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Effects of dietary magnesium supplementation on physiological parameters in captive cheetahs (*Acinonyx jubatus*) at Hoedspruit Endangered Species Centre (HESC)

by

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B.Sc (Agric) Animal Science (Pret.)

Submitted in partial fulfillment of the requirements for the degree

Magister Scientiae Agriculturae

(Production Physiology)

In the Faculty of Natural and Agricultural Sciences

Department of Animal and Wildlife Sciences

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Promoter: Prof. E.C. Webb

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DECLARATION

I, Johan Grobler, declare that the dissertation: **"Effects of dietary magnesium supplementation on physiological parameters in captive cheetah (*Acinonyx jubatus*) at Hoedspruit Endangered Species Centre (HESC)"** which I hereby submit for the degree M.Sc (Agric) Production Physiology at the University of Pretoria, is my own work and that all the sources that I used or quoted have been indicated with complete reference and acknowledgements. This dissertation has not previously been submitted by me for a degree at this or any other tertiary institution.

JOHAN GROBLER **DATE**

DEDICATION

To Lenaldi and my family, for your love and support which fuelled my commitment to this journey and whose involvement motivated me to the end.

ACKNOWLEDGEMENTS

- Most importantly my Heavenly Father who blessed me with the privilege to pursue my studies.
- My study leader, Professor Webb for your creativity and constant support to stay on course.
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- Me. Lente Rhoode and the HESC for financially sponsoring this study.
- The two veterinarians Dr. Brett Gardner and Dr. Greg Simpson and all the other people who helped and guided me along the way.

ABSTRACT

Effects of dietary magnesium supplementation on physiological parameters in captive cheetah (*Acinonyx jubatus*) at Hoedspruit Endangered Species Centre (HESC)

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The last 50 years was characterized by a dramatic decrease in free-roaming cheetah populations and consequently the cheetah now appears on the IUCN Red List for Threatened Species. In order to save cheetahs from extinction, a number of projects were launched to breed cheetahs in captivity. Captive cheetahs, however, receive fundamentally different diets than their free-roaming counterparts, which necessitates feed supplementation to fulfill their unique dietary needs.

The Hoedspruit Endangered Species Centre (HESC) is one such project aiming to breed captive cheetahs. At the HESC, a number of juvenile cubs were diagnosed with a form of relaxed carpal joints, namely metacarpal deformity of the front legs. Literature suggests that the condition is due to a magnesium deficiency, which is a consequence of an unbalanced diet. Supplementing magnesium to the diet of cheetahs can, however, affect the urinary system negatively: such as the formation of urolithiasis.

The aim of this study was to determine whether dietary magnesium supplementation in the diets of captive cheetahs will remedy metacarpal deformity and also to investigate the influence of magnesium supplementation on the formation of urolithiasis.

The study was divided into two phases. Phase 1 was conducted to determine the influence of dietary magnesium supplementation on metacarpal deformity, identified in juvenile cheetahs at the HESC. To determine the degree of deformity, a leg deformity scoring system was developed. On a scale from 1-3, the cheetahs were scored twice to determine the Flexed Deformity Score (FDS) and Rotational Deformity Score (RDS) values before and after dietary magnesium supplementation.

Phase 2 was conducted to determine the influence of magnesium supplementation on different physiological parameters that have an influence on the formation of urolithiasis. Phase 2 was divided into three periods. During each period, the cheetahs received a different diet. During period C, the experimental period, the cheetahs were divided into two groups. One group received a meat-only diet, whereas the other group received a meat-Mg diet. At the end of each of the three periods, blood- and urine samples were collected and analyzed to determine the concentration of minerals in the cheetah's blood plasma and urine.

Based on the FDS and RDS scores, a 25.5% response rate to dietary magnesium supplementation on rotational deformities was found, whereas a 60.8% response rate on flexural deformation was found. It is thus concluded that dietary supplementation of magnesium in juvenile cheetahs that experience metacarpal deformities, will remedy the deformity.

By analyzing the changes of different blood- and urine parameters in the cheetahs it was observed that dietary magnesium supplementation do influence the formation of urolithiasis. The physiological state of the cheetahs can influence these parameters. The results obtained from the study can be utilized by nutritionists, veterinarians and institutions to enhance the health of captive cheetahs.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

A sharp decrease in the number of Cheetah's that is roaming free in its natural habitat was noted. This decline is ascribed to the following factors:

- Loss of habitat due to an increasing commercial agricultural sector that has to provide food for a fast-expanding human population
- Competition with other larger predators as hunting areas are decreasing
- Low viable sperm counts of male cheetahs
- Killing by farmers as cheetahs are often wrongly accused of killing livestock
- High mortality rates of young cubs.

In order to save the Cheetah from extinction, a number of projects were launched o breed cheetahs in captivity. Captive breeding of cheetahs is a difficult and complex process and is characterized by low fecundity of females, poor semen quality in males, reduced growth rates, management problems, behavior problems and dietary complications (Meltzer, 1987). The latter factors have led to reduced rates of reproductive success in captive cheetah populations across the world (Bechert *et al.* 2002). In order to breed cheetahs successfully in captivity, a better understanding of these limitations is necessary. Conservation managers need to continuously improve their knowledge through research, as well as change management practices in order to save the cheetah from extinction.

1.2 PROBLEM STATEMENT

A number of juvenile cheetah cubs at the Hoedspruit Endangered Species Centre (HESC) were identified with deformities in their front legs. By closer visual inspection, these juvenile cubs were diagnosed with a form of relaxed carpal joints or metacarpal deformities in their front legs. According to literature, mainly dogs and horses exhibit this condition. It is suggested that the condition is due to an unbalanced diet especially with regards to magnesium, calcium and phosphorus and their relationship with one another.

It is well documented that minerals in the body interact with one another, as illustrated in Figure1.1 (Vieira, 2008). Thus, when supplementing any mineral to a diet, one has to be very careful not to disturb other processes which can have detrimental effects on the animal's health. The urinary system is especially vulnerable to an excess or deficiency of magnesium, calcium and phosphorous which can lead to various urinary track conditions.

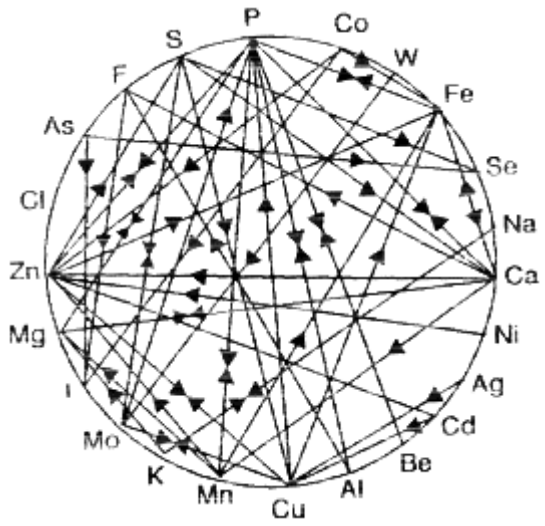


Figure 1.1 Mineral Interactions (Vieira, 2008)

For this reason, it is necessary to monitor the levels of other minerals and parameters when studying dietary supplementation. This is to ensure that by supplementing minerals to treat leg deformity, one does not induce another condition that can negatively affect the cheetah's health.

1.3 AIM OF STUDY

The aim of this research study was to establish whether dietary magnesium supplementation of growing cheetahs will remedy metacarpal deformity without inducing urolithiasis. The research was sub-divided into two parts:

(a) To investigate the possibility that dietary supplementation of magnesium in juvenile cheetah's that experience metacarpal deformities in their front legs, will correct the deformity.
(b) Since magnesium is not usually added to a feline's diets due to interactions with calcium and phosphorous, it is critical to carefully monitor the physiological parameters associated with the formation of urolithiasis as described in domestic cats. By monitoring these parameters, the aim was to investigate the influence of dietary supplementation of magnesium on the formation of urolithiasis

1.4 RELEVANCE OF THE STUDY

1.4.1 Taxonomy of the cheetah

The taxonomy of the cheetah is described by Smithers (1983) as follows:

Domain	*Eukaraya*
Kingdom	*Animalia*
Phylum	*Chordata*
Order	*Carnivora*
Family	*Filidae*
Subfamily	*Felinae*
Genus	*Acinonyx*
Specie	*Jubatus*

The cheetah is on the IUCN's (International Union for Conservation of Nature) Red List for Threatened Species listed as *Vulnerable.* Species are listed as vulnerable when "the best available evidence indicates that it meets any of the criteria A to E for Vulnerable and it is therefore considered to be facing a high risk of extinction in the wild." Criteria A to E are listed below (Anon, 2010):

A. Reduction in population size

B. Reduction in geographic range

C. Population size estimated to number fewer than 10,000 mature individuals

D. Population very small or restricted

E. Quantitative analysis showing the probability of extinction in the wild is at least 10% within 100 years.

According to the IUCN, the known number of adult cheetahs is approximately 7,500 animals. In areas where their population status is poorly known, it was estimated that there were approximately 2500 animals. This amounts to an approximate total of 10,000 free ranging, adult cheetahs left worldwide. In 1975, on the African continent alone, the cheetah population was estimated at approximately 15,000 adult animals. In 1995, this number has decreased to 4,500 free ranging cheetahs. It is an approximate 30% decline in free ranging cheetahs on the African continent over a period of 20 years (3 generations). Although more recently updated numbers are still unavailable, the rate at which the adult cheetah population is decreasing on the African continent alone, without doubt foreshows detrimental effects for the Cheetah specie. One can clearly see the vulnerable state the Cheetah finds itself in (Anon, 2010).

In order to support and increase the worldwide cheetah population, cheetahs are bred in captivity at various institutions, Zoo's and breeding stations across the globe. In terms of nutritional needs, this is however a daunting task which will be discussed in further details in Chapter 2. There is an increasing need among animal nutritionists to develop a Total Mixed Ration (TMR) for captive bred cheetahs which will meet all the specialized nutritional requirements cheetahs has.

This study will aim to extend the knowledge of the role that some minerals play in the health of cheetahs. This will enable nutritionists to get one step closer to the formulation of a complete, well balanced cheetah TMR.

1.5 STRUCTURE OF THE DISSERTATION

This dissertation is divided into the following six chapters:

Chapter 1 – General introduction

Background information and the problem statement is given. It also explains the relevance of the study in the broader scientific field.

Chapter 2 – Feeding Practices of Cheetah

Focuses on the differences between the diets of free ranging and captive cheetahs. It describes the different strategies followed by nutritionists to compensate for differences in diets between free ranging and captive cheetahs.

Chapter 3 – Physiological Conditions

The focus of this chapter is on two physiological conditions namely metacarpal deformities and urolithiasis. In this chapter, the development of the two conditions as well as the influences of mineral interplay and anatomy on the formation and prevention of these conditions will be discussed. Special attention is paid on the influence of dietary magnesium supplementation on these two conditions.

Chapter 4 – Research Methodology

Research design and methodology as well as sampling strategy, data collection procedure and measurement instruments are discussed.

Chapter 5 – Results and Discussion

Experimental findings as well as in-depth discussion of the results with comparisons from the relevant literature are reported.

Chapter 6 – Conclusion

Conclusions are presented and some limitations and recommendations of the study are provided.

List of References

1.6 ETHICAL CONSIDERATION

This research study conforms to the ethical standards of the Animal Use and Care committee of the University of Pretoria. Care was taken to adhere strictly to these ethical principles.

CHAPTER 2

FEEDING PRACTICES

2.1 INTRODUCTION

In this chapter, the focus will be on the feeding practices of free ranging cheetahs versus the feeding practices of cheetahs living in captivity. Furthermore, the influence of these two different feeding practices on the mineral balances of the cheetahs will be discussed. To conclude the chapter, possible physiological conditions that can develop as a consequence of these different mineral balances will be identified.

2.2 FEEDING PRACTICES OF FREE-RANGING CHEETAHS

In its natural environment, cheetahs feed on fresh carcasses of a wide variety of vertebrae prey. They prefer prey generally smaller than 60kg which they can overpower and strangle to death (Phillips, 1993). According to a study done by Mills (1984) in the Kalahari Gemsbok National Park, their most preferred prey is the springbok (*Antidorcas marsupialis*), springhaas (*Pedentes capensis*) and steenbok (*Raphicerus campestris*). In a few cases Mills (1984) also found that cheetahs were able to catch wildebeest- (*Connochaetes taurinus*) and hartebeest (*Alcelaphus buselaphus*) calves.

After killing the prey animal, the cheetahs consumes the whole carcass starting at the ventral surface of the prey by ripping open the stomach (Phillips, 1993). The soft organs (liver, kidneys, heart and lungs) are consumed first followed by the muscles of the back, the hind- and forelimbs and the neck. Skin is consumed with these body parts. Not only do they consume the flesh, organs and skin of the prey, but also partially and wholly digested ingesta that were part of their prey's diet. With the help of the prey's own digestive processes, the cheetahs can obtain valuable nutrients

from various vegetable sources. The intestine of the prey animal is however discarded by the cheetahs (Phillips, 1993).

All the bones of the prey that are less than 15mm in diameter may also be consumed. Especially in smaller prey like steenbok and springbok, all the bones including ribs, long bones and the vertebrae are eaten. Young cheetahs, younger than six months of age have no problem crushing and consuming the ribs of smaller animals like steenbok (Dierenfeld, 1997).

In a quest to obtain information on the nutritional value of whole prey, a study was conducted in the USA by Dierenfeld *et al.* (2002). They investigated the nutritional composition of whole prey fed to Zoo animals and found the following:

- The prey of free ranging carnivores contains enough water to supply in the daily needs of most carnivores including cheetahs. In captivity however, even if animals are fed whole carcasses, the animals must have free access to water *ad lib*.
- Whole prey fed to zoo carnivores supplied an excess of crude protein to the diet of the animals. This is based on the dietary requirements that were established for domestic carnivores.
- The crude fat content in whole prey is also much higher than the recommended minimum dietary levels for domestic carnivores. Depending on the age and sex of the whole prey, body fat concentrations will vary. Neonates have lower fat concentrations than older animals and female animals generally have a higher fat concentration than male animals. Body fat is an essential contributor to vitamin storage. As the body fat varies, so does the vitamin content. By consuming the liver, a major storage site of vitamins, the animal obtains vital amounts of vitamin A and E. According to Dierenfeld *et al.* (2002), the whole prey that was analyzed appears to exceed dietary requirements of domestic dogs and cats for vitamin A and vitamin E. Furthermore, Dierenfeld *et al.* (2002) argues that if there is a vitamin E deficiency, it would have a negative impact on reproduction and the general health of the animal.
- The macro mineral concentrations (Ca, Mg, P, K and Na) of whole prey, meet the established requirements of carnivores.

- Investigating trace minerals are challenging because they are highly variable between and within species. Possible reasons for this are the influence of differing dietary trace minerals, specie specific metabolism as well as varying analytical techniques. Dietary copper, iron, zinc and manganese requirements appear to be met based on domestic canine and feline species.

2.3. FEEDING PRACTICES OF CHEETAHS IN CAPTIVITY

In the early years of keeping and breeding cheetahs in captivity, the diet of the captive cheetahs were based almost exclusively on observed feeding habits of the free ranging cheetahs. In the field of wildlife nutrition, qualitative rather than quantitative information regarding feeding practices was most of the times based on perceived natural feeding habits (Dierenfeld, 1997). Duplicating the free ranging cheetah's diet became the norm. Little consideration was paid to the specie, age, sex or diet of the prey animal as well as the texture and composition of its meat. (Clum *et al*. 1996 as quoted by Dierenfeld *et al.* 2002; Dierenfeld *et al.* 1996; Douglas *et al.* 1994)

Most diets were usually restricted to unsupplemented chunks of meat of certain parts of bovine carcasses from various abattoirs. Although these diets were possibly complete with regard to caloric and protein requirements, they did not meet the cheetah's requirements for vitamins and minerals. The captive animals had to adapt to these suboptimal nutritional diets which, in the long run, had adverse effects on the health of these animals. Zoo-animal nutrition was based on short term production goals mainly for maintenance and exhibition purposes, with fewer emphases on long-term sustainability and reproduction.

Nowadays the emphasis is more on health, reproduction and long-term sustainability of the animals. Institutions and zoos have developed different ways to feed cheetahs in captivity. These different ways of feeding can be divided into two main strategies:

2.3.1 Feeding whole carcasses

The one strategy is to feed the captive cheetahs whole carcasses of various animals but mostly chickens (Barrette, 1988). By feeding cheetahs carcasses of different

animals, the diet of the free ranging cheetah is mimicked. Although feeding whole carcasses to cheetahs would seem to be the answer to the nutritional imbalances experienced by captive cheetahs, there are many factors that complicate it. If one takes into account the unit price, transport, storage and handling of the whole carcass, it becomes a very expensive diet. Also, after death, the mineral composition change in the transformation of muscle to meat. By freezing the meat, some more changes occur especially in mineral composition. The dietary value of the prey thus changes after death due to changes in mineral composition as well as other changes due to preservation procedures such as freezing. Barrette (1988) argues that a free ranging cheetah obtains different nutrients from prey than a cheetah in captivity. Some institutions do feed organs such as livers and hearts to their cheetahs to compensate for mineral losses. The problem however is that there is an unbalanced ratio of calcium to magnesium in these organs. The ratio of calcium:magnesium is 1:50 in the liver and 1:40 in the heart (Barrette, 1988).

2.3.2 Feeding supplemented meat

The alternative is a diet made up of chunks of meat with a supplementation that is added to the meat.

The supplementation that is added to the cheetah's diet is commonly formulated by Zoo animal nutritionists (Allen *et al.*, 1991). It is formulated according to the dietary needs of domestic cats regardless of size. It is however not possible to predict energy needs of exotic cats from those of domestic cats. Allen *et al.* (1991) continues that differences among species are not simply due to body size, but also species-specific differences in metabolic requirements.

The chunks of meat usually come from beef abattoirs where bovine meat is more abundant than meat of wild ungulates. Bovine meat is, however not part of the diet of free-ranging cheetahs. It is only used as a substitute for wild ungulate meat. The chemically composition of bovine meat differs from that found in wild ungulates.

The average fat content of most game species is less than 3%. It is significantly lower than the fat content of domesticated species such as beef and lamb (Table 2.1) (Schonfeldt, 1993 as quoted by Hoffman, 2007).

Table 2.1 Nutritional values for game species compared to that of beef

Species	Protein (%)	Fat (%)
Beef	19.2	12.2
Springbok	20.0	2.20
Nyala	22.2	0.80
Blesbok	22.2	0.92
Impala	23.8	2.45

Adapted from Hoffman (2007)

Fatty acid compositions also differ between beef and game meat. Hoffman (2007) stated that the ratio between polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) is of greater importance than total fat content. Meat from several game species was shown to have high levels of PUFA's. This agrees with a study done on red hartebeest (*Alcelaphus buselaphus caama*), where meat from free-ranging game was found to be richer in polyunsaturated fatty acids compared to bovine meat (Hoffman *et al*. 2010).

Two highly important PUFA's are omega-6 and omega-3. The correct ratio between these two PUFA's is an important factor for animal health. Meat from intensively reared animals (e.g. in feedlots) is high in omega-6, whereas meat from animals raised on grazing has more omega-3 (Simopoulus, 2000 as quoted by Hoffman, 2007). In contrast, the ratio between omega-6 and omega-3 fatty acids in meat from game specie's were all below 4.0 which demonstrate a healthy balance between these fatty acids (Crawford *et al*. (1970) as quoted by Hoffman (2007*).*

Studies from Hoffman *et al*. (2010) and Hoffman (2007) showed that cholesterol levels in game meat are also lower compared to that of beef. (Table 2.2)

Table 2.2 Cholesterol concentration in meat for selected animal species

Species	[Cholesterol] (mg/100g)
Beef	76.0
Nyala	51.0
Alpaca (Lama pacos)	51.1
Blesbok	49.7
Springbok	54.5
Lama (Lama glama)	56.3

Adapted from Hoffman (2007)

Game meat was also shown to have much lower marbling fat compared to meat from other domesticated meat such as beef or mutton (Aidoo & Haworth, 1995 as quoted by Hoffman, 2007).

Protein, moisture and fat content of meat are important determinants of its nutritional value. Water and protein are contained mainly in the lean portion of meat. As game meat is low in fat content, it will cause the moisture and protein portions to be higher than in beef (Table 2.1) (Aidoo & Haworth, 1995 as quoted by Hoffman, 2007).

In light of the above it should be clear that both of these feeding strategies have their unique disadvantages. The biggest common disadvantage is that none of these strategies supply the cheetahs with complete and balanced nutrients that meet all the cheetah's nutritional needs at different stages of maturity. There is a need to design feeding programs and diets that can provide nutritional support for all life-stages of the animal to accordingly minimize nutritional imbalances.

2.4 NUTRITIONAL IMBALANCES DUE TO DIET

Due to insufficient knowledge and information on the dietary requirements of cheetahs, as well as different dietary strategies followed, the diets of captive bred cheetahs are imbalanced. Although most institutions do their utmost best to feed their captive cheetahs a nutritional sound diet with all the required nutrients, there are many shortcomings which can lead to numerous metabolic and structural diseases.

Osteoporosis

Many studies involving Osteoporosis revolves around the role that calcium plays in growth and development. Very few of the studies focus on the effect that magnesium has on calcium and its role in regulating a balanced ratio between magnesium and calcium. One of the causes of Osteoporosis is a dietary imbalance and low levels of magnesium and calcium. It causes brittle bones especially in older individuals.

Osteofibrosis

It is caused by an unbalanced ratio between calcium and phosphorus in the diet. If there is an excess of sulphur-containing amino acids (as it is normally the case with

high meat protein diets) the sulphur is excreted in the form of calcium sulphate which can cause urolithiasis (Barrette, 1988). Primary symptoms of osteofibrosis are anorexia, diarrhea, **painful skeletal deformations** resulting in lameness and other postural abnormalities.

