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EFFECT OF LURE FEEDING ON PHYSIOLOGICAL PARAMETERS IN CAPTIVE CHEETAH (*ACINONYX JUBATUS*) AT HOEDSPRUIT ENDANGERED SPECIES CENTRE

by

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Magister Scientiae Agriculturae Animal Production Physiology in the
Faculty of Natural and Agricultural Sciences,
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DECLARATION

I, Marthie Nickols, declare that this dissertation: **"Effect of lure feeding on physiological parameters in captive cheetah (*Acinonyx jubatus*) at Hoedspruit Endangered Species Centre"** which I hereby submit for the degree M. Sc. (Agric.): Animal Science (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.

_____ _____

MARTHIE NICKOLS **DATE**

DEDICATION

To my parents, grandmother, family and friends, for all your love and support during the good, the bad and the fantastic. Thank you for all your motivation and support throughout my studies.

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ABSTRACT

Effect of lure feeding on physiological parameters in captive cheetah (*Acinonyx jubatus*) at Hoedspruit Endangered Species Centre

by

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Department: Department of Animal and Wildlife Sciences
Degree: Magister Scientiae Agriculturae: Animal Sciences (Production Physiology)

Cheetah are endangered for various reasons. As a result, captive care and breeding of cheetah has been done in an effort to help the survival of the species, however, captive survival rates are low. It is known that cheetah hunt by means of a high speed pursuit of its prey. It is unclear if cheetah in captivity also require a certain amount of exercise as part of their physical activity regimen to mimic natural feeding circumstances. Captive cheetah are fed using various feeding methods at different facilities around the world. The effect of feeding methods have been tested in various circumstances at various facilities. Nutritional management, by means of tried feeding practices, can be implemented to meet physical, physiological and psychological requirements of the captive cheetah. The ideal nutritional management program was not identified at Hoedspruit Endangered Species Centre (HESC) at the beginning of this study. The aim of this research study was to evaluate the effects of physical exercise on the overall physiological status of cheetah in captivity.

The trial with the physical exercise treatments were applied over a period of seven weeks, during January to March, at HESC. The trial had two treatments, with five animals per treatment group. Animals from the two treatment groups, the control and lure group, were fed on feeding days, with physical exercise as the only variable. The two treatments were evaluated, based on physical health evaluations done by observation, and physiological health in the form of blood parameter analyses (one hour postprandial). Blood parameters that were analysed on a feeding day in weeks one, three, five and seven included; albumin, globulin, total protein, packed cell volume, blood urea nitrogen, Ca, P, creatinine, lactic acid/lactate, cortisol, free triiodothyronine (T_3), free tetraiodothyronine (T_4), lipogram, and 25-hydroxy-vitamin D (vitamin D_3). Weekly pooled supplemented meat samples were analysed to show the nutritive value of the food during the seven-week trial. Feed analyses was done for: dry matter content, ash (inorganic matter), crude protein, ether extract, Ca, P, Mg, Cu, Fe, Mn, Zn, K, Na, and Se.

The overall visual health evaluation showed that both the control and lure animals were in good physical health throughout the entire trial. Exercise had an effect on the albumin, creatinine and free T_4 levels over the seven weeks. The higher level of albumin can be an indication that the body produced more albumin in an attempt to transport more oxygen in the blood during exercise. The lower level of creatinine can be an indication that the amount of muscle breakdown in the lure group reduced over time. Free T_4 levels increased over time. This can be an indication of increased activity in the thyroid gland, thus a faster metabolism in the lure group of animals than in the control group. High variability in the mineral and ether extract results from the feed analyses indicates that the CVM-supplement was not used as prescribed. Sampling of supplemented meat could also influence the mineral and ether extract results.

The current feeding method, including feed preparation, should be re-evaluated at HESC. The recommended 60g of the CVM-supplement should be added to the presented food in such a way that each individual receives an adequate amount of supplement for their nutritional requirements. Based on the blood parameters analysed in the two groups, no significant difference was found over the seven-week experimental period. Possible reasons for the lack of significant differences between the two groups can include one or more of the following: the frequency of exercise the lure group was subjected to might have been too short or not

frequent enough, the experimental period was too short, or the sample size of the two groups of animals was too small. One or more of these factors could have resulted in the fact that specific blood parameters were largely unaffected by the treatments and no treatment effect was observed. Research on physical fitness, in other mammals, including humans, indicates that physical fitness improves general health, nutrient metabolism and physiological status. During this trial, the animals showed increased levels of interest and excitation while the lure was set up in their enclosures, in relation to other activities, such as cleaning of facilities. With regard to the psychological wellbeing of the animal it is recommended that the lure should be used as an environmental enrichment at HESC.

ABBREVIATIONS

Δ^6 position	The position of an unsaturated bond in a fatty acid.
AA amyloidosis	A specific form of amyloidosis
AA spectrophotometer	Atomic absorbance spectrophotometer
AAFCO	Association of American Feed Control Officials
ACTH	Adrenocorticotropic hormone
Alb	Albumin
ATP	Adenosine triphosphate
BUN	Blood urea nitrogen
Ca	Calcium
CCr	Endogenous creatinine
CF	Crude fibre
Cort	Cortisol
CP	Crude protein
Creat	Creatinine
Cu	Copper
CVM-supplement	Cheetah vitamin and mineral supplement
DE	Digestable energy
DHBS	3,5-dichloro-2-hydroxybenzenesulfonic acid
DM	Dry matter
EDTA	Ethylenediaminetetraacetic acid
EE	Ether extract
FCRD	Feline central retinal degradation
Fe	Iron
GE	Gross energy
GFR	Glomerular filtration rate
GI	Gastrointestinal
GK	Glycerol kinase
GLM	General linear model
Glob	Globulin
GPO	Glycerophosphate oxidase
Hb	Haemoglobin
$HClO_4$	Perchloric acid
HDL	High-density lipoprotein
HDLD	High-density lipoprotein deficiency
HESC	Hoedspruit Endangered Species Centre
HNO_3	Nitric acid
HPA	Hypothalamic-pituitary-adrenal
HPO	Horseradish peroxidase
HRV	Heart rate variability
IUCN	International Union for Conservation of Nature
K	Potassium
Lac	Lactate or Lactic acid
$LaCl_3$	Lanthanum-III-chloride
LDL	Low-density lipoprotein
LDLD	Low-density lipoprotein deficiency
MANOVA	Multivariate analyses of variance
MBD	Metabolic Bone Disease
Mg	Magnesium
MHC	Major histocompatibility complex
Mn	Manganese
Na	Sodium

$(NH_4)_6MO_7O_{24}.4H_2O$	Ammonium molybdate tetrahydrate
NH_4VO_3	Ammonium vanadate
NRC	National research council
OM	Organic matter
P	Phosphorous
PCV	Packed cell volume
ppm	Parts per million
PTH	Parathyroid hormone
PUFA	Polyunsaturated fatty acid
R^2	A statistical measure of how well the regression line approximates the real data points.
RBC	Red blood cells
SCFA	Short-chain fatty acids
SD	Standard deviation
Se	Selenium
SFA	Saturated fatty acid
T_3	Triiodothyronine
T_4	Tetraiodothyronine or tyroxine
TChol	Total cholesterol
TDF	Total dietary fibre
TG	Triglycerides
TMR	Total mixed ration
TP	Total protein
Type III SS	Type III sum of squares
VLDL	Very low-density lipoprotein
VOD	Veno-occlusive disease
WBC	White blood cell
Zn	Zinc

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CHAPTER 1: INTRODUCTION

1. Background

A rapid decline in the wild cheetah (also known as the hunting leopard) population over the last 50 years has placed the cheetah on the IUCN (International Union for Conservation of Nature) Red List for Threatened Species (Durant *et al*., 2008). This rapid decrease has caused an increased awareness and effort to keep, breed and rear cheetah in captivity (Grobler, 2011). Reasons for the rapid decline in the wild population of cheetah include habitat destruction by humans, decline in number of prey available in their natural habitat, increasing competition from other wildlife as a result of smaller area availability and shooting of cheetah by farmers (Caro *et al*., 1987; Brown *et al*., 1996; Marker *et al*., 2003). Cheetah numbers are also threatened by the sport hunting industry, where they are hunted for trophy, as well as part of the trade industry, both legally and illegally (Durant *et al*. 2008). The captive cheetah population is not growing at expected percentages due to high mortality rates (especially in cheetah cubs), mismanagement and low reproductive efficiency (Brown *et al*., 1996). According to the IUCN, the total number of adult cheetah in southern Africa is estimated at approximately 7,500 (Durant *et al*., 2008). The inclusion of other areas where the population is only estimated would not raise the total number left in the world to over 10 000. Detrimental effects to the cheetah population can be foreshown worldwide, even though newly updated data is unavailable (Durant *et al*. 2008).

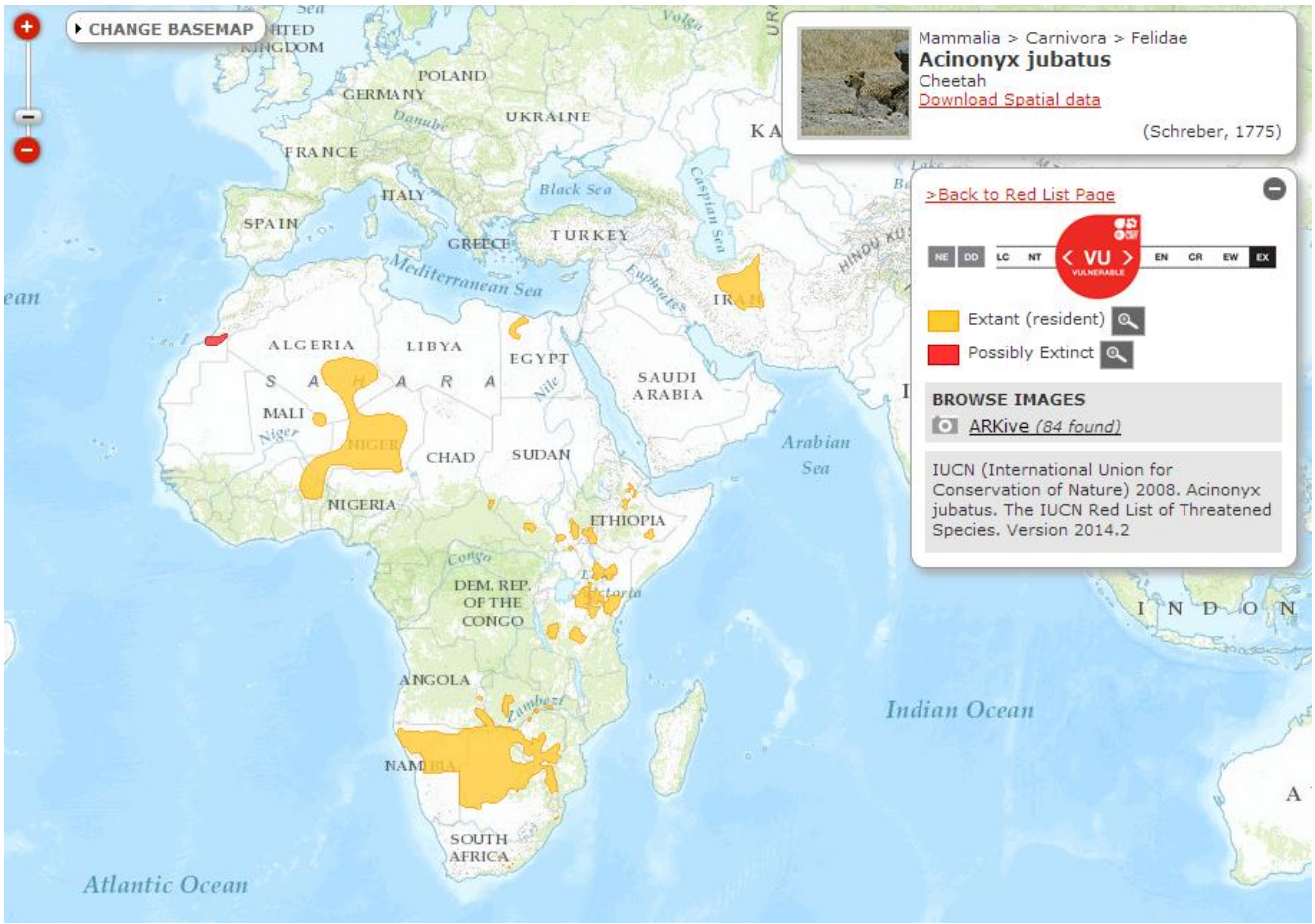


Figure 1.1 A range map of the distribution of cheetah taken from IUCN Red List for Threatened Species website (Durant *et al*. 2008).

Kingdom	Anamalia
Phylum	Chordata
Class	Mammalia
Order	Carnivora
Family	Felidae
Genus	*Acinonyx*
Species	*jubatus*

Figure 1.2 Taxonomy of cheetah (adapted from Campbell *et al*. 2008 and Durant *et al*. 2008).

Cheetah are not only threatened by low numbers but are made more vulnerable to extinction by reduced genetic variation (Wielebnowski, 1996; Marker-Kraus, 1997; Hayward *et al*., 2006). Survival after translocation is also not guaranteed. The combination of MHC complex alleles in an individual can play a role in that animal's ability to adapt to a new area and its potential pathogens (Castro-Prietao *et al*., 2012). Big cat genomic research by O'Brien & Johnson (2005) showed that a population bottleneck most probably occurred in cheetah populations a few thousand years ago. This is the likely cause for the major reduction in effective population size and heterozygosity. The loss in heterozygosity is hypothesized by Caro *et al*. (1987), Marker-Kraus (1997) and Munson *et al*. (2005) to result in lowered ability to fight infectious disease, poor health and lowered reproductivity. O'Brien & Johnson (2005) found that it appears as if cheetah naturally inbred which caused a 90-99% reduction in overall genetic diversity. Analyses of the cheetah major histocompatibility complex (MHC), which specifically determines the class I and II cell surface antigens (responsible for triggering of graft rejections in mammals), revealed that all the alleles were identical (O'Brien & Johnson, 2005).

Cheetah are one of the fastest land mammals (Williams *et al*., 1997), structurally suited to reach speeds of 104-112km.h^{-1} over a short distance (Hayward *et al*., 2006). Their skeletons are described by Marker & Dickman (2003) as light weight and aerodynamically efficient and their bodies have a few anatomical and physiological adaptations (O'Brein *et al*., 1985 as quoted by Williams *et al*., 1996). In pursuit of fast prey, the partly retractable claws aid in driving the cheetah forward at a high speed, almost like spike shoes used by track runners (Londei, 2000). The author further report that the cheetah dewclaw has specialised to take over the hooking function of the entire front paw, whilst the other digits are specialised for traction purposes during the high speed pursuit.

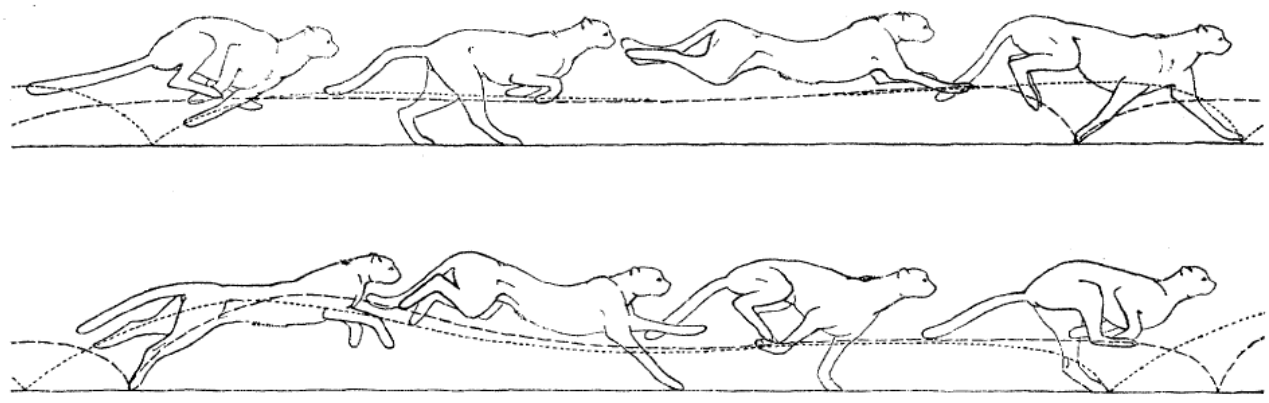


Figure 1.3 Eight positions of a cheetah galloping between 80 and 96km/h as taken from Figure 1 in Hildebrand (1961).

Cheetah body weights range between 30 and 72 kg (Hayward *et al*., 2006). To maintain their body weight and to satisfy their energy needs cheetah have to prey upon large vertebrates, which are more than 45% of their own body mass (Hayward *et al*., 2006). It is not only important to maintain body weight but it is also important to provide the uniquely required nutrients to an animal during all physiological stages. These stages include infancy, new born cub, young and growing cub, adolescent, adult, pregnancy, etc. Domestic cats are

named as an unsuitable cat model for cheetah by Bell *et al*. (2012), especially in predicting energy intake in cheetah.

In a study of the phylogenetic relationships among 37 felid species O'Brien & Johnson (2005) explain that cheetah are in the Puma lineage, but is the only species in the genus *Acinonyx*. Cheetah is significantly different with regards to anatomy and behaviour from any other wild felid species (Marker-Kraus, 1997; Russell & Bryant, 2001; Quirke & O'Riordan, 2011a). For instance, the dewclaw usually refers to a non-functional talon in the case of dogs, but in the case of cheetah the dewclaw is a sharp, curved, large talon which plays a vital role in the cheetah hunting technique to throw prey off balance (Londei, 2000).

1.1 Problem statement

It is known that cheetah hunt by means of a high speed pursuit of its prey. It is unclear if cheetah in captivity also require a certain amount of exercise as part of their physical activity regimen to mimic natural feeding circumstances.

1.2 Objective of the study

The aim of this research study was to evaluate the effects of physical exercise on the overall physiological status of cheetah in captivity. The physical exercise treatments included feeding of cheetah either in their enclosures with no exercise, or feeding in combination with an exercise programme (by means of a lure).

1.3 Layout of the dissertation

Chapter 1: Introduction

Background on the global status of the cheetah, a problem statement and the objective of the study are provided and explained in this chapter.

Chapter 2: Literature review

This chapter considers important research done with regards to different feeding methods and feed analyses. The chapter further highlights key aspects of certain nutrients and components commonly found in feedstuffs. Another aspect covered in this chapter is important research done with regards to physiological conditions or disorders. Most of these conditions or disorders have their root in malnutrition or a lack of environmental enrichment.

Chapter 3: Materials and Methods

The experimental design and research methodology used during this trial are discussed in this chapter.

Chapter 4: Results and discussion

Results of data collected during the trial pertaining to feed, the relevant blood parameters and general physical health will be reported and discussed in this chapter.

Chapter 5: Conclusion

In this chapter conclusions are made based on the aim given in Chapter 1. Further recommendations are made, as well as a statement of problems and important research questions to consider in future.

Bibliography

Appendix

1.4 Ethical and legal consideration

Routine inspections of all animals were done at least twice daily. All sample collections and general health assessments were done during routine feeding with the aid of the curators.

Blood samples were packed for transportation in three layers according to the standard procedure. All shipments of samples were accompanied by a permit issued by the Hoedspruit State Veterinarian, Dr Christiaan Steynman, on the day of transport. Samples and all consumables used during sample preparation, shipment and analyses were destroyed by means of incineration after completion of all analyses. Above mentioned precautions were taken as HESC are situated within the red zone for foot and mouth disease.

This research trial conforms to the ethical standards of the Animal Use and Care Committee of the University of Pretoria (Certificate in Appendix F), as well as HESC. Special attention was taken during experimental planning in order to adhere strictly to all ethical principles.

CHAPTER 2: LITERATURE REVIEW

2. Introduction

This chapter considers important research done with regards to different feeding methods and feed analyses. The chapter further highlights key aspects of certain nutrients and components commonly found in feedstuffs. Another aspect covered in this chapter is important research done with regards to physiological conditions or disorders. Most of these conditions or disorders have their root in malnutrition or a lack of environmental enrichment.

2.1 Feeding methods

Increased pressure on the cheetah natural population of cheetah from various sources increases the demand for successful breeding, rearing and keeping of cheetah in a captive environment (Caro *et al*., 1987; Grobler, 2011). Nutrition plays a major role in the overall physical, physiological and psychological health of animals. Grobler (2011) further reports that an increased need for captive cheetah vigour places more weight on nutritionists to develop a specialized diet, or total mixed ration (TMR), to meet the special nutritional requirements cheetah have in captivity. In the following section (2) the feeding behaviour in wild cheetah will be compared to the feeding methods followed for cheetah in captivity.

2.1.1 Feeding behaviour of free-ranging cheetah

Wild cheetah spends a great amount of time and energy capturing and ingesting food (Bond & Lindburg, 1990). Approximately 50% of all pursuits end in a kill (Hayward *et al*., 2006 & Macdonald & Loveridge, 2010). It is commonly seen that cheetah hunt alone, but they also hunt as a group, depending if the individual is solitary or part of a coalition (Hayward *et al*., 2006). Cheetah can stalk their prey and approach it by walking or slowly jogging out in the open (Williams *et al*., 1997). Cheetah locate prey mostly by relying on sight (Williams *et al*., 1996). Prey on open grasslands is a more prevalent target of cheetah on the hunt (Hayward *et al*., 2006). The characteristic hunting behaviour of cheetah starts with a short (50-60m) high speed pursuit (Macdonald & Loveridge, 2010; Russell & Bryant, 2001; Williams *et al*., 1996; Williams *et al*., 1997) and ends after a blow to the hind leg of the prey (Londei, 2000 & Phillips, 1993). The blow to the prey is of low impact, because the relative speed of the cheetah to its prey is low. The low impact blow is sufficient to hook the prey off balance with either of the dewclaws (Londei, 2000). The prey loses balance which allows the cheetah to overpower its victim and kill it by strangulation (Londei, 2000 & Phillips, 1993). The cheetah starts, according to Phillips (1993) and Vester Boler *et al*. (2009), to feed from the ventral side of the carcass, first consuming the soft flesh and organs, leaving the intestines, which will be abandoned. The authors further reported that cheetah then feed on the muscle and some bone and cartilage (in the case of small prey cheetah may consume all the bone) and abandons prey 1-2 hours after the kill. The build of the cheetah skull, jaws and teeth restricts them from consuming large bones (Phillips, 1993) and limits them to the feeding of mostly flesh and softer tissues (Hayward *et al*., 2006). It was long believed that cheetah can be at risk of overheating during a long distance hunt. The theory was that cheetah abandoned the hunt to stop and cool down in order to prevent thermal compromise to the brain and other vital organs. This entire theory was based on a study done by Taylor & Rowntree (1973) with two cheetah that ran on a treadmill. There were a couple of flaws to this experiment, including that the cheetah never reached maximum speed or distance on the treadmill. A recent study by Hetem *et al*. (2013) showed that wild cheetah had a higher temperature build-up during the killing of the prey after the initial sprint, than during the sprinting pursuit. The author further reported that after a successful hunt and killing of prey cheetah do cool down before starting to consume the prey.

