattern. Percentage crude fibre varied from 29.51% during the month of January to 39.94 during the month of December.

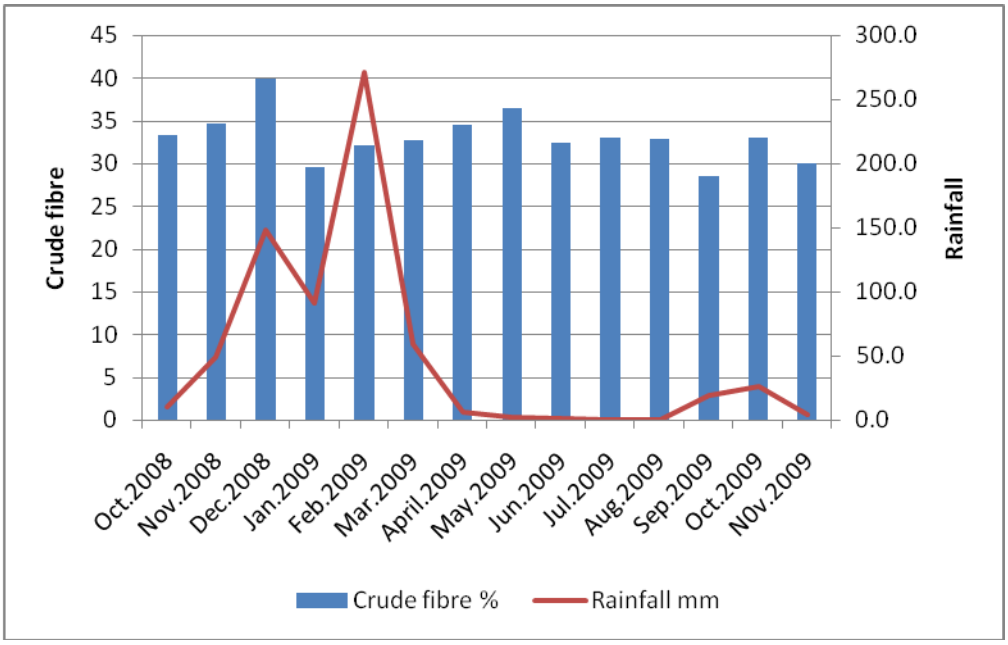

Figure 4.10 Plant crude fiber and rainfall of different months in 2008-2009.

CHAPTER 5

DISCUSSION

5.1 Effect of seasonality on the nutritional status of zebra and springbok.

5.1.1. Faecal Crude Protein (CP)

Year 2006 had significantly higher crude protein content compared to 2009. The reason for 2006 having the higher crude protein could be because there was high rainfall in that year since Erasmus and Penzhorn (1978) confirmed that crude protein content follows rainfall pattern.

Springbok had a higher CP content compared to zebra in all seasons. This observation is similar to that of Mbatha (2008). This observation could be due to the fact that springbok is a mixed-feeder with a small mouth and can easily select forage with higher nutritive value compared to zebra. Another explanation could be that the digestive system of zebra is less efficient than that of springbok, but in compensation the zebra eats greater quantities of food (Van Hoven, 1983). Additionally, springbok can switch to high-quality browse when grass quality is low in the dry season (Bigalke 1972).Therefore springbok (a mixed feeder) had higher CP level than zebra (a grazer) in this study.

5.1.2 Faecal Crude Fibre (CF)

Faecal CF was significantly higher in 2009 compared to 2006. High fibre content tends to be associated with low protein content and vice versa (Erasmus and Penzhorn, 1978).

Zebra had significantly higher CF level than springbok in both seasons of 2006, and wet of 2009. The significant difference between zebra and springbok could be due to the fact that zebra eats less selectively than springbok and therefore consume more grasses that are high in fibre (Erasmus and Penzhorn, 1978). Another explanation is that springbok being a ruminants can digest cellulose and hemicelluloses because of microbial action in the rumen while zebra cannot digest cellulose and hemicelluloses because they only have hydrolytic digestion (Erasmus and Penzhorn, 1978). A non significant difference in faecal CF between springbok and zebra in the dry season of 2009 could be due to the fact that both animals consumed forages that are high in fibre because there was relatively high rainfall in this year which encourages grass growth that leads to high moribund grass (Scholes, 1997).