Muscle contraction

Muscle contraction is another physiological mechanism that is affected by a nutritional imbalance. This imbalance is between calcium and magnesium. Magnesium competes with calcium for binding to the troponin molecule found at regular intervals along the actin filaments. Troponin undergoes a conformational change upon the binding with calcium. This allows the binding of actin and myosin filaments prior to muscle contraction. Magnesium competes with this process of binding and can prevent the mechanism of contraction if there is an excess of magnesium available.

Focal-palatine erosion

A study was conducted by Phillips (1993) where they examined the feeding behavior of wild and wild-caught captive cheetahs. The focus was on the type and amount of bone consumed by the wild cheetahs at killings. They found that a condition known as focal-palatine erosion developed in the captive cheetahs. This, according to Phillips (1993) was due to diets lacking sufficient hard materials such as bone that were needed by the captive cheetahs.

Essential fatty acid deficiencies

Most mammalian species, including *Felidae*, do not have the ability to synthesize the essential fatty acid linoleic acid. It needs to be provided by their diet (Bauer, 1997). Together with this, felines have a limited capacity to synthesize arachidonic acid from linoleic acid, and eicosapentaenoic- & docosahexaenoic acid from α-linolenic acid. Felines rely on other mammals (usually ungulates[1]) to produce these important fatty acids for them which are important not only for their health, but also for their reproductive performance. Fat, the most concentrated form of energy, is highly efficiently used by large feline species. It has also been estimated that 60% of total energy derived from dietary fats. The apparent digestibility of crude fat by felines was

[1] Ungulates - Any animal with hooves

reported as between 95-99%. When only meat is fed, these essential fatty acids are not ingested. This leads to detrimental health effects (Bauer, 1997).

2.5 SUMMARY

Free ranging cheetahs have the advantage of constructing their own diet depending on their nutritional needs. By consuming all the different parts of the prey animal, free-ranging cheetahs receive a balanced nutritional diet.

In order to feed captive cheetahs properly, their diet needs to be formulated as close as possible to what they receive in nature. As explained above, various factors complicate pure dietary duplication. The free-ranging cheetah's diet can therefore not only be duplicated. One has to take into account the nutritional value of the diet as well as right concentrations of the different minerals needed.

Both feeding strategies followed to feed captive cheetahs are not sufficient in providing a complete, nutritional, balanced diet. There are many nutritional shortcomings that can have detrimental effects on the health of the captive cheetah. As wildlife nutritionists gain more insight into the nutritional needs of captive cheetahs, there is an increasing need for a complete TMR to supply these animals with a complete, well balanced diet which can prevent some structural- and metabolic conditions that negatively influence the health of the cheetahs.

CHAPTER 3

PHYSIOLOGICAL CONDITIONS

3.1 INTRODUCTION

In Chapter 2, the differences between the diets of free-ranging and captive cheetahs were discussed. The dilemma of supplying a well balanced nutritional diet to captive cheetahs was also mentioned and the main nutritional differences were identified.

In this chapter, the focus will be on the anatomy of the forelimb, its muscles and basic muscle contraction. The physiology and mechanism of the leg deformity that juvenile cheetahs were diagnosed with will then be discussed. The chapter will also look into its causes and formation as well as possible dietary supplementation of magnesium to rectify the leg deformity.

Furthermore, the effect of dietary magnesium supplementation on some other minerals will be discussed. Attention will also be paid to the regulation and interplay of these minerals.

In order to rectify the leg deformity by supplementing dietary magnesium, the effect of magnesium on another physiological condition namely urolithiasis, should be monitored carefully. To conclude this chapter, urolithiasis will be discussed in great detail. Parameters will then be identified to monitor how the mineral dietary supplementation affects the formation of urolithiasis.

3.2 METACARPAL DEFORMITY

The term metacarpal deformity was introduced to describe a leg deformity in animals. Metacarpal refers to the specific region of the deformity. In some literature, the condition is called *flexural deformity;* this refers to the leg that has been over flexed.

3.2.1 Anatomy of the forelimb

3.2.1.1 Skeleton of the forelimb

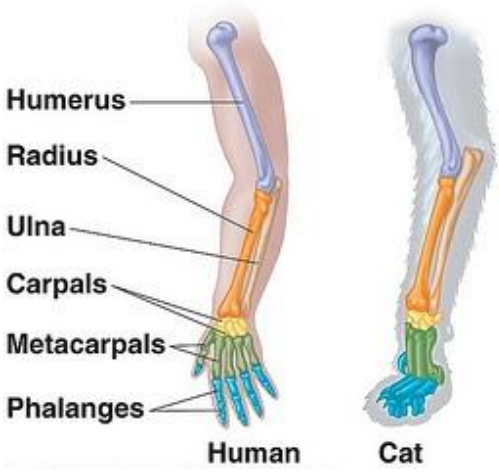


Figure 3.1 Skeleton of forelimb (Gwen, 2011)

As described by Dyce *et al*. (2002), Evans (1993) and Frandson *et al.* (2003), the skeleton of the carnivore forelimb starts at the *scapula* or shoulder-blade which is a flat bone that lies over the laterally part of the thorax. It is held in place by means of muscles without forming articulation with the trunk. Proximally attached to the scapula is the *humerus* which forms the skeleton of the arm. The skeleton of the forearm consists of two bones, the *radius* and *ulna* with the ulna as the main weight-supporting bone of the forelimb. In a standing position, the radius and ulna are arranged with the ulna caudal to the radius in the upper part of the forearm but lateral in the lower part.

Figure 3.1 illustrates the different bones of the forelimb of a cat in comparison to the forearm of a human being. Externally, there might be some confusion as to what animal region fits with what human region, but when the internal skeletons are viewed, the different parts can be compared with ease.

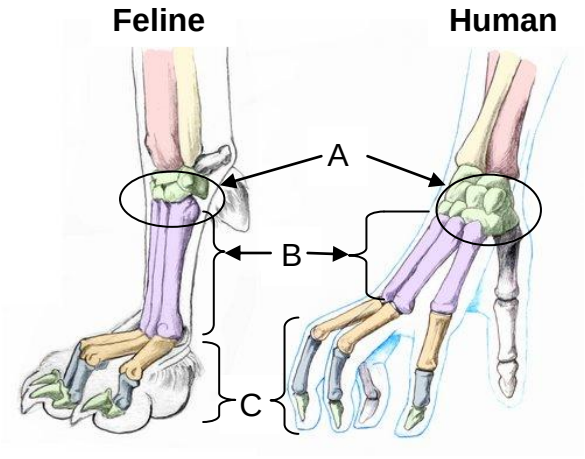


Figure 3.2 Skeleton of human hand and feline front paw
(Adapted from Anon, 2009)

A - Carpus; B - Metacarpus; C - Phalanges

Figure 3.2 illustrates the anatomy of the feline forepaw in comparison to the same structures in the human hand.

The forepaw includes the carpus (wrist), metacarpus and the digits (Dyce *et al.* 2002; Evans, 1993). The short *carpal bones* consist of seven bones that are clearly arranged in two transverse rows to form the wrist. The proximal row comprises the *radial*, *intermediate*, *ulnar* and *accessory* bones. The radial and intermediate carpals fuse in the dog and cat. The distal row is numbered from one to five with the fifth fused with the fourth carpal bone. The five *metacarpal bones* have been greatly modified and have been specialized for running in the cat. The cat supports itself only by the digits. Each digit comprises of 3 bones namely the *proximal-, middle- and distal phalanx* (Dyce *et al*. 2002; Evans, 1993; Frandson *et al*. 2003).

3.2.1.2 Joints of the forelimb

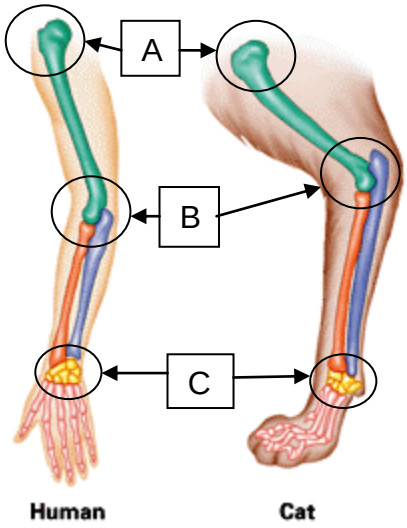


Figure 3.3 Joints of human- and cat forelimb
(Adapted from Anon, 2009)
A - Shoulder Joint; B - Elbow Joint; C - Carpal Joint

The *shoulder joint* is a ball-and-socket joint between the scapula and the head of the humerus of the forelimb. It is capable of movement in any direction, but its main movements are flexion and extension (Dyce *et al*. 2002). The *elbow joint* combines the hinge joint between the humerus, radius and ulna within a single capsule. In carnivores, a pivot joint pairs the bones of the radius and ulna (Evans, 1993). The *carpal joint* comprises of three different levels of articulation namely the *antebrachiocarpal-*, *midcarpal-* and *carpometacarpal*. In dogs and cats, the proximal joint can be regarded as an ellipsoidal joint[2] with restricted movement. At antebrachiocarpal- and midcarpal level, hinge movement is quite free but at carpometacarpal level no movement is allowed (Dyce *et al*. 2002; Evans, 1993). A number of ligaments around the carpal joint are responsible for holding the joint in place while allowing some movement.

[2] Ellipsoidal joint – Movement are in two planes at right angles to each other; small amount of rotation possible

3.2.1.3 Muscles of the forelimb

The muscles of the forelimb consist of the girdle musculature, which passes between the trunk and the limb, and the intrinsic musculature, which are grouped by their common location, actions and innervations. Looking at the intrinsic muscles of the forelimb, one can differentiate between muscles acting on the shoulder joint, muscles acting on the elbow joint, muscles of the forearm and muscles of the carpal- and digital joints (Dyce *et al*. 2002; Evans, 1993; Frandson *et al*. 2003).

The scope of this discussion will be on the carpal- and digital joints. The muscles of the carpal- and digital joints are simply classified as extensor- or flexor muscles.

One can differentiate between five different *extensor* muscles of the carpus and digits (Evans, 1993; Frandson *et al*. 2003). The *extensor carpi radialis* is the most medial muscle of the group. Its origin is on the proximal part of the third or fourth metacarpal bone. The *ulnaris lateralis* is the most lateral muscle of the group and run parallel to the outer aspect of the limb. It may extend an already extended carpus and further flexes the joint that is in a flexed position or deviate the paw laterally. The *extensor carpi obliquus* functions as an extensor of the carpus with a potential for medial deviation of the paw. The long digital extensor muscles can be subdivided into two muscle groups. The *common digital extensor* inserts on the distal phalanges of each functional digit and therefore divides into five in the cat. The *lateral digital extensor runs* along the lateral edge of the common extensor and is divided into four in the cat. It inserts on the dorsal surface of the proximal phalanx and divides into four in the cat (Dyce *et al*. 2002; Evans, 1993; Frandson *et al*. 2003).

When looking at the *flexor muscles* of the carpus and digits in the forelimb of the cat, the *flexor carpi radialis* is the most medial and runs directly to the border accessory carpal bone. It ends in the second or third metacarpal bone. The *flexor carpi ulnaris* ends in the accessory bone. Both of these flexor bones are only carpal flexors. The *superficial digital flexor* is situated in the caudo-medial part of the forearm; it divides into a branch for each digital flexor. The *deep digital flexor* lies deeper in the forearm and passes the carpus through the carpal canal before dividing into one to four digital branches. Moving to the short digital muscles, the *interosseous muscles*

support the metacarpophalangeal joints (Dyce *et al*. 2002; Evans, 1993; Frandson *et al*. 2003).

3.2.2 Skeletal muscle anatomy and contraction

3.2.2.1 Structure

According to Farah and Reinach (1995), a muscle consists of numerous bundles of muscle fibers which are long cells with visible striations and many nuclei. Each fiber consists of sarcoplasmic reticulum (SR) that surrounds the elongated protein strands (myofibrils). The SR is the major intracellular source of Ca^{2+}.T-tubules forms invaginations into the SR and aid in signal transportation. The myofibrils are characterized by a highly organized network of parallel thick and thin filaments, polymers of the proteins myosin and actin, respectively. Each thick filament consists of an elongated body with an enlargement at the end which forms a flexible “head”. The three principal regulatory components of the thin filament are actin, tropomyosin and the troponin complex (Farah & Reinach, 1995).

3.2.2.2 Muscle contraction

The *sliding filament model* was proposed in the 1950’s by Hugh and Andrew Huxley (Solomon *et al.* 2005). According to this model, skeletal muscle contraction is triggered by the generation of an action potential on the sarcolemma. Acetylcholine is released by a motor neuron and combines with receptors on the muscle fiber. This causes depolarization and an action potential develops. The invagination of the T-tubules enables the action potential to spread through the muscle fiber and stimulates the release of Ca^{2+} from the SR. The Ca^{2+}-ions bind to the troponin and cause a conformational change in the shape of tropomyosin, it also exposes active sites on the actin filaments. The myosin head binds to the exposed active site on the actin filaments forming a cross bridge. During the power stroke, ADP is converted to ATP and the actin filament slides over the myosin filament causing the muscle to contract.

This model was modified over the years into a swinging lever-arm model as new insight, mostly about the cross bridge orientation and functioning, was found (Holmes, 1997).

3.2.3 Flexural deformities

Leg flexural deformities are common deformities that are often found in dogs and horses of especially young age. The deformities are usually in the sagittal[3] plane where a joint is held in a flexed- or extended position (Auer, 2006).

The deformity is named according to the joint involved and one can differentiate between contractual (hyperflexed)- or hyperextension deformities that are either of congenital- or acquired (developmental) origin (Auer, 2006).

Congenital deformities could be present at, or soon after birth in several species. There are various reasons for its formation: intrauterine malpositioning (Auer, 1999), teratogenic effects from ingesting locoweeds and hybrid Sudan grass by the mare during gestation (Mcllwraith & James,1982 as quoted by Kidd & Barr, 2002), a dominant gene mutation in a stallion (Hutt, 1968 as quoted by Kidd & Barr, 2002), a neuromuscular disorder (Mayhew, 1984 as quoted by Kidd & Barr, 2002) or due to an influenza outbreak (Fessler, 1977 as quoted by Kidd & Barr, 2002). The precise cause of the majority of flexural deformities is however unknown and therefore presently assumed that it is of multi-factorial origin (Auer, 2006). The carpus and metacarpophalangeal joints are the most common congenitally affected joints (Fackelman, 1980 as quoted by Kidd and Barr, 2002).

Acquired deformities develop during the remainder of the animal's life and are due to reasons such as trauma and nutrition (Auer, 1999; Hay & Mueller, 1999 as quoted by Altunatmaz & Ozsoy, 2006). In horses, this type of deformity is influenced by the age of the animal as indicated by the different groups that Munroe and Marrr (1989) as quoted by Kidd and Barr (2002) devised. The three groups consist of: 1) foals between six weeks to six months 2) yearlings between nine to eighteen months and 3) mature horses older than eighteen months. This type of deformities usually occur

[3] Saggital plane – Any plane parallel to the median plane

in the distal interphalangeal- or metacarpophalangeal joints of the forelimbs (Adams, 2000 as quoted by Adams 1999).

There is thus an indication that congenital deformities are more likely to occur in the proximal part of the distal forelimb whereas acquired deformities are more likely to occur in the more distal part of the distal forelimb.

The deformity that the juvenile cheetahs in this study were diagnosed with can be classified as an acquired deformity because they haven't been born with the deformity but it developed soon after birth.

Various reasons have been proposed as the causes for the development of acquired deformities in animals. Auer (2006) stated that "*balancing nutritional intake is the key in the prevention of flexural deformities.*"

One of the proposed causes for the development of acquired flexural deformities is a mismatch in bone and tendon/ligament growth. The bone growth rate is either faster or slower than the associated tendons and ligaments (Kidd & Barr, 2002). Auer (2006) reports that bone growth rate is influenced by nutrition, especially the balance of minerals and trace minerals, which is vitally important for skeletal development as well as bone growth.

Related to this is that of post-weaning nutritional stress. In a study done by Osthoff *et al. (*2006), milk of two cheetahs was analyzed shortly after their cups were separated from their mothers for hand rearing. As reported by Osthoff *et al. (*2006), the milk had a very high concentration of nutrients and minerals (99.6g protein, 64.8g fat and 40.21g lactose). After weaning, the young cheetahs need to adapt to the change in diet and growth rate is consequently reduced. This is because nutrients and minerals now need to be obtained from a different source of diet to enable optimum skeletal development and bone growth. This abrupt change in nutrition after weaning has an influence on the balance between bone- and tendon growth. Kidd and Barr (2002) argue that the shift to an alternative diet after weaning is the reason that flexural deformities in horses are found shortly after weaning.

Based on the findings of Osthoff *et al. (*2006) and Kidd and Barr (2002), a study was done by Altunatmaz and Ozsoy (2006). In this study, they investigated carpal flexural

deformities of 31 puppies of different dog breeds. All the puppies in the study were between 6 to 24 weeks old and of 10 different breeds. The puppies were divided into two groups. The experimental group was separated from their mothers 10 to 15 days before and was fed cow's milk and various dog foods. This group showed signs of the deformity in the form of contraction in the flexor tendons, particular that of the *flexor carpi ulnaris* muscle. The control group was not separated from their mother and did not show any signs of the deformity. In the Anatolian Sheepdog litter, only the three puppies that were separated from the mother 15 days before showed the deformity. This reaffirms the post-weaning nutritional stress hypotheses proposed by Osthoff *et al. (*2006).

Stressing the importance of balanced mineral nutrition of young animals around weaning, Kidd and Barr (2002) stated in a reviewed article that the mineral supplementation a mare and her young foal receive shortly after birth till weaning, should be balanced with respect to calcium and phosphorus. This is also emphasized in research done by Frape (1989) on Thoroughbred horses. Especially during winter when the diet is high in phosphorus and low in calcium, it can cause the formation of flexural deformities.

In order to establish which minerals are mostly affecting the formation of flexural deformities, Stahlmann *et al.* (2000) studied the leg deformities in four juvenile Beagle dogs. In the study, Stahlmann *et al.* (2000) analyzed the effects of a magnesium deficient diet on skeletal growth and development. The dogs were deliberately fed a magnesium deficient diet for 40 to 46 days. Stahlmann *et al.* (2000) reported that after 4 weeks on the magnesium deficient diet, all dogs were limping. One of the dog's front legs was hyperextended at an angle of 90°.

Vaughan (1992) as quated by Altunatmaz and Ozsoy (2006) argued that certain dog breeds may be predisposed to deformities of the carpal joints and that the deformity is inherited. Altunatmaz and Ozsoy (2006) however do not agree as their research indicated that 10 different breeds all showed carpal joint deformity with no dominant breed. Altunatmaz and Ozsoy (2006) also argued that only the puppies separated from their mother showed carpal joint deformities. Auer (1999) and Wagner *et al.*

(1985) further showed that carpal deformities that developed in foals might be related to nutritional unbalanced diets.

3.2.4 Summary

In light of the above it should be clear that there is a direct relationship between leg deformity and nutrition. The transition from mother milk to a solid diet (weaning), play an important role in formation of deformities. Also, as reported in the study by Stahlmann *et al.* (2000), that magnesium supplementation can have a positive effect on rectifying the deformity in other animal species.

3.3 ROLE, CONTROL AND REGULATION OF CALCIUM, MAGNESIUM AND PHOSPHOROUS IN THE BODY

After establishing the positive effect of balanced nutrition and especially magnesium on front leg deformities in animals, this section will discuss the relationship between minerals. The focus will be on magnesium and its interaction with calcium and phosphorous.

The reason for investigating the relationship of magnesium with other minerals, is to study how these minerals will influence the concentration of magnesium, calcium and phosphorous in the urinary- and blood system. It is also important to determine how the dietary supplementation of magnesium will affect the formation of urolithiasis. Urolithiasis will then be discussed in the following section.

The relationship between dietary mineral concentrations and animal health is highly correlated. Animals can tolerate mineral concentrations that are in excess or shortage up to a certain level. When this excess or shortage is not rectified, these minerals will either become toxic to the animal or the animal might be in deficit of a specific mineral. This will have detrimental effects on the health of the animal. This relationship is explained in Figure 3.4

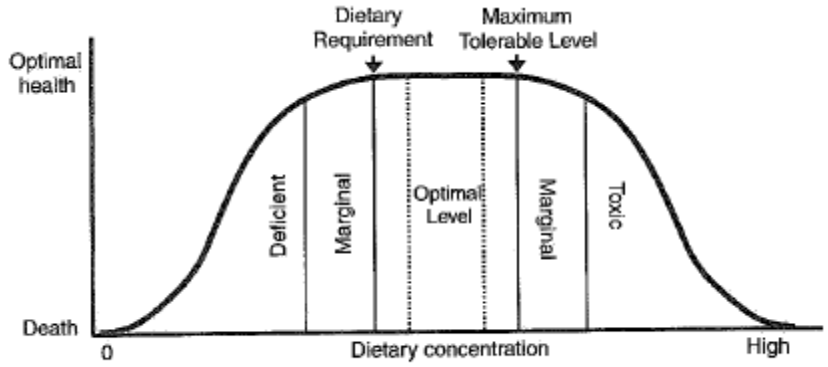


Figure 3.4 Mineral concentrations in animal health (NRC, 2005)

3.3.1 Role and regulation of magnesium

Magnesium is one of the most abundant minerals in the body with bone as its major site of storage (Creedon *et al*. 1998 as quoted by Anast & Gardener, 1981; Wallach, 1990). Other storage sites include the inside of cells of body tissues and organs.

Although very little magnesium is found in blood, the body needs to keep blood levels of magnesium constant as it plays a very important role in a variety of biochemical reactions. These reactions include the maintenance of normal muscle- and nerve function, a healthy immune system as well as maintenance of blood sugar and blood pressure levels. It also plays a vital role in energy metabolism and protein synthesis. There is an increasing interest in its preventative effects on disorders such as cardiovascular disease, diabetes, arthritis, osteoporosis and hypertension.