Cheetah prey range from 2 kg hares (*Lepus sp.*) and warthog (*Phacochoerus africanus*) piglets to zebra (*Equus quagga burchellii*) and adult wildebeest (*Connochaetes taurinus*) weighing up to 270kg, including giraffe (*Giraffa camelopardalis*) and buffalo (*Syncerus caffer*) calves (Hayward *et al*., 2006; Macdonald & Loveridge, 2010; Marker *et al*., 2003). Owen-Smith & Mills (2008) also added kudu (*Tragelaphus strepsiceros*), waterbuck (*Kobus ellipsiprymnus*), impala (*Aepyceros melampus*) and other small antelope species to this list. It was documented by Phillips (1993) that wild cheetah preferred prey weighing less than 60 kg. Owen-Smith & Mills (2008) marked that the most favoured prey size appears to be once or twice the size of the predator. The prey differs between seasons, depending on availability (Macdonald & Loveridge,

2010). This variation may be due to seasonal migration of prey species forcing cheetah to migrate to different habitats with varying types, numbers and age distribution of prey. This variation in prey must also be considered when formulating a diet for captive cheetah. One means of adapting the formulation of the diet to the season, may be adaptation of the concentrate given with the meat chunks to the seasonal changes and deficiencies in fat, minerals and vitamins found in meat. This will, however, also differ between sources of meat as fat, mineral and vitamin content depend on feeding methods of the meat source animal example extensive feeding on the veld vs. feedlot fed animals. Although it is not recommended to use domestic cats as a model animal for cheetah (Allen *et al*., 1995), the cat food standards by the Association of American Feed Control Officials (AAFCO) can be used as an indication of the nutritional requirements of cheetah. The AAFCO Cat Food Profile published in 2008 is given below.

Table 2.1 AAFCO Cat Food Nutrition Profiles Published in 2008[a] as adapted from Foster *et al*. 2012.

Nutrient	Unit on Dry Matter (DM) basis	Growth and Reproduction Min	Adult Maintenance Min	Adult Maintenance Max
Protein (CP) [b]	g/kg	300.00	260.00	-
Taurine {canned}	%	0.20	0.20	-
Fat	g/kg	90.00	90.00	-
Calcium (Ca)	g/kg	10.00	6.00	-
Phosphorous (P)	g/kg	8.00	5.00	-
Ca:P	Ratio	1.25:1.00	1.20:1.00	-
Potassium (K)	g/kg	6.00	6.00	-
Sodium (Na)	g/kg	2.00	2.00	-
Chloride (Cl)	g/kg	3.00	3.00	-
Magnesium (Mg) [c]	g/kg	0.80	0.40	-
Iron (Fe) [d]	g/kg	0.08	0.08	-
Copper (Cu){canned} [e]	g/kg	5.00×10^{-3}	5.00×10^{-3}	-
Manganese (Mn)	g/kg	7.50×10^{-3}	7.50×10^{-3}	-
Zinc (Zn)	g/kg	0.075	0.075	-
Selenium (Se)	g/kg	1.0×10^{-4}	1.0×10^{-4}	-
Vitamin D	IU/kg	750.00	500.00	10 000.00

[a] Presumes an energy density of 3.50kcal ME/g DM, as determined in accordance with regulation PF9, which is based on the 'modified Atwater' values of 3.50, 8.50, 3.50kcal/g for protein, fat, and carbohydrate (nitrogen-free extract, NFE). Respectively.
Rations greater than 4.00kcal/g should be corrected for energy density; rations less than 3.50kcal/g should not be corrected for energy.
Rations of low-energy density should not be considered adequate for growth or reproductive needs based on comparison to profiles alone.
[b] Although a true requirement for fat per se has not been established, the minimum level was based on recognition of fat as a source of essential fatty acids, as a carrier of fat-soluble vitamins, to enhance palatability, and to supply adequate caloric density.
[c] If the mean urine pH of cats fed ad libitum is below 6.4, the risk of struvite urolithiasis increases as the magnesium content of the diet increases.
[d] Because of very poor bioavailability, Fe from carbonate or oxide sources that are added to the diet should not be considered as components in meeting the minimum nutrient level.
[e] Because of very poor bioavailability, Cu from oxide sources that are added to the diet should not be considered as components of meeting the minimum nutrient level.

Cheetah hunt diurnally with prey sizes ranging from small to medium (Hayward *et al*., 2006; Quirke & O'Riordan, 2011a; Quirke & O'Riordan, 2011b). Cheetah have been reported to appear nervous at kills and consume feed rapidly (Phillips, 1993). The rapid consumption of prey is hypothesized by Hayward *et al*., (2006) to be a means to avoid the loss of the catch to potential kleptoparasites (an animal that steals food from an individual who has just obtained it). Rapid feeding was expected to be induced (Williams *et al*., 1996) by using a pulley enrichment device, as it may be responsible for inducing a natural defence mechanism. Hayward *et al*., (2006) further reported that adult cheetah sometimes (but rarely) scavenge the ca 2.8kg meat they require for daily maintenance, as they are competitively inferior in size and strength to most other predators. The author also hypothesises that cheetah are diurnal hunters as an adaptive measure against the kleptoparasites

(most competing predators are active during night time). Focusing on smaller prey also allow cheetah to consume larger portions of the animal before losing it to potential kleptoparasites (Hayward *et al*., 2006).

2.1.2 Feeding methods for captive cheetah

Captive cheetah is fed food (usually commercially prepared) that require no or little effort (or energy) to obtain or ingest (Allen *et al*., 1995; Bond & Lindburg, 1990; Vester Boler *et al*., 2009). It is not only important to meet the physiological and nutritional needs of captive cheetah, but also their physical and psychological needs (Depauw *et al*., 2012). Furthermore, Bond & Lindburg (1990) found that the texture, temperature and palatability of the food should be taken into account, over and above the nutritional composition of the diet, to better meet nutritional and psychological needs of cheetah in captivity. In many captive situations carnivores are generally fed a basic meat diet regardless the species or age of the predators. Allen *et al*. (1995) suggested that this approach is incorrect because the diet should cater for a specific body size and built, as well as the activity level of the individual animal being fed. Bond & Lindburg (1990) and Depauw *et al*., (2012) hypothesised that the provision of a more natural diet to captive cheetah will increase their physical and psychological welfare. Feeding and food allowance of captive wild felids are mainly based on a trial-and-error-approach, where the body condition of the animals is used to judge the wellbeing of the animal (Robbins, 1993 as quoted by Clauss *et al*., 2010). Bond & Lindburg (1990) conducted further studies to determine the impact of the type of food provided to the captive cheetah (commercial diet or carcasses) on their eating style, level of interest and time taken to consume the food presented. Feeding of live prey is possibly the ultimate environmental enrichment that a conservation centre or zoo can offer captive carnivores, including cheetah. However, it is against the ethical code of many bodies, including zoo federations (Lemmer *et al*., 2008), and is regarded as illegal in the United Kingdom (Williams *et al*., 1996). It was recommended by Lemmer *et al*. (2008) that alternatives should be investigated, such as whole carcass feeding. The authors also named other studies that showed feeding of whole carcasses which can be used to allow the expression of a more natural feeding behaviour in captivity. It may be possible to stimulate the animals in captivity by implementing enrichment programmes to encourage natural levels of energy expenditure and opportunity for development and expression of more natural behaviour (Williams *et al*., 1996).

2.1.3 Feeding whole carcasses

Captive cheetah is completely dependent on the feed that they receive from the institution they belong to (Grobler, 2011). The author further reported that feeding whole carcasses of various animals, most commonly chickens, to captive cheetah, are some of the strategies followed to formulate a complete diet for cheetah in captivity. Feeding carcass or portions of carcasses to cheetah is an expensive practice especially if the costs of the carcasses, transportation, storage and handling are considered. The nutritional value and how wholesome the prey provided would be to the captive cheetah are further influenced by the origin, handling and storage methods performed (Dierenfeld *et al*., 2002). After the death, the mineral content of the prey changes with the freezing and the natural processes where muscle is converted into meat (Grobler, 2011). Another feeding strategy mentioned by Grobler (2011) is feeding of large portions of meat with added supplements. This approach relies on the feeding of large portions of bovine meat, as wild ungulate meat is scarce; however, bovine meat is not part of the wild cheetah diet and differs in chemical composition to that of the wild ungulates (Grobler, 2011; Vester Boler *et al*., 2009). In the process of formulating a diet for cheetah in captivity, it is important to take the chemical composition and the type of diet consumed in the wild into account (Vester Boler *et al*., 2009).

2.1.4 Feeding commercially prepared (“processed”) diets or supplemented meat

These diets may be frozen, canned, extruded or pelleted. It is recommended that the energy content of these diets should be projected by a process where fibre, crude fibre (CF) or total dietary fibre (TDF), are used to estimate energy digestibility (Clauss *et al*., 2010). Whole carcasses, without supplement, can be fed when the viscera (including the heart, lungs, liver, spleen and kidneys and may exclude the intestinal tract), skin, fur and bones are included to supply the essential hassle factor, vitamins and minerals (Depauw *et al*., 2012). It has been found that a diet of supplemented muscle meat met all the requirements, although oversupplying in vitamins A and E (Vester Boler *et al*., 2009). It is, however, vital to supplement a raw diet (Allen *et al*., 1995; Depauw *et al*., 2012). The exclusive use of a commercially prepared diet, may have a negative influence on

behaviour, as well as overall health (Depauw *et al*., 2012). An example of a commercially prepared diet fed in a trial by Depauw *et al*. (2011) includes the Nebraska Brand Special beef feline (frozen) diet. Ingredients of this diet listed in order by weight: beef, meat by-products, fish meal, soy bean meal, dried beet pulp, calcium carbonate, dried egg, brewers dried yeast, Nebraska Brand feline vitamin premix, salt, Nebraska Brand trace element premix (Central Nebraska Packing, Inc.). See Table 2.2 for the guaranteed analysis of this specific diet of the Nebraska Brand.

Table 2.2 Guaranteed analysis of special beef feline (frozen) diet adapted from Central Nebraska Packing, Inc.

Guaranteed analyses	Min or max	DM (%)
Crude Protein	(min)	48.15
Crude Fat	(min)	31.60
Crude Fibre	(max)	3.90
Ash	(max)	11.80
Calcium	(min)	1.57
Phosphorus	(min)	1.31
Vitamin A (IU/kg)	(min)	10 512
Vitamin D (IU/kg)	(min)	1 025

2.1.5 Recommendations: Diet formulation for captive wild felids

The first step in the process of formulating a species specific diet is to determine the nutritional requirements of the animal. This can be done by determining the energy content of the diet (Clauss *et al*., 2010). The energy content gives an indication of the amount of food that should be consumed in a specific timeframe and the required nutrient composition of such a feed. The energy content of a diet can be used as a measure to determine the amount of energy required for maintenance, as well as other physiological reports of the animal. This is possible as energy content of the diet is empirically known to maintain body condition. Animals fed to maintenance level will not lose or gain weight (Allen *et al*., 1995). What we also need to keep in mind is that "Animals eat grams, not percentages" which refers to the capacity for feed consumption of the animal that needs to be taken into account (Clauss *et al*., 2010).

Even though Bell *et al*. (2012) advise against using domestic cats as a model in predicting energy intake in cheetah, both of these, cheetah and domestic cats, are obligated carnivores (Allen *et al*., 1995; Depauw *et al*., 2011). Domestic cats are usually used to extrapolate feeding recommendations for large exotic felids from, but no direct comparisons between domestic and exotic felids fed the same basic diet have been done before Vester *et al*. (2010). Previous studies did measure apparent total tract digestibility and faecal fermentative end products (Vester *et al*., 2010). It might be useful to use a well-studied model animal (such as domestic cats) to determine the digestion correlations and eventually nutrient requirements for cheetah, as this will aid in completing step one of diet formulation (Clauss *et al*., 2010). It is further reported that cheetah and some other animals from the study required less food to maintain body weight than most other wild felids (Vester *et al*., 2010). It was reported in the same paper and also in Allen *et al*. (1995) that metabolisable energy (ME) requirements cannot be extrapolated from domestic cats or from other known exotic felids as there are too high variability between and among species.

2.2 Feed analyses

During any trial where the influence of feed or feeding methods are studied, representative samples of the feed provided to the animals in the trial, during the whole trial period, must be taken. These samples must be marked and stored identically (to prevent bias and errors) and either pooled for testing or tested individually. Vester *et al*. (2010) analysed dry matter (DM), organic matter (OM), crude protein (CP) and gross energy (GE) in the studied diets. CP, GE and DM was also analysed by Depauw *et al*. (2012). The authors also did other analyses that can aid in comparison and quality evaluation of diets, which include: crude fat or ether extract (EE), crude fibre (CF), total dietary fibre (TDF), minerals (including taurine, Ca, Fe, K, P, Mg, Na, Cu, Mn and Zn), vitamins, fatty acids and dietary lipids.

2.2.1 Energy

Cheetah prey upon large vertebrates (sometimes even more than 45% of their own body mass) to meet their body's energy demands (Hayward *et al*., 2006). The authors further report that morphological limitations

may exist that limits the cheetah in prey size where energetic benefit must be weighed against energetic cost of obtaining the prey. According to NRC (2006) energy cannot be regarded as a nutrient itself but is rather a property of the contribution of fats (lipids), proteins and carbohydrates, the three basic nutrients of diets. After consumption of food, the animal's body digest the food, in order to extract different nutrients, and these nutrients are then absorbed and transported to different areas in the animal's body where it can be either processed further, used or stored (McDonald *et al*., 2002). Digestible energy (DE) was expressed relative to metabolic body weight (kcal/kg $BW^{0.75}$) as 150-185 in cheetah (Allen *et al*., 1995). According to Bell (2010) DE is the energy left over after the GE lost via faeces was deducted from the GE gained from food (McDonald *et al*., 2002). Cheetah have similar energy needs for maintenance of body weight to that of domestic cats (Vester *et al*., 2010) but require higher levels of energy from their diets (Vester Boler *et al*., 2009). No specific relationship was found between DE intake and season. New studies on the relationship of body weight and DE suggest that these two parameters may be more complexly connected (Allen *et al*., 1995).

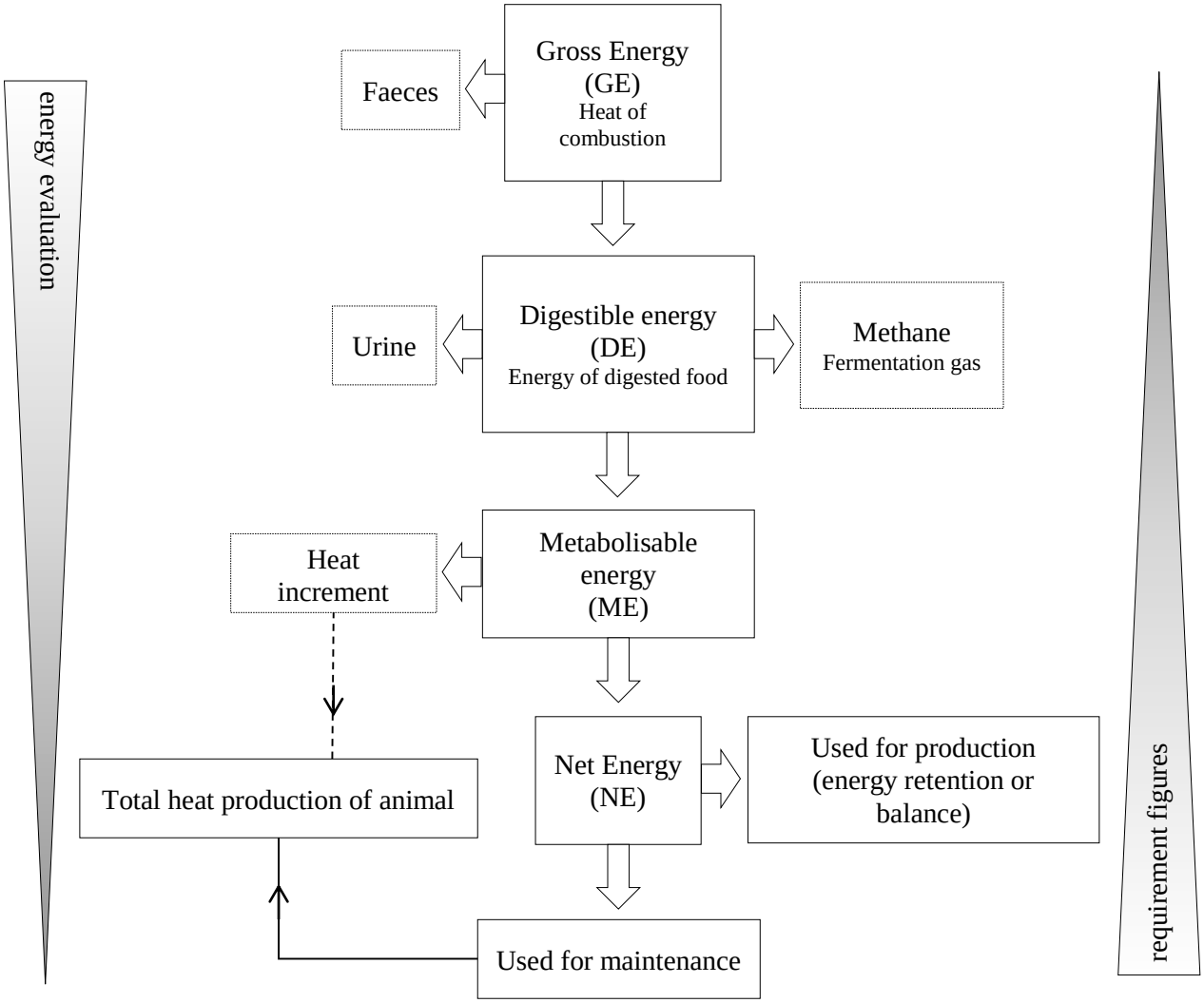


Figure 2.1 The partition of food energy in the animal. Losses of energy are shown as the items boxed in dashed boxes. (Adapted from McDonald *et al*., 2002 & NRC, 2006).

ME utilization does not occur equally efficient in all stages of production. Maintenance is the stage during which energy utilization is at its most efficient, whereas protein deposition, fat deposition, growth in general, production of milk (lactation), foetal growth (growth of conceptus) and work (e.g. draught animals) are all less efficient than the one preceding it in this list (McDonald *et al*., 2002). This means that a value of ME requirement may not be efficient but specific ME and other energy requirements for each of these

processed must be taken into account. In a study that evaluates the activity level of cheetah, enclosure design, as well as ambient temperature must be taken into account in energy expenditure (Allen *et al*., 1995).

2.2.2 Fibre

Dietary fibre has become increasingly important in the nutrition of both humans and animals (Depauw *et al*., 2011). Fibre have many properties including working as a pre-biotic, aiding in weight loss, as a healing medium for gastrointestinal (GI) disorders or act as a substrate to form short-chain fatty acids (SCFA) in the colon (Depauw *et al*., 2011). In cheetah the most likely substrate for hindgut fermentation is (glyco) protein as the amount of plant material intake is negligible (Depauw *et al*., 2011).

2.2.3 Fat

In conjunction with domestic cats, cheetah and lion (*Panthera leo*) also lack the ability to efficiently desaturase fatty acids at the Δ^6 position (NRC, 2006). The authors further found that cheetah require their polyunsaturated fatty acid supply not to be spoiled by oxidation, or in other words they prefer their meat fresh. Triglyceride fats is an important source of energy. Although inferior to proteins, it has a higher gross energy value than carbohydrates (McDonald *et al.*,2002). Higher fat content diets are more energy dense.

2.2.4 Carbohydrates

Carbohydrates can be subdivided into the following groups according to function: absorbable carbohydrates (monosaccharides), digestible carbohydrates (disaccharides, some oligosaccharides, and non-structural polysaccharides), fermentable carbohydrates (lactose, some oligosaccharides, dietary fibre and resistant starch) and non-fermentable carbohydrates such as some dietary fibre (NRC, 2006).

2.2.5 Protein

With the decrease in digestibility of the protein source, the amount of protein reaching the large bowel increases (Vester *et al*., 2010). Fermentation of proteins occurs in the large bowel. This fermentation process causes an increase in the putrefactive compounds, a product of the fermentation, and an increase in putrefactive smell of the faeces results (Vester *et al*., 2010).

2.3 Blood chemistry

Nutritional status is defined by Mosby (2009) as the degree to which nutrients are available to meet metabolic requirements. Nutritional status is an important tool to evaluate whether the diet presented to an animal, such as captive cheetah, can meet in all the physiological needs of the animal during different production stages. One means of collecting data on the nutritional status of animals is by using blood parameters (Depauw *et al*., 2011). When blood is collected, for analytical studies, it is very important to note the amount required for a specific test and if blood can be stored or if it is essential to immediately analyse the samples. If samples are stored, specific procedures to prepare samples of blood before storage, as well as specific storage conditions must be followed. Blood samples can either be prepared as whole blood samples or blood serum samples (Bechert *et al*., 2002). Serum samples were used by Depauw *et al*. (2011) to determine overall serum biochemistry, mineral and vitamin concentrations and the lipogram of cheetah. The authors also analysed the fasting serum T_3 and T_4 concentrations, as well as taurine concentrations.

Animals can either be immobilised (Depauw *et al*., 2011; Bechert *et al*., 2002) or conscious when blood samples are taken. During trials by Depauw *et al*. (2011) and Bechert *et al*. (2002) cheetah were fasted and immobilised before sample collection commenced. The Hoedspruit Endangered Species Centre (HESC) in South Africa have specially designed cages within each cheetah holding pen, allowing caretakers to safely withdraw blood (taking into account the safety of both caretakers and animals) while animals are conscious. Tubes used for blood sample collection by Bechert *et al*. (2002) are noted in Table 2.3.