5.1.3 Faecal Phosphorus (P)

Year 2006 had significantly higher crude phosphorus content compared to 2009. The reason behind this could be that there was higher rain fall in 2006 compared to 2009 since Bothman, (1989) stated that phosphorus appears to be present in higher levels when rainfall is high.

Faecal P was significantly higher in springbok than zebra in 2006 and 2009 in both seasons. This result is in agreement with Mbatha (2008), who also observed that springbok had the higher faecal phosphorus than that of zebra. Springbok had higher phosphorus because they are capable of selecting plants (shrubs and trees) high in phosphorus throughout the year (Meissner et al. 1996). This is in agreement with Bothman (1989) and McDonald *et al*. (1996); who observed that phosphorus appears to be present in higher levels when rainfall is high. Rainfall can affect the mineral composition of grasses (McDonald *et al*., 1996).

5.2 Faecal silicate differs between seasons and species within the year.

The results indicate that zebras had a significantly higher silicate (soil) value compared to springbok. This implies that zebra ingested more soil compared to springbok. Wong *et al.,* (1999) stated that the rate of silica excretion is proportional to the rate of ingestion. Zebras may have ingested more soil because they are mainly grazers (McClintock, 1976), they grazed very close to the ground and in the process ingest more soil, while springboks tend to have more selective diets, and are mixed feeders (Churcher, 1993) and ingest less soil. Results indicate that there is an association between the season of increased faecal silicate ingested and anthrax occurrence. Thus, the diet selection of zebra and springbok may indicate that zebra have a higher rate of contact with *B. anthracis* than springbok. Grazing species (i.e.

zebra) feed closer to the soil level more often than do browsing or mixed feeding species (i.e. springbok) feeding on trees and shrubs. Ingestion of soil may expose animals to anthrax as grazing is the frequent mode of uptake of soil (WHO, 2008). This result is in agreement with Lindeque and Turnbull (1994); they observed that there is higher incidence of anthrax in zebra than springbok.

The results show that the wet season had significantly higher crude silicate compared to dry season in both animals. Animals must have ingested more soil in the wet season than in dry season. This could be why anthrax occurrence peaks in the wet season (Lindeque and Turnbull 1994).

The major goal of this study was to quantitatively approach the hypothesis of a direct correspondence between silica content of feaces and the incidence of anthrax. The positive correlation ($r^2=0.33$, $P<0.05$) between these traits was true for both seasons, and these results suggest a considerable influence of ingested silica on anthrax. It could be that as faecal silicate content increases anthrax occurrence increases or as faecal silicate decreases anthrax occurrence decrease.

5.3 Nutrient content of *Enneapogon desvauxii* and it relationship with rainfall.

5.3.1 Plant crude fibre

Crude fibre did not show strong seasonal difference the reason behind this could be that E. desvauxii is such a shot grass, and never builds up much fibre as other tall grasses that need fibre for structural support. This could explain why there is no much of a seasonal pattern in this one grass species examined.

5.3.2 Plant phosphorus

Rainfall can affect the mineral composition of grass (McDonald *et al.*, 1996). Phosphorus concentration in the grass fluctuated throughout the year. Peaks , however occurred during the months of Novermber, December, January, February, and March (wet season). This is the period of highest precipitation in Namibia. Phosphorus concentration becomes low during the months of April, June, July, August and October which is during dry season. This result is in agreement with Bothman, (1989) and McDonald *et al*., (1996). NRC (1997) gave dietary phosphorus recommendations of 0.10-0.44% for springbok, and 0.15-0.34% for zebra. In this study phosphorus concentration of *E. desvauxii* fluctuated between 0.06% for drier months-0.19% for the wet months. Interestingly, *E. desvauxii* had insufficient levels of P in both seasons