To investigate the role of magnesium on muscle contraction and relaxation, D'Angelo *et al.* (1992) did an experiment on swine carotid arteries. D'Angelo *et al.* (1992) examined the mechanism that is responsible for magnesium induced smooth muscle relaxation. They found that myoplasmic calcium decreased when extracellular magnesium was increased. This is consistent with the hypothesis that increased magnesium relaxes arterial smooth muscle primarily by decreasing calcium. There appears to be an interaction between magnesium and calcium in which elevated magnesium induces muscle relaxation through reductions in myoplasmic calcium.

The homeostasis of magnesium is essentially regulated by the kidneys. Most of the plasma magnesium that crosses the glomerulus forms part of renal fluids. Plasma magnesium will either be excreted or absorbed depending on the body's nutritional needs.

When plasma magnesium levels are below normal, the Parathyroid hormone (PTH) will induce magnesium reabsorption from the renal fluids. PTH is however not under the control of renal magnesium levels. PTH secretion is induced in response to hypocalcaemia through a negative feedback mechanism. Unless the concentration of calcium is also below normal, PTH will not be secreted. In the absence of PTH secretion, magnesium levels remain below normal and the animal will be in a state of negative plasma magnesium. The animal will therefore start to show signs of a magnesium deficiency (NRC, 2005). These signs include retarded growth rate, spreading toes, soft tissue calcification, enlargement of the long bones and hyperextension of the carpus and tarsus (Pulse, 1994).

When there is an excess of plasma magnesium, magnesium in the renal fluids will not be reabsorbed. The magnesium will form part of the urine and will be excreted. The animal is thus in a positive state of magnesium. When, however, the kidneys can't keep up with the abnormal high levels of magnesium, the animal will be in risk of the formation of urinary calculi (NRC, 2005). Pulse (1994) stated that high magnesium in the diet directly relates to urinary crystal formation (alkaline urine) in cats. Cats fed higher amounts of magnesium were found to have greater amounts of struvite stones in their bladder and urethra. It is suggested that the magnesium oxide used, acted as an alkalinizing agent and that the magnesium was not the true cause of the stones. It is also stated that the uroliths are more dependent on the urine pH than the dietary magnesium (NRC, 2005).

Animals have no hormonal mechanisms that enable the animal to adapt to a low magnesium diet by increasing intestinal magnesium absorption. The animal must receive a relatively constant supply of dietary magnesium to avoid developing hypomagnesaemia and deficiency problems (NRC, 2005).

3.3.2 Role and regulation of calcium

About 98% of the calcium in the body is located within the skeleton. Calcium and phosphorus provide structural strength and hardness to the bone. The other 2% of calcium in the body is found in extracellular fluids of the body. Extracellular calcium is essential for the formation of skeletal tissue (bone), nerve impulses, the primary messenger of contractile activation in muscle, blood clotting etc. (Somlyo & Himpens, 1989). Intracellular calcium plays a vital part in second messenger information between the surface and interior of the cell. For growth and reproduction, the animal requires more calcium compared to maintenance requirements alone.

Whenever there is a loss of calcium from the extracellular fluids by means of digestive secretions or from sweat and urine, the body will try to rectify this deficit firstly by means of an increase of dietary calcium. If dietary calcium supply is however inadequate, the body surrenders to state of hypocalcaemia. In this state, the body will reabsorb calcium from either bone or renal fluids, which consequently leads to bone loss. The body will sacrifice bone calcium in an attempt to normalize blood calcium concentrations (NRC, 2005).

The reabsorption of calcium from bone or renal fluids is regulated by PTH which is secreted when there is a decrease of blood calcium (hypocalcaemia). This hormone increases renal calcium reabsorption and consequently reduces urinary calcium excretion.

If the body is not able to rectify the need of calcium, the animal will show signs of calcium deficiency (NRC, 2005). These signs include among others: enlarged epiphysis of the ribs and long bones, splayed toes, spontaneous fractures and hyperextension of the carpus and tarsus as well as abnormal deviations of the leg. These signs also occur with phosphorus and magnesium deficiencies (Pulse, 1994).

An excess of blood calcium (hypercalcemia) will stimulate the secretion of another hormone, Calcitonin[4], and suppress the secretion of PTH. As already stated, PTH enhances calcium release from the bones. By suppressing PTH, calcium is inhibited

[4] Calcitonin – Hormone produced by the parafollicular cells of the thyroid gland.

from being released from the bone. The interplay between calcitonin and PTH regulates calcium levels in the body, by working antagonistic to one another.

Although very few studies focus on the effects of elevated dietary calcium in cats, the NRC (2005) showed that it resulted in hypomagnesaemia in kittens. This is because the hormonal control (PTH and Calcitonin) of magnesium and calcium both are determined by ionized ionized plasma calcium concentrations. Thus, when the animal is in a state of hypomagnesaemia and hypercalcemia, calcitonin will be secreted and the uptake of magnesium will be suppressed. The state of hypomagnesaemia will remain.

Diets that are high in calcium also interfere with the absorption of other minerals such as phosphorous and can cause a deficiency of these minerals despite their inclusion in diets at sufficient levels (NRC, 2005).

3.3.3 Role and regulation of phosphorus

Phosphorus is a highly abundant mineral in the body and is involved in most aspects of the metabolism. Dietary nutrient requirements of phosphorus can be affected by age, physiological stage, performance as well as dietary levels of calcium (McDowell, 2003 as quoted by NRC, 2005).

In bone, phosphorus serves as a structural component, whereas it plays a structural and metabolic role as components of phospholipids, DNA, RNA and nucleotides in soft tissue.

Body phosphate homeostasis is maintained primarily by three organs or tissues namely the intestine, kidney and bone. As with magnesium and calcium, PTH and Calcitonin are the two main hormones that assist in its regulation.

Littledike and Goff (1987) as quoted by the NRC (2005) points out that the regulation of phosphorous in the body requires adequate levels of dietary calcium as well as the correct ratio between calcium and phosphorus. When there is an excess of dietary phosphorous available in relation to calcium, insoluble complexes high in phosphorous are formed. The phosphorus is then unavailable for absorption

resulting in insoluble phosphorous complexes which may induce urolithiasis (NRC, 2005).

3.3.4 Interaction between magnesium, calcium and phosphorus

The functions of minerals in animals are interrelated. They can seldom be considered as single elements with independent and self sufficient roles (Swenson & Reece, 1993). The relationship between calcium, magnesium and phosphorus is of great importance. More important than consuming enough of one or the other is finding the correct ratio between these minerals. When the ratio between minerals is unbalanced, certain minerals might not be available to the body; this might lead to signs of deficiency even if some minerals are available in excess.

The relationship between calcium to phosphorous for example plays an important role in the formation and maintenance of skeletal tissue. Both calcium and phosphorous serve as major structural elements of skeletal tissue. Most of the calcium and phosphorous in the body are found in bones and teeth (Swenson & Reece, 1993). According to Pulse (1994), phosphorus toxicity may be produced by an excess of phosphorus in relationship to low calcium levels, which can result in weak bones.

According to Pulse (1994) and the NRC (2005), plasma phosphorus levels are inversely related to plasma calcium levels. Low calcium levels will stimulate the release of PTH. This will increase plasma calcium concentration but decrease plasma phosphorous levels. This effect is also seen in dogs where inadequate dietary phosphorus levels and an excess of dietary calcium, leads to reduced plasma levels of phosphorous.

The interplay between calcium to magnesium is yet another complicated interaction where both minerals can influence one another. By supplementing one, it has an influence on the other mineral.

Magnesium is vital for the reabsorption of calcium from bone. If there is a deficiency of magnesium in the body, calcium will be deposited in the soft tissue and can cause

arthritis (Fuchs, 2009). It is argued by Pulse (1994) that excessive supplementation of calcium will reduce plasma magnesium levels.

In order to investigate the effect of dietary magnesium supplementation on calcium- and magnesium levels in the body, experiments were conducted by the International Clinical Nutrition Review (Fuchs, 2009) on humans. A number of volunteers on a low-magnesium diet were given calcium supplements. All but one of the volunteers showed deficient levels of calcium even though they received a dietary calcium supplement. The volunteers were then given calcium intravenously. They showed an increase of calcium concentration in their blood only for as long as the calcium supplementation lasted. When they were however given a supplementation, the calcium deficiency disappeared.

This effect of magnesium supplementation on calcium was also investigated by Creedon *et al.* (1998). Rats were fed different diets of different concentrations of magnesium. The rats that received moderate to severe magnesium-restricted diets, showed an increased concentration of calcium in the kidneys, which is a sign of severe magnesium deficiency. According to Bunce and King (1978) as well as Koh *et al.* (1989) as quoted by Creedon *et al.* (1998), this is due to inefficient absorption of calcium by the bone.

3.3.5 Summary

As outlined above: The same hormonal mechanism regulates calcium, magnesium as well as phosphorous levels in the body. PTH is secreted when plasma calcium and magnesium concentrations are low. Calcitonin is secreted when calcium and magnesium concentrations in serum are high. Phosphorous levels are highly dependent on the correct relationship with calcium and magnesium.

Supplementing magnesium will influence the levels of phosphorous and calcium. These influences should be monitored carefully to ensure that the right proportions of these minerals are present as the absence of correct mineral proportions can lead to unhealthy conditions.

3.4 UROLITHIASIS

Although very little is known about the development of urolithiasis in captive cheetahs, it is suspected that the condition is mainly due to nutritional imbalances. What complicates the situation is that urolithiasis is not a condition that is affected only by a few predisposing factors. Numerous factors work together simultaneously in the formation of urolithiasis. Bacterial infections, viruses, drugs given to the animals, diseases, stress, water quality, hydration status of the animal as well as the diet, is just a few of the suspected predisposing factors.

The diet of the animals is at the top of the cascade that eventually leads to the formation of urolithiasis. Dietary ingredients influence volume, pH and the concentration of solutes found in the urine. Type and composition of the diet given, amount of water included, interaction between different metals (mostly Ca, Mg, P, and K), sulphur-containing amino acids and fatty acids may all have an influence.

Markwell (1998) argued that modification of the diet that the animals receive might play a big role in preventing the formation of urolithiasis in the lower urinary tract.

3.4.1 Lower urinary tract diseases (LUTD)

Lower urinary tract diseases (LUTD) are common in most feline species. It is a term used to describe not a specific disease, but a group of diseases occurring in the lower urinary tract. They are characterized by the occurrence of the same clinical signs such as haematuria[5], pollakiuria[6], stranguria[7] and or abdominal pain. According to Gerber *et al.* (2005), LUTD can be classified into four broad categories namely: urinary tract infections, urethral plugs, idiopathic LUTD and urolithiasis.

Urinary tract infections are amongst others, caused by *Escherichia Coli* and *Staphylococcus spp.* These different types of bacteria infect the lower urinary tract causing different kinds of infections. The urinary pH of animals with urinary tract infections varied between 5.0 and 8.0 (Gerber *et al,* 2005).

[5] Haematuria - Presence of red blood cells (erythrocytes) in the urine.
[6] Pollakiuria - Frequent daytime urination
[7] Stranguria - Painful excretion of urine due to spasmodic contraction of urethra and bladder

Urethral plugs are classified as any plug forming material that obstructs the urethra. These plugs can be composed of anything that close or obstruct the passageways or ducts of the urinary system. The urinary pH of animals with urethral plugs varied between 5.5 and 7.0 (Gerber *et al,* 2005).

In the absence of a specific cause for the clinical signs, the animal is diagnosed with Idiopathic LUTD. The urinary pH of animals diagnosed with Idiopathic LUTD varies between 5.0 and 7.5 (Gerber *et al,* 2005).

Urolithiasis is described by Gerber *et al.* (2005) as the presence of uroliths in the bladder and/or the urethra and can be composed of several different salts and or minerals.

Most uroliths are composed of minerals. When these minerals are present in high concentrations, they tend to crystallize, aggregate and then develop into uroliths in the urine. The formation and growth of crystals depend on the degree of supersaturation as well as the degree of promoters and inhibitors in the urine (Houston *et al*. 2003).

Finlayson (1978) divide the physicochemical features of urolithiasis into four interrelated categories namely the driving force (supersaturation), nucleation, growth of crystals and particles and lastly aggregation.

Supersaturation of urine salts is the driving force behind the formation of uroliths. This driving force can be explained as free energy (ΔG) and is expressed as: (Finlayson, 1978)

$$\Delta G = RT \ln \left(\frac{A_i}{A_o} \right) \quad \textbf{(1)}$$

In (1), R is the gas constant (8.314), T the temperature and A_i & A_0 the activities of the unionized salt species in the solution.

In equation (1), when A_i/A_0 is less than one for any given salt (A) in the urine, ΔG will be smaller than zero. The urine is then undersaturated with respect to the stone salt (A) and any existing stones that are present in the urine can dissolve.

If A_i/A_0 equals one in equation (1), ΔG will be zero and the urine is thus saturated. Old existing stones will not dissolve and new ones will not form. Old existing stones can, however, still grow in size.

As the concentration of a given salt(s) increases, the relationship between A_i and A_0 will also increase in equation (1). When A_i/A_0 is larger than one, the urine is supersaturated and ΔG will be larger than zero. In this case, there is available free energy (ΔG). Present stones may still grow in size, but if stone crystals are not present, precipitation will not occur unless A_i/A_0 exceeds an experimental limit called the metastable limit. Above this metastable limit, new stones can form and old existing stones can grow.

It is Important to be able to measure the relationship between A_i and A_0 because it makes it possible to identify individuals who are at an increased risk for urolithiasis. Anti-stone therapies such as magnesium oxide, operates by reducing A_i/A_0 and thus the availability of free energy.

Nucleation is the event of phase formation e.g. stone-salt precipitation, and is determined by energy that is given up in the formation of the particle as well as the energy that is required to form the new particle. In his article, Finlayson (1978) divided nucleation into two main categories based on research done by Nielsen (1964), Walton (1961), Sears (1961) and Walton (1969. If the new particle is well defined in space, it is characterized as Classical nucleation where the surface energy is related to the liquid and newly formed solid particle. If the new particle is not well defined in space and thus diffuses, it is characterized as Non-classical nucleation. The surface energy is now related to the gradient of the two phases that is most likely still in liquid form. The standard free-energy change (ΔG°) can be calculated by equation (2):

$$\Delta G^\circ = \frac{\pi l^3}{6} \Delta G_v + \pi l^2 \sigma \quad \textbf{(2)}$$

In (2), *l* is the sphere diameter and will determine whether the new phase will dissolve. If *l* is too small, the sphere will stay the same size. If *l* is large enough, the sphere will grow. The critical value of *l* needed for a new particle to remain stable or grow can be calculated by equation (3):

$$l^* = 4\sigma/\Delta G_v \quad \textbf{(3)}$$

The growth of the particle refers to the way in which the particle will increase in size. If there is a high concentration of particles in a supersaturated solution, the particles will increase in size and thus grow.

If the product of the particle growth rate and particle transit time through the lumen is small, relative to the diameter of the lumen, then the particle will pass harmless and no stone will develop.

Lastly, aggregation is when the crystal particles attach to each other to form calculi stones. After the particles have increased in size, about one millimeter in diameter, gravitational forces become greater than the adhesion forces and the particles tend to be drawn to one another and aggregate. The mechanisms by which the aggregates are held together are: electrostatic attraction, van der Waal forces, liquid bridges, capillarity, viscous binder and solid bridge (Rumpf, & Schubert, 1977 as quoted by Finlayson, 1978).

There are a few points worth mentioning regarding calculi formation. Firstly, there need to be an abundance of crystals at a high concentration for the formation of calculi to occur. Crystals consists of minerals. When high concentrations of minerals are available, minerals will form crystals and increase the risk of calculi formation. Secondly, for nucleation to take place there has to be an exchange of energy. Thirdly, a large, well defined particle further increases the probability of nucleation. Last but not least, for particle grow to occur there has to be an interaction between the particles and/or the medium in the urine. In light of the above it should be clear that the properties of the particle play a vital role in the formation of calculi.

3.4.2 Different types of stones

The composition of the calculi determines the type of calculi. This is the single most important factor determining dietary modifications and treatment for specific types of urolithiasis. There are many factors that contribute to the formation of crystals and consequently the formation of the uroliths. Griffith (1978) proposed five primary different types of uroliths namely calcium oxalate, calcium phosphate, struvite, uric acid and cystine.

Based on calculi- composition and -properties the different types of uroliths have been grouped together into two groups namely struvite and calcium oxalate.

3.4.2.1 Struvite

This crystal was first identified by a Swedish geologist in 1845. He named it in honor of the Russian diplomat and naturalist H.C.G. von Stuve (1772-1851) who published one of the first scholarly geological works "*Mineralogical Memoirs*" in 1807 (Griffith, 1978). Struvite chrystal has also been named "infection stones" or "triple phosphate" due to its chemical composition (Seaman & Bartges, 2001;Griffith, 1978).

Struvite uroliths are one of the most common calculi that are found in dogs (Seaman & Bartges, 2001) and cats (Maede *et al.* 1987 from Edfors *et al.* 1989). It is also found in *Mustelidae* like the mink (*Mustela vision)* (Sompolinsky, 1950 from Edfors *et al.* 1989), ferret *(Mustela putorius furo) (*Nguyen *et al.* 1979 and Williams, 1976 from Edfors *et al.* 1989), some other weasels (Leoschke *et al*. 1949 from Edfors *et al.* 1989) as well as in cheetahs and lions (*Panthera leo)* (Corpa, *et al.* 2003; Keymer *et al.* 1981 from Edfors *et al.* 1989).

Struvite consist of mainly three substances namely magnesium (Mg), ammonium (NH_4^+) and phosphate (PO_4^{3-}) as well as water which combines to give struvite its formula: $MgNH_4PO_4.6H_2O$.

The formation of struvite stones is highly dependent on urinary pH. A normal pH for cats ranges between 5.0 and 7.0 (Kaneko, 1980 as quoted from Edfors *et al.* 1989), for minks between 6.8 and 7.5 (Leoschke & Ziberia, 1949 as quoted from Edfors *et al.* 1989), and for ferrets between 6.5 and 7.5 (Thornton *et al*. 1979 as quoted from

Edfors *et al.* 1989). In a pH above 6.6, urine tends to crystallize (Rich & Kirk, 1969 from Edfors *et al.* 1989). Cat-like species, within their normal pH range, are thus automatically at risk of urolithiasis. It should be clear that there is a very fine line between a normal pH and a pH that is alkaline which puts the cheetah at risk for developing urolithiasis like other cat species.

Magnesium has been identified as one of the diet components that induce the formation of struvite crystals (Buffington, 1994). In domestic cats, the consumption of diets high in magnesium showed an increased risk of developing urolithiasis (Corpa *et al.* 2003). In a study conducted in Spain, Corpa *et al.* (2003) found two lions with severe cases of urolithiasis. These lions had extremely high concentrations of minerals in their urine due to drinking water contaminated by sea water. High calcium diets in rats have also shown decreases in urinary phosphates that subsequently inhibit struvite stone formation (Parivar *et al.* 1996).

3.4.2.2 Calcium oxalate

Oxalic acid in the body is produced naturally. The endogenous metabolism of glycine, glycolate and hydroxyproline are the main reasons for the development of oxalic acid. Other reasons for the development of oxalic acid are as the end products of dietary ascorbate metabolism and dietary oxalate. Endogenous oxalate production cannot be decreased (Hagler & Herman, 1973 as quoted by Parivar *et al.* 1996). Low levels of oxalic acid that are formed in the body are excreted in the urine in low amounts and are harmless to the body. When there is an excess of oxalic acid in the body, the kidneys are first to be effected. The excess oxalic acid binds to calcium ions, which then forms a salt known as calcium oxalate that is deposited in the kidneys.

As with struvite, the formation of calcium oxalate stones is highly dependent on urinary pH. Although struvite crystal formation is inhibited at a urine pH < 6.6, further reduction of urine pH below 6.0 increases the risk of calcium oxalate formation.