Table 2.3 Tubes used in blood collection for specific analyses (Bechert *et al*., 2002).

Tubes	Analyses
Heparinised, Blue-top and non-heparinised, Grey-top	Plasma and whole blood taurine analyses
Heparinised, Blue-top	Vitamin and mineral analyses
Plain, Red-top	Serum chemistry and ceruloplasmin activity determinations
EDTA, Purple-top	Complete blood counts (CBC) evaluations

2.4 Physiological conditions (or disorders)

Cheetah in captivity have been reported to suffer from stress, muscle atrophy, boredom, and other aspects of poor health (Bond & Lindburg, 1990). Two of the main contributors to poor health are nutrition and feeding methods (Depauw *et al*., 2012). Gut health plays a major role in the health of animals and is influenced by the diet consumed by the animal (Depauw *et al*., 2011).

Nutritional disorders in captive cheetah discussed in this section are: metabolic bone disease, gastritis, renal disease, taurine deficiency, vitamin and mineral deficiencies and toxicities, and protein and fat.

2.4.1 Metabolic bone disease

Calcium (Ca) would be one of the first minerals to consider as it has high metabolic importance in cheetah (Bell, 2010). Bell (2010) reports that Ca not only plays a vital role in physiological functions, but is crucial for the skeleton of the animal, as it acts as the foundation on which the skeleton is built. The Ca to phosphorus (P) ratio of the body can be used as an indication of the ratio that Ca should be to P in the diet (usually 1:1 to 2:1). Mono-calcium phosphate (insoluble compound) forms when the P intake exceeds that of the Ca. This leads to a continuation in the absorbing of P but not of Ca resulting in hypocalcaemia. In reaction to the hypocalcaemia higher volumes of parathyroid hormone (PTH) are released. PTH stimulates bone demineralisation and finally rickets, osteoporosis or osteomalacia (collectively known as metabolic bone disease (MBD)) sets in. Pathological fractures, bowing of the limbs, cataract formation and strabismus all occur as a result of MBD. Beef meat (no bones or fur) contains too much P and the Ca:P balance are out (Bell, 2010). To supplement the Ca, carcasses of animals such as rodents (naturally high in Ca) are recommended above mineral premixes (Bell, 2010).

Grobler & Webb (2011) conducted a study in which a dietary magnesium supplement was added to the feed to determine the effect this added magnesium had on leg deformities. Results from the trial indicated that the occurrence of leg deformities reduced with the magnesium supplement. Improvement of 25.5% in rotational deformities and 60.8% in flexural deformities were found in the individuals that received dietary magnesium supplementation.

2.4.2 Gastritis

Vester *et al*. (2008) reports that due to longevity of cheetah in captivity it is vital to maintain gut-health in these animals to ensure their guts can support them throughout their long life. Progressive gastritis associated with *Helicobacter* infection causes vomiting, weight loss, and failure to succeed in most captive cheetah globally (Terio *et al*., 2005). Eaton *et al.* (1993) isolated four new *Helicobacter* species from the gastric biopsies of four captive cheetah infected with gastritis. The authors distinguished the isolates from known *Helicobacter* species (such as *H. pylori*). *Helicobacter acinonyx* was proposed by the authors as a possible name for the new *Helicobacter* species. Terio *et al*. (2005) found that the newly discovered *Helicobacter spp.* was named *Helicobacter acinonychis* and is related to *Helicobacter pylori*, which is associated with gastritis and other conditions in humans. The authors further report that gastritis in the captive cheetah population of South Africa was identified as one of the main reasons for deaths or euthanasia.

A study was conducted by Terio *et al*. (2005) with gastric samples of 10 wild cheetah and 23 captive cheetah (housed in different facilities, infected with *Helicobacter* organisms and with various degrees of gastritis), where the *Helicobacter* organisms were isolated from the gastric samples and identified. The results of the analysis of all the gastric samples showed that no single strain of *Helicobacter* could be associated with gastritis in captive cheetah and it was found that although the same *Helicobacter* spp. was found in most captive cheetah, the severity of infection had marked differences. No specific types of *Helicobacter* were found to be associated to any one facility (Terio *et al*., 2005). In further studies where host factors important for development of gastritis are determined, *Helicobacter* organisms can be used to act as an interesting natural disease model.

Lane *et al.* (2011) conducted a study to establish if the incidence of gastritis, renal disease and subsequent death related to these disorders was partly dietary induced in captive cheetah. The authors showed in their study that diet did not have any significant influence on gastritis levels in cheetah but healthy cheetah remained gastritis free for a longer period when fed a diet formulated for cats compared to cheetah fed the supplemented meat-based diet. It was further concluded by the authors that mortality caused by gastritis or renal disease in captive cheetah can be reduced by applying proper dietary and/or therapeutic management (Lane *et al*., 2011). Nutritional management of the diet with regards to quality of feed presented and supplementation to improve the overall physiological status and immune system of the animals. Other methods of treatment and prevention are management and enrichment of the surrounding environment, to lower the exposure to pathogens and stressful situations.

2.4.3 Renal disease

Renal disease is one of the main causes for morbidity and mortality in captive cheetah (Holder *et al.*, 2004). The author further reports that cheetah may have a predisposition to potentially fatal diseases, such as renal amyloidosis and glumerulosclerosis, because of the small genetic diversity in the cheetah gene pool. It is also difficult to characterize and control the afore mentioned fatal diseases as they can in many cases only be confirmed by means of post-mortem examinations (Holder *et al*., 2004). Glumerulosclerosis was identified by Munson (1993) as the most predominant pathological process that affect the kidneys of cheetah in captivity.

Holder *et al*. (2004) found that there was a strong correlation between glomerular filtration rate (measured as urinary clearance of inulin) and endogenous creatinine (CCr). The authors report that CCr can be used as a measure of glomerular filtration rate (GFR), which is a means to measure renal function. Measuring CCr is a less invasive way of determining renal function. This measure can detect renal damage at a much earlier stage with lowered GFR than with serum creatinine and blood urea nitrogen (BUN). The authors report that the values in this study can be used as a starting point in renal evaluation of captive cheetah, however, the small population size did not allow the authors to propose a definite reference range for the normal values of GFR and CCr in cheetah.

Lane *et al.* (2011) and Terio *et al*. (2005) report that recurrent gastritis predisposes cheetah for renal amyloidosis as it causes systemic AA amyloidosis, which results in renal failure. Lane *et al.* (2011) also reports that a high percentage of all captive cheetah suffer from glumerulosclerosis. Bell (2010) reports that the feeding of a high protein diet may be a contributing factor to the development of chronic renal disease in captive cheetah. For the recommended protein to fat ratio see the discussion with regards to protein and fat.

2.4.4 Taurine deficiency

Ofri *et al*. (1996) describes feline central retinal degradation (FCRD), a disorder related to dietary taurine deficiency in domestic cats. Bechert *et al*. (2002) reports that taurine can prevent FCRD, myocardial disease and other abnormalities and concludes that taurine is an essential amino acid. FCRD occurs when taurine levels are too low and the structural integrity of the cells of the inner and outer segments of the retinal photoreceptors cannot be kept in place. The photoreceptor function is subsequently lost and most damage is found on the central retina. Ofri *et al*. (1996) reports that the zoo fed a diet of raw beef, which was occasionally substituted with raw poultry, supplemented with a powdered mix of a variety of vitamin and mineral supplements. However, this powder, did not contain any supplementary amino acids. FCRD was diagnosed in captive cheetah at the Jerusalem Zoological Gardens in the study by Ofri *et al*. (1996) after a legion associated with FCRD was revealed during a retina examination. Ofri *et al*. (1996) found that after nutritional supplementation of taurine to four cheetah females the lesions can be prevented. The authors also proved that low levels of dietary taurine cause a fall in retinal taurine levels. Muscle meat contains only small amounts of taurine and not merely the levels required by cheetah (Ofri *et al*. 1996). Bechert *et al.* 2002 found that the highest levels of dietary taurine can be found in turkey and the lowest levels in cattle.

2.4.5 Vitamin and mineral deficiencies and toxicities

Some vitamins need to be activated to be utilised by the body (Bell, 2010). The author further report that cheetah are carnivores and, unlike herbivores or omnivores, do not possess the enzymes or have too low levels of them to convert some of the precursors into active forms, for example vitamin A (a fat soluble vitamin). Adequate levels of fat intake are required to ensure sufficient and effective vitamin A absorption.

Vitamins can be oversupplied and cause toxicities. Hypervitaminosis A is linked to hepatic fibrosis (veno-occlusive disease (VOD)) (Bell, 2010). Hypervitaminosis A is related to a number of VOD cases. Carnivores have a unique method of urinary secretion of excess vitamin A, giving them a relative high level of tolerance to excess levels of vitamin A. (Bell, 2010).

Vitamin B1 (thiamine) is regarded as an important vitamin to supplement in cheetah (Bell, 2010). Severe symptoms of vitamin B1 deficiency in cheetah include lethargy, ventroflexion of the neck, and hind limb ataxia (Bell, 2010).

Unlike other animals, vitamin D_3 (or 25-hydroxy-vitamin D) is not synthesized in the skin of cheetah after exposure to UV-light (Zoran, 2002 & How *et al*., as interpreted by 1994 Bell, 2010). Vitamin D_3 plays an important role in Ca absorption during bone formation and is required as a dietary source (Zoran, 2002 & How *et al*., 1994 as interpreted by Bell, 2010).

When cheetah are fed high-fat diets it has been reported that they suffer from vitamin E deficiencies. This vitamin has antioxidant qualities, and for this reason the partial degradation of animal muscle and fat tissue may cause the requirement for vitamin E increase (Bell, 2010).

Crissey *et al*. (2003) reports vitamins A, D and E, and lipids are needed for normal growth and reproduction in animals. For the preservation of various immune functions of the animal, in addition to the fore mentioned, the animal needs the carotenoids β-carotene and lutein (Crissey *et al*., 2003).

A study by Depauw *et al*. (2011) discusses the impact of dietary factors on the incidence of hepatic, renal and gastrointestinal diseases in captive cheetah. This study by Depauw *et al*. (2011) was conducted to evaluate the difference in nutritional status of animals fed a supplemented beef diet or whole rabbit diet. Even though the beef diet did meet adequate Ca and Cu intake with supplements, the needed Ca:P ratio was not met. The required Ca:P level recommended by Fowler (1986) (as referred to in Bell, 2010) is 1:1 to 2:1. It is said that this Ca:P ratio represents the composition of the body (Fowler, 1986 (as referred to in Bell, 2010)). The use of a single exclusive food source in feeding of cheetah is strongly discouraged until further research data are available (Depauw *et al*., 2011).

2.4.6 Protein and fat

Dietary protein is composed of amino acids and contains nitrogen (Bell, 2010). Protein is required by animals to generate energy, for structural (muscle and organ) and for other functional (hormones and enzymes) purposes (Bell, 2010). The author also reports that carnivores have a high requirement for protein, especially essential amino acids. It is also reported that the enzymes required for the nitrogen metabolism in the liver cannot be up-or down-regulated in relation to dietary protein intake changes transferring breakdown activity of excess amino acids to the kidneys. A recommended level of protein to fat ratio of 3:1 is made (even if most prey species have a protein to fat ratio of 1.5:1). Dietary protein intake influences the incidence of chronic renal disease (Bell, 2010 as adapted from Fowler, 1986). Whole prey may be either an over- or underestimation of fat content of the diet. The variation in the actual content of the consumed diet by either free ranging or captive cheetah feeding on large prey may not be accountable. The consumed feed will vary in protein to fat ratio, depending on the size of prey and the area of prey consumed (Bell, 2010), for example a cheetah in captivity feeding on the rump area vs. one feeding on the stomach area (including omentum and peri-renal fat). An imbalance of protein to fat ratio can be found in captive cheetah fed stock animals, as they tend to have a higher fat content than wild animals (Bell, 2010). As mentioned previously, cheetah need to consume small amounts of fat to enable normal absorption of fat soluble vitamins.

2.5 Psychological welfare

Phillips (1993) conducted a field study on wild cheetah to collect data on the consumption of their prey with special focus on the amount of food consumed, time spent feeding and which, if any, bones were ingested. Hayward *et al*. (2006) reports that the relatively fragile teeth in conjunction with the skull and jaw musculature of the cheetah, restrict them to feeding on flesh. Phillips (1993) showed that some bone and/or cartilage consumption is necessary for the maintenance of good oral health.

Another study was conducted by Bond & Lindburg (1990) to see what the influence of the feeding of a commercial diet and a carcass-fed diet would be on the oral health and psychological welfare of captive cheetah. In the study by Bond & Lindburg (1990) the following findings were made: Carcass-fed cheetah spent more time sniffing their food, fed longer, chewed more and slice their food more using their molars than those

fed the commercial diet. Those on the commercial diet showed lower levels of possessiveness, approached their food promptly and showed decreased interest in feeding. Bond & Lindburg (1990) further reported that the commercial diet's texture did not lend itself to be ripped and did not possess the necessary hassle factor. Carcass fed cheetah used their whole body in the feeding process, changing position, restraining the carcass, shaking their head, while those fed the commercial diet did not restrain the food nor reposition themselves with respect to the feeding pans while feeding. Bond & Lindburg (1990) reports that a more naturalistic diet may result in improved oral health and psychological welfare. This statement is supported by the evidence of increased masticatory exercise, as well as the improved interest in feeding associated with carcass-fed diets. Phillips (1993) showed in a field study, with provision of fresh goat and sheep carcasses, the feeding behaviour of the cheetah in captivity to be similar to that of a wild cheetah feeding on springbok. The author noted that the portions consumed, the order in which they were consumed, and the gnawing and cracking bone during feeding to be similar in the two situations. Phillips (1993) concludes that feeding of sheep and goats appear to be a viable substitute to springbok. Bond & Lindburg (1990) concludes that the feeding of a diet with closer relation to the wild prey of cheetah may take all the nutritional and psychological needs of captive cheetah into account. Additional benefits of such a diet may include taking into account the texture, temperature, palatability and variability that occur in natural prey.

2.5.1 Family structure

Cheetah have almost a unique family or social structure from other wild felids (Caro, 1994 as quoted by Quirke & O'Riordan, 2011a; Macdonald & Loveridge, 2010). Cheetah females are usually solitary except when being part of a litter still running with their mother or having her own litter running with her (Macdonald & Loveridge, 2010; Williams *et al*., 1996). Females do not keep specific territories and tolerate range sharing with other female cheetah. Males can be territorial but can also share their territory with other males and females (being social). It was found that more than half the cheetah males found in the Serengeti form livelong coalitions with two or three other cheetah, usually brothers from the same litter. These coalitions make the males more successful in obtaining food, as well as defending their range. Males usually keep to a smaller range than females. A single female would cover the range of several males or male coalitions annually while the males keep to a small specific territory. This phenomenon is unique to cheetah among felids and other mammals (Macdonald & Loveridge, 2010).

2.6 Stress

Terio *et al*. (2005) reports that by means of adrenal and sex hormone evaluation physiological, reproductive and health status can be determined in captive and wild cheetah. The authors report that both management and physical environment can cause changes in endocrine function. It is further reported that evaluation of these changes in endocrine functions and hormone levels under various circumstances would be an important tool in quantification of the impact that changes have or may have on cheetah wellbeing. The role of different hormones in stress of animals must be investigated, as well as different means of quantification and sampling methods. Hormones and groups of hormones that will be discussed in this review include catecholamines (epinephrine & nor-epinephrine), corticosteroids (cortisol), adrenocorticotropic hormone (ACTH), mineralocorticoids (aldosterone), androgens and dopamine.

2.6.1 Potential stressors of captive cheetah

Homeostasis must be maintained at all times and the body will launch a series of physiological and behavioural changes in an attempt to restore balance upon disturbance (Piñeiro *et al*. 2012). The authors further state that animals launch an adaptive physiological response to a sudden change causing stress in the short term. If the stress occurs in the long term ('chronic stress') immune and reproductive suppression may be among the negative outcomes. According to Touma & Palme (2005) the hypothalamic-pituitary-adrenal (HPA) axis is one of the main components of the stress response. HPA is involved in stress response, as well as other emotional excitement such as found as a part of sexual behaviour (Touma & Palme, 2005).

According to Cuomo-Benzo *et al*. (1997), the placement of animals in an environment other than their natural can induce stress on the animal and it may also remove natural stressors from the animal. The authors report that in the captive environment the pressure of natural selection, natural predators, search for food (hunting) and territorial defence are removed. External stressors include change of social conditions

(Wilebnowski *et al*., 2002; Jurke *et al*., 1997), activity by visitors (Piñeiro *et al*., 2012), nutrition, thermal comfort (Jurke *et al*., 1997). According to Piñeiro *et al*. (2012) stress can suppress reproductivity in both males and females, although affecting the females more than males.

Wielebnowski *et al*. (2002) found that placing females in pairs or groups caused lowered reproduction and hypothesized that it may cause some means of immunosuppression as a result of the stress. The authors further hypothesize that the immunosuppressive report induced by stress will in turn increase the vulnerability of these individuals to disorders. Wielebnowski *et al*. (2002) reports that it should be taken into account when housing cheetah that the natural social conditions of males and females differ and that males form alliances.

2.7 Hormone quantification techniques

Hormone concentrations can be used as indicators of stress and glucocorticoids (one class of hormones) can be measured in urine, faecal samples, saliva or plasma. These methods can be categorized as either invasive or non-invasive, of which faecal samples are non-invasive and the rest (especially blood collection) can be classified as invasive (Piñeiro *et al*., 2012). The authors describe non-invasive techniques as techniques which avoid additional stress on the animals in the form of handling, sedation, capturing, or transport (e.g. faecal samples). The adrenal gland secretion of glucocorticoids exhibits a pulsatile secretory pattern and the use of blood samples (plasma) to measure glucocorticoids would only represent the concentration of the hormones in the blood at that specific moment in time (Valk, 2012). It is further reported by Valk (2012) that a sequence of blood samples over time will, however, produce a reliable representation of chronic changes in the hormones after the stressor was introduced.

From literature it seems that the most popular technique of monitoring changes in hormonal output are to analyse faecal corticoids (Valk, 2012, Piñeiro *et al*., 2012; Terio *et al*., 2005; Touma & Palme, 2005; Wielebnowski *et al*., 2002). It is reported by Wielebnowski *et al*. (2002) that monitoring of faecal corticoids have shown to be useful for assessing adrenal activity in animals in captivity. The use of faecal hormone metabolite analysis is described as being beneficial. The gut microbes cannot break down the sterane skeletal structure whereas the steroid hormones are broken down more extensively on the route to the urine via the kidneys and the liver (Touma & Palme, 2005). The authors further report that faecal samples indicate concentration of circulating hormone over a set period of time and, unlike blood samples, are not sensitive to sporadic changes of pulsation of hormone secretion. Unlike blood samples a downside to faecal samples is that they record hormonal changes over long periods of time that can have a dampening effect on short spurts of hormonal release, which may be vital to make conclusions in certain studies (Touma & Palme, 2005). Another advantage of faecal measurements of glucocorticoids according to Touma & Palme (2005) is that the levels of glucocorticoid metabolites measured in the faecal samples might be a reflection of the biological active fraction of glucocorticoids circulating in the bloodstream as only the unbound fraction is readily metabolized by the liver to bile (excreted in faeces). Touma & Palme (2005) conclude that it is important to validate glucocorticoid metabolite measurements physiologically and biologically for each individual species in order to be able to make conclusions from data collected. It is also important according to Touma & Palme (2005) and Wielebnowski *et al*. (2002) to lay out sampling, storage and analytical materials and methods precisely before onset of the experiment.

A challenge may be to collect faecal samples and correctly identify each sample, especially in pair and group housed animals. This problem can be resolved according to Wielebnowski *et al*. (2002) by means of adding uncooked rice (1tablespoon), green food colouring (~1-2 ml) or uncooked corn (2 tablespoons) to the diet fed to each individual. In a study by Terio *et al*. (2005) where different drying and storage methods of faecal samples were investigated and it was found that steroid hormone levels are not significantly altered by preservation in ethanol and storage at ambient temperature for more than 14 days prior to extraction of the steroid hormones. The authors also found that drying samples by means of a solar or conventional oven resulted in steroid hormone concentration variation with the exception of androgens. In a study done by Valk (2012) the mean basal concentration of glucocorticoids metabolite was established for each individual before any experimental data can be interpreted. Wielebnowski *et al*. (2002) found that no specific conclusion can be made with regards to stress in cheetah as individual stress levels are highly variable. Baseline faecal corticoids for captive cheetah ranged from 196.08± 36.20ng/g dry faeces and for wild cheetah ranged from 71.40±14.35ng/g dry faeces in a study by Terio *et al*. (2005). Wielebnowski *et al*. (2002) found mean baseline concentration for faecal corticoids to range from 28.9±1.74 to 59.2±1.8ng/g, which is much different from

those found by Terio *et al*. (2005). These baseline faecal corticoid levels can be used in future studies where corticoid levels are used as measures of chronic stress in captive and wild cheetah.

2.8 Faecal analyses

Faecal analyses can yield a lot of information. It is a non-invasive means of collecting data on animals. Collection of faecal samples opens up a means of preventing additional stress on the animals in the form of handling, sedation, capturing, or transport during sample collection (Piñeiro *et al*., 2012).

2.8.1 Putrefaction of faeces

Animals are housed together in small enclosures in high numbers and in close proximity to visitors. With the increase in putrefaction of faeces, the air quality declines. With higher degrees of protein fermentation in the large bowel, the production of putrefactive compounds increases and the risk of lowered gastrointestinal health and disease increases (Vester Boler *et al*., 2009; Vester *et al*., 2010). The faecal ammonia levels in cheetah are much higher than other species of felids (Vester Boler *et al*., 2009).

2.8.2 Faecal corticoids

In the section above on the hormone quantification techniques (2.7) it is described in detail how faecal samples can be used in the evaluation and monitoring of hormones in animals, especially captive cheetah.

2.9 Physical exercise

The influence of physical exercise was explored during this trial. A lure system, developed and built by staff at HESC, was used to pull a luring agent (mop, piece of meat, plastic pom-pom, etc.) to induce physical exercise. A constant speed was used over a distance of 80m. The induced exercise was done in conjunction with the normal feeding as a treatment to see what the influence of the lure based feeding method can have on specific physiological aspects of cheetah in captivity. Creating a feeding method to simulate the natural hunting process of cheetah, can act as a means of environmental enrichment of captive cheetah. This enrichment may not only have an influence on their physical health but also their psychological wellbeing.