5.3.3 Plant crude protein

Highest dietary crude protein was from January to March (wet season). The CP content was higher in the wet season because of more regrowth available than during the drier months. This result is in agreement with Bodenstein, *et al*., (2000) they observed that dietary crude protein content follows rainfall patterns. The dietary crude protein requirements for maintenance of adult mammals range from 5.5-9% for wild ruminants (Van Soest, 1982), and the dietary crude protein in this study range from 4.19 in the dry season to 12.74 in the wet season.

5.4 Relating diet nutritional content to faecal nutritional content and rainfall.

Springbok and zebra eat different types of forages, but in this study data is only available for the main grass eaten (*Enneapogon desvauxii)* by zebra. It appear that the protein content of the faeces reflects the protein content of the diet and protein content of diet follows rainfall pattern, this result is in agreement with (Wrench *et al.,*1997).

The result shows that phosphorus content of the diet is higher in the wet season. This result is in agreement with Bothman, (1989) and McDonald *et al*., (1996). Also diet P reflects feacal P (Karn et al. 1997).

5.5 Seasonality of nutrition and soil ingestion in relation to anthrax occurrence.

Faecal CP and P in springbok were compared to zebra in both seasons, and springbok had a lower faecal silicate levelcompared to zebra in both seasons. Faecal silicate was also higher in the wet season compared to the dry season.

The faecal CP of springbok range from 9.74 – 12.62% in most of the study area while that of zebra is between 6.92-8.51%. Faecal CP higher than 6-8% is required to maintain rumen fermentation (Grant *et al*. 1995). Faecal CP is higher than the critical value of 6% in all the seasons (wet and dry). The faecal P of springbok ranges from 0.21 – 0.41% in most of the study area while that of zebra ranged 0.26- 0.41 %. Faecal phosphorus below 0.2% is considered to be indicative of phosphorus limitation (Dorgeloh *et al*. 1998). In this study faecal phosphorus was above the critical value of 0.2%. This implies that these animals are getting enough nutrients from the forage they are consuming in Etosha National Park. It also means that the nutrients they are getting are likely sufficient to support their immune systems in either season.

Anthrax occurrence in the wet season is unlikely to be as a result of nutritional stress. However, if the soil is saturated with water, it may be easier for grazers to pull up root with soil on the root while grazing. Soil may also splash onto grasses during the rainy season, since the types of grasses found in Etosha National Park are very short. This result is not in agreement with Dragon *et al*. (1999) and Hugh-Jones and de Vos (2002) who observed that anthrax occurs in dry seasons, which they suggest is because animals are nutritionally stressed, and because of decrease in forage nutrient and water resource. Low nutrition may not be the cause of anthrax occurrence in Etosha National Park because nutrition is high enough to support healthy animal population in both seasons, but their mode of grazing might have something to with the way animals contact anthrax, and the seasons in which they do so.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

From the results of this study, it can be concluded that:

Springbok or zebra feeding style was reflected in the silica content of their faeces. The major goal of this study was to quantitatively approach the hypothesis of a direct correspondence between silica content of ingested material and incidence of anthrax. There may be higher incidence of anthrax in zebra than springbok**,** because zebra ingested more soil content compared to springbok. The results show that the wet season had significantly higher crude silicate compared to dry season. This implies that the animals ingested more soil in wet season than in dry season. Result show that there could be an influence of ingested soil on anthrax seasonality.

Springbok had significantly higher faecal crude protein and phosphorus than zebra, while zebra had higher faecal crude fibre compared to springbok. The difference in chemical composition of springbok and zebra faeces seems to reflect a real difference in the type of feed eaten by each species. Chemical composition of faeces can be used as an additional indicator of the type of forage available to each species at different times of the year. It can also be stated that low nutrition may not be the cause of anthrax occurrence in Etosha National Park because nutrition is high enough to support healthy animal population in both seasons, but their feeding style may have something to do with the way animals contact anthrax.