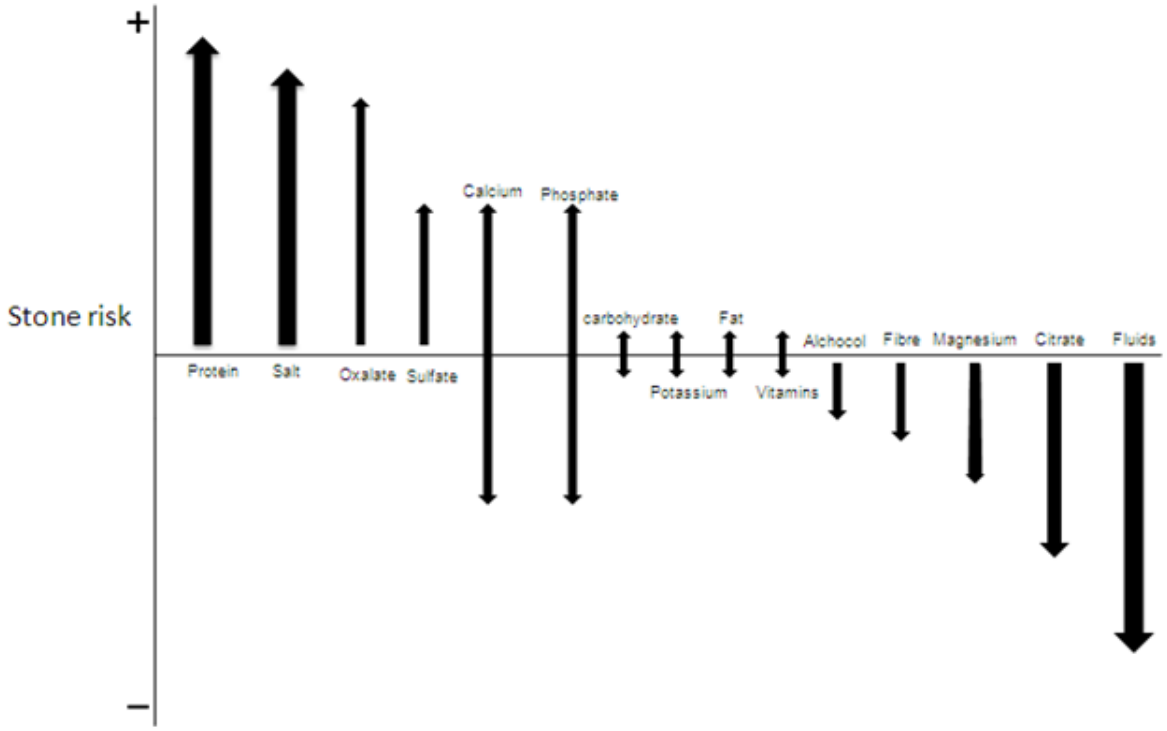


Figure 3.5 Relative calcium oxalate stone risk with different dietary components

(Adapted from Parivar et al, 1996)

In Figure 3.5, Parivar *et al.* (1996) identify the factors that have the biggest influence on calcium oxalate stone formation. Proteins together with salts (sodium) are the dietary components that play the biggest role in the process of nucleation. This agrees well with findings of Trinchieri *et al.* (1991) as quoted by Parivar *et al.* (1996) that showed that a vegetarian diet containing little or no animal protein lowers the risk of urolithiasis despite being high in oxalate. In Figure 3.5 sulfates are also one of the main dietary components, with calcium and phosphate acting either as inhibitors or inducing factors of calcium oxalate formation, depending on the stone type. In Figure 3.5, Parivar et al. (1996) indicated that magnesium has an inhibiting effect on calcium oxalate stone formation. This inhibiting effect of magnesium on calcium oxalate has also been demonstrated by Trinchieri *et al.* (1991) as quoted by Parivar *et al.* (1996) showing that magnesium supplementation decreases nucleation and growth of calcium oxalate crystals.

3.4.3 Factors that influence stone formation

3.4.3.1 Urinary pH

Struvite crystal formation is inhibited at a urine pH of less than 6.6. Further reduction of urine pH can however lead to the formation of calcium oxalate calculi. If acid intake exceeds excretion, the animal is in a state of metabolic acidosis. This can lead to mineral imbalances and calcium oxalate urolithiasis (Buffimgton, 1994).

3.4.3.2 Mineral imbalances

Minerals are interrelated. If minerals do not stand in the right relation towards each other urolithiasis can develop.

In a study conducted by Pastoor *et al.* (1995), the influences of dietary magnesium on urinary excretion of calcium, magnesium and phosphorus were investigated.

Pastoor *et al.* (1995) found that in rats, an elevated level of dietary magnesium increased the excretion of calcium in the urine. In another study done in cats, an elevated level of dietary magnesium also increased urinary excretion of calcium (Mars *et al*.1988, Sterck *et al*. 1992, Bergstra *et al*. 1993 as quoted by Pastoor *et al.*1995). According to Pastoor *et al.* (1995) this phenomenon, where there is an increase of urinary calcium excretion when dietary magnesium is supplemented, is due to the competition between calcium and magnesium for tubular reabsorption. The increased load of magnesium that needs to be filtered by the kidneys depresses reabsorption of urinary calcium and consequently increases urinary calcium excretion.

There is an interplay between dietary intake of magnesium or phosphorous and its effect on urinary excretion of magnesium or phosphorous.

In the study conducted by Pastoor *et al.* (1995), it is shown that when dietary magnesium increases, urinary magnesium excretion also increases while urinary phosphorous on the other hand decreases. The excess magnesium

binds to available phosphorous and forms an insoluble $MgPO_4$-complex in the lumen of the intestine. The decrease in urinary phosphorous excretion can thus be explained by the formation of the $MgPO_4$-complex. This $MgPO_4$-complex lowers the availability of phosphorous to be absorbed in the body (Pastoor *et al,* 1995). The $MgPO_4$-complex is already part of the building material for the struvite calculi. An increase in urinary formation of $MgPO_4$, thus promotes struvite stone formation.

Parivar *et al.* (1996) reports that a high phosphate intake increases urinary phosphate excretion while at the same time decreasing urinary calcium. Parivar *et al.* (1996) stated that it is mostly due to a decrease in vitamin D that decreases intestinal calcium absorption.

In light of the above mentioned observations, a direct relationship between an increase in dietary magnesium and urolithiasis should be clear.

However, in a reviewed article, Parivar *et al.* (1996) challenges the view of Pastoor *et* al. (1995). In contrast to Pastoor *et* al. (1995), Parivar *et al.* (1996) argues that the supplementation of magnesium decreases the rate of stone formation through two mechanisms: The first mechanism is that an increased excretion of citrate (a natural inhibitor of calculi formation) decreases the rate of stone formation. Parivar *et al.* (1996) explains that the citrate chelates the calcium in solution. It then forms a highly soluble calcium-citrate complex that decreases ionic concentrations of calcium and subsequently decreases urinary calcium. This explains why hypocitraturia is a predisposing factor to calcium oxalate stone formation.

The second mechanism proposed by Parivar *et al.* (1996) is the binding of magnesium to intestinal and urinary oxalate in order to produce a more soluble magnesium-oxalate complex than the calcium-oxalate complex.

There are thus conflicting studies, one reporting that dietary magnesium promotes urolithiasis while the other study arrived at the conclusion that dietary magnesium prevents urolithiasis in animals. However, all these studies agree that magnesium affects other minerals that play roles in urolithiasis formation.

3.4.3.3 Protein

A vegetarian diet that consists of a minimal of animal proteins was shown to decrease the risk of urolithiasis (Robertson *et al.* 1980 as quoted by Parivar *et al*. 1996). This is especially true if the protein is high in amino acids that contain sulfates such as methionione. When there is a high concentration of sulfates in the urine, the excess sulfates bind to calcium in the urine to form a complex that prevents the absorption of calcium by the tubular cells (Arora *et al.* 1985 as quoted by Parivar *et al*. 1996). As protein is ingested, endogenous acid production is increased. This leads to an increase in acid excretion in the urine that makes the urine more acidic and consequently decreases the urine pH. Together with this, a diet high in animal protein also contributes to the formation of urolithiasis by increasing oxalate and uric acid which are constituents of calcium oxalate calculi (Parivar *et al*. 1996).

3.4.3.4 Bacterial infection

Bacteria were identified as contributing factors in the formation of struvite urolithiasis. The specific types of bacteria that contribute to struvite urolithiasis are: *Staphylococcus spp., Proteus spp.* and *E.coli communis spp.* (Houston, 2003; Seaman & Bartges, 2001; Griffith, 1978)

These species are urease-producing bacteria which mean that this bacteria metabolizes urea to ammonia and carbon dioxide in the presence of water (Griffith, 1978; Seaman & Bartges, 2001). There are thus increasing amounts of ammonia present in the urine. If the ammonia combines with water or a hydrogen ion (H^+), it forms ammonium which causes the urinary pH to become alkaline. Seaman and Bartges (2001) continues that if this H^+ originates from H_3PO_4. The PO_4^{3-} will form a part of the calculi by binding to excess magnesium (if available) and together with the available ammonium, form $Mg_2NH_4PO_4$ (Srutvite) (Fig. 3.6)

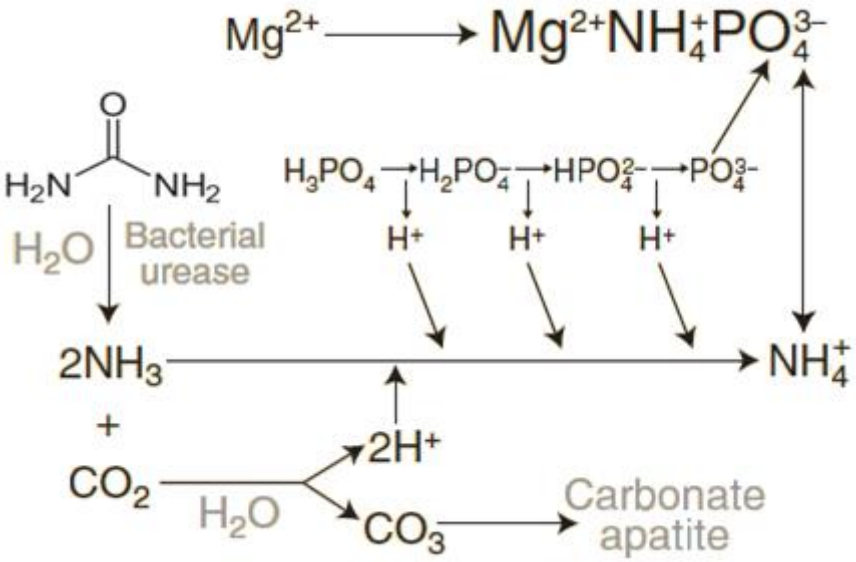


Figure 3.6 **Diagrammatic representation of formation of struvite due to bacterial infection by urease-producing bacteria**
(Seaman & Bartges, 2001)

Urease bacteria, as explained above, are responsible for increasing levels of ammonia. Add to these excess levels of magnesium and the formation of urolithiasis greatly increases.

3.4.3.5 Chemicals

Ethylene glycol commonly known as “Antifreeze” is a common substance used in automobiles to prevent engine water from freezing. Ethylene glycol is a byproduct that is formed during the process of fermentation of molasses into citric acid. Ethylene glycol is however also used as a poison to induce calcium oxalate urolithiasis. When ethylene glycol is oxidized by the body, it forms oxalate. When there is an excess amount of ethylene glycol in the body (of which one cause is high amounts of antifreeze in the diet) the body becomes overwhelmed and consequently unable to detoxify the high amounts of oxalate. This leads to calcium oxalate urolithiasis formation (Goudas and Lusis, 1970).

3.5 SUMMARY

From this chapter it is evident that an in depth understanding of mineral interplay- especially the interplay between calcium, magnesium and phosphorus- is vital to prevent adverse physiological conditions namely metacarpal deformity and urolithiasis.

On the one hand, dietary magnesium supplementation was shown to remedy metacarpal deformity in cats and dogs.

On the other hand however, dietary magnesium supplementation affects the relationship between magnesium, calcium and phosphorus in the body. Changes in this mineral interrelationship may have a negative effect on the formation of urinary calculi and thus increase the animal's risk of developing urolithiasis.

The delicate mineral balance between calcium, magnesium and phosphorus still needs to be quantified. When supplementing dietary magnesium to remedy metacarpal deformity, one has to constantly monitor this mineral balance in order to prevent the induction of urolithiasis.

CHAPTER 4

RESEARCH METHODOLOGY

4.1 INTRODUCTION

In this chapter the experimental design is presented and the methodology used is explained.

4.2 EXPERIMENTAL DESIGN

The experimental design of a scientific experimental study is a complete layout of the methods to be used to investigate the aim of the study in order to obtain results that can be compared to the hypothesis.

In this study, two different research designs were used to investigate the two different phases of the study.

In phase 1, the *between-subjects design* (Completely randomized design) was used. In phase 2, the *between*-subjects design as well as the *within*-subjects design was used. The details of each of these designs are discussed under the relevant sections.

4.3 RESEARCH METHODOLOGY

Keppel (1991) stated that the experimental method is the contrast between two treatment conditions. The subjects of both these conditions are treated identical, except for one feature, the independent variable that is different. Some aspects of the performance of the subjects in the two treatment conditions are measured and recorded after the administration of the treatment. These aspects are referred to as

the dependent variables. Any difference between the two conditions that are observed on the dependent variable, are referred to as the treatment effect.

4.3.1 Experimental layout

The study was divided into two phases.

Phase 1 was to investigate the aim as stated in 1.3 a) *Does magnesium supplementation correct metacarpal deformities* by means of a *within*-subjects design.

Following phase 1, phase 2 was designed to investigate the aim as stated in 1.3 b) *The influence of magnesium supplementation on the risk of urolithiasis* by making use of a *between*-subjects design together with a within-subjects design. Table 4.1 and Table 4.2 give the experimental layout of each of the two phases in table format.

Table 4.1 Experimental design and layout of phase 1

PHASE 1		
Period	Period 1	Period 2
Experimental Design	Within-subject design	Within-subject design
Subjects	Cheetahs	Cheetahs
Treatment Groups	Control group	Experimental Group
Treatment Conditions	Dietary Supplementation	Dietary Supplementation
Treatment Levels	0g Mg/kg	1g Mg/kg
Independent Variable/Diet	Meat-Only	Meat-Mg
Dependent Variables	RDS, FDS	RDS, FDS

*RDS - Rotational Deformity Score
**FDS - Flexural Deformity Score

Table 4.2 Experimental design and layout of phase 2

PHASE 2				
Period	Period A	Period B	Period C	
Experimental Design	Within-subject design	Within-subject design	Between-subjects design	Between-subjects design
Subjects	Cheetahs	Cheetahs	Cheetahs	Cheetahs
Treatment Groups	All cheetahs remained in same treatment group	All cheetahs remained in same treatment group	Cheetahs used in Period A & B divided into 2 groups:	
			Group CE	Group CC
Treatment Conditions	Dietary Supplementation	None	Dietary Supplementation	Dietary Supplementation
Treatment Levels	1g CVM	0g	2g Mg	0g Mg
Independent Variable/Diet	Meat-CVM	Meat-Only	Meat-Mg	Meat-Mg
Dependent Variables	Plasma Ca	Plasma Ca	Plasma Ca	Plasma Ca
	Plasma Mg	Plasma Mg	Plasma Mg	Plasma Mg
	Plasma P	Plasma P	Plasma P	Plasma P
	Urine pH	Urine pH	Urine pH	Urine pH
	Urine NH_4^+	Urine NH_4^+	Urine NH_4^+	Urine NH_3
	Urine NO_3^-	Urine NO_3^-	Urine NO_3^-	Urine NO_2
	Urine Total N	Urine Total N	Urine Total N	Urine Total N
	Urine K	Urine K	Urine K	Urine K
	Urine Na	Urine Na	Urine Na	Urine Na
	Urine S	Urine S	Urine S	Urine S

4.3.1.1 Phase 1: *Within*-subjects design

In the *within*-subjects design, *all* subjects are assigned to *all* the treatment conditions. Any differences in behavior observed among the treatment conditions are based on the *same* set of subjects. The treatment effects are represented by differences *within* a single group of subjects.

The advantage of this type of design, as stated by Keppel (1991) is that fewer subjects are required and that it is more sensitive to the treatment conditions. The disadvantage however is that the subjects can change while they are receiving the different treatment conditions. For this reason, it is vital that all other variables (nuisance variables[8]) that can influence the treatment effects must remain constant throughout the whole experiment.

Phase 1 comprises of 2 different periods. In each period, the subjects and treatment conditions remain constant. The independent variable and treatment level, however, alter between the two different periods.

4.3.1.2 Phase 2: *Within*-subjects design and *between*-subjects design (Completely randomized design)

In the *between*-subjects design, the subjects are only assigned to *one* of the different treatment conditions. This design has the advantage that it is a relative simple design that is easy to analyze and is relatively free from restrictive statistical assumptions. The disadvantage however is that it requires a relatively large number of subjects which can make the experiment costly.

Phase 2 consists of three different periods. In periods A and B, a *within*-subjects design was used. In period C, a *between*-subjects design was used.

During period A, the pre-trial period, all subjects were examined and their dependent variables recorded. All the subjects received the same independent variable (Meat-CVM; see Appendix B) and treatment levels.

[8] Nuisance variables – Potential independent variables which, if left uncontrolled, could have an influence on the treatment conditions and treatment effects. (Keppel, 1991)

During period B, the withdrawal period, the subjects received a different independent variable (Meat-Only) than in period A. This period served to exclude any carry-over effects that might act as nuisance variables.

During period C, the experimental period, subjects were randomly divided into two treatment groups. Treatment groups received the same treatment condition but with two different treatment levels. The independent variable remains constant throughout this whole period. The same dependent variables are compared between the two different treatment groups.

4.3.2 Subjects used and Treatment groups

The subjects of an experiment are the experimental units that are subjected to the treatment conditions. In this study, the subjects used in both phases were cheetahs from the Hoedspruit Endangered Species Centre (HESC).

Phase 1

The subjects used in phase 1 remained the same in period 1 and 2. The subjects were four juvenile cheetahs aged between 0 to 18 months and included both males and females. The juvenile cheetahs of phase 1 had been diagnosed with front leg deformities.

Phase 2

Twenty-four male as well as female cheetahs, between ages 16 months to 13 years with a weight variation between 18 to 53kg were used.

During period A and B of phase 2, all twenty-four cheetahs remained together as one sample group.

During period C, the cheetahs were randomly divided into two different treatment groups namely group CC (control group) and group CE (experimental group). These groups received different treatment levels.

4.3.3 Treatment conditions and –level

Treatment conditions are the conditions that the subjects are objected to. In this study, the cheetahs (the subjects) were all subjected to different treatment conditions (dietary supplementation). The different treatments were added to portions of beef chunks (2-5kg for adults; 500g-2kg for juveniles).

Treatment levels are the quantitative value of the independent variable that the cheetahs had been objected to. The treatment level varies between the different treatment conditions and two different phases of the study.

Phase 1

During period 1 of phase 1, the cheetahs received a treatment level of 0g of the independent variable (magnesium) that was added to their diet. In period 2 of phase 1, they received a treatment level of 1g of the independent variable (magnesium) that was added to their diet.

Phase 2

During period A (pre-trial period) of phase 2, all cheetahs received the same treatment condition (dietary supplementation) and treatment level of 1g of the independent variable (CVM supplementation) that was added to their diet.

During period B (withdrawal period) of phase 2, all cheetahs received the same treatment condition (dietary supplementation) and treatment level of 0g of the independent variable (no supplementation) that was added to their standard diet.

During period C (experimental period) of phase 2, all cheetahs received the same treatment condition (dietary supplementation). Treatment group CC received a treatment level of 0g of the independent variable (magnesium supplementation) added to raw beef chunks. Group CE received a treatment level of 2g of the independent variable (magnesium supplementation).

4.3.4 Independent variable

This variable is under direct control of the researcher and can be manipulated by the researcher. In both periods 1 and 2 of phase 1 of the study, the independent variable was magnesium which was supplemented to the diet of the cheetahs (the subjects) at different levels.

In phase 2, the independent variable of period A was a CVM-supplementation; period B had no supplementation while the independent variable of period C was magnesium. The independent variables were supplemented to the diets of the cheetahs (the subjects) at various levels.

In this study, the focus was on the *relationship* between the two treatment levels of the independent variable. This type of independent variable is described by Kirk (1982) as a *quantitative* independent variable where the different treatment levels constitute different amounts of the independent variable. With a quantitative independent variable, there is little interest in the exact values of the treatment levels used in the experiment.

4.3.5 Dependent variable

The variable that reflects any effects associated with manipulation of the independent variable (Kirk, 1982).

Phase 1

Two different categories of leg deformities were identified during phase 1: flexural- and rotational- deformities. For the two deformities identified, each cheetah was scored and given a numerical value. This numerical value was named either RDS (Rotational Deformity Score) or FDS (Flexed Deformity Score). Thus, the dependent variables of phase 1 are the RDS and FDS values of each cheetah.

Phase 2

During phase 2, plasma- and urine samples were obtained for each individual cheetah. These samples were analyzed for different levels of minerals as well as

urine pH. These different mineral levels as well as the urine pH are the dependent variables of phase 2.

4.3.6 Treatment effect

Treatment effect refers to the dietary supplementation and the different treatment levels were magnesium or CVM levels. The treatment effect will be discussed in greater detail in Chapter 6.

4.3.7 Feed Preparation

Adult cheetahs (older than 18 months) received between 2 to 5kg meat chunks whereas juvenile cheetahs received between 500g to 2kg per day. Each cheetah received its own chunk of meat from which excessive subcutaneous fat was removed. Each chunk of meat was grilled on the surface for a few seconds to kill any surface bound bacteria. To add supplementations (both magnesium and CVM), two to three cuts were made into each meat chunk into which the supplement was carefully inserted as indicated in Figure 4.1. The cheetahs were fed on Mondays, Wednesdays and Fridays between 8h00-10h00 with water supplied *ad lib*.

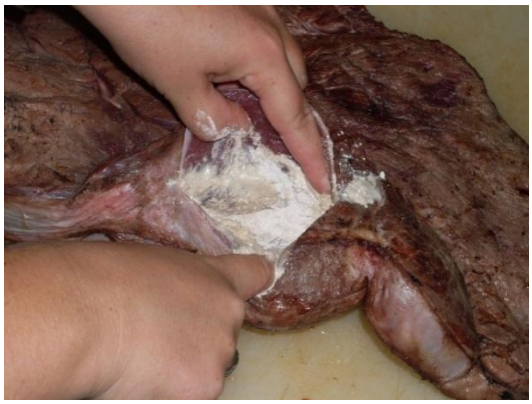


Figure 4.1 Insertion of CVM-supplementation into meat chunk fed to the cheetahs

4.3.8 Available Resources

All cheetahs included in the research project were the property of the (HESC). Cheetahs were kept in large camps either in small groups, or as individual animals depending on the age and sex of the animals. Camps were equipped with trap cages, used to trap animals to enable the veterinarian to administer the anesthetics. The HESC Clinic was used for all medical procedures done on the cheetahs and also for the collection of blood -and urine samples. The clinic was a very well organized and fully equipped animal clinic that was well suited for the required medical procedures. Samples were analyzed at the laboratories at the Faculty of Natural Sciences of the University of Pretoria.

4.4 FLEXURAL DEFORMITY

The leg deformities of the cheetahs' frontal paws were investigated during phase 1 of the study. Although most of the methods described in literature were developed specifically to be used on equine, these methods can be adapted to be used on most animals.