2.9.1 Fitness

The high speed chase frequently leads to fatigue and an extended recovery period (Schaller, 1972; Eaton, 1974 as quoted by Williams *et al*., 1997). Cheetah naturally roam over vast areas of up to 1651.1km^2 (Marker *et al*., 2008) and may even cover a distance of 13.8km to 26.0km per day (Marker *et al*., 1996). In captivity this type of exercise is not possible. Can exercise and fitness level of cheetah have an influence on their physiological and psychological welfare?

2.9.2 Means of measuring fitness

In a study on horse fitness by Voss *et al*. (2002), two separate methods were used to determine the metabolic consequences of aqua-treadmill exercise. The first was metabolic parameters such as lactate and haemoglobin levels in the blood. These values are described as reliable markers for exercise induced changes to be detected. The second is the measurement of heart rate variability (HRV). HRV can be used to characterize changes in the central control mechanisms during exercise.

Voss *et al*., (2002) further explain that the presence of lactic acid in blood (lactacidaemia) may indicate that the specific individual was not able to compensate for the increased oxygen demand on a muscular level during exercise. The lower the value of lactic acid in the blood, it is presumed, that the individual had high levels of aerobic respiration on a muscular level.

Both histological and biochemical differences of the muscles need to be considered when evaluating the fitness of an animal on muscular level. Skeletal muscles consist of two major types of muscle fibres. The two types are fast twitch or white muscle fibres and slow twitch or red muscle fibres (Campbell *et al*., 2008, Cunningham, 2002, Frandson *et al*., 2006 & Junqueira & Carneiro, 1983). Muscles needs energy in the form of adenosine triphosphate (ATP) to contract (Campbell *et al*., 2008).

Red muscle fibres are higher in myoglobin content, have a rich blood supply and have many mitochondria. Mitochondria supplies the much needed ATP to the muscle fibres, where the higher blood supply

and myoglobin content supplies high levels of oxygen. Aerobic respiration is mainly used to perform steady contractions over longer periods in red muscle fibres. These fibres also tend to be darker, because of the higher myoglobin content and blood supply. Another name used for the red muscle fibres are slow twitch muscle fibres. This is based on the slower rate of contraction which these muscles exhibit. The slow twitch is also a more durable means of contraction and allows these muscles to contract for longer periods (Campbell *et al*., 2008; Cunningham, 2002; Frandson *et al*., 2006). An example of red muscle fibres are the muscles in the human back, which keeps the back straight and in good posture (Junqueira & Carneiro, 1983).

White muscle fibres can either be glycolytic or oxidative (Campbell *et al*., 2008). White muscle fibres rely on anaerobic glycolysis to generate ATP for contraction, when the stored ATP supplied by the mitochondria are depleted. The amount of blood supply, mitochondria and myoglobin in and around the red muscle fibre sarcoplasmic reticulum is less than at the red muscle fibres. This gives the white muscle fibre a 'lighter' appearance than the red muscle fibres (Campbell *et al*., 2008). White muscle fibres tend to fatigue quicker than red muscle fibres. The white muscle fibres have short contraction times and is sometimes referred to as fast twitch muscle fibres (Campbell *et al*., 2008; Cunningham, 2002; Frandson *et al*., 2006 & Junqueira & Carneiro, 1983). These muscle fibres are well adapted for brief, powerful actions/movements such as jumping or sprinting (Cunningham, 2002).

A study by Williams *et al*. (1997) showed that cheetah have more adaptations for anaerobic respiration on muscular level than other mammalian sprinters. This was based on their performance. This adaptation in cheetah was in the higher anaerobic enzyme capacities of the locomotor muscles, rather than the fibre composition of the muscles.

The authors also established that a higher level of haemoglobin was measured in the blood as a result of more red blood cells being mobilized from the splenic reservoir during hard work or high levels of physical exercise. The higher level haemoglobin results in larger volumes of oxygen reaching the working muscles, allowing muscles to perform at higher aerobic levels and less lactic acid to be produced.

In this trial it was not possible to measure heart rate before, during or after the lure run because it would have been necessary to sedate the cheetah and sedation can have influences on heart rate. It was further not practical to do sedation in relation to this study as it is time consuming. During this trial, however, the lactate levels was measured in both blood serum and plasma one hour post-prandial (after the run and feeding). No measure of haemoglobin (Hb) was possible. Samples should be collected in EDTA tubes for Hb determination. The maximum time from collection to analysis cannot be more than 24 hours and it was impossible to collect samples at HESC and have it at the laboratories for analysis within this timeframe.

2.10 Environmental enrichment

It is vital to provide enrichment in the environment of captive animals (Depauw *et al*., 2012). Captive cheetah that lack proper environmental enrichment, typically express stereotypical pacing. These animals may even go to the exact same area at the usual time of feeding to wait for food to be delivered (Quirke & O'Riordan, 2011a). Institutions are increasingly looking into ways of enriching the environment for animals in captivity (Depauw *et al*., 2011). The aim of environmental enrichment for captive carnivores is to increase the exploratory behaviour and reduce pacing (Quirke & O'Riordan, 2011a; Quirke & O'Riordan, 2011b). The enrichment must induce physical, physiological and psychological stimulation (Williams *et al*., 1996). Even applying the same kind of environmental enrichment at set times may induce the forming of habits (Quirke & O'Riordan, 2011b). The authors further report that if animals explore more, they exhibit more natural behaviour and they are more active. Bait movement systems to increase hunting behaviour were used in an early study (Williams *et al*., 1996). Studies have been done in many other species such as a study (Quirke & O'Riordan, 2011a) where feed was delivered at different times and different locations with red fox (*Vulpes vulpes*). All animals respond differently to different enrichment techniques. It is therefore important to investigate possible enrichment techniques that will have maximum effect on cheetah in captivity (Quirke & O'Riordan, 2011a). A reduction in pacing was seen with environmental enrichment by means of olfactory stimulation, spatial and timing variation of food delivery (Quirke & O'Riordan, 2011a; Quirke & O'Riordan, 2011b). Enrichment can be done with either food items or non-food items (Quirke & O'Riordan, 2011a; b). A range of olfactory enrichments can be used, including perfumes (Quirke & O'Riordan, 2011b). In house experiments may be the best means of establishing what kind of enrichment programme will work the best for the animals from a specific facility (Quirke & O'Riordan, 2011b). It is also essential that the enrichment

technique should not take up a lot of time or be expensive to implement and maintain (Williams *et al*., 1996). One such in house study was conducted with a gravity driven pulley system to test the effect of moving bait on the behaviour of cheetah in captivity (Williams *et al*., 1996).

In our study the lure was investigated as a potential means of environmental enrichment.

2.11 Summary

Contrary to common believe, captive cheetah are not better off than their wild counterparts. The numerous detrimental consequences on the health of captive cheetah are rooted in a number of nutritional shortcomings. Many studies showed that nutritional management of tried feeding practices can be implemented to meet the physical, physiological and psychological needs of captive cheetah. Studies also emphasised the fact that feeding beef, chicken and other commonly fed diets will not meet all the requirements of captive cheetah. Therefore, the type and chemical composition of the wild-type diet needs to be considered when planning a diet for captive cheetah. Changing the management practices in facilities with captive cheetah, especially with regard to nutrition and feeding, may alleviate or even eradicate many of these disorders. This review emphasized the absence of proper guidelines for nutritional requirements of cheetah and further research is warranted. Further there are also uncertainties of the true causative factors of many of the disorders discussed in this review, and further research will be required.

CHAPTER 3: MATERIALS AND METHODS

3. Introduction

The experimental design and research methodology used during this experiment are discussed in this chapter.

3.1 Experimental design

The experimental design of a controlled experiment is the sum total of all the steps to be taken during the investigation of a specific research question. During the design of such an experiment all variables need to be considered. The experimental design should include all vital variables to the aim in order to produce results that can be compared to the original hypothesis.

The research design used in this experiment included both between subjects and within subject comparisons, which are also referred to as mixed within- and between-subjects experimental designs (Seltman, 2014). The present study was based on a completely randomized design.

3.1.1 Experimental layout

This study was conducted to investigate the aim as indicated in 1.2; Will captive cheetah show change in overall physiological status with induced exercise added to the feeding method by means of a mixed within- and between-subjects design? This experiment qualifies as a mixed within- and between-subjects design because the subjects are assigned to different treatments and are evaluated against themselves over time. The experimental design, and layout of this study is given in Table 3.1. The animals of the two groups where chosen from the same groups of parents (parent effect) and thus the same genetic variation exist within and between the two treatment groups.

Table 3.1 Experimental design and layout

Duration of Study	Seven weeks		Seven weeks	
Experimental Design	Within- and between-subjects		Within- and between-subjects	
Subjects	Cheetah		Cheetah	
Treatment groups	Control group		Experimental/test group	
Treatment conditions	Feeding method		Feeding method	
Treatment levels	0 m		ca. 80 m	
Number of subjects per group	Five		Five	
Independent variable	No exercise		Exercise by means of lure	
Dependent variables	*Blood* Serum Albumin Serum Urea Serum Calcium Serum Creatinine Serum Lactate Serum Phosphorous Serum Total Protein Serum Globulin Serum Pack Cell Volume Calcium to Phosphorous Ratio Plasma Lactate Serum Cortisol Serum Free T_4 Serum Total Cholesterol Serum Triglycerides Serum HDL Serum LDL Serum Free T_3 Serum Vitamin D_3	*General Health Assessment* Response to lure Gate Lameness Appetite Vomit Coat Quality Coat Condition Eye Colour Eye Brightness Faecal scoring	*Blood* Serum Albumin Serum Urea Serum Calcium Serum Creatinine Serum Lactate Serum Phosphorous Serum Total Protein Serum Globulin Serum Pack Cell Volume Calcium to Phosphorous Ratio Plasma Lactate Serum Cortisol Serum Free T_4 Serum Total Cholesterol Serum Triglycerides Serum HDL Serum LDL Serum Free T_3 Serum Vitamin D_3	*General Health Assessment* Response to lure Gate Lameness Appetite Vomit Coat Quality Coat Condition Eye Colour Eye Brightness Faecal scoring

3.1.2 Within-subjects design and between-subjects design (Completely randomised design)

In the between-subject design each of the cheetah in the experiment will be randomly assigned to a single treatment. The benefit of using the between-subject design is that it is reasonably simple to design such an experiment. The relative freedom from limiting statistical assumptions can be seen as a further benefit of using this type of design. Unfortunately, the between-subject design requires a large population of subjects, which in experiments on an endangered species such as cheetah in this case, can be limiting. It is also more expensive when more subjects need to be included for statistical significance.

The experiment also has a within-subject design aspect. This can be justified because the differences of subjects are measured against data collected on that same subject over time. A benefit of the differences over time within one animal is that it can be measured and a comparison can be made to the same animal at a different time. According to Keppel (1991) as reported by Grobler (2011) the advantage of this type of design is that less subjects are required in the experiment. Grobler (2011) further reports that subjects can, however, change over time and to reduce this variability all other potential independent variables should be controlled throughout the entire experiment.

3.1.3 Subjects used in treatment groups

According to the Oxford advanced Learner's dictionary a subject of an experiment is the person or thing being treated in a certain way. Kuehl (2000) reports that an experimental unit is the subject or physical unit subjected to treatment independent of other entities. By further adapting these definitions to the language of experimental design and statistics, one can say that a subject is the person, physical entity or organism subjected to a specific treatment and the conditions pertaining to that treatment.

Two groups of captive cheetah (five animals per group) were selected from the cheetah housed at Hoedspruit Endangered Species Centre (HESC), Kapama Reserve, Hoedspruit, South Africa (24°31'42.2"S 31°02'00.4"E). A completely randomized design was used to allocate the cheetah to either the sedentary group (control) or physical exercise group (test). The animals in the two treatments of this trial were also chosen from the same pool of parents, reducing the genetic variation between the two groups of animals.

The two groups of animals remained in the same treatments throughout the entire trial (seven weeks). The subjects of the control and test group consisted out of four juvenile cheetah aged between 0 to 18 months and eight sub-adults to adult cheetah aged between one year nine months and three years seven months. These groups included both males and females.

The cheetah were randomly assigned to either the control- or test treatment groups. Appendix C contains a table with the details of the animals used in the trial.

3.1.4 Treatment conditions and -levels

Treatment conditions, included the specific circumstances that the experimental subjects were exposed to for the duration of the treatment. In this trial the cheetah (subjects) were all exposed to different treatment conditions (physical exercise levels). The two treatments were applied as part of the normal daily feeding routine.

The cheetah in the test group only received their food after physical exercise, in the form of running with a lure (HESC) inside their enclosure over a distance of ±80m. The cheetah in the control group received their feed without any induced physical exercise.

3.1.5 Independent variable

In the present study the independent variable was induced physical exercise, by means of a lure, with the feeding method of the cheetah (subjects) at two levels. The different levels of the independent variable created the different treatment levels.

3.1.6 Dependent variable

After each feeding all the cheetah were trapped and blood collections were done. During the feeding and blood collection different general health aspects were observed and scored and different blood parameters were tested on the blood samples taken on test days one hour post-prandial. The observed general health aspects included; response to lure, gait, lameness, appetite, vomit, coat quality, coat condition, eye colour, and eye brightness. Blood parameters tested included albumin, urea, Ca, creatinine, lactate, P, total protein, globulin, pack cell volume, Ca:P ratio, Free T_4, total cholesterol, triglycerides, HDL, LDL, Free T_3, Vitamin D_3 in blood serum, and blood plasma lactate. These above listed general health aspects and blood parameters were the dependent variables during this trial for each cheetah.

3.1.7 Treatment effect

The treatment effect included the effect of the two different levels of induced physical exercised on the independent variables as listed in 3.1.5. The treatment effects are discussed in detail in Chapter 5, conclusion.

3.1.8 Feed preparation

All cheetah were fed an individual meat chunk of approximately 10% of the body weight of the cheetah. This varied between 3kg and 5kg for an adult cheetah on each feeding day (feeding days were only every second day). Chunk size may vary because of the inclusion of bone. At the onsite butchery on feeding day, a few cuts were made into the chunk of meat to supplement it with a cheetah CVM-supplement developed for the cheetah at HESC. The preparation was done by volunteer students supervised by HESC staff. The preparation procedures was followed as stipulated by the HESC regulations. Each animal's meat was

supplemented with a pre-weighed bag of 60g CVM-supplement for each 2kg of meat (bone included). The mineral composition of the supplement is given in Table 3.2. Meat chunks were prepared fresh, the morning of each feeding, for all animals at HESC. Each animal received a random piece of meat from the feeding crate as selected by a HESC staff member during the routine morning feeding according to body size and physiological status.

Table 3.2 CVM-supplement mineral composition

Ingredient	± Mineral contribution (m/m)
Calcium carbonate	36.00% Ca
Magnesium oxide	51.00% Mg
Salt (NaCl)	39.30% Na
Copper glycinate	24.00% Cu

Meat comes from healthy livestock slaughtered at abattoirs. Spoiled meat (either due to bacteria or freezer burn) is destroyed or fed to vultures at the vulture restaurant. HESC staff previously believed that excessive subcutaneous fat should be removed from the surface of the meat to reduce the incidence of vomiting due to nausea. More recently the practice has been stopped at HESC in order to improve the fat to protein ratio in the meat as fed. Fat is a very important nutrient that forms part of the energy supply to the cheetah. Feeding of experimental animals occurred as illustrated in Table 3.3.

Table 3.3 Feeding and sampling roster used during the experimental period

	Week 1		**Week 2**	**Week 3**		**Week 4**	**Week 5**		**Week 6**	**Week 7**	
	Feeding	Samples taken	Feeding only	Feeding	Samples taken	Feeding only	Feeding	Samples taken	Feeding only	Feeding	Samples taken
Monday	1, 3, 6, 8	1, 6	1, 3, 6, 8	1, 3, 6, 8	1, 6	1, 3, 6, 8	1, 3, 6, 8	1, 6	1, 3, 6, 8	1, 3, 6, 8	1, 6
Tuesday	2, 4, 5, 7, 9, 10	2, 7	2, 4, 5, 7, 9, 10	2, 4, 5, 7, 9, 10	2, 7	2, 4, 5, 7, 9, 10	2, 4, 5, 7, 9, 10	2, 7	2, 4, 5, 7, 9, 10	2, 4, 5, 7, 9, 10	2, 7
Wednesday	1, 3, 6, 8	3, 8	1, 3, 6, 8	1, 3, 6, 8	3, 8	1, 3, 6, 8	1, 3, 6, 8	3, 8	1, 3, 6, 8	1, 3, 6, 8	3, 8
Thursday	2, 4, 5, 7, 9, 10	4, 9	2, 4, 5, 7, 9, 10	2, 4, 5, 7, 9, 10	4, 9	2, 4, 5, 7, 9, 10	2, 4, 5, 7, 9, 10	4, 9	2, 4, 5, 7, 9, 10	2, 4, 5, 7, 9, 10	4, 9
Friday	1, 3, 6, 8	None	1, 3, 6, 8	1, 3, 6, 8	None	1, 3, 6, 8	1, 3, 6, 8	None	1, 3, 6, 8	1, 3, 6, 8	None
Saturday	2, 4, 5, 7, 9, 10	5, 10	2, 4, 5, 7, 9, 10	2, 4, 5, 7, 9, 10	5, 10	2, 4, 5, 7, 9, 10	2, 4, 5, 7, 9, 10	5, 10	2, 4, 5, 7, 9, 10	2, 4, 5, 7, 9, 10	5, 10

3.1.9 Available resources

All cheetah were selected from the cheetah housed at Hoedspruit Endangered Species Centre (HESC), Kapama Reserve, Hoedspruit, South Africa (24°20'35.974" S, 30°56'58.261" E). An aerial view of HESC is supplied in Appendix A. This photo was amended from figures provided by HESC onto a screenshot from Google Maps. Cheetah were housed for the duration of the experimental period in the same housing and groupings (specific individuals per enclosure), depending on age and sex, as at the onset of the experimental period. Enclosures consist of uncovered camps with vegetation (including trees, shrubs and grass), size depending on the number of animals housed per camp. It also includes a feeding area, which is cleared of any vegetation.

The entrance for cheetah to the feeding camp, from the main enclosure, is a trapping cage. Cheetah at HESC are regularly trapped using these trapping doors for procedures such as sedation/immobilisation or blood collection. During this trial cheetah were trapped and restrained, with aid of wooden poles, for the purpose of blood collection and physical health evaluation. A diagram (not to scale) showing the design of a typical enclosure for cheetah housed at HESC is given in Figure 3.1.

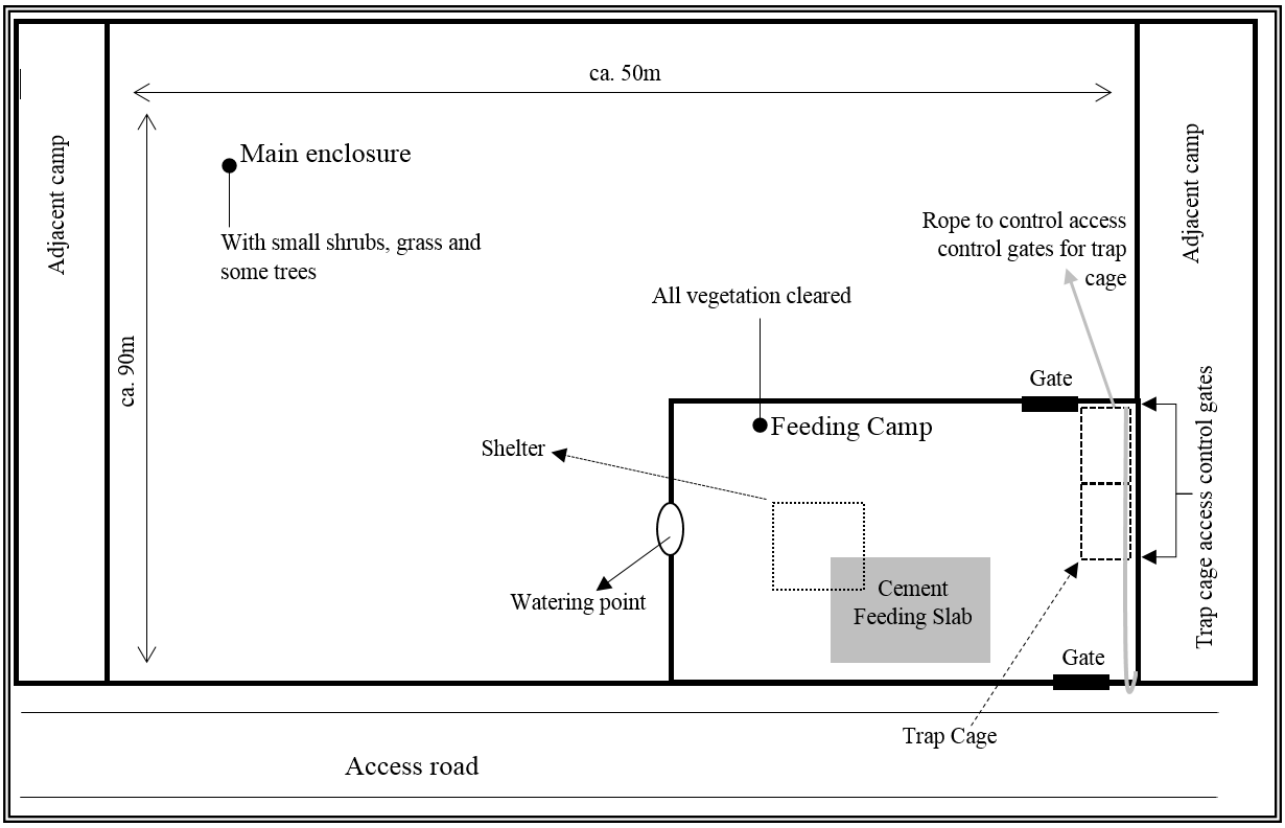


Figure 3.1 A diagram representing the design of the enclosures (not to scale).

3.2 Physical health evaluation

During every feeding each of the experimental animals were visually evaluated on a few health aspects that can be evaluated by observation. A form was completed for each animal for the following aspects; response to lure, gait, lameness, appetite, presence/absence of vomit, coat quality, coat condition, eye colour, eye brightness and faecal score. The scoring system worked in such a way that the lowest value was awarded to the preferred health indicator. An example of this form can be seen in the Appendix B. An overall health value of nine was the perfect score for an animal where no faecal sample was available for scoring. Animals with an overall health value of ten to 13 were animals with either a faecal sample available or animals that fell in the further monitoring category. Animals in the further monitoring category were monitored at least three times per day. In the case of diarrhoea or vomiting being present, a veterinarian was consulted to make a recommendation about the animal's health and nutrition during the study.