The protein and phosphorus content of the faeces reflects the protein and phosphorus content of the diet. Protein content of diet follows rainfall seasonality.

There is similarity between some of the chemical components of the feed when compared with the faeces but the degree and nature of this similarity/relationship needs to be quantified in future, including more species present in their diets.

I recommend that:

Faecal analysis should be used as an adjunct conventional vegetation monitoring method to determine whether an area will fulfil the nutritional requirements of different herbivores.

When monitoring seasonal changes in herbivores, faecal crude protein and faecal phosphorus need to be measured.

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APPENDICES

APPENDIX 1: Mean faecal crude protein, crude fibre and phosphorus of springbok and zebra in wet and dry season within years.

			Animal				
Year	Variable	Season	Springbok	Zebra	LSD	Fr	Pr
2006	CP	Wet	$12.62\pm0.35^{b}_{A}$	$8.51\pm0.37^{b}_{B}$	1.07	64.89	<0.0001
		Dry	$11.73\pm0.33^{a}_{A}$	$6.97\pm0.\ 33^{a}_{B}$	0.98	104.56	<0.0001
	P	Wet	$0.48\pm0.02^{a}_{A}$	$0.41\pm0.02^{a}_{B}$	0.07	4.60	0.46
		Dry	$0.36\pm0.03^{b}_{A}$	$0.21\pm0.02^{b}_{B}$	0.07	20.45	0.0003
	CF	Wet	$23.76\pm0.45^{b}_{B}$	$28.9\pm0.91^{b}_{A}$	2.13	26.64	<.0001
		Dry	$25.95\pm0.49^{a}_{B}$	$34.01\pm1.31^{a}_{A}$	2.95	104.56	<.0001
2009	CP	Wet	$10.96\pm0.23^{a}_{A}$	$8.03\pm0.23^{b}_{B}$	0.90	82.10	<.0001
		Dry	$9.74\pm0.17^{b}_{A}$	$6.92\pm0.20^{a}_{B}$	0.54	119.40	<.0001
	P	Wet	$0.44\pm0.03^{a}_{A}$	$0.37\pm0.02^{a}_{B}$	0.07	5.90	0.03
		Dry	$0.26\pm0.02^{b}_{A}$	$0.21\pm0.01^{b}_{B}$	0.05	5.70	0.03
	CF	Wet	$24.35\pm0.85^{b}_{B}$	$32.35\pm1.07^{a}_{A}$	2.87	32.01	<.0001
		Dry	$31.66\ \pm0.73^{a}_{A}$	$33.62\pm0.68^{a}_{A}$	2.10	3.90	0.06

Means with common scripts (row - A, B) and (.column- a, b) for each variable do not differ significantly (P>0.05).

APPENDIX 2: Correlation between silicate ingestion and anthrax in zebra and springbok.

Variable	r	Probability
Faecal silicate x anthrax outbreak	0.33	0.0010

Variable	(r)wet	(r)dry	(P)wet	(P)dry	Probability
Faecal silicate x anthrax outbreak	0.34	0.17	0.02	0.23	

Variable	(r) springbok	(r)zebra	(P)springbok	(P)zebra
Probability				
Faecal silicate x anthrax outbreak	0.22	0.37	0.15	0.0075

APPENDIX 3: Silicate content of the faeces of zebra and springbok with season in 2009.

			Season				
Year	Variable	Animal	Wet	Dry	LSD	Fr	Pr
2009	Silicate	Zebra	$21.94\pm0.30^{a}_{A}$	$14.85\pm1.05^{b}_{A}$	2.52	42.12	0.0002
		Springbok	$18.05\pm1.15^{a}_{B}$	$5.82\pm1.33^{b}_{B}$	4.05	48.56	0.0001
		LSD	2.74	3.90			
		Fr	10.72	28.48			
		P	0.0113	0.007			