Several different methods were proposed by various authors. Some of these methods are designed to be used on specific animals only, whereas other methods can be adapted to be used on a variety of animals.

Radiographs are one method that is widely used. It has the benefit of not only confirming the specific location of the deformity, but also providing objective values for the degree of angulations. They are very helpful when assessing forelimb deviations (Fretz, 1980 as quoted by Witte & Hunt, 2009; Bertone *et al*. 1985; Jansson & Ducharme, 2005). The problem, however, with this type of assessment, is that it is quite costly especially when a large number of animals have to be assessed.

A more commonly used method is that of observation. This method has the advantage that each individual animal can be assessed in different dimensions (Love *et al.* 2006) with minimal extra costs. It is done when the animal is at rest (static

evaluation) or when the animal walks towards and away from the observer (Dynamic evaluation), as described by Witte and Hunt (2009). Static evaluation is performed 1 or 2 meter in front of the animal in line with the carpus. Dynamic evaluation is used to assess the influence of weight bearing and is mostly used in animals carrying weights e.g. horses. This method of observation has the disadvantage of subjective observation and can therefore not be used when there is a need to differentiate between very fine levels of deformities. It is however a relative uncomplicated and cost effective method if there has to be differentiated between broad classifications of deformities.

4.4.1 Leg deformity scoring

Since no leg scoring method for cheetahs could be found in the literature, methods used in horses and dogs (Witte & Hunt, 2009) were investigated to develop a unique scoring system for analyzing leg deformities specifically for cheetahs.

Depending on the joint examined, different angles between different bones were observed. These angles were used to compare the level of deformity between the different cheetahs.

Each cheetah was scored twice. First the lateral view was used to give the deformity a FDS (Flexed Deformity Score) based on the degree of deformity observed. Each cheetah's FDS value was used to determine the degree of flexion of the frontal paw. Second the frontal view was used to score the RDS (Rotational Deformity Score) based on the degree of rotation of the frontal paw.

Scores were scaled from 1-3. A score of 1 indicates a normal leg with no observed deformities. A score of 2 indicates moderate signs of deformity, and a score of 3 indicates severe forms of deformity. This scoring system is very similar to the system used to describe the body condition of dairy cows.

4.4.2 Scoring method

Each cheetah was observed as follows:

- The cheetah was observed in a standing position on a smooth, level, surface. The cheetah was observed from a frontal view with the observer standing about 1 meter away.

- Two joints were observed: (Figure 4.2 & Figure 4.3)
    - *Carpal joint:* Between the medial planes of the radius/ulna and the metacarpal bones (Figure 4.2 A).
    - *Metacarpal joint:* Between the medial planes of the metacarpal bones and the proximal phalanges (Figure 4.2 B).

- Each individual cheetah was scored according to these angles. (Figure 4.2)

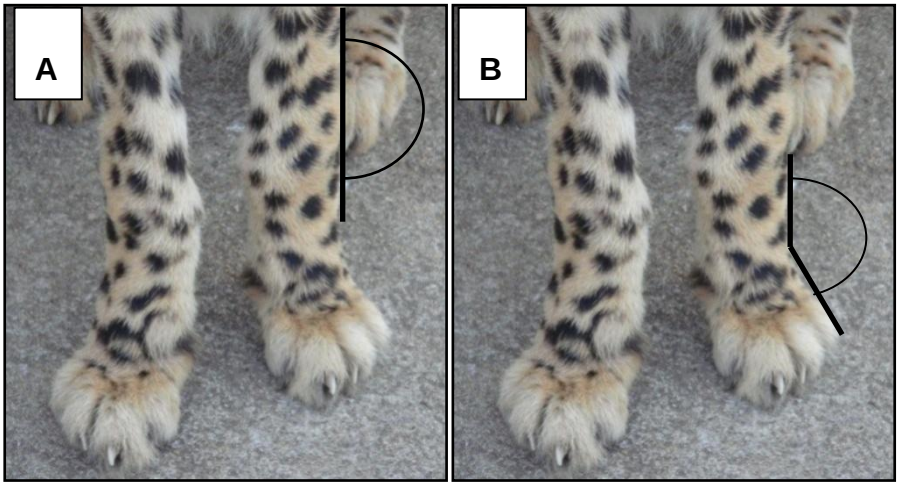


Figure 4.2 Two angles used to score the cheetah for a RDS value
A - Carpal joint; B - Metacarpal joint

- With the cheetah still in the same position, the observer changed position so that the cheetah was now observed from a lateral view.

- Only the *metacarpal joint* between the palmar planes of the metacarpal bones and proximal phalanges was observed. If the angle was normal, the leg was

scored: FDS=1. If the front paw either over-flexed or overextended, the leg was scored: FDS=2 (intermediate) or FDS=3 (severe). (Figure 4.3)

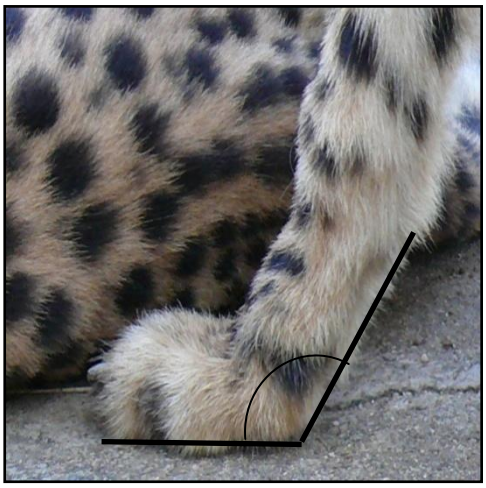


Figure 4.3 Angle used to score the cheetah for a FDS value

4.5 MINERAL ANALYSES

4.5.1 Sampling procedures

During phase 2 of the study, blood and urine samples were obtained to investigate the concentration of minerals in the cheetah's blood plasma and urine.

The cheetahs were sampled three times during phase 2. The first sampling was done at the end of period A (pre-trail sampling); the second sampling was done after period B (withdrawal period) and the third sampling was done after period C (post-trail sampling).

Depending on weight, cheetahs were immobilized and anesthetized. During each sampling, the cheetahs were identified by name (by the curator) and scanned for its universal identity microchip number. They were also weighed and visually inspected by a veterinarian. Where possible, kidney measurements were also taken by use of ultrasound.

4.5.1.1 Blood samples for magnesium and calcium analyses

Samples were obtained from each cheetah by venepuncture of the medial saphenous vein (*v. saphena medialis*).

The blood samples for magnesium and calcium analysis were collected into 5ml heparinised vacutainers. The samples were centrifuged at 3000rpm for fifteen minutes to separate plasma and blood cells. Plasma samples were then frozen and stored below 0°C for laboratory analysis.

4.5.1.2 Blood samples for phosphorous analysis

In order to correctly measure inorganic phosphorus concentration in blood or blood plasma, samples need to be preserved in order to prevent the hydrolysis of the inorganic phosphorus. A protein precipitate, tri-chloro acetic acid (TCA) was used as buffer to prevent the hydrolysis. The degree of hydrolysis, which affects the analysis, is a function of both the concentration of TCA and the length of blood storage before protein precipitation and analysis (Little *et al.* 1971).

Prior to blood sample collection, 10ml of a TCA-buffer was added to 25ml glass sampling bottles. The 10% m/v TCA buffer was prepared as follows:

- Exactly 10g of Tri-chloro acetic acid (TCA) was weighed.
- It was made up to a total of 100ml with distilled water in a volumetric flask.
- 40g of Sodium tri-chloro acetate (NaTCA) was weighed.
- It was made up to a total of 400ml with distilled water in a volumetric flask.
- The 100ml TCA-solution was added to the 400ml NaTCA-solution in a measuring cylinder.
- It was stirred thoroughly. The pH should be 1.25 ± 0.05

Blood samples were obtained from each cheetah by venepuncture of the medial saphenous vein (*v. saphena medialis*) and collected into 5ml heparinised vacutainers. The samples were centrifuged at 2500rpm for 16 minutes to separate plasma and blood cells.

A pipette was used to obtain 2ml plasma from the separated blood samples of the heparinised vacutainers. The 2ml plasma was added to the 10ml buffered TCA-solution and mixed thoroughly for good precipitation. It was stored below 0°C for laboratory analysis.

4.5.1.3 Urine samples

Urine samples were collected either in 25ml glass bottles or 15ml clear tubes by method of cystocentesis as described by Kurien *et al. (2004).* The cheetah was either in lateral recumbence (laid on its side) and palpated by hand to locate the bladder or dorsal recumbence where the bladder was located with the aid of ultrasound. A needle (22 guage) was inserted into the bladder to collect the urine sample. If the bladder could not be allocated or no urine could be collected, a catheter was used to collect a urine sample. To avoid injury, both methods were only attempted two to three times. If the method was done to no avail, no sample was collected from the cheetah. Samples were refrigerated below 0ºC and stored for laboratory analysis.

4.5.2 Laboratory serum and plasma analyses

The analysis of the cheetahs plasma samples were done by lab technicians at the Nutrilab of the Department of Animal- and Wildlife Science of the University of Pretoria.

The plasma samples were thawed and kept at room temperature for the lab analysis to be done.

4.5.2.1 Magnesium and calcium analyses

The magnesium- and calcium concentration of the cheetahs plasma were determined by the AOAC Method 935.13 as described by Pybus (1968), Savory *et*

al. (1969) and Zettner & Seigson (1974). After the samples were thawed, 2ml of the plasma samples were transferred to test tubes in duplicate form. One 2ml test tube of the plasma was analyzed for magnesium while the other 2ml test tube was tested for calcium.

The one test tube with 2ml plasma for magnesium analysis was placed in an Atomic Absorption Spectrophotometer (Varian SpectroAA 50). For each plasma sample, two readings were taken, and the average was recorded to be analyzed.

The other test tube also containing 2ml plasma was used to determine the calcium concentration of the plasma. The test tube was placed in a different Atomic Absorption Spectrophotometer (Perkin-Elmer 5100pc) and the reading was recorded for analysis.

4.5.2.2 Phosphorus analysis

The phosphorous concentration of the cheetah's plasma was determined by the AOAC Photometric Method 965.17 as described by Little *et al.* (1971). After the plasma-TCA-solutions were thawed, the samples were placed in a Spectrophotometer (Analytip jena Spekol 1300SPM) and the phosphorus readings were recorded for analysis.

4.5.3 Laboratory urine analysis

After the urine samples were collected at the HESC clinic, the samples were kept below 0ºC, using a well insulated container. The samples were then transported to the storage facilities at the University of Pretoria. These facilities were well equipped to store samples at a constant temperature of below 0ºC.

When samples were ready for analysis, the samples were removed from the storage facility and thawed to room temperature.

The analysis of the cheetah's urine samples was done by a lab technician at the laboratory of the Department of Soil Science at the University of Pretoria.

4.5.3.1 pH

The pH of the urine samples were determined by using a digital pH meter (Mettler Toledo MP230). The pH meter was calibrated by using two solutions of pH 4 and pH 7. The pH of each sample was recorded for each available sample.

4.5.3.2 Minerals

All the minerals (Ca, Mg, P, K, Na and S) were analyzed at the same time, using an Optical Emission Spectrometer with Inductively-Coupled Plasma excitation (ICP-OES). This is a well suited technique which is widely used for elemental analyses. ICP-OES uses the intensity of light, emitted from a flame or plasma at a particular wavelength to determine the quantity of an element in a sample. It utilizes UV and visible spectrometry to image the plasma at the exact wavelength of ionic excitation of the element.

The process consists of the conversion of the molecules in the sample to individual atoms and ions by using high temperature, radio frequency induced argon plasma. The plasma consists of a hot, partially ionized gas and contains an abundant concentration of cations and electrons. This makes the plasma a conductor.

The readings were taken by a lab technician of the Department of Soil Science at the University of Pretoria. The data was recorded separately for each sample and documented to be statistically analyzed.

4.5.3.3 Nitrogen

Urinary nitrogen concentrations (NH_4^+; NO_3^+ and Total Nitrogen) were determined by the Kjeldahl-method. This method was developed by Johan Kjeldahl in 1883. Although the technique and apparatus have been altered considerably over the past 100 years, the basic principles still apply today.

The Kjeldahl-method can be broken down into three main steps. In the first step, digestion, the nitrogen in the urine samples was decomposed by using a concentrated acid solution. In the next step, distillation, excess base was added to

the acid mixture to convert NH_4^+ to NH_3. The last step, titration was then used to quantify the amount of nitrogen in the sample.

The readings were taken by a lab technician of the Department of Soil Science at the University of Pretoria. The data was recorded separately for each sample and documented for statistical analysis.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 INTRODUCTION

Statistical data obtained by the scoring system for metacarpal deformity and analyzing plasma and urine concentrations are presented and discussed.

5.2 METACARPAL DEFORMITY

Metacarpal deformity was investigated in phase 1 of this study.

As shown in the literature, metacarpal deformity is a condition where the front paw of the animal is in an over-flexed position. This can be either a congenital deformity, which are present at birth, or an acquired deformity that develops after birth during the remainder of the animal's life and are due to reasons such as trauma and nutrition (Auer, 1999; Hay & Mueller, 1999 as quoted by Altunatmaz & Ozsoy, 2006).

The cheetahs at the HESC were weaned early and start to develop this deformity soon after birth. It was thus believed that this was an acquired deformity that could be rectified by nutrition in the form of dietary magnesium supplementation.

Chapter 4 explained in detail the method used to score the front paws of the cheetah cubs. Two scores were recorded to quantify the extent of the deformity and to monitor the effects of the dietary magnesium supplementation the cheetahs received. These scores are presented in Table 5.1 and Table 5.2.

Table 5.1 Rotational Deformity Scores (RDS) for cheetahs in phase 1

	RDS Score		
Cheetahs	Before Treatment	After Treatment	Response (%)*
Tristan	3.0	2.0	33.3
Thea	2.5	2.0	20.0
Sarel	2.0	1.0	50.0
Vrede	2.0	2.0	0.00
Average**	2.4	1.8	25.8

*Response: Percentage differences between before- and after treatment scores

**Average: Mathematical average of four cheetahs

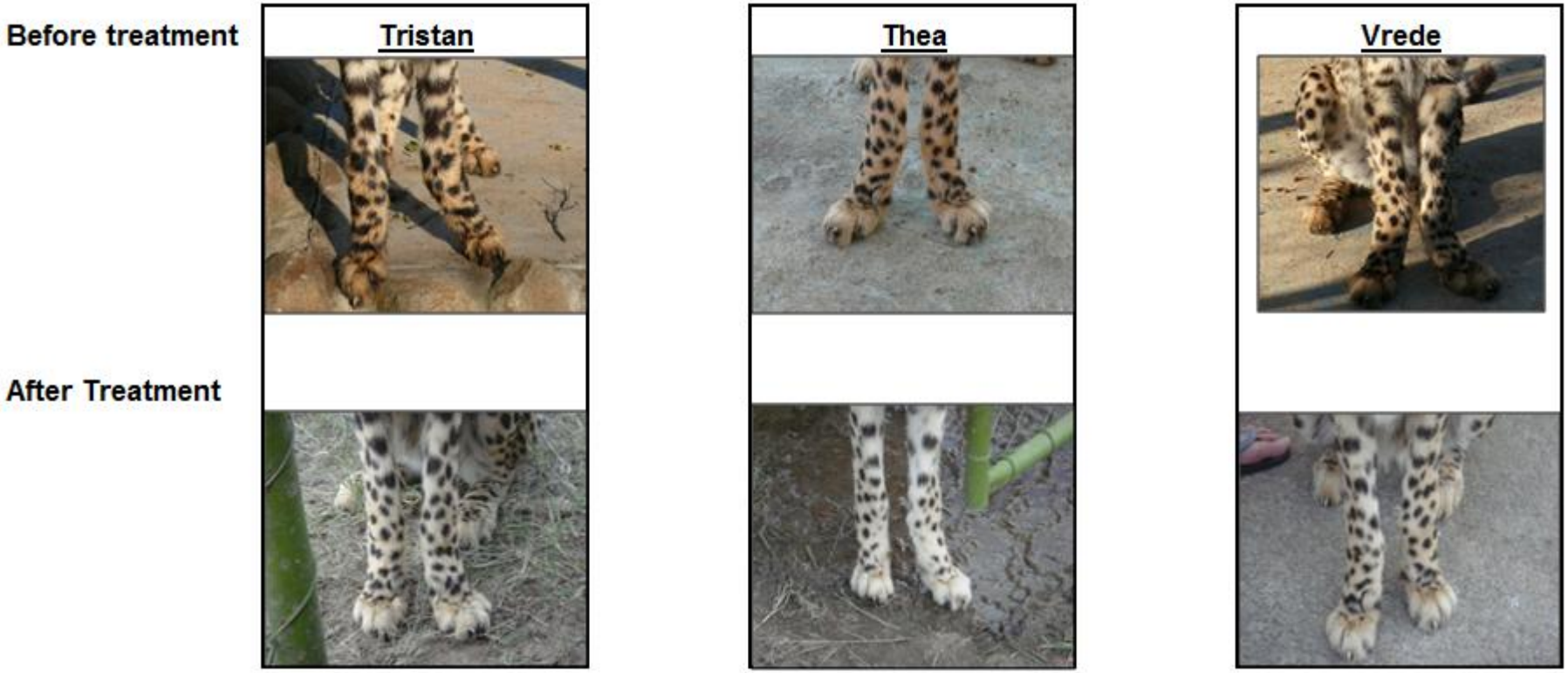
Table 5.2 Flexural Deformity Scores (FDS) for cheetahs in phase 1

	FDS Score		
Cheetahs	Before Treatment	After Treatment	Response (%)*
Tristan	2.5	1.0	60.0
Thea	3.0	1.0	66.6
Sarel	3.0	1.0	66.6
Vrede	2.0	1.0	50.0
Average**	2.6	1.0	60.8

*Response: Mathematical differences between before- and after treatment scores

**Average: Mathematical average of four cheetahs

Figure 5.1 Photos of cheetahs before- and after treatment



5.2.1 Rotational Deformity

Table 5.1 presents the Rotational Deformity Scores (RDS) of the four juvenile cheetahs used in the study. The RDS scores for the cheetahs, before they received the magnesium treatment, varied from 2 to 3 with an average of 2.4. RDS scores for the cheetahs after they received the dietary magnesium supplementation varied from 1 to 2 with an average of 1.8. The response between the before- and after treatment RDS scores varied from 50% to 0% with an average response of 25.8%.

Rotational leg deformities in the front paw of juvenile cheetahs thus showed a small respons to dietary magnesium supplementation. When these four response values were compared to one another statistically, by using a proc-logistic analysis, no significant response ($p > 0.05$) was however observed. As described in Chapter 4, the experimental design used for this phase (phase 1) was a *within*-subjected design. This design has the advantage that not many subjects were needed. Due to financial as well as logistic implications in this study, only four subjects were included; this made the *within*-subject design particularly appropriate for this study. In order to conduct a proc-logistic analysis however, more subjects were needed to indicate whether the average response of 25.8% was significant or not. There were thus too few subjects in this study to allow for the indication of whether 25.8% was statistical significant or not.

5.2.2 Flexural Deformity

In Table 5.2 the Flexural Deformity Scores (FDS) of the four juvenile cheetahs included in the study are presented. The FDS scores for the cheetahs, before they received the magnesium treatment varied from 2 to 3 with an average of 2.6. FDS scores for the cheetahs, after they have received the dietary magnesium supplementation was 1. The response between the before- and after-treatment FDS scores varied from 66.6% to 50% with an average response of 60.8%.

An average response of 60.8% to treatment indicated that the supplementation of dietary magnesium to a meat-only diet did have a positive effect on the deformity. Before the cheetahs received magnesium treatment, they all had very high FDS scores. This indicates that all of them were experiencing front leg flexural

deformities. After treatment, all the cheetahs had FDS scores of 1 which indicated that they did not experience any leg-deformities and that their front legs were normal.

As with the RDS scores, FDS scores did not vary significantly ($p > 0.05$) between before- and after-treatment values. FDS scores were also compared statistically by using a proc-logistic analysis. This analysis, as mentioned previously, needs more values in order make a meaningful comparison to subsequently indicated whether they vary significantly. The response was drastic: from an average of 2.6 which indicated severe deformity to 1, which indicated normality or no deformity.

5.2.3 Summary

From the data presented in Table 5.1 and Table 5.2, it could be seen that only dietary magnesium supplementation to an all meat diet had a positive effect on the two types of leg deformities in growing cheetahs.

The average response to RDS was 25.8% whereas the average response to FDS was 60.8%. Dietary magnesium supplementation thus had a higher response on flexural deformity than on the rotational deformity. Rotational deformities might have an element of inheritance as we observed that some of the parents of the juvenile cheetahs included in this study had rotational deformities of some degree.

The flexural deformity was cured with a response rate of 60.8%. Dietary magnesium supplementation thus had a positive influence on rectifying the flexural deformities. This reaffirms Auer's (2006) statement that "*balancing nutritional intake is the key in the prevention of flexural deformities.*"

5.3 UROLITHIASIS

Urolithiasis was investigated in phase 2 of this study.

Literature has shown that magnesium supplementation influences the formation of struvite- and/or calcium-oxalate calculi. Magnesium thus has the capacity to either induce or prevent urolithiasis in cheetahs.

In this section, the results of magnesium supplementation on certain blood and urine minerals that influence urolithiasis are presented and discussed. The interactions within these minerals and the interactions of these minerals with different ages and sexes of the cheetahs are also discussed in order to arrive at some conclusions.

5.3.1 Urinary pH and Body weight

Table 5.3 presents the effects of age and diet on the urine pH and bodyweight of the cheetahs. The values of the Meat-Mg diet are pooled values for *all* cheetahs of both group CC and group CE during the experimental period (period C) of phase 2, regardless of whether they received a Meat-only diet or a Meat-Mg diet. Data were only compared between columns within the same rows (between age groups). No data were compared between rows within the same column (between diets).