3.2.1 Response to lure

The response to lure was done on a three scale evaluation. A normal response (scored as one) was noted when the animal followed the lure with enthusiasm. A lowered interest but still following the lure to some extent (even for a part of the trial) received a score of two. A score of three was awarded to the event of no interest in the lure.

3.2.2 Gait

Gait was measured on a two-point scale. A value of one was awarded to a run with a co-ordinated gait. If the gait was not co-ordinated a value of two was awarded.

3.2.3 Lameness

Lameness can have many causes. The level of lameness was evaluated to ensure animals were not uncomfortable when using the lure during the trial. Lameness was evaluated while animals were roaming their enclosures prior to the application of any treatment. No lameness was preferred and awarded a value of one. Moderate lameness (two) was awarded if the animal showed some stiffness in one or two of the four limbs. If the animal was not bearing weight on one of the limbs, it would be awarded a value of three. The animal will rest until an evaluation on the next running/testing day showed that the lameness was at a level one.

3.2.4 Appetite

The appetite of a cheetah can be an indicator of the general wellbeing of the animal. Animals will, however, not consume meat if it is not fresh enough. When animals showed lowered feed intake it would be noted, and with the next feeding the intake will be monitored very carefully. Close monitoring of individual intake levels is especially important in enclosures where more than one cheetah are housed. The reason for lowered intake should be investigated. A good appetite was awarded a value of one. If the intake was moderate but not poor a value of two was awarded. In cases where animals did nibble on the meat (poor) but most are left over a value of three was awarded. No feed intake was awarded a value of four.

3.2.5 Vomit

Presence or absence of vomit can be considered as an indicator of the overall health of the animal's gut and it also relates to appetite. The feeding of cheetah in the feeding enclosure allowed us to see clearly if vomit was present or not after each feeding. All animals were kept in these feeding enclosures for at least one hour post-prandial. Absence of vomit is preferred and a value of one was awarded. If vomit was present a value of two was awarded.

3.2.6 Coat quality and condition evaluation

Coat quality and the condition of the coat (how shiny) were evaluated on a two scale system. It is a very subjective evaluation and for consistency the same person did all the measures for the duration of the trial period. The two categories for measurement were either shiny or dull. As shiny is the preferred condition, it was awarded a value of one and dull a value of two. The coat can also be evaluated for being groomed or not groomed. The coats of animals that are in good health are in a well-groomed and neat condition. We awarded groomed a value of one not groomed a value of two. Rain did, however, influence the accuracy of this measure. It caused hair to look not groomed and mud also made the coat look dirty and dull.

3.2.7 Eye colour and brightness evaluation

The initial observation of the eye colour differences between healthy and ill animals was made at HESC by a previous resident veterinarian, Dr. Nina Kisch, and her supporting staff. Their observations are shown in Figure 3.2. During the experimental period of this study, this observation was used to develop some added criteria for evaluating the physical health status of the animals. Eye colour of animals was rated for both brightness and colour. There were two categories for each evaluation. A lower value was given to the preferred condition. For brightness 1: Bright ("healthy") or 2: dull ("sick"). For iris colour 1: lighter orange ("healthy") and 2: dark orange ("sick"). The use of eye colour evaluation can be subjective and was done by the same individual for the duration of the experimental period.

Figure 3.2 Cheetah eye colour, specifically referring to the iris, can be seen here. On the left the eye colour of a "healthy cheetah" can be seen, which appears to be a yellow to light orange colour. On the right the eye colour of a "sick" cheetah can be seen, which appears to be a deep orange in relation to the "healthy" cheetah. (These photos were taken at HESC).

3.2.8 Faecal scoring

A score was given to the faeces whenever it was possible to detect faecal droppings. In enclosures with more than one individual, rice was added to the feed as an indigestible agent to act as a means of identifying their faeces. A five level scale was used to award a faecal score. Level two was the preferred score. Scores were awarded as follow; 1: hard, dry pellets, 2: dry well-formed stool, 3: soft, moist formed stool, 4: soft unformed stool and 5: Watery, liquid that can be poured.

3.3.9 Weighing of animals

All cheetah in the experiment were weighed during the experiment by means of a standard Obaru livestock scale. The scale was set up in the feeding camp of the trial animals on the feeding days. Feed was used to lure animals onto the scale. A reading of the weight was taken as the monitor displaying the weight reading reached a stable reading. HESC make use of an Obaru livestock scale to weigh immobilised cheetah. This method of weighing mobile cheetah was first used during this experiment at HESC.

3.4 Feed sample collection and storage

A representative sample of ca 250g supplemented feed was taken from the feed presented to the animals on each feeding day. Samples were placed in marked plastic bags, vacuum sealed and kept at ca -4°C until preparation for analyses. Feed analyses were included to monitor variation in feed composition over the duration of the study.

3.5.1 Analyses of feed samples

Frozen meat samples were booked in at UP Nutrilab, Room 10-12, Agricultural Sciences Building, University of Pretoria, 2 Lynnwood Road, Pretoria, South Africa (25°45'23.2" S, 28°14'4.7" E).

3.5.1.1 Sample preparation

In preparation for proximate analyses meat samples were thawed and chopped using a food processor (if samples were too small they were diced with a scalpel by hand into roughly ¼cm cubes). During the mincing process the samples were pooled per week. Pooled samples were freeze dried in a custom made freeze drier, after which they were grinded in a food processor. Ground samples were subsequently analysed in duplicate.

3.5.1.2 Initial dry matter

Initial dry matter (DM) was done in duplicate for all the pooled meat samples. Approximately 3g representative sample was weighed out in crucibles from the pooled meat samples, before freeze dried. It is important to do the initial DM before the samples are placed in the freeze drier for the initial moisture content to be determined. Samples were placed in an oven at 105°C for 24h. All samples were then cooled in

desiccators and weighed back. The whole DM process was according to the official method of analysis 934.01 (AOAC, 2000). Equation 3.1 was used to determine the DM% and Equation 3.2 was used to convert DM% to DM on an "as is" basis.

Equation 3.1 Calculation of DM%.

$$\%DM = 100 - \left(\frac{weight\ loss\ during\ drying}{initial\ sample\ weight} \times 100\right)$$

Equation 3.2 Calculation of DM (g/kg) "as is".

$$DM\ \left({}^{g}/_{kg}\right)"as\ is" = 1000 - (\%DM \times 10)$$

3.5.1.3 Second dry matter and ash

A second dry matter determination was done after the freeze dried samples were milled. Approximately 2g representative sample were weighed out in duplicate into ceramic crucibles. To determine the ash content, the same samples were placed into an ASH-oven at 250°C for two hours. The temperature of the ASH-oven was then increased to 600°C for a further four hours. The oven was turned off and crucibles were left to cool down overnight. The next day samples were cooled further in desiccators and crucibles were weighted back. The second DM was done according to the official method of analysis 934.01 (AOAC, 2000) and the dry matter ash were done according to the official method of analysis 942.05 (AOAC, 2000).

3.5.1.4 Crude Protein (CP)

Freeze dried meat samples were analysed for CP using the Leco crude protein analyses with a Leco TruMac® N analyser. 0.50g samples were weighed into boat crucibles and loaded into the analyser. All samples were analysed for their nitrogen (N) content. The N content of a sample can be multiplied with the Kjeldahl factor of 6.25 (Equation 3.3 & Equation 3.4) to calculate the approximate CP content of that sample on DM basis. This analyses were done according to the official method of analyses 968.06 (AOAC, 2000).

Equation 3.3 Calculation of CP%.

$$CP\ \% = N\% \times 6.25$$

Equation 3.4 Calculation of final CP (g/kg) DM.

$$CP\ \left({}^{g}/_{kg}\right) DM = \frac{CP\%}{Second\ DM} \times 1000$$

3.5.1.5 Ether extract (EE)

EE analyses were done according to the official method of analyses 920.39 (AOAC, 2000). Approximately 2.00g of every milled freeze dried sample was weighed out in duplicate on a piece of filter paper. Each filter paper was folded six times and placed in its own extraction thimble. The thimbles were loaded in a Soxtec System HT with a 1043 Extraction unit and 1046 Service Unit and a Foxx Soxtec™ with a 2043 Extraction Unit and a 2046 Control Unit, with the aid of a thimble support.

Units were switched on. Water taps were opened for the cooling of the extraction unit during extraction. The fume hoods were then switched on. Thimbles were pulled into the tubes by moving the levers from 'boiling' to 'rinsing'. The heating lever was then moved to the on position. Extraction cups were then weighed in using thongs (immediately after being dried in an oven overnight at 60°C, followed by cooling in a desiccator for a minimum of ten minutes).

The extraction cups were then filled to ca. ¾ full with Petroleum Ether (60-80°C) in the fume hood, wearing gloves for protection. Either a cup holder or a thong was then used to handle cups. Cups were placed on the heating pads using a cup holder. Cups were then fixed into position by depressing the fastening mechanism lever (on the left hand side of extraction unit) until it locks. The levers were then moved to the 'boiling' position. The timer was set for one hour. After the one hour expired, the levers were moved to the 'rinsing' position and the timer was set for a further 30 minutes. At the end of the rinsing period, the recovery knobs were turned clockwise (not over tightened) to close the extraction unit. Ether were collected for 20 minutes before the 'heating' lever was switched off. The extraction cups were then removed using the cup

holder and placed in an oven at 60°C for 30 minutes, or until all excess ether evaporated. The thimbles were removed from the extraction unit and the samples with their filtration paper were disposed of. New extraction cups were placed in the extraction unit, using a cup holder. The recovery knob was turned anti-clockwise (until open) and ether were collected. After the collection were completed, ether was poured into a waste 1x used ether bottle. This ether can be used to clean the extraction cups later on. After all excess ether evaporated, the extraction cups were removed from the oven and placed in desiccator until cooled down completely. Extraction cups were then weighed back. The EE% was determined using Equation 3.5. Equation 3.6 were used to calculate the final EE (g/kg) DM content of the sample.

Equation 3.5 Calculation of EE%.

$$EE\% = \frac{(Cup + Ether\ extract) - Cup\ mass\ only}{Sample\ mass} \times 100$$

Equation 3.6 Calculation of final EE (g/kg) DM.

$$EE\ \left(g/kg\right) DM = \frac{EE\%}{Second\ DM} \times 1000$$

3.5.1.6 Mineral analyses

Acid digestion as sample preparation for calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), potassium (K), sodium (Na), iron (Fe), phosphorous (P) and zinc (Zn) determination.

An acid digestion should be done for all samples in duplicate, prior to mineral content readings being made, in order to get minerals into a solution. A representative 0.5000g of each sample was weighed off in duplicate into 250ml digestion tubes. Samples were digested in a heating block set at 240°C. During the digestion process nitric acid (HNO_3) and perchloric acid ($HClO_4$) were added at timed intervals. After digestion samples were made up to 50ml and transferred to 50ml medicine bottles for storage and mineral readings. The acid digestion was based on the official method of analysis 935.13 (AOAC, 2000).

Ca content

Samples were diluted with 1% Lanthanum-III-chloride ($LaCl_3$) solution to a concentration within the standard range (depending on the type of sample). These dilutions were done for each of the 50ml duplicate samples in the medicine bottles (as a product of acid digestion). A 100ppm Ca solution were prepared from the certified 1 000ppm Ca solution (Merck CertiPUR Calcium ICP Standard) using 0.1% HNO_3. From the 100ppm Ca solution standards of 1.00ppm, 3.00ppm and 5.00ppm were made, using $LaCl_3$. The PerkinElmer 5100 PC Atomic Absorption (AA) Spectrophotometer were switched on and allowed to warm up. The AA was then blanked and calibrated using a blank and the standard Ca solutions. The Ca content of the samples were determined with a method based on the standard method described by Giron (1973) at a wavelength of 422.7nm. The Ca percentage in the sample was determined by using Equation 3.7. Equation 3.8 was used to calculate the Ca (g/100g) content of feed samples on DM basis.

Equation 3.7 Calculation of Ca% of a sample.

$$Ca\% = \frac{\left(\frac{Reading\ value \times End\ volume\ *}{Mass\ of\ dry\ sample\ for\ acid\ digestion}\right)}{10\ 000}$$

$* End\ volume = End\ volume\ at\ acid\ digestion \times Dilution$

Equation 3.8 Calculation of Ca (g/kg) DM.

$$Ca\ \left(g/kg\right) DM = \frac{Ca\ \%}{Second\ DM} \times 1000$$

Mg, Cu, Fe, Mn, K and Na content

A Varian AA 50 was used for mineral analyses of Mg, Cu, Fe, Mn, K and Na by the standard methods described by Giron (1973). Equation 3.9 was used to calculate the Mg, K and Na% of each analysed sample. The final content of Mg, K and Na in the feed sample (g/kg) on DM basis were calculated using Equation 3.10. Equation 3.11 was used to calculate the Cu, Fe and Mn level (ppm) of each analysed sample. The calculation of the final Cu, Fe and Mn content (g/kg) on DM basis was done using**Error! Reference source not found.** Equation 3.12.

For Mg, 0.20ppm, 0.40ppm and 0.80ppm standard Mg solutions with 0.1%$LaCl_3$ were used to calibrate the AA before readings were done. Mg readings were done at a wavelength of 285.2nm.

For Cu, 0.50ppm, 1.00ppm and 2.00ppm standard Cu solutions with 0.1% HNO_3 were used to calibrate the AA before readings were done. Cu readings were done at a wavelength of 324.7nm.

For Fe, 0.50ppm, 1.00ppm and 2.00ppm standard Fe solutions with 0.1% HNO_3 were used to calibrate the AA before readings were done. Fe readings were done at a wavelength of 248.3nm.

For Mn, 0.20ppm, 0.40ppm and 0.60ppm standard Mn solutions with 0.1% HNO_3 acid were used to calibrate the AA before readings were done. Mn readings were done at a wavelength of 279.5nm.

For K, clean deionised water, and 0.50ppm, 1.00ppm and 1.50ppm standard K solutions with deionised water were used to calibrate the AA before readings were done. K readings were done at a wavelength of 766.5nm. **Error! Reference source not found.**

For Na, clean deionised water, and 0.10ppm, 0.20ppm, 0.40ppm and 1.00ppm standard Na solutions with deionised water were used to calibrate the AA before readings were done. Na readings were done at a wavelength of 589.0nm. **Error! Reference source not found.**

Equation 3.9 Calculation of Mg, K and Na % of a sample.

$$Mg, K\ and\ Na\% = \frac{\left(\frac{Reading\ value \times End\ volume}{Mass\ of\ dry\ sample\ for\ acid\ digestion}\right)}{10\ 000}$$

Equation 3.10 Calculation of Mg, K and Na (g/kg) DM.

$$Mg, K\ and\ Na\left(^{g}/_{kg}\right) DM = \frac{Mg, K\ and\ Na\ \%}{Second\ DM} \times 1000$$

Equation 3.11 Calculation of Cu, Fe and Mn ppm of a sample.

$$Cu, Fe\ and\ Mn\ ppm = \frac{Reading\ value \times End\ volume}{Mass\ of\ dry\ sample\ for\ acid\ digestion}$$

Equation 3.12 Calculation of Cu, Fe and Mn (g/kg) DM.

$$Cu, Fe\ and\ Mn\left(^{g}/_{kg}\right) DM = \frac{Cu, Fe\ and\ Mn\ ppm}{Second\ DM} \times 10$$

P content

P content of the samples were determined on samples after acid digestion. A colorimetric method was used. A yellow colour development that is time sensitive occurs during this analyses. A 5.00ppm, 10.00ppm, 15.00ppm, and 20.00ppm standard P solutions were used to calibrate the spectrophotometer at a wavelength of 400nm. A reagent for the colour development was prepared using ammonium molybdate tetrahydrate ($(NH_4)_6MO_7O_{24}.4H_2O$), ammonium vanadate (NH_4VO_3), deionised water and perchloric acid ($HClO_4$). After a 1.00ml sample to 7.00ml deionised water dilution was made for each of the duplicate samples in the 50ml medicine bottles, 2.00ml of the colour reagent was added. The samples were mixed well and allowed to develop colour for 30 minutes. Reading commenced for the absorbance at 400nm with an Analytic Jena Specol 1300 spectrophotometer. All samples and other solutions containing the colour reagent was disposed of in a marked waste bottle. Calculations were done using Equation 3.13 and Equation 3.14. The factor is the mean factor calculated from absorbance reading of each of the standards, divided by its concentration in ppm. This analyses are based on the official method of analyses 965.17 (AOAC, 2000).

Equation 3.13 Calculation of P % of a sample.

$$P\ \% = \frac{\left(\frac{Absorbance\ \times factor \times End\ volume}{Mass\ of\ dry\ sample\ for\ acid\ digestion}\right)}{10\ 000}$$

Equation 3.14 Calculation of P (g/kg) DM.

$$P\left({}^{g}/_{kg}\right) DM = \frac{P\ \%}{Second\ DM} \times 1000$$

Zn content

Zinc (Zn) content was determined with a GBC 905 AA by the standard method described by Giron (1973). For Zn, 0.50ppm, 1.00ppm and 1.50ppm standard Zn solutions with 0.10% HNO_3 were used to calibrate the AA before readings were done. Zn readings were done at a wavelength of 213.90nm. Equation 3.15 and Equation 3.16 were used to do the calculations of the Zn content in the feed samples on DM basis.

Equation 3.15 Calculation of Zn ppm of a sample.

$$Zn\ ppm = \frac{Reading\ value \times End\ volume}{Mass\ of\ dry\ sample\ for\ acid\ digestion}$$

Equation 3.16 Calculation of Zn (g/kg) DM.

$$Zn\left({}^{g}/_{kg}\right) DM = \frac{Zn\ ppm}{Second\ DM}\ 10^{-1}$$

Wet digestion for selenium (Se) determination and reading of Se content

A wet digestion process was used to prepare samples in duplicate for Se content determination. A representative 0.1000g of each sample was weighed in, into the digestive tubes, in duplicate. Only 0.1000g of each sample was used as it was expected that due to the samples being meat they will have a high Se-value. 5ml of the digestive acid solution was added (perchloric acid ($HCLO_4$) and nitric acid (HNO_3)). Tubes were placed in a digestive block for 16 hours (recommended to digest overnight). Se was then converted from SeVI to SeIV by adding 2.50ml of 20% HCl. The tubes were then further heated in the digestion block for 40 minutes. The AA was heated and when the gain reading read ca. 80 an adjustment of the gain dial was done downward until the gain reading was 74-75, the ideal gain to read Se concentration. The PerkinElmer 2380 Atomic Absorption Spectrophotometer (AA) were then blanked at a wavelength of 196nm. The standard solutions were used to calibrate the AA. Samples were made up to 20ml with 10% HCl. All samples were then read on the AA. Equation 3.17 was used to calculate the Se content of the samples from the reading on the AA. The whole analysis was done based on the official method of analysis 996.16 (AOAC, 2000). Equation 3.18 was used to calculate the Se (µg/kg) content of feed samples on DM basis.

Equation 3.17 Calculation of Se (ng/g).

$$Se\left({}^{ng}/_{g}\right) = \frac{Reading\ (ng) \times Dilution\ (20)}{Mass\ (g)}$$

Equation 3.18 Calculation of Se (g/kg) DM.

$$Se\ \left({}^{g}/_{kg}\right) DM = \frac{Se\left({}^{ng}/_{g}\right)}{Second\ DM} \times 10^{-4}$$

3.6 Blood sample collection and blood chemistry

During this trial blood samples were collected and prepared for analyses and/or shipment on site in the HESC animal hospital laboratory.

3.6.1 Blood sample collection

Blood samples were collected from cheetah in both groups on the test days. Samples were collected one hour post-prandial. Animals were lured into the trap cage designed and regularly used at HESC for blood collection. The animals were restrained with the help of HESC curators in a standard stress free method

developed and routinely applied at the HESC. This is done by pushing down on the shoulder and pelvic area of the cheetah using two wooden sticks with a diameter of ca 40mm. The person who draws the blood stabilised the cheetah by holding on to its tail and the leg from which the blood sample was taken. A single blood sample was taken from the medial saphenous vein (*v. saphena medialis*) by means of a 5ml syringe and 20gauge needle. A single drop of blood was used to create a blood smear on a glass slide. After the slide was air dried the sample was placed in the field crate to be transported back to the animal hospital. The needle was removed from the syringe before the blood was transferred into plasma (fluoride oxalate) and serum vacutainers, to prevent haemolysis. 1ml of blood was transferred into the plasma vacutainer. The sample was inverted 9 times to allow thorough mixing of the anti-coagulant and the blood. The remaining blood was transferred into a serum vacutainer. The sample was inverted 5 times to ensure the blood is mixed well. Both the serum and plasma vacutainers were placed on ice and transport back to the animal hospital.

All tested blood parameters have been selected in order to collect data. From this data we will attempt a physiological evaluation of animals from the two treatment groups. A physiological evaluation will aid us in the discussion around the treatment of a group of animals by means of running with the lure have been to the benefit of the test animals (receiving the lure as treatment) of this group over the control group (no lure as part of feeding). It will also aid us to make either conclusions or recommendations pertaining to the one or both of the treatments.

3.6.2 Blood sample preparation

Allow the vacutainers to stand at least 20 minutes, but not more than 60 minutes, to allow for sufficient clot formation in the serum tube, before placing the tubes in the centrifuge. The tubes were loaded into the Universal 320 centrifuge and centrifuged at a speed of 4000 RPM for 10 minutes. Blood slides were fixated by placing them in a Coplin jar, filled with 100% methanol for 60 seconds. Slides were removed and place on draining paper to allow to air dry.

3.6.3 Analyses, packing and storage of blood samples

3.6.3.1 On-site analyses

After air drying, the glass slides were individually wrapped in parafilm for shipment to IDEXX Laboratories at the end of the test week. Vacutainers were removed from the centrifuge. A pack cell volume percentage (PCV%), also called a haematocrit (Cunningham, 2002), was measured and calculated using Equation 3.19 for each serum sample. The volume of the cells was measured using a standard ruler with mm increments. The same ruler was used during all measurements pertaining to the PCV%.

Equation 3.19 Calculation of PCV%.

$$PCV\% = \frac{Volume\ of\ packed\ cells}{Total\ sample\ volume} \times 100$$

Plasma was transferred using a pipette and 5ml Eppendorf® pipette tip into a marked 5ml Eppendorf® tube. Eppendorf® tubes were sealed with parafilm and placed in the freezer until shipment to IDEXX Laboratories, Unit 2, Woodmead Willows Office Park, 196 Morris Street East, Woodmead, Johannesburg, South Africa.

The IDEXX VetTest® 8008 analyser were calibrated with the quality control kit in week 1 and week 4, as recommended in section F of the VetTest® user manual.

Serum was transferred into a marked serum transfer Eppendorf® tube using a pipette and 5ml Eppendorf® pipette tip. Serum samples were allowed to reach room temperature before analysing. Analyse the serum sample using the IDEXX VetTest® 8008 (Software update 8.37 A) according to the method stipulated below taken from the IDEXX VetTest® Chemistry Analyzer Operator's Manual.

- Switch on the VetTest® and allow for sensor warmup cycle to complete (approximately 20 minutes).
- Enter patient species from menu given, in this case feline.
- Enter patient identification. (e.g. 0801ROSY).
- Insert slides for albumin, BUN, Ca, creatinine, lactate, P and total protein. Insert a single slide at a time into the slide loading tray with the bar code facing up and the notch on the left. Gently push the

loading tray forward as far as it would go, after slide was placed into position, and then pull it back out. Repeat for each slide. The analyser can take a maximum of 12 slides.
  - Slides can be used directly out of the freezer.
  - Open foil package only when ready to load the slide into the slot (should be used within 15 minutes of opening).
  - Do not let your fingers or any other surface come into contact with the membrane of the slide. Always handle a slide by grasping it by the outer plastic edge.
- Press **E** after all the desired slides were loaded and follow the onscreen instructions.
- Remove the pipettor from its holder on the VetTest®.
- Firmly place a new, disposable pipette tip over the metal end of the pipettor.
- Replace the pipettor in its holder and follow onscreen instructions.
- Place the tip of the pipette into the serum sample that needs to be analysed. (Important: Ensure that sample number corresponds with the animal entered as patient.)
- With the **Single Beep** press and release the pipettor button. (Ensure pipette tip remains submerged in sample.)
- With the **Double Beep** lift the tip of the pipettor out of the sample.
- With the **Triple Beep** carefully wipe the tip of the pipettor, with a twisting motion, using a lint-free Kimwipe®.
- Immediately replace the pipettor into its holder in the analyser and the analyses process will continue.
- The analyses will be completed within 5 to 6 minutes (depending on the chemistries that will be done).
- After the analyses the VetTest® will eject the used slides. Empty the used-slide drawer.
- Remove the pipettor and discard the used pipette tip. Replace the pipettor in its holder.
- Check the printing paper supply. Follow the onscreen instructions.
- Read the results. (See Figure 3.3 below explaining the result reading.)

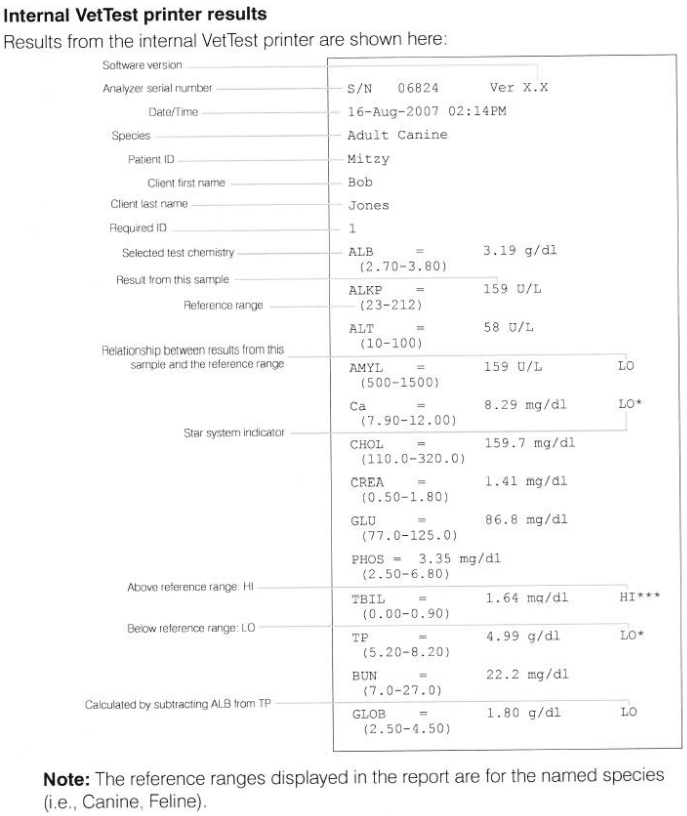


Figure 3.3 Example of a result from the internal printer of the IDEXX VetTest® and how to read the results. (Taken from the IDEXX VetTest® 8008 Chemistry Analyzer Operator's Manual).

3.6.3.2 Packing, storage and shipment

Remaining serum was transferred into marked Eppendorf® tubes for shipment to IDEXX and AMPATH laboratories at the end of the test week. IDEXX Laboratories did blood smear evaluation and serum analyses for creatinine, cortisol, free T_4 and urea. AMPATH Laboratories did serum analyses for lipogram, vitamin D_3 and free T_3. All Eppendorf® tubes were sealed with parafilm and stored at ca -4°C until shipment. With shipment the samples of the week were packed, with the appropriate documentation, and couriered to IDEXX laboratories.

3.6.3.3 Analyses done at IDEXX Laboratories

Urea

A quantitative measurement of urea (reported either as Urea Nitrogen (BUN) or as Urea (UREA)) in Serum was analysed using a VITROS 350 (supplied by Ortho Clinical Diagnostics – Johnson and Johnson). The test was a colorimetric measurement at a wavelength of 670nm. The test involves two reaction sequences, the first enzymatic forming ammonia which selectively passes through the colour-forming layer to react with an indicator to form a dye. The reflection density of the dye is measured and is proportional to the concentration of Urea in the sample. The instrument is based on dry slide technology.

Creatinine

A VITROS 350 was used to make a quantitative measurement of the serum creatinine level. It was a two-point rate measured at a wavelength of 670nm based on dry slide technology. Four reaction sequences driven by enzymatic oxidation result in changes to be measured in the reflection density at two different time points. The difference in reflection densities is proportional to the concentration of creatinine in the serum.

Lactate

Lactate was quantitatively measured in plasma using a VITROS 350. The test is a colorimetric test at a wavelength of 540nm. Enzymatic oxidation drives a two reaction sequence and results in a dye complex. The intensity of the dye complex is assessed spectrophotometrically after the incubation of the slide. This test is based on dry slide technology slide technology.

Cortisol

A quantitative measure of cortisol was done in serum samples of the experimental animals. An IMMULITE 1 (Upgraded with IMMULITE 1000 software and supplied by Siemens Healthcare Diagnostics) was used to do the measurement. The principle of the test is a solid-phase, competitive chemiluminescent enzyme immunoassay.

Free T_4

Referred to as a veterinary Free T_4 test IDEXX laboratories. An IMMULITE 1 was used to quantitatively measure non-protein-bound Thyroxine (free T_4) levels in serum samples. The principle of the test is a solid-phase, competitive chemiluminescent enzyme immunoassay.

3.6.3.4 Analyses done at AMPATH Laboratories

Free triiodothyronine (Free T_3)

Serum samples were analysed using a UniCel® Immunoassay System (supplied by Beckman Coulter) with the Access Immunoassay Systems to determine quantitatively the free triiodothyronine levels (Free T_3). The test is a competitive binding immune-enzymatic assay. A serum sample is added to a reaction vessel containing an anti-T_3 monoclonal antibody conjugated to alkaline phosphatase. The free T_3 in the sample reacts with the anti-T_3 antibody during incubation. Particles coated with streptavidin and biotinylated T_3 analogue are then added to the mixture. Unoccupied binding sites on the anti-T_3 antibody are bridged to the particle through the T_3 analogue. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while the unbound materials are rinsed away. Then, the chemiluminescent substrate Lumi-

Phos* 530 (*Lumi-Phos is a trademark of Lumigen, Inc, a subsidiary of Beckman Coulter, Inc.) is added to the vessel. Light generated by the reaction is measured with a luminometer. This value of light generated is inversely proportional to the concentration of free T_3 in the serum sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

25-hydroxy-vitamin D (Vitamin D_3)

A serum sample was analysed using a COBAS® E 411 analyser with the Elecsys 2010 Vitamin D Assay (Supplied by ROCHÉ) to do a quantitative determination of total 25-hydroxyvitamin D. The test is an electrochemiluminescence binding assay and comprises out of three stages of incubation:

1) Vitamin D released from vitamin D binding protein.
2) Incubation with ruthenium labelled vitamin D. A complex is formed between ruthenylated vitamin D binding protein and vitamin D.
3) After the above mentioned steps streptavidin-coated micro particles and vitamin D labelled with biotin are added and this results in free ruthenium labelled vitamin D binding proteins becoming occupied. A complex consisting of the ruthenylated vitamin D binding protein and the biotinylated vitamin D is formed and becomes bound to the solid phase via interaction of biotin and streptavidin.

Total Triglycerides (Total TG)

Quantitative determination of the total triglyceride (total TG) concentration in blood serum samples was done by using a SYNCHRON LX® system, UniCel® DxC 600/800 system and Synchron® Systems Multi Calibrator. A timed endpoint method is used with Triglycerides GPO reagent to measure the triglycerides concentration.

Glycerol and free fatty acids are released after hydrolysis of triglycerides by the action of lipase. A three part coupled enzyme reaction sequence involving glycerol kinase (GK), glycerophosphate oxidase (GPO), and horseradish peroxidase (HPO) causes the oxidative coupling of 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) with 4-aminoantipyrine to form quinoneimine, a red dye. The appropriate amount of reagent and sample are automatically measured into the cuvette by the SYNCHRON® System. A ratio of 1:100 of sample to reagent is used. The change in absorbance is measured at 520nm by the system. The concentration of TG in the sample and the change in absorbance is directly proportional and the system uses this to calculate and report the TG concentration.

a) $Triglycerides \xrightarrow{Lipase} Glycerol + Fatty\ Acids$

b) $Glycerol + ATP \xrightarrow[Mg++]{GK} Glycerol - 3 - phosphate + ADP$

c) $Glycerol - 3 - phosphate + O_2 \xrightarrow{GPO} Dihydroxyacetone + H_2O_2$

d) $2H_2O_2 + 4 - Aminoantipyrine + DHBS \xrightarrow{HPO} Quinoneimine\ Dye + HCL + 2H_2O$

Figure 3.4 Chemical reaction scheme for TG analyses.

HDL Cholesterol

The quantitative determination of HDL cholesterol in the high density lipoprotein fraction of serum samples with the use of the HDLD reagent used in combination with the SYNCHRON LX® system, UniCel® DxC 600/800 System and SYNCHRON® System Lipid Calibrator.

It has been found that in humans an inverse relationship between HDL cholesterol level and the risk of developing coronary artery disease exists. There is also an association between high levels of HDL cholesterol and the "longevity" syndrome in humans (Beckman Coulter Synchron (a), 2013).

With this direct HDL cholesterol method there is no need for any centrifugation or pre-treatment of samples offline. It is a homogenous assay. During this method a colour product is produced by a reaction. During this reaction a unique detergent solubilises only the HDL lipoprotein particles and this then free the

HDL cholesterol to react with cholesterol esterase and cholesterol oxidases in the presence of chromogens. The reaction of cholesterol enzymes with LDL, VLDL, and chylomicrons lipoproteins are inhibited by the same detergent mentioned above, by adsorbing to their surfaces. The selectivity for the HDL cholesterol assay is enhanced by a part of the reagent. A poly-anion contained in the reagent acts specifically by complexing LDL, VLDL, and chylomicrons lipoproteins.

A timed-endpoint method is used to measure the cholesterol concentration with the HDLD reagent. The appropriate amount of reagent and sample are automatically measured into the cuvette by the SYNCHRON® System. A ratio of 1:93 parts sample to reagent is used in the analyses. The change in absorbance is measured by the system at 560nm. There is a direct proportionality between the cholesterol concentration in the sample. The System uses this to calculate and give a result of the HDL-cholesterol concentration in the sample.

$$LDL, VLDL, Chylomicrons \xrightarrow[Polyanion]{Detergent} Stable\ Complexes$$

$$HDL\ Cholesterol \xrightarrow[Cholesterol\ Oxidase]{Cholesterol\ Esterase} \Delta^4\ Cholesterone + H_2O_2$$

$$H_2O_2 + DSBmT + 4 - AA \xrightarrow{Peroxidase} Colour\ product$$

DSBmT: N,N-bis (4-sulphobutyl)-m-toluidine-disodium
4-AA: 4-aminoantipyrine

Figure 3.5 Chemical reaction scheme for HDL analyses.

LDL Cholesterol

The direct quantitative determination of low-density lipoprotein in serum samples is done by using the LDLD reagent in combination with the SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and Synchron® Systems Multi Calibrator (Supplied by Beckman Coulter. A timed-endpoint method is used to determine a cholesterol concentration in serum samples.

A direct correlation between the risk of developing coronary heart disease and higher levels of LDL in relation to HDL exists. This increased level of LDL is the initial target of cholesterol-lowering treatment.

With this direct LDL Cholesterol method there is no need for any centrifugation or pre-treatment of samples offline. All non-LDL lipoprotein particles are solubilised by the exclusive detergent. This reaction releases cholesterol. A non-colour producing reaction occurs where cholesterol reacts with cholesterol esterase and cholesterol oxidase. The remaining LDL particles are solubilised by a second detergent. During this reaction a chromogenic coupler permits the colour formation.

A timed-endpoint method is used to measure the cholesterol concentration with the LDLD reagent. The appropriate amount of reagent and sample are automatically measured into the cuvette by the SYNCHRON® System. A ratio of 1:93 parts sample to reagent is used in the analyses. The change in absorbance is measured by the system at 560nm. There is a direct proportionality between the cholesterol concentration in the sample. The System uses this to calculate and give a result of the LDL-cholesterol concentration in the sample.

Detergent 1

$$HDL - C, VLDL - C, CM - C \xrightarrow[Cholesterol\ Oxidase]{Cholesterol\ Esterase} Non - Colour\ Product$$

Detergent 2

$$LDL\ Cholesterol \xrightarrow[Cholesterol\ Oxidase]{Cholesterol\ Esterase} \Delta^4\ Cholesterone + H_2O_2$$

$$H_2O_2 + DSBmT + 4 - AA \xrightarrow{Peroxidase} Colour\ Product$$

C:	Cholesterol
DSBmT:	N, N-bis (4-sulphobutyl)-m-toluidine-disodium
4-AA:	4-aminoantipyrine

Figure 3.6 Chemical reaction scheme for LDL analyses.

3.7 Data management and statistical analyses

Data from laboratory results and observations and/or recordings in the field was recorded in Microsoft Excel.

A consultant, Mr. R. Coertze, did the statistical analyses, which were done in SAS by multivariate analyses of variance (MANOVA) in a general linear model (GLM) (Carey, 2006). A repeated measures analysis method was used. Frequency distribution was analysed and tested for normality of treatment groups analysed differences with a p-value of $p<0.05$ (Carey, 2006). A Fisher test was used to test the different blood parameters values against the following independent variables; Treatment, time (week), the combined effect of treatment and time (week) and weight.

An overall R^2 value was used to determine what proportion of the variance is described by the model fitted to the data and if the model is suitable for the analyses of this specific type of data.

CHAPTER 4: RESULTS AND DISCUSSION

4. Introduction

Results of data collected during the trial pertaining to general physical health, feed and the relevant blood parameters will be reported and discussed in this chapter.

4.1 Physical health evaluation

In this section the physical health evaluation that was done in the veld on each feeding day is discussed. The raw data sheets are summarised into tables per animal in Appendix E. No statistical analyses were done for any of the physical health parameters.

4.1.1 Response to lure

All animals in the test group showed good interest in the lure during the experimental period. Animal 1 and 3 had exposure to the lure before the onset of the experimental period. Initially animal 5 ignored the lure, but with some encouragement she started to follow it and continued to run very well on all subsequent runs. We also experienced some difficulty with animal 5 and getting his full attention at the lure. He ran during each feeding, although he showed lower enthusiasm during some of the runs. He never scored below 2 (lowered interests) for the response to lure category. In wet weather animals was not allowed to run with the lure, because of the risk that animals might slip and injure themselves. Wet weather only caused the lure to be excluded from the feeding method on days where no blood samples were taken.

4.1.2 Gait

The gait of all animals were carefully evaluated, especially for the test group animals. No animals showed lowered co-ordination during the experimental period.

4.1.5 Lameness

The level of lameness was evaluated to ensure animals were not uncomfortable when they ran on the lure. Animal 1 showed a moderate level of lameness on 1 February 2014, although she still showed a good response to the lure. She showed no noticeable level of discomfort on the next feeding day (3 February 2014). No other animals showed lameness during the experimental period.

4.1.6 Appetite

Appetite is a good indicator of the physical wellbeing of an animal. A lowered appetite was recorded when animal 6 had leftovers during week 5. She consumed the entire piece of meat presented to her on the next feeding day. No other animals showed lowered appetite during the experimental period.

4.1.7 Vomit

Vomiting, in conjunction with appetite, can be used as a good indication, of the overall physical wellbeing of an animal. Vomiting may indicate some infection or an upset stomach in animals, thus the absence of vomit is preferred. All enclosures were vomit free after every meal for animals from both treatment groups for the extent of the experimental period.

4.1.8 Coat quality and condition evaluation

A protein deficiency can result in a dry and unthrifty coat (Bechert *et al*., 2002). On rainy days the coat quality was lowered, although it was taken into consideration when the scoring was done. No animal showed a lowered physical wellbeing from coat quality or condition over the experimental period.

4.1.9 Eye colour and brightness evaluation

Eye colour and brightness scores was good colour and brightness for all animals, except animal 10 during week 2. Animal 10 most probably had an encounter with a snake, which spit her in the eye. This caused some swelling and a slight discharge. It cleared up before the next feeding.

4.1.10 Faecal scoring

It was not possible to do regular evaluations for faeces, as the faecal samples were either removed from enclosures by vultures or destroyed by the wet conditions.

4.1.9 Weighing of animals

Weighing of animals without sedation posed a challenge. It was only possible to weigh the animals once during the entire experimental period. Weights are given in Table 4.1 below.

Table 4.1 Animal weights during the experimental period.

Animal number	Weight (kg)
1	42
2	21
3	39
4	18
5	39
6	41
7	22
8	42
9	21
10	37

4.1.18 Summary of physical health evaluation

The physical health evaluation, of each animal during each feeding, showed that the animals were in good health and not adversely affected by either of the two treatments. No animals had to exit the trial due to health problems.

4.2 Feed analyses

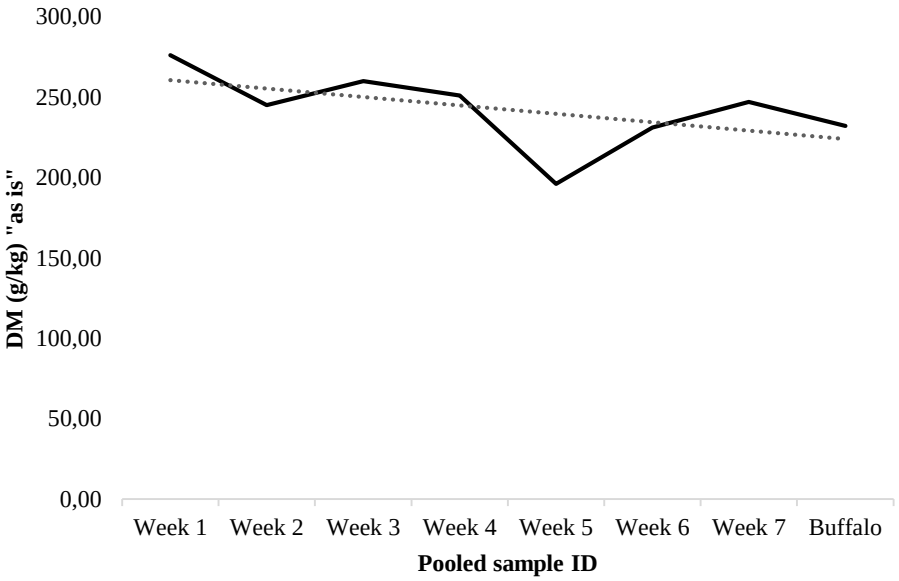
The results of the nutrient concentrations determined by UP Nutrilab for the primary feed that cheetah received during the experimental period are given in Table 3.3. All values are based on DM basis. This enables us to compare different pooled feed samples with each other. The feed samples were pooled per experimental week. A separate analysis was done for buffalo meat fed for two feeding days during the trial. This was done in order to determine if supplementation to game meat, presented to cheetah in captivity, is required or not. The perception among the staff at HESC is that when feeding game meat to the cheetah at the facility, it is not necessary to do nutrient supplementation. They presumed that the game meat, which is close to what the cheetah consumes in the wild, has a good enough nutrient profile to permit the exclusion of the standard CVM-supplement when feeding game to the captive cheetah. No supplement was added to buffalo meat presented to animals during this trial.