Table 5.4 presents the effects of sex and diet on the urine pH and bodyweight of the cheetahs. Data were only compared between columns within the same rows (between sexes). No data were compared between rows within the same column (between diets).

Table 5.5 presents the influence of different levels of dietary magnesium supplementation on urinary pH and body weight of the cheetahs. Data were only compared between columns within the same rows (between different levels of magnesium supplementation). No data were compared between rows within the same column (between parameters).

Table 5.6 and Table 5.7 present the interaction between age (Table 5.6) or sex (Table 5.7) and different levels of dietary magnesium supplementation on the urine pH of the cheetahs. Data were compared between columns within the same rows (different age groups) and between rows within the same column (between magnesium supplementation levels).

Table 5.3 The effects of age and diet on urine pH and body weight of cheetahs (n=24)

		Age (months)		
		0 - 47	48-71	> 71
	Diet	(LS Mean ± SD)	(LS Mean ± SD)	(LS Mean ± SD)
Urine pH	Meat-CVM	5.90 ± 0.330	6.20 ± 0.193	6.40 ± 0.772
	Meat-Only	6.10 ± 0.035	6.10 ± 0.098	6.10 ± 0.152
	Meat-Mg*	5.90 ± 0.352	6.00 ± 0.177	6.00 ± 0.216
Body Weight	Meat-CVM	$31.1 \pm 12.83_a$	$38.5 \pm 5.715_b$	$38.6 \pm 4.243_b$
(kg)	Meat-Only	$32.6 \pm 10.52_a$	$36.2 \pm 5.776_b$	$37.7 \pm 4.610_b$
	Meat-Mg*	$33.7 \pm 7.991_a$	$37.9 \pm 4.491_b$	$38.1 \pm 3.822_b$

$_{a,b,c}$ Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

*Pooled data. Mean value of all cheetahs receiving either a Meat-Only or Meat-Mg diet during experimental period

Table 5.4 The effects of sex and diet on urine pH and body weight of cheetahs (n=24)

		Sex	
	Diet	Female (LS Mean ± SD)	Male (LS Mean ± SD)
Urine pH	Meat-CVM	6.20 ± 0.625	6.00 ± 0.293
	Meat-Only	6.10 ± 0.111	6.00 ± 0.000
	Meat-Mg*	5.90 ± 0.269	6.00 ± 0.271
Body Weight	Meat-CVM	$32.7 \pm 6.360_a$	$39.4 \pm 10.78_b$
(kg)	Meat-Only	$33.9 \pm 5.662_a$	$36.1 \pm 8.522_b$
	Meat-Mg*	$33.2 \pm 3.679_a$	$38.6 \pm 6.481_b$

$_{a,b}$ Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

*Pooled data. Mean value of all cheetahs receiving either a Meat-Only or Meat-Mg diet during experimental period

Table 5.5 Influence of Mg supplementation levels on urine pH and body weight of cheetahs (n=24)

	Mg supplementation	
	0g Mg	2g Mg
Parameter	(LS Mean ± SD)	(LS Mean ± SD)
Urine pH	$6.10 \pm 0.193_{a}$	$5.80 \pm 0.211_{b}$
Body Weight (kg)	33.0 ± 6.325	34.8 ± 5.514

$_{a,b}$ Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

Table 5.6 The interaction between age and Mg supplementation level on urine pH in cheetahs*

		Age (months)		
		0 - 47	48-71	> 71
Parameter	**Mg supplementation**	(LS Mean ± SD)	(LS Mean ± SD)	(LS Mean ± SD)
Urine pH	0g Mg	6.20 ± 0.270[1]	6.10 ± 0.130	6.10 ± 0.120
	2g Mg	5.60 ± 0.140[2]	6.00 ± 0.190	5.90 ± 0.220

[1,2] Means within the same column with different superscripts differ significantly ($p \leq 0.05$)

[a,b,c] Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

* Body weight was excluded since no significant interaction was observed

Table 5.7 The interaction between sex and Mg supplementation level on urine pH in cheetahs*

		Sex	
		Female	Male
Parameter	**Mg supplementation**	(LS Mean ± SD)	(LS Mean ± SD)
Urine pH	0g Mg	6.10 ± 0.080[1]	6.20 ± 0.260[1]
	2g Mg	5.80 ± 0.270[2]	5.80 ± 0.140[2]

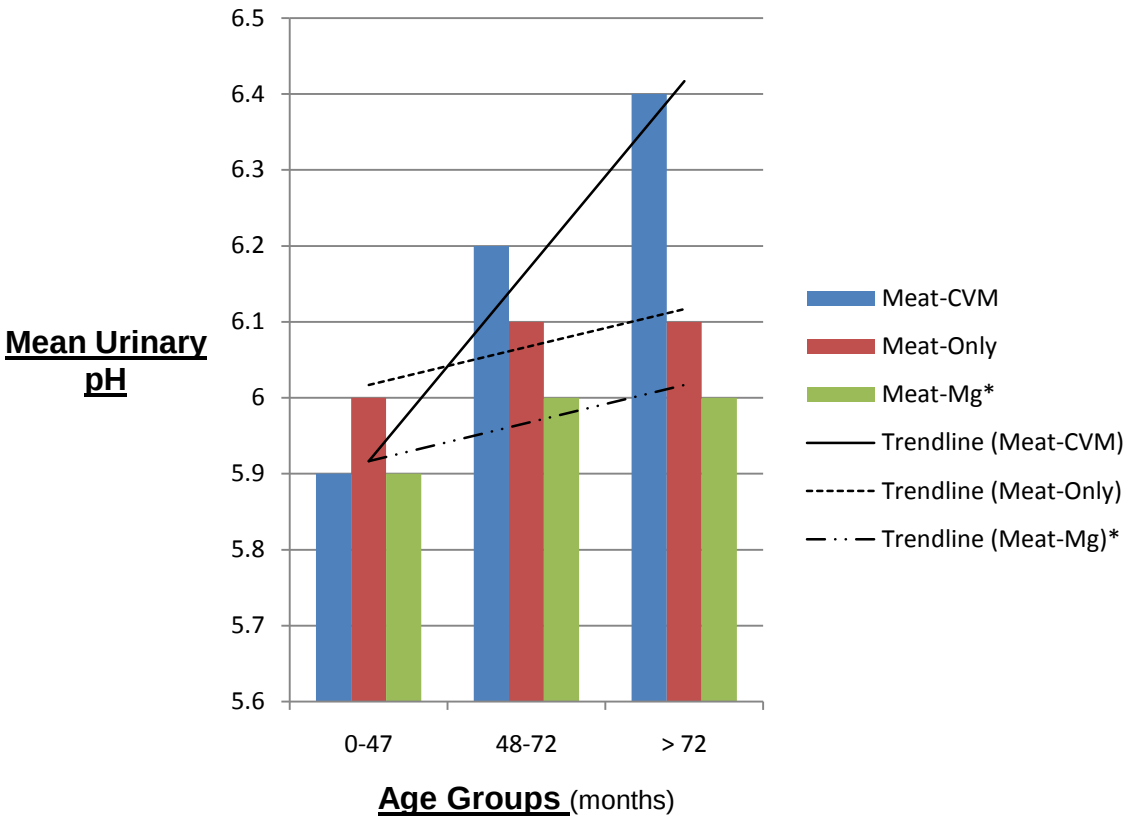
[1,2]Means within the same column with different superscripts differ significantly ($p \leq 0.05$)

[a,b]Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

*Body weight was excluded since no significant interaction was observed

The pH of urine plays a critical role in the formation of urolithiasis, as explained previously in section 3.3. It is one of the most important factors that determine whether struvite- or calcium-oxalate stones will develop or not. If the urinary pH is below 6.6, the cheetah is at risk of developing struvite urolithiasis. If the pH is below 6.0, the animal's risk for developing calcium-oxalate urolithiasis is very high.

Table 5.3, indicates that there was no urinary pH differences ($p > 0.05$) between the different age groups for the different diets. When these values were however represented graphically (Graph 5.1), some trends between the different diets were observed with regards to their urinary pH.



Graph 5.1 **Influence of age group and diet on urinary pH of cheetahs**

*Pooled data. Mean value of all cheetahs receiving either a Meat-Only or a Meat-Mg diet during experimental period.

In Graph 5.1, the urinary pH values of the Meat-CVM diet showed a sharp increase as the age of the cheetahs increased. Urinary pH started at 5.9 for juvenile cheetahs on the Meat-CVM diet and then sharply increased to a pH of 6.4 for cheetahs older than 71 months. On the other hand only a slight increase in urinary pH was observed for the Meat-Only and Meat-Mg diets as the age of the cheetahs increased.

When the three diets (in Graph 5.1) were compared to one another with respect to the three age groups, a sharp increase was observed from the pH of the juvenile cheetahs to the pH of the cheetahs older than 71 months.

The Meat-CVM diet was thus accompanied by a sharp increase in urine pH as the cheetah's age increased. The Meat-only and Meat-Mg[9] diets however only showed a minimal increase in urinary pH. The CVM supplementation thus had a much greater effect on the urinary pH of the older cheetahs. The urine pH value of cheetahs on the Meat-CVM diet was indicated as 6.4 that consequently put the cheetahs at risk of developing struvite urolithiasis. This, to a certain extent, explained why older cheetahs on a Meat-CVM diet showed signs of urolithiasis.

Table 5.3 also indicated differences in body weight ($p \leq 0.05$) between the different age groups of the different diets. This was because the cheetahs younger than 47 months were still in a growing phase and had therefore not yet reached adult body weight.

Table 5.4, indicates that sex did not had any effect ($p > 0.05$) on urinary pH for the different diets the cheetahs received. Differences in body weight were however observed between the sexes, mainly because of the heavier bodyweight of male cheetahs compared the bodyweight of female cheetahs.

In Table 5.5, the influence of different levels of magnesium supplementation on urine pH and bodyweight are presented and some differences ($p \leq 0.05$) are reported.

[9] Data pooled together. Mean value of all cheetahs receiving either Meat-Only or Meat-Mg diet during experimental period.

Cheetahs that received 0g magnesium supplementation had higher urinary pH levels than the cheetahs that received 2g magnesium supplementation. Dietary magnesium supplementation decreased the urinary pH levels of the cheetahs. This decrease of the urinary pH to a pH of less than 6 increased the cheetah's risk of developing calcium-oxalate urolithiasis. It is thus of extreme importance to monitor urine pH carefully when dietary magnesium is supplemented. No influence was however observed in body weight when different levels of dietary magnesium were supplemented.

Table 5.6 shows an interaction ($p \leq 0.05$) between urinary pH and different levels of dietary magnesium supplementation, especially for the cheetahs between 0-47 months. The urinary pH level of cheetahs between 0-47 months that received 0g magnesium supplementation was significantly higher than the cheetahs between 0-47 months that received 2g of magnesium supplementation. There was however no difference in urinary pH ($p > 0.05$) with respect to the two different levels of dietary magnesium supplementation within the age groups 48-71 months as well as cheetahs older than 71 months. Magnesium supplementation's ability to lower urinary pH levels, as observed in Table 5.5 was only seen in cheetahs younger than 47 months. Dietary magnesium supplementation thus only influenced urinary pH values of the juvenile cheetahs younger than 47 months.

Table 5.7 show that different treatment levels of dietary magnesium affected the sexes equally. No difference between the sexes was observed within a single treatment level. Sex thus did not have any influence on urinary pH levels; while however, the urinary pH of both the sexes was influenced by different levels of dietary magnesium supplementation.

5.3.1.1 Summary for urine pH and body weight

To summarize the influence and or interactions of the different diets, age groups, sexes and different treatment levels of magnesium supplementation on urinary pH and body weight, the following conclusion can be made:

CVM supplementation had a much greater effect on the urinary pH of older (>71 months) cheetahs than younger cheetahs. The pH value of older cheetahs on a Meat-CVM diet was 6.4. This urinary pH of 6.4 put the cheetahs at a very high risk

of developing struvite calculi. This might explain why older cheetahs often demonstrate symptoms of urolithiasis.

Dietary magnesium supplementation decreased the urinary pH levels of juvenile cheetahs (< 47 months) in this study. This decrease in urinary pH to a pH of less than 6 increased the juvenile cheetahs risk for developing calcium-oxalate urolithiasis.

5.3.2 Urinary Nitrogen

Nitrogen in the urine is excreted by animals in one of three ways: urea, uric acid and ammonia (as ammonium ion). As discussed in section 3.3, ammonia (NH_3) in urine can play a role in the formation of urolithiasis, especially in the formation of struvite stones. The Ammonium ion (NH_4^+) is one of the building blocks of struvite stones ($MgNH_4PO_4.6H_2O$). It forms during the conversion of NH_3 to NH_4^+ in the presence of water.

Table 5.8 presents the effects of age and diet on the concentrations of the different nitrogen fractions (NH_4^+, NO_3^- and Total Nitrogen) in the urine of the cheetahs. Nitrogen fraction concentrations were compared amongst the three different age groups within each diet. Within each age group, a deviation was calculated from the benchmark Meat-Only diet. This deviation is expressed as a percentage deviation from the Meat-Only diet. Data were only compared between columns within the same rows (between age groups). No data were compared between rows within the same column (between diets).

Table 5.9 presents the effects of sex and diet on the concentrations of the different nitrogen fractions in the urine of the cheetahs. Data were only compared between columns within the same rows (between sexes). No data were compared between rows within the same column (between diets).

Table 5.10 presents the influence of treatment with different levels of dietary magnesium supplementation on the different nitrogen fractions. Data were only compared between columns within the same rows (between different levels of

magnesium supplementation). No data were compared between rows within the same column (between nitrogen fractions).

Table 5.11 presents the interactions between age and magnesium supplementation on the concentration of different nitrogen fractions in the urine of the cheetahs. In this table, no nitrogen fractions were compared to one another. Interactions were only compared within each nitrogen fraction individually. Each nitrogen fraction was first compared to the different age groups within each level of magnesium supplementation treatment. It was followed by a comparison between the two levels of magnesium supplementation within each age group.

Table 5.12 presents the interactions between the different sexes and magnesium supplementation on the concentration of nitrogen fractions in the urine of the cheetahs. In this table, no nitrogen fractions were compared to one another. Interactions were only compared for each nitrogen fraction individually. Each nitrogen fraction was first compared to the different sexes, within each level of magnesium supplementation. It was then followed by a comparison between the two sexes and the two different levels of magnesium supplementation.

Table 5.8 The effects of age and diet on the concentrations of different nitrogen fractions in the urine of cheetahs

Urinary Nitrogen fractions	Diet	**Age** (months) 0 - 47 (LS Mean ± SD)	Deviation[#]	48-71 (LS Mean ± SD)	Deviation[#]	> 71 (LS Mean ± SD)	Deviation[#]
NH_4^+	Meat-CVM	4.0 ± 1.83	35%	4.5 ± 1.42	40%	4.4 ± 1.90	37%
(g/l)	Meat-Only	6.2 ± 1.43	0%	7.5 ± 1.92	0%	7.0 ± 1.32	0%
	Meat-Mg*	3.5 ± 1.49	44%	4.7 ± 1.10	37%	3.8 ± 1.47	46%
NO_3^-	Meat-CVM	1.9 ± 1.35	55%	2.7 ± 1.36	45%	3.0 ± 1.39	35%
(g/l)	Meat-Only	4.2 ± 1.24	0%	4.9 ± 1.54	0%	4.6 ± 1.23	0%
	Meat-Mg*	2.4 ± 1.16	43%	3.3 ± 1.49	33%	1.4 ± 1.56	70%
Total Nitrogen	Meat-CVM	5.1 ± 1.11	28%	6.1 ± 2.71	26%	6.4 ± 2.24	23%
(g/l)	Meat-Only	7.1 ± 1.67	0%	8.2 ± 3.29	0%	8.3 ± 1.49	0%
	Meat-Mg*	5.8 ± 1.60	18%	6.8 ± 2.41	17%	5.2 ± 2.29	37%

$_{a,b,c}$ Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

*Pooled data. Mean value of all cheetahs receiving either a Meat-Only or Meat-Mg diet during experimental period

[#]Deviation from benchmark; compared to values of Meat-Only diet

Table 5.9 The effects of sex and diet on the concentrations of different nitrogen fractions in the urine of cheetahs

		Sex	
Urinary Nitrogen fractions	**Diet**	Female (LS Mean ± SD)	Male (LS Mean ± SD)
NH_4^+	Meat-CVM	3.7 ± 1.53	5.0 ± 1.16
(g/l)	Meat-Only	6.0 ± 1.12	7.7 ± 2.28
	Meat-Mg*	3.7 ± 1.03	4.3 ± 1.75
NO_3^-	Meat-CVM	1.5 ± 1.04	3.5 ± 1.17
(g/l)	Meat-Only	3.7 ± 7.75	5.5 ± 1.90
	Meat-Mg*	2.4 ± 1.11	2.3 ± 1.35
Total Nitrogen	Meat-CVM	4.8 ± 2.50	7.1 ± 2.18
(g/l)	Meat-Only	9.7 ± 1.69	1.3 ± 4.15
	Meat-Mg*	6.1 ± 1.94	6.6 ± 3.04

$_{a,b}$ Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

*Pooled data. Mean value of all cheetahs receiving either a Meat-Only or Meat-Mg diet during experimental period

Table 5.10 The influence of Mg supplementation levels on the concentrations of different nitrogen fractions in the urine of cheetahs

	Mg supplementation	
Urinary Nitrogen fractions	0g Mg (LS Mean ± SD)	2g Mg (LS Mean ± SD)
NH_4^+ (g/l)	$6.4 \pm 1.06_a$	$3.8 \pm 1.55_b$
NO_3^- (g/l)	$4.4 \pm 0.96_a$	$2.3 \pm 1.31_b$
Total Nitrogen (g/l)	$7.6 \pm 1.72_a$	$5.4 \pm 2.81_b$

$_{a,b}$ Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

Table 5.11 The interaction between age and Mg supplementation on the concentrations of different nitrogen fractions in the urine of cheetahs

		Age (months)		
Urinary		0 - 47	48-71	> 71
Nitrogen fractions	**Mg supplementation**	(LS Mean ± SD)	(LS Mean ± SD)	(LS Mean ± SD)
NH_4^+	0g Mg	$6.7 \pm 1.42_1$	$6.4 \pm 6.12_1$	$6.5 \pm 1.23_1$
(g/l)	2g Mg	$3.4 \pm 1.60_2$	$4.8 \pm 1.64_2$	$3.2 \pm 1.41_2$
NO_3^-	0g Mg	$4.1 \pm 9.14_1$	$4.1 \pm 1.13_1$	$4.6 \pm 10.0_1$
(g/l)	2g Mg	$1.9 \pm 1.12_2$	$3.3 \pm 2.10_2$	$1.7 \pm 8.70_2$
Total Nitrogen	0g Mg	$7.3 \pm 2.30_1$	$7.5 \pm 9.84_1$	$7.8 \pm 2.23_1$
(g/l)	2g Mg	$5.2 \pm 2.68_2$	$6.1 \pm 3.68_2$	$4.9 \pm 2.25_2$

^{1,2} Means within the same column with different superscripts differ significantly ($p \leq 0.05$)

$_{a,b,c}$ Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

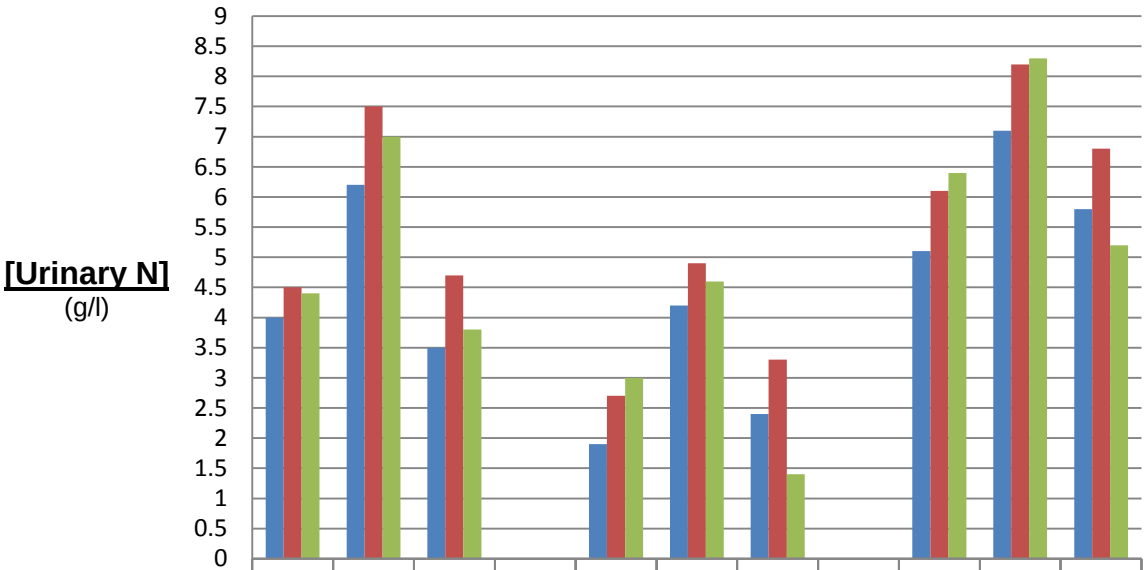
Table 5.12 The interaction between sex and Mg supplementation on the concentrations of different nitrogen fractions in the urine of cheetahs

		Sex	
Urinary		Female	Male
Nitrogen fractions	**Mg supplementation**	(LS Mean ± SD)	(LS Mean ± SD)
NH_4^+	0g Mg	$6.8 \pm 1.36_1$	$6.0 \pm 0.16_1$
(g/l)	2g Mg	$3.8 \pm 8.99_2$	$3.7 \pm 2.08_2$
NO_3^-	0g Mg	$4.8 \pm 1.20_1$	$4.0 \pm 8.27_1$
(g/l)	2g Mg	$2.0 \pm 1.06_2$	$2.5 \pm 1.60_2$
Total Nitrogen	0g Mg	$6.9 \pm 2.09_1$	$8.3 \pm 1.31_1$
(g/l)	2g Mg	$5.9 \pm 1.92_2$	$6.2 \pm 3.65_2$

^{1,2} Means within the same column with different superscripts differ significantly ($p \leq 0.05$)

$_{a,b}$ Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

When evaluating the effects of the three different age groups and diets on the concentrations of the different nitrogen fractions in the urine in Table 5.8, no differences ($p > 0.05$) were observed for any of the three nitrogen fractions (NH_4^+, NO_3^- or Total Nitrogen). When the Meat-Only diet was used as a benchmark for comparing the diets within a specific nitrogen fraction, however, some numerical differences were observed in Table 5.8. The differences between the diets are expressed as percentage deviations from the benchmark (Meat-Only diet). For all three nitrogen fractions, the Meat-CVM and Meat-Mg diets significantly deviated from the Meat-Only diet used as a benchmark. These deviations are also presented graphically in Graph 5.2.