Table 4.2 Feed analyses of supplemented beef presented to experimental animals pooled per week, and a sample of buffalo meat on DM basis (except DM-analysis that is given in g/kg on "as is" basis)

Sample	DM (g/kg "as is")	Ash	CP	EE	CP:EE ratio	Ca	P	Ca:P ratio	Mg	Cu	Fe	Mn	Zn	K	Na	Se
Buffalo meat	232.00	39.80	891.00	112.00	7.98:1	0.40	8.60	0.04:1	1.20	5.36×10^{-3}	0.139	1.07×10^{-3}	0.114	13.90	2.50	1.65×10^{-4}
Beef Week 1	276.00	72.30	803.00	114.00	7.02:1	8.10	7.40	1.09:1	3.80	6.67×10^{-3}	0.885	7.18×10^{-3}	0.135	13.50	5.10	3.42×10^{-4}
Beef Week 2	245.00	59.40	734.00	185.00	3.98:1	6.40	7.20	0.89:1	3.80	5.71×10^{-3}	0.880	7.27×10^{-3}	0.131	14.30	5.20	5.54×10^{-4}
Beef Week 3	260.00	53.90	831.00	124.00	6.72:1	3.60	7.70	0.47:1	2.10	4.11×10^{-3}	0.419	3.60×10^{-3}	0.119	12.70	3.40	2.93×10^{-4}
Beef Week 4	251.00	78.40	888.00	49.00	18.24:1	5.90	7.90	0.75:1	3.20	7.29×10^{-3}	0.744	6.77×10^{-3}	0.095	24.50	8.10	2.94×10^{-4}
Beef Week 5	196.00	66.40	883.00	46.00	19.32:1	6.00	8.90	0.68:1	3.10	6.22×10^{-3}	0.746	6.22×10^{-3}	0.207	17.90	7.30	2.76×10^{-4}
Beef Week 6	231.00	64.20	889.00	61.00	14.63:1	2.30	8.70	0.27:1	2.20	5.14×10^{-3}	0.365	3.08×10^{-3}	0.116	8.80	6.50	4.07×10^{-4}
Beef Week 7	247.00	48.90	855.00	88.00	9.66:1	2.20	8.20	0.27:1	1.80	5.15×10^{-3}	0.301	2.58×10^{-3}	0.115	13.70	3.80	4.27×10^{-4}
$\bar{x}$ *beef pooled samples*	243.71	63.36	840.43	95.29	11.37:1	4.93	8.00	0.69:1	2.86	5.76×10^{-3}	0.620	5.24×10^{-3}	0.131	15.06	5.63	3.70×10^{-4}

4.2.1 Dry Matter Content (DM)

According to McDonald *et al*. (2002), to determine the moisture content of a sample, a known weight of the sample is dried at 100°C. After drying the sample in the oven at 100°C, the sample is weighed back and the moisture content can be determined from the sample weight loss. In order to compare different feeds on a fair and unbiased basis, we convert all the nutrient concentrations determined by analyses to a dry matter basis. It is, however, important to graphically show (Graph 4.1) how the moisture content of the different feed samples differed. Graph 4.1 shows that there was variation in the moisture content of the samples between weeks. The variation in the moisture content can most probably be attributed to sampling area in the feeding crate. If sampling was done from meat at the bottom of the crate it would have been lying in the exudate of bloody water. This might give a higher moisture content. Meat samples taken from meat pieces at the top of crate might give lower moisture content. In future sampling should be done with care to have samples that represent the entire crate and not only a few pieces. Table 4.2 contains the precise moisture content for each of the pooled samples.

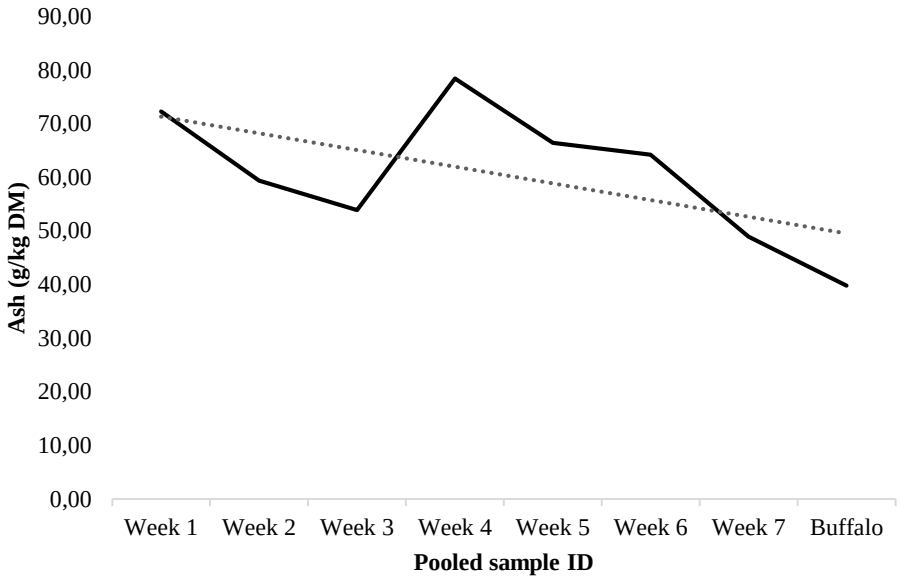


Graph 4.1 Moisture content (g/kg) "as is" in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.2 Ash (Inorganic Matter)

The mineral matter of a feed is in the ash fraction of the feed. Minerals are of high importance in the nutrition of all living organisms (Kleyn, 2012). The ash content varied between 39.80g/kg DM to 78.40g/kg DM. Table 4.2 presents values for ash content of pooled meat samples per week, as well as the pooled buffalo sample. In the ash content of all meat samples are graphically presented. It can be seen from Graph 4.2 that the ash content in week three, week seven and the buffalo sample were much lower than in the other samples. This could be possibly an indicator that in the case of week three and week seven the supplemented minerals were not mixed in well with the sample. This error can be an indicator of poor mixing of CVM-supplement and meat presented to the animals. It was reported that some of the supplement was seen falling out when meat was presented to the animals. These values can also possibly indicate sampling error. Sampling error results in samples not containing a representative amount of the supplement and meat combination as it is presented to the animals. When workers supplement the meat, supplement is added to parts of the meat where it is possible to place the supplement into grooves cut into the meat. The distribution of the supplement is not even throughout the chunk of meat. It is nearly impossible to get a representative sample of a meat and supplement combination if one sample from any area of the meat. The lowest value for ash was in the buffalo sample. This value of 39.80g/kg DM indicates the importance of supplementation of buffalo meat, and potentially other

game meat, presented to animals. Another possible solution is to add the supplement into a minced meatball that is presented to the animal separately from the main chunk of meat.



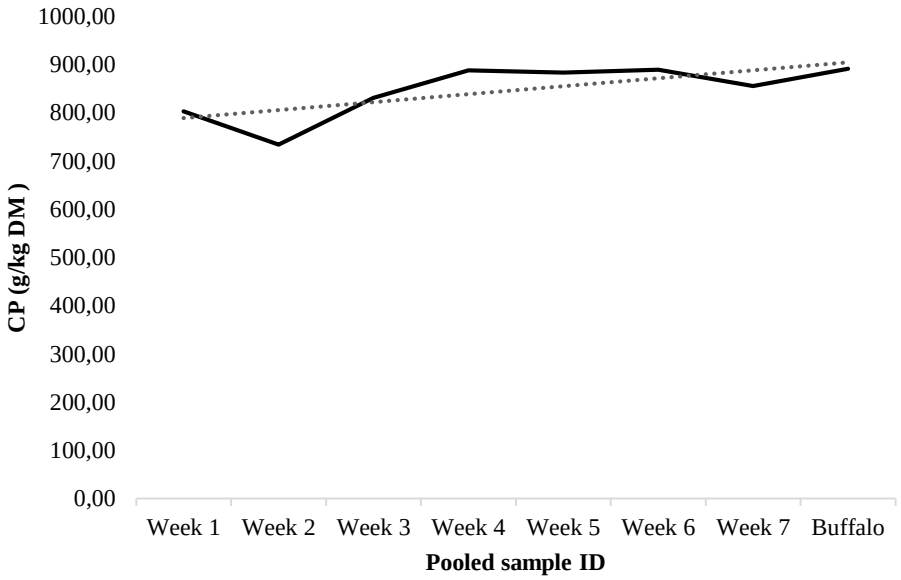
Graph 4.2 Ash content (g/kg DM) in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.3 Crude Protein (CP)

In the determination of the different concentrations of CP of feeds, the nitrogen (N) content of a feed is determined using the Leco TruMac®N. The CP value is then calculated using Equation 3.3 and Equation 3.4, where *N content*, the reading from the Leco TruMac®N, is multiplied with the Kjeldahl factor of 6.25. According to McDonald *et al*. (2002) it is presumed that the reading for nitrogen, we get using this system, is nitrogen derived from protein with a nitrogen content of approximately 16%. According to the author it is further assumed that an appropriate value for protein content, based on nitrogen content, can be calculated (Equation 3.3) by multiplying the N content with the Kjeldahl factor of 100/16 (or 6.25).

In Graph 4.3 it can be seen that the CP content did not show a lot of variation between weeks. Due to the lack of degrees of freedom in feed analyses no statistical analyses were done on the feed analyses data. It is for the aforementioned reason unfortunately not possible to determine if there is a significant or non-significant difference in the CP content of the different samples. Table 4.2 contains the mean values of duplicate protein analyses done on all the samples.

No protein concentrate was supplemented in the CVM-supplement presented to the animals. This variability in the CP of the meat can entirely be due to the origin of the meat. Meat for the animals at HESC comes from various sources. Some are collected, with a refrigerated truck, from far and wide. Just to name one area, some meat comes from Bronkhorstspruit, Gauteng, South Africa (approximately a five-and-a-half-hour drive by truck). Cheetah are obligated carnivores and can only use meat as a source of feed. It is difficult to get meat at a reasonable price when meat is produced for human consumption. HESC is a non-profit organisation and are dependent on donations for the most part of the meat supply. Some weeks a shortage are experienced and losses during transport occur. No pre-presentation analyses are done on meat, which is presented to animals, because it is not realistic with regards to time cost.



Graph 4.3 CP content (g/kg DM) in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

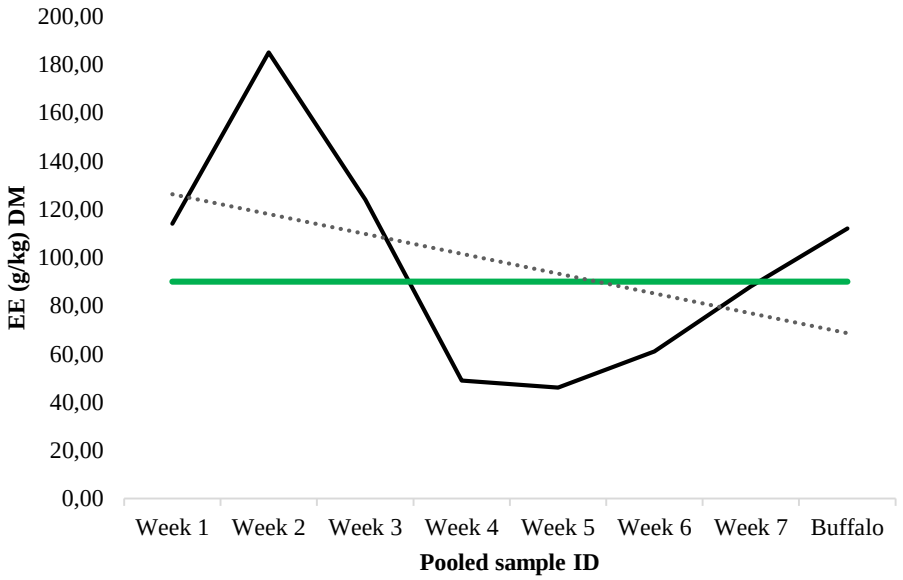
4.2.4 Ether extract (EE)

During the EE method the amount of fat and oil of the feed, in this case meat from either beef or buffalo, are determined (Kleyn, 2012). In Table 4.2 the mean values of the duplicate EE analyses for all samples are given. In Graph 4.4 it can be seen that there was a high variability in the EE content of the feeds between weeks. It is, however, not possible to say if it is statistically significant or not as the degrees of freedom did not allow for statistical analyses on the data of feed samples.

Feed preparation was monitored to ensure that meat was prepared according to the method discussed in Chapter 3. It was important that no excessive fat was removed in order to maintain a good protein to fat ratio (CP:EE); a ratio of 3:1 (Bell, 2010) is ideal. The ideal protein to fat ratio is discussed below in section 4.2.5.

The beef used for feed differed in region of origin and in age. According to Berg & Butterfield (1968) different genetic, nutritional and other experimental factors can influence carcass composition, including the content and overall distribution of fat.

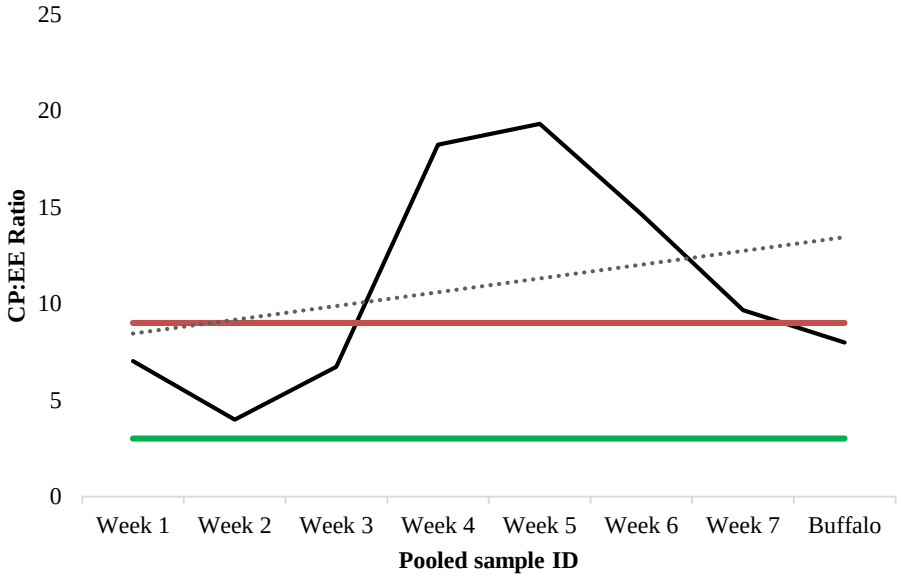
Another factor to consider is the variation of fat content in the pooled samples. Sampling of meat for the EE analyses can skew the results if the sample is not truly representative of the entire meat chunk fed to the animal. It is, however, difficult to sample a true representative sample from a crate of meat. The green line in Graph 4.4 indicates AAFCO minimum fat inclusion in cat food for growth, reproduction and adult maintenance.



Graph 4.4 EE (g/kg DM) of supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.5 CP to EE ratio

According to Bell (2010) an ideal ratio of protein to fat is 3:1 (green line in Graph 4.5). The author further reports that in supplemented meats CP:EE ratios of up to 9:1 (red line in Graph 4.5) have been found. In Table 4.2 the CP:EE ratio exceeds this recommended value in each sample (average of 11.37:1). Graph 4.5 shows the variation in the CE:EE ratio between weeks. The average ratio obtained for the total sample period was higher than the maximum ratio of 9:1 reported by Bell (2010). This emphasises the importance of not removing any excess fat from meat presented to cheetah, because this might increase the CE:EE ratio. Cheetah require an energy dense diet and the fat content is positively correlated to the energy density of the diet. Sampling error can also attribute to the higher ratio seen in the meat samples. In future the individual who takes samples from crates should make an effort to include a representative portion of fat in the sample. It is, however, not possible to say if the differences in the CP:EE ratio is statistically significant between weeks or not. The degrees of freedom did not allow for statistical analyses on the data of feed samples.



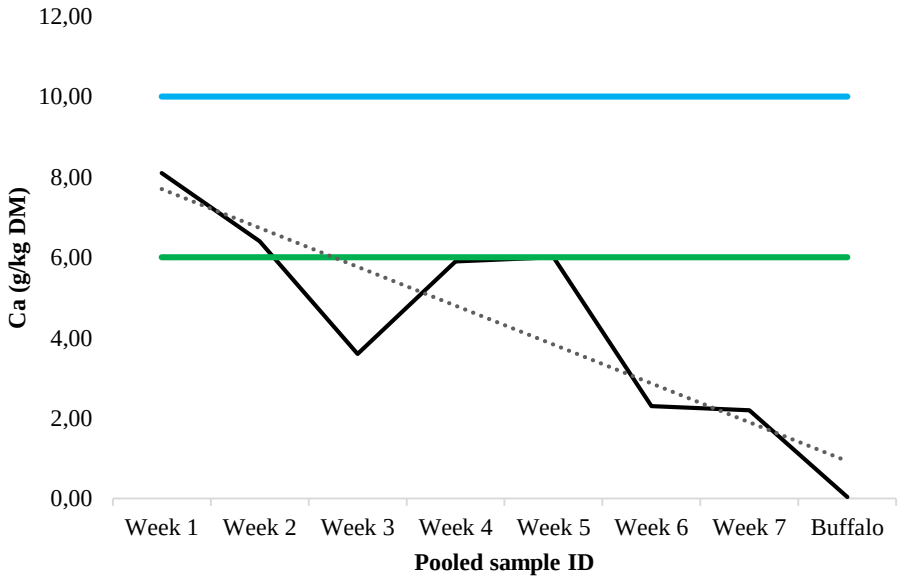
Graph 4.5 CP:EE ratio of supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.6 Calcium (Ca)

Ca together with P are two of the most important macro minerals (Kleyn, 2012). Ca is one of the most abundant mineral elements in the animal body and is one of the most important building blocks of the skeleton (McDonald *et al*., 2002). According to Kleyn (2012) most of the Ca and P are contained in the skeleton. Kleyn (2012) and McDonald *et al*. (2002) reports that 99% of all the Ca and 80-85% of the total amount of P in the body are stored in the skeleton and teeth. Kleyn (2012) and McDonald *et al*. (2002) further reports that Vitamin D_3 (Vit D_3) plays a vital role in the way both Ca and P are metabolised. The two authors agree that a decreased level of Vit D_3 increases the requirements for Ca and P and *vice versa*. McDonald *et al*. (2002) further reports that a large amount of Ca can be mobilised from bone to restore the Ca level in blood plasma. The signal for resorption comes from the parathyroid gland in reaction to low Ca levels in the plasma. Many sources of Ca are available. The Ca used in the CVM-supplement is described as a bioavailable Ca source. Bioavailability also plays a role in the inclusion rate of the Ca source. Ca also plays a key role in muscle contraction (Hopkins, 2006).

The Ca in the diet comes from both the meat and the CVM-supplement. Ca is one of the main nutrients that is deficient in meat. Looking at the data presented in both Table 4.2 and Graph 4.6, it can be seen that there was a variation in Ca content between the different weeks in the beef samples, as well as between the beef and the buffalo samples. The Ca value for buffalo was 0.40g/kg DM and the mean Ca value for all the beef samples was 4.93g/kg DM. This high variation between beef and buffalo supports the recommendation to feed the CVM-supplement with game meat as well. AAFCO recommends a minimum Ca level of 10.00g/kg (blue line on Graph 4.6) for growth and reproduction and a minimum level of 6.00g/kg (green line on Graph 4.6) for adults for maintenance. Cheetah are sensitive for Ca deficiencies and the calcium carbonate in CVM-supplement is added to contribute ca. 36% Ca m/m. If the dosage of CVM-supplement is correct, the minimum required level of 6.00g/kg Ca can easily be achieved.

The high variation in Ca levels of the supplemented beef meat shows that the CVM-supplement is not used correctly or it may be because of meat sampling error. It can be recommended that during the mixing of the CVM-supplement with the meat, it must be ensured that the supplement is mixed in, in such a way that it will not fall out during feeding. Another means of presenting the supplement to the animals might be by mixing the supplement into a meat ball with some minced meat. This is to ensure that each individual receives its daily requirement of the supplement. Sampling can also be adapted to ensure that the sample taken from a feed crate is truly representative for the entire feeding crate. Another recommendation is to supplement the Ca using carcasses of animals, that are naturally high in Ca (such as rodents) and it can even be recommended above mineral premixes (Bell, 2010), but it might pose a threat to spread of disease.

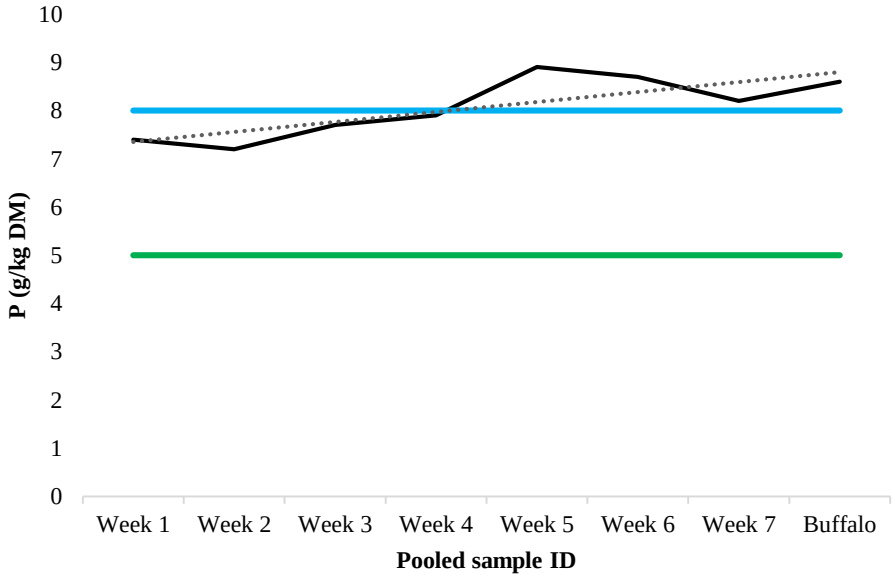


Graph 4.6 Ca (g/kg DM) levels in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.7 Phosphorous (P)

It is already mentioned in the discussion of Ca that Kleyn (2012) and McDonald *et al*. (2002) reports that 80-85% of all P in the mammalian body is found in the skeleton and teeth of the organism. McDonald *et al*. (2002) further reports that P is the one mineral element in the animal body with more functions than any other mineral element. The author further reports that it is not only important in association with Ca in building of bone, but also occurs in nucleic acids, phospholipids and phosphoproteins. According to McDonald *et al*. (2002) P also plays a role in many aspects of metabolism, including the energy metabolism such as the formation sugar-phosphates. According to McDonald *et al*. (2002) and Kleyn (2012) Vit D_3 plays a vital role in the requirements of the Ca to P ratio. When giving a P supplement, McDonald *et al*. (2002) reports that it is important to take the Ca:P ratio into consideration. The Ca:P ratio is discussed in section 4.2.8 below. P are well absorbed (McDonald *et al.*, 2002) even if the levels in the body are in excess of the requirements. Excess P can be excreted, mainly through the kidneys, in monogastric animals, such as cheetah, to restore the balance. During resorption of Ca from bone, which is under the control of the parathyroid gland, P is also released, but unlike the Ca the excess P is excreted and lost. This loss leads to a lack of P when Ca is rebuilt into bone.