	NH4+				NO3-				Total Nitrogen		
	Meat -CVM	Meat -Only	Meat -Mg*		Meat -CVM	Meat -Only	Meat -Mg*		Meat -CVM	Meat -Only	Meat -Mg*
1 - 47 months	4	6.2	3.5		1.9	4.2	2.4		5.1	7.1	5.8
48-72 months	4.5	7.5	4.7		2.7	4.9	3.3		6.1	8.2	6.8
> 72 months	4.4	7	3.8		3	4.6	1.4		6.4	8.3	5.2

Diets within different nitrogen fractions and age groups

Graph 5.2 The effect of age and diet on the urinary nitrogen fractions of cheetahs

Graph 5.2 graphically presents the effect of age and diet on the urinary nitrogen fractions in the urine of the cheetahs. As seen in the deviations in Table 5.8, it is also

noted in Graph 5.2 that nitrogen levels for the Meat-Only diet was much higher than nitrogen levels for the Meat-CVM and Meat-Mg diets, for all three nitrogen fractions (NH_4^+, NO_3^- and Total Nitrogen). Both the Meat-CVM and Meat-Mg diets affected either the primary or secondary metabolism of nitrogen in the body of the cheetahs. When dietary calcium or magnesium was thus supplemented, the urinary excretion of NH_4^+, NO_3^- and Total Nitrogen decreased. When there is less NH_4^+ or NO_3^- in the urine, risk for the formation of struvite calculi decrease since NH_4^+ is a compound of struvite and NO_3^- is a precursor.

Table 5.9, indicates that sex and diet did not had a significant effect ($p > 0.05$) on any of the three different urinary nitrogen fractions.

In Table 5.10, differences were observed for all three urinary nitrogen fractions, when compared between the two different levels of dietary magnesium supplementation. Cheetahs that received 0g magnesium supplementation had significantly higher ($p \leq 0.05$) nitrogen fraction levels than the cheetahs that received 2g magnesium supplementations. This same pattern repeated itself in examination of urinary nitrogen fraction in comparison to different age groups (Table 5.11) as well as different sexes (Table 5.12). This reaffirms the observations made in Graph 5.2 that magnesium supplementation did influence the nitrogen metabolism of the cheetahs.

5.3.2.1 Summary for Nitrogen

Dietary magnesium- or calcium supplementation (CVM-supplementation) had a preventative effect on the formation of struvite calculi by decreasing the concentration of stuvite building blocks when added to the cheetah's diet.

5.3.3 Urine- and plasma minerals

Minerals are the building blocks of urolithiasis. Mineral interaction forms an intertwined web, where each and every mineral affects other minerals and where each and every mineral is in turn affected. Minerals can therefore not be studied in isolation, but need to be studied in relationship to one another.

Table 5.13(A) and Table 5.13(B) presents the effects of age and diet on the mineral concentrations of the urine and plasma of the cheetahs. All mineral concentrations were compared to the three different age groups within each particular diet for urine and plasma separately. Data were only compared between columns within the same rows (between age groups). No data were compared between rows within the same column (between diets).

Table 5.14(A) and Table 5.14(B) presents the effects of sex and diet on the mineral concentrations of the urine and plasma of the cheetahs. All mineral concentrations were compared to the sexes within each particular diet for urine and plasma separately. Data were only compared between columns within the same rows (between sexes). No data were compared between rows within the same column (between diets).

Table 5.15 presents the influence of different levels of dietary magnesium supplementation on the concentrations of minerals in the urine and plasma of the cheetahs. All mineral concentrations were compared to the two levels of magnesium supplementation for urine and plasma separately. Data were only compared between columns within the same rows (between different levels of magnesium supplementation). No data were compared between rows within the same column (between different minerals).

Table 5.16 presents the interactions between age, magnesium supplementation and the concentration of minerals in the urine and plasma of each cheetah. In Table 5.16, no minerals were compared to one another. Each mineral is discussed as follows:

1st: The *urinary* concentrations of the mineral under discussion are compared to the different age groups that received **0g** magnesium supplementation.

2nd: The *urinary* concentrations of the mineral under discussion are compared to the different age groups that received **2g** magnesium supplementation.

3rd: The *urinary* concentrations of the mineral under discussion are compared to the different magnesium supplementation levels between ages **0-47 months**.

4th: The *urinary* concentrations of the mineral under discussion are compared to the different magnesium supplementation levels between ages **48-71 months**.

5th: The *urinary* concentrations of the mineral under discussion are compared to the different magnesium supplementation levels for ages **71 months and older**.

6th: The *plasma* concentrations of the mineral under discussion are compared by same method as followed in steps 1-5 above.

Table 5.17 presents the interactions between sex, magnesium supplementation and the concentration of minerals in the urine and plasma of the cheetahs. In Table 5.17, no minerals were compared to one another. Each mineral is discussed as follows:

1st: The *urinary* concentrations of the mineral under discussion are compared to the different sexes that received **0g** magnesium supplementation.

2nd: The *urinary* concentrations of the mineral under discussion are compared to the different sexes that received **2g** magnesium supplementation.

3rd: The *urinary* concentrations of the mineral under discussion are compared to the different magnesium supplementation levels of **female** cheetahs.

4th: The *urinary* concentrations of the mineral under discussion will be compared to the different magnesium supplementation levels of **male** cheetahs.

5th: The *plasma* concentrations of the mineral under discussion are compared by same method as followed in steps 1-4 above.

For Table 5.16 and Table 5.17, when no differences are observed in some steps, these steps are discussed as one.

Table 5.13(A) The effects of age and diet on mineral concentrations in the urine and plasma of cheetahs

			Age (months)		
			0 - 47	48-71	> 71
Minerals		**Diet**	(LS Mean ± SD)	(LS Mean ± SD)	(LS Mean ± SD)
Phosphorus	Urine	Meat-CVM	3.70 ± 2.090	3.00 ± 1.840	3.20 ± 1.750
(g/l)		Meat-Only	–	4.10 ± 0.890	5.40 ± 1.160
		Meat-Mg*	4.20 ± 1.910	4.80 ± 1.640	3.40 ± 1.750
	Plasma	Meat-CVM	$0.09 \pm 0.036_a$	$0.05 \pm 0.007_b$	$0.05 \pm 0.006_b$
		Meat-Only	$0.09 \pm 0.024_a$	$0.04 \pm 0.006_b$	$0.04 \pm 0.011_b$
		Meat-Mg*	$0.09 \pm 0.022_a$	$0.07 \pm 0.010_b$	$0.07 \pm 0.011_b$
Magnesium	Urine	Meat-CVM	88.0 ± 50.78	60.4 ± 21.87	57.5 ± 20.00
(mg/l)		Meat-Only	–	–	–
		Meat-Mg*	$295 \pm 48.33_a$	$206 \pm 34.75_b$	$137 \pm 49.38_c$
	Plasma	Meat-CVM	0.56 ± 0.108	0.57 ± 0.112	0.49 ± 0.042
		Meat-Only	0.51 ± 0.094	0.49 ± 0.061	0.47 ± 0.043
		Meat-Mg*	0.62 ± 0.204	0.51 ± 0.147	0.54 ± 0.058
Calcium	Urine	Meat-CVM	19.0 ± 1.300	17.0 ± 1.000	18.0 ± 1.300
(mg/l)		Meat-Only	–	18.0 ± 1.300	20.0 ± 4.930
		Meat-Mg*	17.0 ± 5.470	21.0 ± 5.490	17.0 ± 8.560
	Plasma	Meat-CVM	1.80 ± 0.194	1.90 ± 0.387	1.70 ± 0.276
		Meat-Only	1.70 ± 0.148	1.80 ± 0.251	1.60 ± 0.299
		Meat-Mg*	2.10 ± 0.198	2.20 ± 0.746	2.00 ± 0.231

$_{a,b,c}$Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

*Pooled data. Mean value of all cheetahs receiving either a Meat-Only or Meat-Mg diet during experimental period

Table 5.13(B) The effects of age and diet on mineral concentrations in the urine of cheetahs

Minerals		Diet	**Age** (months) 0 - 47 (LS Mean ± SD)	48-71 (LS Mean ± SD)	> 71 (LS Mean ± SD)
Potassium (g/l)	Urine	Meat-CVM	2.10 ± 1.110	1.90 ± 9.350	1.60 ± 0.810
		Meat-Only	–	2.10 ± 1.460	3.00 ± 0.370
		Meat-Mg*	1.40 ± 1.300	1.70 ± 0.750	1.30 ± 0.710
Sodium (g/l)	Urine	Meat-CVM	0.05 ± 0.119$_a$	0.15 ± 0.120$_a$	0.44 ± 0.331$_b$
		Meat-Only	–	0.25 ± 0.216	0.41 ± 0.247
		Meat-Mg*	0.20 ± 0.299	0.25 ± 0.259	0.29 ± 0.168
Sulphur (g/l)	Urine	Meat-CVM	2.20 ± 0.460	2.20 ± 0.746	1.50 ± 0.660
		Meat-Only	–	2.60 ± 0.854	2.60 ± 0.379
		Meat-Mg*	2.10 ± 0.977	2.10 ± 0.723	1.90 ± 0.701

$_{a,b,c,}$Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

*Pooled data. Mean value of all cheetahs receiving either a Meat-Only or Meat-Mg diet during experimental period

Table 5.14(A) The effects of sex and diet on mineral concentrations in the urine and plasma of cheetahs

			Sex	
			Female	Male
Minerals		**Diet**	(LS Mean ± SD)	(LS Mean ± SD)
Phosphorus	Urine	Meat-CVM	3.50 ± 1.750	3.20 ± 2.200
(g/l)		Meat-Only	4.60 ± 1.240	4.90 ± 0.000
		Meat-Mg*	4.80 ± 1.910	3.50 ± 1.630
	Plasma	Meat-CVM	0.07 ± 0.025	0.06 ± 0.032
		Meat-Only	0.07 ± 0.018_a	0.05 ± 0.023_b
		Meat-Mg*	0.08 ± 0.021	0.08 ± 0.024
Magnesium	Urine	Meat-CVM	56.8 ± 33.99	80.4 ± 56.70
(mg/l)		Meat-Only	–	–
		Meat-Mg*	274 ± 49.24	205 ± 45.92
	Plasma	Meat-CVM	0.53 ± 0.095	0.55 ± 0.104
		Meat-Only	0.47 ± 0.054	0.51 ± 0.086
		Meat-Mg*	0.55 ± 0.182	0.56 ± 0.133
Calcium	Urine	Meat-CVM	23.0 ± 3.820	29.0 ± 7.000
(mg/l)		Meat-Only	25.0 ± 4.800	23.0 ± 6.510
		Meat-Mg*	18.0 ± 6.860	18.0 ± 6.950
	Plasma	Meat-CVM	1.90 ± 0.259	1.70 ± 0.287
		Meat-Only	1.80 ± 0.289	1.60 ± 0.369
		Meat-Mg*	2.10 ± 0.242	2.10 ± 0.515

$_{a,b,}$Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

*Pooled data. Mean value of all cheetahs receiving either a Meat-Only or Meat-Mg diet during experimental period

Table 5.14(B) The effects of sex and diet on mineral concentrations in the urine of cheetahs

			Sex	
			Female	Male
Minerals		**Diet**	(LS Mean ± SD)	(LS Mean ± SD)
Potassium	Urine	Meat-CVM	1.80 ± 0.920	1.90 ± 1.140
(g/l)		Meat-Only	1.50 ± 0.670	1.60 ± 1.420
		Meat-Mg*	1.60 ± 0.870	1.40 ± 1.070
Sodium	Urine	Meat-CVM	0.20 ± 0.244	0.23 ± 0.104
(g/l)		Meat-Only	0.20 ± 0.216	0.46 ± 0.220
		Meat-Mg*	0.26 ± 0.218	0.24 ± 0.266
Sulphur	Urine	Meat-CVM	1.80 ± 0.855	2.10 ± 0.604
(g/l)		Meat-Only	2.00 ± 0.535	3.20 ± 0.600
		Meat-Mg*	1.90 ± 0.709	2.10 ± 0.935

$_{a,b,}$Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

*Pooled data. Mean value of all cheetahs receiving either a Meat-Only or Meat-Mg diet during experimental period

Table 5.15 The influence of Mg supplementation on the concentration of minerals in the urine and plasma of cheetahs

		Mg supplementation	
		0g Mg	2g Mg
Mineral		(LS Mean ± SD)	(LS Mean ± SD)
Phosphorus	Urine	4.90 ± 1.110$_a$	3.40 ± 1.750$_b$
(g/l)	Plasma	0.07 ± 0.008	0.08 ± 0.028
Magnesium	Urine	99.3 ± 19.73$_a$	279 ± 41.62$_b$
(mg/l)	Plasma	0.49 ± 0.038$_a$	0.63 ± 0.173$_b$
Calcium	Urine	10.0 ± 5.230$_a$	14.0 ± 7.530$_b$
(mg/l)	Plasma	1.80 ± 0.430	2.20 ± 0.382
Potassium	Urine	1.30 ± 0.656	1.60 ± 1.140
(g/l)			
Sodium	Urine	0.32 ± 0.270	0.18 ± 0.212
(g/l)			
Sulphur	Urine	1.85 ± 0.809	2.20 ± 0.850
(g/l)			

$_{a,b,}$Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

Table 5.16 The interaction of age, Mg supplementation and the concentration of minerals in the urine and plasma of cheetahs

Parameter		Mg supplementation	Age (months) 0 - 47 (LS Mean ± SD)	48-71 (LS Mean ± SD)	> 71 (LS Mean ± SD)
Phosphorus (g/l)	Urine	0g Mg	5.80 ± 0.410[1]	5.00 ± 1.840	4.60 ± 1.300
		2g Mg	3.00 ± 1.980[2]	4.60 ± 1.830	2.90 ± 1.440
	Plasma	0g Mg	0.08 ± 0.003[1]	0.08 ± 0.006	0.06 ± 0.005
		2g Mg	0.12 ± 0.009[2]$_a$	0.07 ± 0.013$_b$	0.06 ± 0.014$_b$
Magnesium (mg/l)	Urine	0g Mg	73.4 ± 9.861[1]	55.9 ± 7.931[1]	32.1 ± 2.662[1]
		2g Mg	559 ± 27.22[2]$_a$	342 ± 81.00[2]$_{ab}$	94.5 ± 15.05[2]$_b$
	Plasma	0g Mg	0.47 ± 0.053[1]	0.44 ± 0.0263	0.47 ± 0.021
		2g Mg	0.76 ± 0.187[2]$_a$	0.66 ± 0.180$_{ab}$	0.52 ± 0.065$_b$
Calcium (mg/l)	Urine	0g Mg	16.0 ± 3.300[1]	14.0 ± 3.030[1]	19.0 ± 3.630[1]
		2g Mg	21.0 ± 4.780[2]	22.0 ± 5.510[2]	24.0 ± 5.970[2]
	Plasma	0g Mg	2.10 ± 0.103	2.10 ± 0.122	2.20 ± 0.235
		2g Mg	2.40 ± 0.183	2.20 ± 0.765	2.30 ± 0.116
Potassium (g/l)	Urine	0g Mg	1.90 ± 0.850	1.10 ± 0.069	1.70 ± 0.329
		2g Mg	1.20 ± 1.620	2.40 ± 0.367	1.40 ± 0.817
Sodium (g/l)	Urine	0g Mg	0.39 ± 0.363	0.11 ± 0.125	0.43 ± 0.018
		2g Mg	0.13 ± 0.196	0.40 ± 0.298	0.10 ± 0.099
Sulphur (g/l)	Urine	0g Mg	1.90 ± 0.835	1.60 ± 0.810	2.60 ± 1.250
		2g Mg	2.30 ± 1.180	2.50 ± 0.403	1.70 ± 0.588

[1,2]Means within the same column with different superscripts differ significantly ($p \leq 0.05$)

$_{a,b,c}$Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

Table 5.17 The interaction between sex, Mg supplementation and the concentration of minerals in the urine and plasma of cheetahs

Parameter		Mg supplementation	Sex: Female (LS Mean ± SD)	Sex: Male (LS Mean ± SD)
Phosphorus	Urine	0g Mg	5.90 ± 0.980	4.40 ± 1.060
(g/l)		2g Mg	3.80 ± 1.930	3.30 ± 1.620
	Plasma	0g Mg	0.07 ± 0.011	0.07 ± 0.004[1]
		2g Mg	0.08 ± 0.026	0.09 ± 0.031[2]
Magnesium	Urine	0g Mg	64.3 ± 16.10[1]	43.3 ± 16.20[1]
(mg/l)		2g Mg	472 ± 27.32[2]	326 ± 29.12[2]
	Plasma	0g Mg	0.05 ± 0.022[1]	0.05 ± 0.050[1]
		2g Mg	0.65 ± 0.209[2]	0.60 ± 0.144[2]
Calcium	Urine	0g Mg	16.0 ± 3.190[1]	15.0 ± 6.220[1]
(mg/l)		2g Mg	21.0 ± 6.330[2]	21.0 ± 6.930[2]
	Plasma	0g Mg	1.80 ± 0.252[1]	1.90 ± 0.127[1]
		2g Mg	2.10 ± 0.166[2]	2.10 ± 0.518[2]
Potassium	Urine	0g Mg	1.50 ± 0.455	1.50 ± 0.840
(g/l)		2g Mg	1.70 ± 1.080	1.70 ± 1.270
Sodium	Urine	0g Mg	0.29 ± 0.115	0.33 ± 0.372
(g/l)		2g Mg	0.24 ± 0.267	0.18 ± 0.156
Sulphur	Urine	0g Mg	1.50 ± 0.895	2.60 ± 0.636
(g/l)		2g Mg	2.40 ± 0.490	2.00 ± 1.110

[1,2]Means within the same column with different superscripts differ significantly ($p \leq 0.05$)

[a,b,]Means within the same row with different subscripts differ significantly ($p \leq 0.05$)

5.3.3.1 Phosphorus

No differences ($p > 0.05$) for *urinary* phosphorus concentration were observed (Table 5.13(A)) when age groups were compared within each of the specific diets. When *plasma* phosphorus concentrations between the different age groups were compared (Table 5.13(A)) within each specific diet, differences ($p \leq 0.05$) were observed. In all three diets, *plasma* phosphorous concentrations for the age group 0-47 months were significantly higher than the *plasma* phosphorous concentrations for the other two age groups (48-72 months and >72 months). Juvenile cheetahs younger than 48 months consequently had an overall higher *plasma* phosphorous concentration than the older cheetahs irrespective of the different diets.

Table 5.14(A), indicated no difference ($p > 0.05$) for *urinary* phosphorus concentrations between the sexes within each of the specific diets. When *plasma* phosphorous levels between the different sexes were compared within each of the specific diets, one difference ($p \leq 0.05$) was be observed: When all the cheetahs received a Meat-Only diet, there was a decrease in *plasma* phosphorus concentration for the male cheetahs in comparison to the female cheetahs. The female cheetahs did not show a decrease of *plasma* phosphorus in any of the three diets. Male and female cheetahs thus reacted differently to a Meat-Only diet in terms of *plasma* phosphorus concentrations when receiving a Meat-Only diet.

Table 5.15 indicated that the two different magnesium supplementation levels influenced ($p \leq 0.05$) *urine* phosphorus concentrations. When the cheetahs received 0g magnesium supplementation, the *urine* phosphorus concentration was higher than when they received 2g magnesium supplementation. This reaffirms previous research by Buffington (1994) who stated that an increase in dietary magnesium supplementation will decrease *urinary* phosphorus concentrations. When there is a supersaturation of minerals (e.g. magnesium and phosphorus) the minerals aggregate and form an insoluble $MgPO_4$-complex. This insoluble $MgPO_4$-complex lowers the body's ability to absorb phosphorus. This leads to the formation of nuclei that induces the development of calculi. By increasing urinary formation of $MgPO_4$, the formation of struvite stones ($MgNH_4PO_4.6H_2O$) is promoted. It is thus very important to monitor the effects of dietary magnesium supplementation on urinary

phosphorus concentrations. No differences ($p > 0.05$) were observed when the two different levels of dietary magnesium supplementation were examined for *plasma* phosphorus concentration.

In Table 5.16, the only interaction ($p \leq 0.05$) for *urinary* phosphorus concentrations when age and magnesium supplementation level was compared occurred in the age group 0-47 months. In this age group, the *urinary* phosphorous levels for treatment level of 0g magnesium supplementation was higher than for the same age group that received treatment level of 2g magnesium supplementation. This relates to what has been observed in Table 5.15 and also reaffirms existing literature (Buffington, 1994) that states that a decrease in urinary phosphorus is anticipated due to the formation of $MgPO_4$-complex. Juvenile cheetahs (0-47 months) showed the only significant decrease in *urinary* phosphorus concentration when they received a magnesium supplementation. Juvenile cheetahs were thus the group that was most affected by dietary magnesium supplementations in terms of *urinary* phosphorus concentrations.

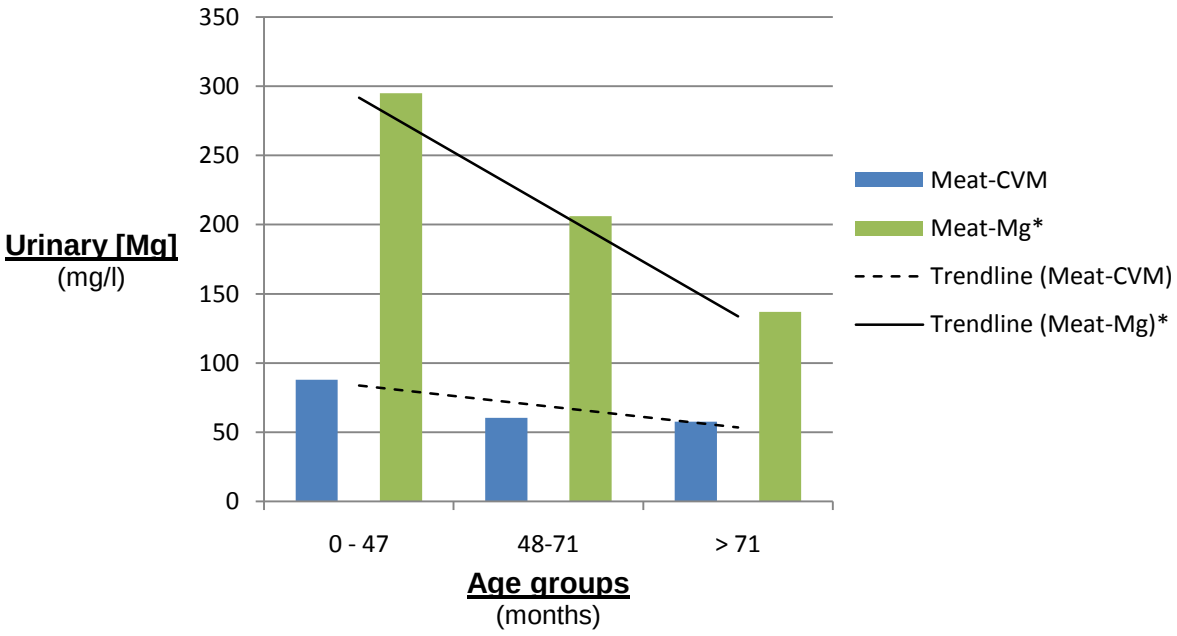
For *plasma* phosphorus concentrations however, two interactions ($p \leq 0.05$) between age and magnesium supplementation levels were observed. Although no interaction for overall *plasma* phosphorus concentrations was observed between the two different levels of magnesium supplementation in Table 5.15, an interaction was observed within the age group 0-47 months in Table 5.16. When the cheetahs between 0-47 months received a treatment level of 0g magnesium supplementation, *plasma* phosphorous levels proved to be lower than the same age group that received a treatment level of 2g magnesium supplementation. The effects of dietary magnesium supplementation on *plasma* phosphorous levels were thus only observed in cheetahs younger than 47 months.

The other interaction that is visible in Table 5.16 occurs between the three age groups within a treatment level of 2g magnesium supplementation. *Plasma* phosphorous concentrations for 0-47 month old cheetahs were significantly higher than the *plasma* phosphorous concentrations of cheetahs older than 47 months. Thus, when supplemented with dietary magnesium, more phosphorous was available in juvenile cheetahs than in cheetahs older than 47 months.

In Table 5.17, only one interaction ($p \leq 0.05$) between sex and treatment level was observed for *plasma* phosphorous concentrations. Male cheetahs that received a 0g dietary magnesium supplementation had significantly lower *plasma* phosphorus concentrations than male cheetahs that received 2g magnesium supplementation.

5.3.3.2 Magnesium

In Table 5.13(A), a difference ($p \leq 0.05$) in *urinary* magnesium concentration was observed between the different age groups within the Meat-Mg diet. There was a sharp decrease in *urinary* magnesium concentration as the ages of the cheetahs on the Meat-Mg diet increased. The younger cheetahs (0-47 months) had the highest concentration of magnesium in their *urine* when on the Meat-Mg diet. This is graphically illustrated in Graph 5.3.



Graph 5.3 Effect of age and diet on *urinary* magnesium levels in cheetahs

(No data available for Meat-only diet)

No difference ($p > 0.05$) in *plasma* magnesium concentration was observed when the different age groups were compared within each particular diet. Although larger amounts of magnesium were administered to the older cheetahs, no extra

magnesium was observed in the plasma; it can thus be concluded that the excess *plasma* magnesium was excreted in the urine.

In Table 5.14(A) no differences ($p > 0.05$) between the sexes within each specific diet were observed for *urinary*- as well as *plasma* magnesium concentrations. Sex thus did not affect *urinary*- or *plasma* magnesium concentrations in any of the respective diets.

In Table 5.15, differences ($p \leq 0.05$) were observed between the two levels of magnesium supplementation. When the cheetahs received 2g magnesium supplementation, there was an increase in both *urinary*- and *plasma* magnesium concentration as compared to the cheetahs that received 0g magnesium supplementation. An excess of *plasma* magnesium caused magnesium in the renal fluids not to be reabsorbed. The excess was then excreted in the urine and thus explains the increase of urinary magnesium concentration. This agrees with existing literature: Buffington (1994) stated that an increase of dietary magnesium will lead to an increase of *urinary* magnesium due to the higher intake of magnesium. This higher concentration of *urinary* magnesium decreases the rate of stone formation by binding to intestinal and urinary oxalate that induces magnesium-oxalate formation (Parivar *et al*. 1996). The more soluble magnesium-oxalate prevents the binding of calcium and thus prevents calcium-oxalate stone formation.

In Table 5.16, some interactions ($p \leq 0.05$) for *urinary*- and *plasma* magnesium concentrations was observed. When 2g dietary magnesium was supplemented, cheetahs aged between 0-47months showed not only significantly higher *urinary* magnesium levels, but also significantly higher *plasma* magnesium levels than the cheetahs older than 71 months. Cheetahs in the age group 48-72 months did not show any significant differences for *urinary*- as well as *plasma* magnesium concentrations when compared to the other age groups.

When the two levels of magnesium supplementation were compared in Table 5.16 to the three different age groups, differences in urinary magnesium concentrations ($p \leq 0.05$) were observed for all ages. Cheetahs that received the higher level of magnesium supplementation had higher *urinary* magnesium concentrations than the cheetahs that received 0g magnesium supplementation. This proved to be true

across all of the age groups. By increasing the level of dietary magnesium supplementation, the concentration of magnesium in the *urine* was thus also increased. This reaffirms the observations made in Table 5.13 that an increase in dietary magnesium also increases magnesium concentrations in the urine.

When the plasma magnesium concentrations in Table 5.16 were compared to the two different levels of magnesium supplementation for the various age groups, a difference ($p \leq 0.05$) only for the age group of 0-47 months could be observed. Cheetahs between 0-47 months that received 2g magnesium supplementation had significantly higher *plasma* magnesium levels than cheetahs in the same age group that received 0g magnesium supplementation. This difference in *plasma* magnesium concentration when cheetahs received different levels of magnesium supplementation was however only seen for cheetahs between 0-47 months. Table 5.16 thus indicated that younger cheetahs were more susceptible to changes in *plasma* magnesium concentrations when magnesium was administered to their diet than older cheetahs. When supplementing the diets of cheetahs with magnesium, it is thus vitally important to carefully monitor younger cheetahs to prevent any negative consequences of dietary magnesium supplementation.

In Table 5.17, no differences ($p > 0.05$) were observed when *urinary*- and *plasma* magnesium concentrations were compared within each level of dietary magnesium supplementation between the two sexes. Sex did not influence *urinary*- or *plasma* magnesium concentrations at all.

In Table 5.17, differences were observed ($p \leq 0.05$) for *urine*- and *plasma* magnesium concentrations when the two levels of dietary magnesium supplementation was compared within a single sex between different levels of magnesium supplementation. When 2g magnesium was supplemented to the diets of the cheetahs, *urine*- as well as *plasma* magnesium concentrations were higher than without (0g) any magnesium supplementation. Although no difference for *urinary* and *plasma* magnesium concentrations between the sexes were observed, the level of dietary magnesium supplementation did influence the *urinary* and *plasma* magnesium concentrations of both sexes.

5.3.3.3 Calcium

Urinary calcium serves as an indication as to whether the animal is in a state of hyper- or hypocalcaemia. Calcium and magnesium constantly interacts with one another. It is therefore necessary to evaluate these minerals together as the one mineral can influence the other. Both of the minerals need to be taken into consideration when determining excesses or deficits because although an animal might be in a state of hypocalcaemia, it might be due to an excess of magnesium.

In Table 5.13(A), no differences ($p > 0.05$) for either *urinary*- or *plasma* calcium concentrations, were observed when the three different age groups were compared with one another within each particular diet.

In Table 5.14(A), no differences ($p > 0.05$) for either *urinary*- or *plasma* calcium concentrations were observed when the two sexes were compared to one another within each diet.

In Table 5.15, when the influence of different levels of magnesium supplementation on *urinary*- and *plasma* calcium concentrations was examined, differences ($p \leq 0.05$) were observed. *Urinary* calcium levels, for cheetahs that received 0g magnesium supplementation were lower than the *urinary* calcium levels of cheetahs that received 2g magnesium supplementation. No difference ($p > 0.05$) was observed for *plasma* calcium concentrations in cheetahs when different levels of magnesium supplementation were administered. This reaffirms the studies done on rats and cats, which stated that an increase in dietary magnesium concentrations led to an increase in the concentration of *urinary* calcium. This, as stated by Pastoor *et al*. (1995), is due to the competition between calcium and magnesium to be reabsorbed by the tubular cells. Due to the increased concentration of magnesium in the urine, calcium reabsorption was depressed and consequently excreted in the urine. Parivar *et al*. (1996) also stated that diets high in protein, especially sulphate containing amino acids such as methionine, influence *urinary* calcium concentrations. The excess urinary sulphates binds to calcium to form a calcium-sulphate complex which prevents calcium absorption by the tubular cells. PTH and Calcitonin secretion are under the control of calcium concentrations. If the cheetah is in a state of hypomagnesia and hypercalcaemia, the cheetah will still excrete magnesium in the

urine due to its hormonal control of calcium. The increased concentrations of *urinary* calcium and magnesium can lead to the formation of calcium-oxalate stone formation.

In Table 5.16, no differences were observed for urinary- or *plasma* calcium concentrations when compared between the two magnesium supplementation levels for the various age groups.

When, in Table 5.16, *urinary* calcium concentrations was compared to the two levels of magnesium supplementation between the different age groups, differences was observed in all three age groups. *Urinary* calcium concentrations were higher when 2g of magnesium was supplemented to the diets of the cheetahs than when 0g magnesium was supplemented. No differences were however observed for *plasma* calcium levels when different levels of dietary magnesium were supplemented. Plasma levels were not affected by the two levels of magnesium supplementation. Urinary levels however were affected. The body of the cheetahs thus maintained blood calcium levels by secreting excess calcium in the urine.

In Table 5.17, no differences ($p > 0.05$) for *urinary*- or *plasma* calcium concentrations were observed between the sexes when compared to each of the two levels of magnesium supplementation. When *urinary*- or *plasma* calcium concentration were however compared within each of the sexes, differences ($p \leq 0.05$) between the levels of magnesium supplementation for both sexes was observed. *Urine* plasma calcium concentrations of both sexes were higher when 2g magnesium supplementation was administered to the diets of the cheetahs. When higher dietary magnesium concentration were supplemented to the diets of the cheetahs, urinary- and *plasma* calcium concentrations of both sexes were influenced.

5.3.3.4 Potassium, sodium and sulphur

When the *urinary* potassium, -sodium and -sulphur concentrations between the different age groups in Table 5.13(B) were compared, only one difference ($p \leq 0.05$) could be observed. The *urinary* sodium concentrations of cheetahs on the Meat-CVM diet increased as the cheetahs' ages increased. Cheetahs on the Meat-CVM diet excreted more sodium as their age increased. Sodium in the urine can act as calculi and when it is combined with other minerals, the risk of urolithiasis can

increased. In the Meat-Mg diet, no difference ($p > 0.05$) for *urinary* sodium concentrations between the various age groups could be observed. It could thus be concluded that the exclusion of CVM supplementation had a positive effect on urinary sodium levels especially in the older cheetahs. This consequently lowered the risk of urolithiasis.

In Table 5.14(B), no differences ($p > 0.05$) for *urinary* potassium, -sodium and -sulphur concentrations between the two sexes, when compared within each of the diets, could be observed. Sex did not have an influence on any of the three *urinary* mineral concentrations.

In Table 5.15, no treatment effect on *urinary* potassium, -sodium and -sulphur concentrations ($p > 0.05$) were observed.

In Table 5.16, no interactions between age, treatment level and *urinary* concentrations of potassium, sodium and sulphur were observed ($p > 0.05$).

In Table 5.17, no interactions between sex, treatment level and *urinary* concentrations of potassium, sodium and sulphur were observed ($p > 0.05$).

5.3.3.5 Summary for Minerals

<u>Phosphorous</u>

- Juvenile cheetahs showed overall higher levels of *plasma* phosphorous than the older cheetahs, irrespective of the 3 different diets fed. (Table 5.13(A)
- When the cheetahs received a Meat-Only diet, male *plasma* phosphorous levels decreased.(Table 5.14(A)
- By supplementing 2g dietary magnesium to the diets of the cheetahs, *urine* phosphorous concentrations (Table 5.15) of the cheetahs younger than 47 months (Table 5.16) was decreased. This decrease was due to the formation of an insoluble $MgPO_4$-complex. The formation of struvite calculi was thus promoted.
- An increase of *plasma* phosphorus levels could be seen in cheetahs younger than 47 months when 2g dietary magnesium was supplemented (Table 5.16).

- Male cheetahs that received 2g dietary magnesium supplementation had higher *plasma* phosphorus concentrations than when they received 0g magnesium supplementation (Table 5.17).

Magnesium

- Younger cheetahs (0-47 months) had the highest concentration of magnesium in their *urine* when on a Meat-Mg diet. No difference in *plasma* magnesium concentration was however observed and it can thus be concluded that the excess *plasma* magnesium was excreted in the urine of the younger cheetahs.
- Younger cheetahs excreted more magnesium than the older cheetahs when on a Meat-Mg diet. (Table 5.13(A)
- When 2g dietary magnesium was administered to the cheetahs, the *urine* magnesium of cheetahs (Table 5.15) of all ages (Table 5.16) and both sexes (Table 5.17) increased. The increase was however more visible in younger cheetahs than in older cheetahs (Table 5.16). The higher concentration of *urinary* magnesium decreased the rate of stone formation by forming magnesium-oxalate. This prevented the formation of calcium-oxalate stone formation.
- An increase in *plasma* magnesium levels when 2g of dietary magnesium was supplemented (Table 5.15) only in juvenile cheetahs younger than 47 months (Table 5.16). It is thus of vital importance to monitor young cheetahs carefully when magnesium is supplemented to their diets to prevent any negative influences that the supplementation might have.

Calcium

- *Urinary* calcium levels were lower for cheetahs (Table 5.15) of all ages (Table 5.16) and both sexes (Table 5.17) when they received 0g magnesium supplementation. Cheetahs that received a 2g magnesium supplementation, however showed higher levels of *urinary* calcium. This increase in *urinary* calcium increased the risk for calcium-oxalate calculi formation. (Table 5.15 & Table 5.16)
- *Plasma* levels were not affected by the two levels of magnesium supplementation (Table 5.15) in any of the age groups (Table 5.16) or by sex (Table 5.17). *Urinary*

levels however were affected. The body of the cheetahs thus maintained blood calcium levels by secreting excess calcium in the urine.

Potassium, Sodium and Suplher

- Exclusion of CVM supplementation had a positive effect on *urinary* sodium levels and consequently lowered the risk for urolithiasis.

In Table 5.18, a summary of the effects of dietary magnesium supplementation on the physiological parameters is presented. Evident from the table is the complex nature of influence of these parameters on urolithiasis and their interactions and influences on one another.

Table 5.18 Summary of the effect of two levels of dietary magnesium supplementation on physiological parameters

Age (Months);Sex	Mg Supplementation		Effect on the risk for urolithiasis
	2g	0g	
0-47;Both	↓ Urine pH		Induce or prevent
All; Both	↓NH_4		Decrease struvite (Decrease building block)
All; Both	↓NO_3		Decrease struvite (Decrease calculi precursor)
0-47;Both	↓ $[P]_{urine}$	↑ $[P]_{urine}$	$MgPO_4$-complex; Inc. risk struvite calculi
0-47;Both	↑ $[P]_{Plasma}$	↓ $[P]_{Plasma}$	
0-47;Both	↑ $[Mg]_{Urine}$	↓ $[Mg]_{Urine}$	Excess Mg excreted in urine
0-47;Both	↑ $[Mg]_{Plasma}$	↓ $[Mg]_{Plasma}$	Mg-oxalate formation decrease Ca-oxalate calculi Young cheetahs more affected
All; Both	↑ $[Ca]_{Urine}$	↓ $[Ca]_{Urine}$	Calcium-oxalate formation
All; Both	No effect $[Ca]_{Plasma}$	No effect $[Ca]_{Plasma}$	Body maintains Ca levels although Mg supplemented

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 INTRODUCTION

The aim of this research study was to establish whether dietary magnesium supplementation of juvenile cheetahs will remedy metacarpal deformity without inducing urolithiasis.

Conclusions are herewith presented with regards to the two aims set in Chapter 1.

6.2 CONCLUSIONS

6.2.1. Conclusions regarding the aim stated in 1.3a): Will dietary supplementation of magnesium in juvenile cheetahs that experience metecarpal deformity in their front legs, remedy the deformity.

Based on scores to quantify the severity of the deformity, a 25.5% response rate to dietary magnesium supplementation on rotational deformities was found. A 60.8% response rate to dietary magnesium supplementation on flexural deformation was found.

It can be concluded that dietary supplementation of magnesium in the form of MgO in juvenile cheetahs that experience metacarpal deformity on a meat-only diet, will remedy the flexural deformity with less effects on the rotational deformities.

6.2.2. Conclusions regarding the aim stated in 1.3b): To investigate the influence of dietary supplementation of magnesium on the formation of urolithiasis.

Based on the changes of mineral and nitrogen concentrations in the urine and plasma of cheetahs as well as their body weight and urinary pH, it was observed that dietary magnesium supplementation did have either a positive or negative influence on the formation of urolithiasis. Depending on the physiological state of the cheetahs with regards to the parameters identified, dietary magnesium supplementation did either induce or prevent urolithiasis.

It can thus be concluded that magnesium supplementation in the diets of cheetahs in this study did remedy metacarpal deformity while at the same time predispose the cheetahs to the formation of urolithiasis. If the urinary- and plasma- parameters are monitored carefully, induction of urolithiasis can be prevented.

6.3. LIMITATIONS

- Much research (Kidd and Barr, 2002) has been conducted to show the effects of mineral interplay in other (mostly domestic) animals. Limited literature is available on the mineral profile and interactions of minerals in cheetahs.

- The limited numbers of subjects in phase 1 limited statistical procedure. This can be attributed to costly procedures to study the animals.

6.4. RECOMMENDATIONS

- An in depth understanding of mineral interplay- especially the interactions between calcium, magnesium and phosphorus- is vital to counteract urolithiasis formation in captive cheetahs. Although the present results provide good reference values, more research need to be done in cheetahs in order to gain a better understanding of the relationship between minerals and the mineral profile in the captive cheetah as well as their specie-specific needs.

- Some important questions that still need to be covered by research: Do the age and sex of cheetahs play a role in urolithiasis formation? What are the “safe” values for certain mineral concentrations and at what stage do these minerals become problematic in terms of the development of urolithiasis? Last but not least: What are the physiological parameters for different types of minerals in the body of the cheetah?

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APPENDIX A

Cheetahs used

Cheetah Name	Sex	Age (months)	Micro Chip #	Avg Weight (kg)
Amber	Female	65	00-0668-5D6F	34
Buehla	Female	64	00-0689-8E22T	35
Duma	Male	19	4A6B587753	33
Flenters	Female	72	00-066C-0959	30
Habana	Male	64	00-06B8-E373	37
Khula	Male	51	00-0689-9DB5	47
Lana	Female	28	00-06B8-BD35	36
Lance	Male	28	00-06B8-C436	45
Lex	Male	28	00-06B8-EAEB	49
Louis	Male	106	00-0611-CA85T	41
Michael (King)	Male	41	00-0689-D8D2	30
Mogli	Male	74	00-0611-BEDC	42
Moise	Female	72	00-06B8-C241	35
Naledi (King)	Female	156	985140000669110	35
Sarel	Male	18	4A634F7C1B	24
Selati	Male	94	00-01C5-40BF	39
Spies	Male	64	00-0689-D153	41
Spotti	Female	106	00-0669-1JA4	33
Thea	Female	17	4A5E1D527D	21
Tilla	Female	64	00-0671-DC05	32
Toffee	Female	75	00-0659-14BAT	34
Tristan (King)	Male	17	4A6C073BIC	25
Trudie	Female	75	00-0659-4F5A	34
Vrede	Female	18	4A6D390F58	21

APPENDIX B

CVM- Supplementation

A dry composition (1kg) was prepared by mixing:

Ingredient	(g)
Absorbable calcium	0.1600
Vit A	5.0000
Vit E	5.0000
Tiamien (Vit B1)	0.2500
Riboflavien (Vit B2)	0.0068
Pantothenic Acid	0.3500
Choline	0.2500
Niasin	0.4900
Taurine	0.0006
Brewer's yeast	sufficient to produce 1kg