The phosphorous content of the meat remained between 7.20g/kg DM and 8.90g/kg DM, with a mean of 8.00g/kg DM, for the pooled beef samples (Table 4.2). The P level for the buffalo sample, 8.60g/kg DM, was not numerically significantly different from the P level in the beef. From Graph 4.7 it can be seen that there is little variation between the P levels in the pooled supplemented beef and buffalo meat fed during the experimental period. The degrees of freedom in the feed analyses results did not allow for statistical analyses to be done and it cannot be said with certainty if this difference between the buffalo and beef samples is significantly different or not. It is, however, known that no specific P supplementation was done by means of the CVM-supplement. P levels remained constant during the entire experimental period. AAFCO recommends a minimum P level of 8.00g/kg (blue line on Graph 4.7) for growth and reproduction and a minimum level of 5.00g/kg (green line on Graph 4.7) for maintenance in adult cats.



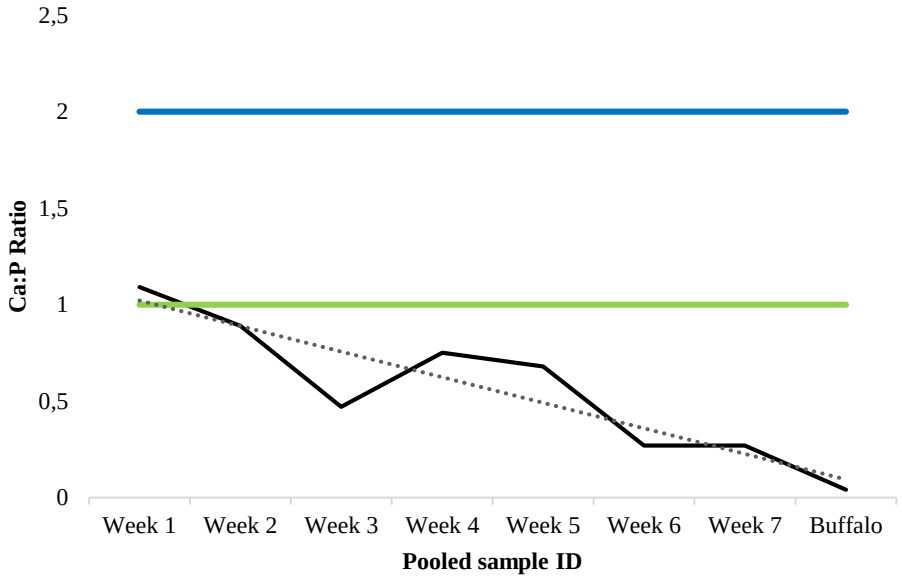
Graph 4.7 P (g/kg DM) levels in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.8 Calcium to Phosphorous ratio (Ca:P)

Ca and P cannot realistically be discussed independently. It is important to consider the Ca:P ratio when formulating a feed for any animal, regardless of the species. Kleyn (2012) reports that the Ca and P interacts with each other both before and after the absorption from the digestive tract. McDonald *et al*. (2002) reports that when considering the Ca:P ratio in a supplement an abnormal ratio can be more harmful than a deficiency of either Ca or P in the diet. As previously reported, Kleyn (2012) and McDonald *et al*. (2002) emphasised that Vit D_3 plays a vital role in the requirements for both Ca and P. The bioavailability of the source from

which the Ca and P are supplemented also play a role in the amount of the source to be added to the supplement (Kleyn, 2012).

The required Ca:P level recommended by Fowler (1986) (as referred to in Bell, 2010) and McDonald *et al*. (2002) is 1:1 to 2:1. The requirement levels of 1:1 (green line) and 2:1 (blue line) Ca:P are indicated on Graph 4.8. From Table 4.2 can be seen that only week one of the beef pooled samples had a ratio larger than the minimum requirement (1.09:1) of 1:1 Ca:P. The mean Ca:P ratio for all the beef samples were 0.69:1, which is well below the lower recommended Ca:P ratio. It can clearly be seen that the buffalo meat had the lowest Ca:P ratio (0.04:1). It is, however, not possible to say if any of these values differs significantly or not, as it was impossible to do a statistical analysis on the data of the nutritional analyses of the feeds. The variation in Ca:P ratio is an indication that CVM-supplement was not used according to regulation, as the Ca level in the feed (from calcium carbonate in the CVM-supplement) is varies, while the P level stays constant. The steady P level suggests that the overall meat mineral level. The low values might also be a result of sampling error. It is recommended that the sampling should be done to try and get a good representation of the whole feeding crate in the sample. The CVM-supplement can also possibly be presented by means of mixing the supplement with minced meat into a meatball. This might help to ensure that each animal receives the recommended amount of supplement per day.



Graph 4.8 Ca:P ratio in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

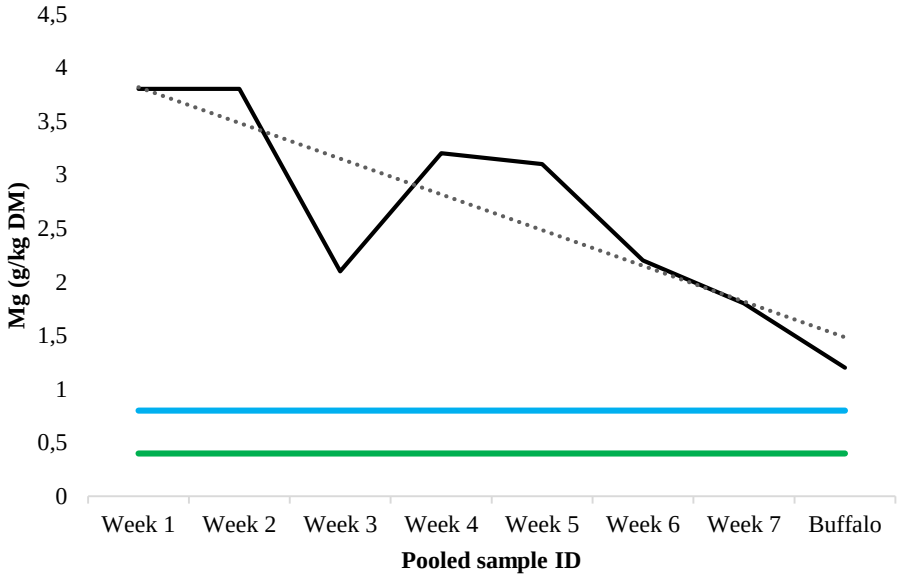
4.2.9 Magnesium (Mg)

Mg, a major element, is closely associated with Ca and P levels (McDonald *et al*., 2002). According to Kleyn (2012) and McDonald *et al*. (2002) approximately 70% of all Mg in the body of mammals can be found in the skeletal structure. The authors further report that Mg is very important for the overall wellbeing of the animal, in particular with regards to the ionic balance of the body and for the role in some enzyme systems.

To date no recommendations for the mean Mg intake have been established for cheetah in captivity. It can, however, be seen from Graph 4.9 that there was variation in the amount of Mg between the different beef samples. The mean Mg level in the different beef samples were 2.86g/kg DM, with week one and two as a maximum at 3.80g/kg DM and week seven as a minimum at 1.80g/kg DM. The buffalo sample had a value of 1.20g/kg DM. It is, however, not possible to say whether there was a significant difference between samples, as the degrees of freedom of the feed analysed did not allow for statistical analyses. AAFCO recommends minimum level of 0.80g/kg Mg for growth and reproduction (blue line on Graph 4.9) and a minimum level of 0.40g/kg for adult maintenance requirements (green line on Graph 4.9). The nutritional levels of Mg are not established for cheetah, but Grobler (2010) recommends that a high level of Mg should be supplemented to the feed to reduce the incidence of metacarpal deformity. The author also reports that excessive levels of Mg may increase the incidence of kidney stone formation. The level of Mg in the total feed analysed was higher

than the requirement for growth, reproduction and maintenance, as recommended by AAFCO, during the entire experimental period.

Mg is added in the CVM-supplement in the form of magnesium oxide contributing ca. 51% Mg m/m. The variation in the feed Mg also suggests that the CVM-supplement was not added consistently. The variation can also be attributed to sampling error.

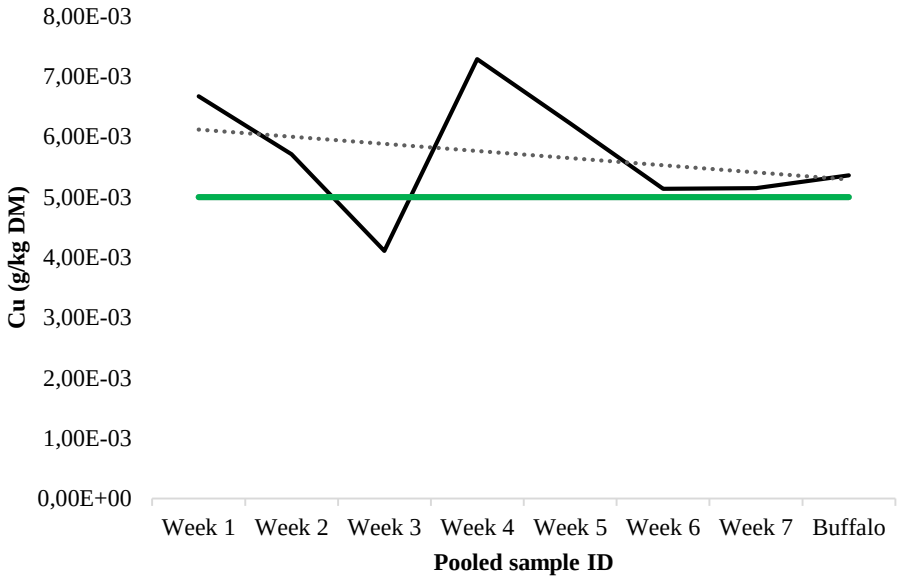


Graph 4.9 Mg (g/kg DM) level in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.10 Copper (Cu)

Cu plays a role in the synthesis of haemoglobin (Hb) and an animal with a Cu deficiency has an impaired ability to absorb Fe (McDonald *et al*., 2002). Cu and Fe is discussed by Kleyn (2012) as a single point of importance and reports that approximately five to ten times less Cu is required in the diet in relation to Fe. Toxicity can easily occur. McDonald *et al*., (2002) reports that Cu is also part of the structure of other proteins found in blood. It is further reported by the author that it also plays a role in some enzyme systems and is important for normal pigmentation of hair and fur. According to McDonald *et al*., (2002) the liver of the animal is the main storage site for Cu. It is possible to use the liver of animals such as beef as a natural means of supplementing Cu to the diet.

The results of the analyses for beef and buffalo samples over the seven week experimental period are listed in Table 4.2. Graph 4.10 also illustrates the different levels of Cu found between the weeks. The mean Cu level in the beef samples were $5.76x10^{-3}$g/kg DM and for the buffalo sample it was 5.36 $x10^{-3}$g/kg. It can be seen that there was only a variation of 3.18 $x10^{-3}$g/kg, although it is not possible to report whether this variation is significant or not, as the data did not comply with the basic requirements for statistical analyses. AAFCO recommends a minimum Cu value of $5.00x10^{-3}$g/kg for growth, reproduction and adult maintenance (green lien on Graph 4.10). The diet sufficiently supplied high enough levels of Cu to meet these requirements. Cu glycinate is used to supply the CVM-supplement with ca. 24% Cu m/m.

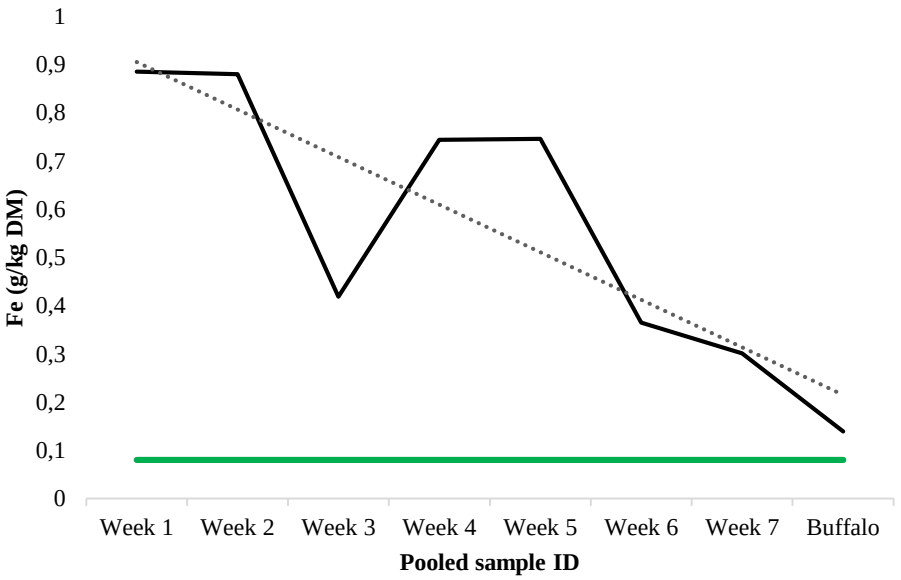


Graph 4.10 Cu (g/kg DM) level in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.11 Iron (Fe)

Iron is a trace element (McDonald *et al*., 2002). According to Kleyn (2012) Fe and Cu is important for the production of red blood cells (RBC) and a deficiency of Fe will lead to anaemia and irregular RBC. Fe is important in the production of Hb and is important for oxygen transport by the blood during respiration (Kleyn, 2012). The author further reports that approximately five to ten times more Fe is required in the diet than Cu. Fe also play a major role in some biochemical reactions and forms part of structures such as the proteins transferrin and ferritin (McDonald *et al*., 2002).

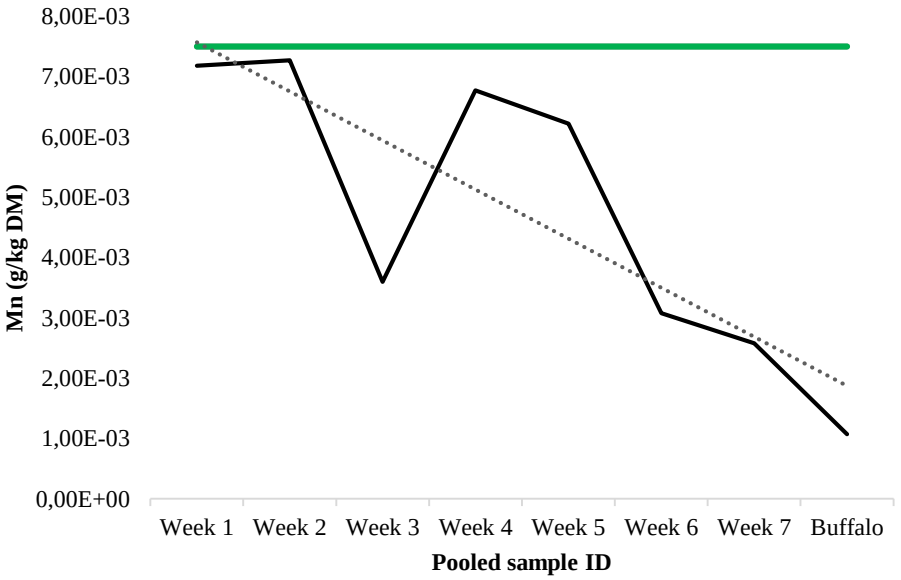
No recommendations for Fe inclusion are available for cheetah in captivity. AAFCO recommends an Fe level of minimum 0.08g/kg (green line on Graph 4.11) for growth, reproduction and adult maintenance. Fe levels exceeds this minimum by far. As reported above, the ratio of Fe to Cu is within the recommendation by Kleyn (2012). Not all Fe is not necessarily bioavailable. Fe are found in two different forms throughout the body tissues (Duke *et al*., 1942). It can either be hematin iron or non-hematin iron. The latter is the only form that can be used to produce Hb. It is, however, important to ensure the amount of Fe given to cheetah through their diet is sufficient to support all the different metabolic functions for which Fe is required. The results of the feed analyses are shown in Table 4.2. In Graph 4.11 it can be seen that a high variation of Fe existed in the meat samples. This high variation might be attributable to sampling error or an error made during analyses of the feed sample. Furthermore, 90% of all Fe is combined with proteins in the body, of which the most important is Hb (McDonald *et al*., 2002). Most Hb is found in blood. During the slaughter process animals are bled and this may cause a loss of Fe from the meat, due to blood loss. Lower levels of Fe might, for the afore mentioned reason, be attributed to different levels of blood left over in the muscle after bleeding of animals.



Graph 4.11 Fe (g/kg DM) level in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.12 Manganese (Mn)

The total amount of Mn in the animal body is very low (McDonald *et al*., 2002). Kleyn (2012) reports that Mn is related to bone formation. McDonald *et al*., (2002) describes Mn as an activator for many enzymes in the animal body. The author further reports that high levels of Ca and P can lead to increased Mn deficiency symptoms. The levels of Mn were highly variable over the experimental period as illustrated by Graph 4.12. The specific values found in the samples of both beef and buffalo meat are given in Table 4.2. It is, however, not possible to report whether this variation is significant or not, as the data did not comply with the basic requirements for statistical analyses. There is a mineral interaction between Mn and Fe (O'Dell, 1989). This interaction explains the similarity in the change of Mn and Fe levels found in the meat over time. AAFCO recommends a Mn level of $7.50x10^{-3}$g/kg for growth, reproduction and adult maintenance (green line in Graph 4.12).

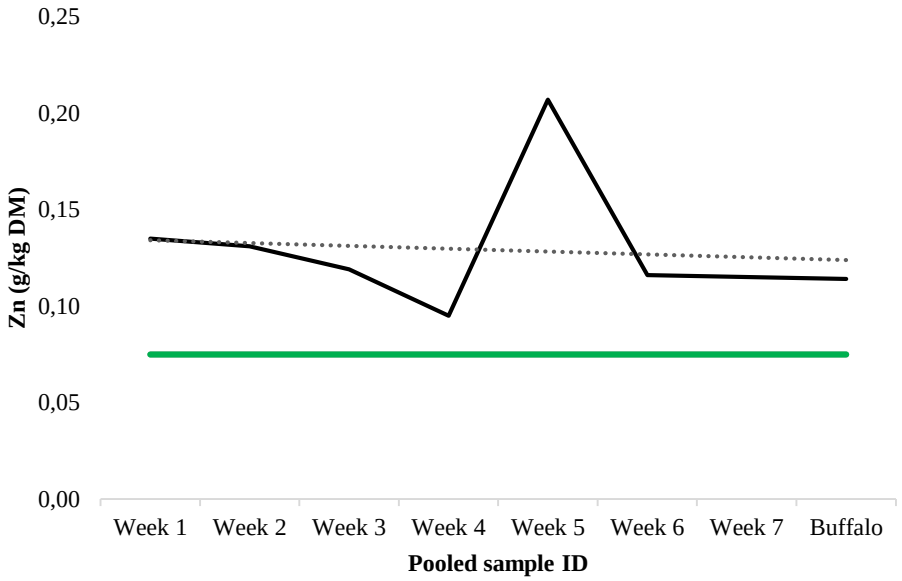


Graph 4.12 Mn (g/kg DM) level in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.13 Zinc (Zn)

Kleyn (2012) reports that Zn is important to the overall immune response in the animal body and an improved immune response has been reported with increased levels of Zn. Zn is incorporated into the structure of many enzymes in the animal body (Kleyn, 2012 & McDonald *et al*., 2002). It is found in trace amounts in all tissues in the animal body. Zn is important for cell replication and maturation, especially in nucleic acid metabolism (Thompson *et al.*, 2008 & McDonald *et al*., 2002). Thompson *et al*. (2008) further reports that Zn plays a role in sexual maturation. Sperm quality and fertility of cheetah have been significantly correlated with plasma concentrations of Cu, Zn and Ca (unpublished data by Dierenfeld and Howard as referenced by Bechert *et al*., 2012).

The levels of Zn were variable for the beef samples over the experimental period, as well as for the buffalo sample in relation to the beef (Graph 4.13). Table 4.2 contains specific values of all samples. The significance of the differences is unknown as no statistical analyses were possible. AAFCO recommends a Zn level of 0.075g/kg for growth, reproduction and adult maintenance requirements (green line Graph 4.13). It is clear from Graph 4.13 that the level of Zn was sufficient to meet the requirements of all the animals in the trial.

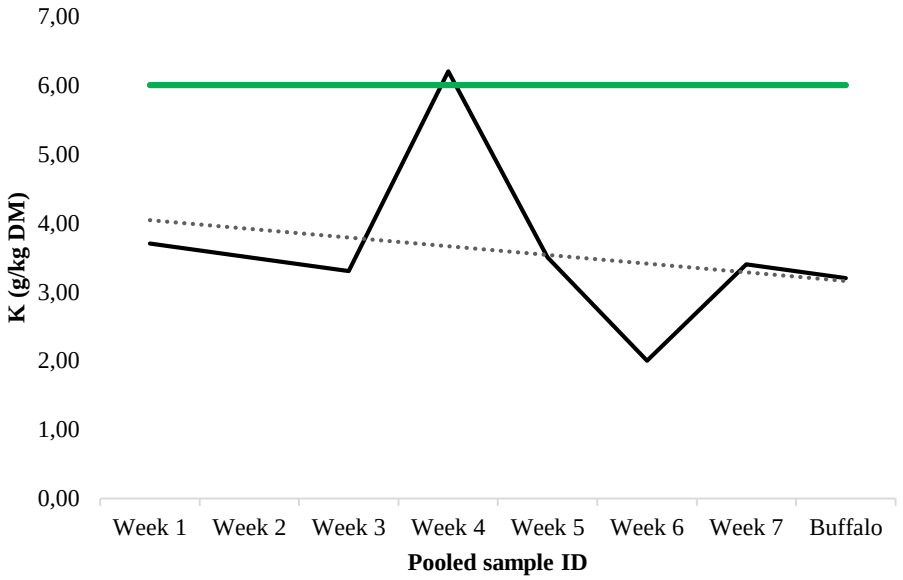


Graph 4.13 Zn (g/kg DM) level in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.14 Potassium (K)

K is involved in the osmotic regulation of the body fluids and acid-base equilibrium of the animal body, together with Na and Cl (Kleyn, 2012; McDonald *et al*., 2002). According to McDonald *et al*., (2002) K functions mainly as the cation of the body cells and plays a key role in nerve and muscle excitability. Kleyn (2012) reports that there exists a relationship between Na and K and if the levels of one in the feed is increased, the requirement for the other ion automatically increases.

The levels of K were variable for the beef samples over the experimental period, with a mean of 15.10g/kg DM. The buffalo sample had a K level of 13.90g/kg DM. Graph 4.14 depicts these variations. Table 4.2 contains specific K values of all samples. AAFCO suggests a K value of 6.00g/kg (green line on Graph 4.14) for growth, reproduction and adult maintenance. The significance of the differences is unknown as no statistical analyses were possible.

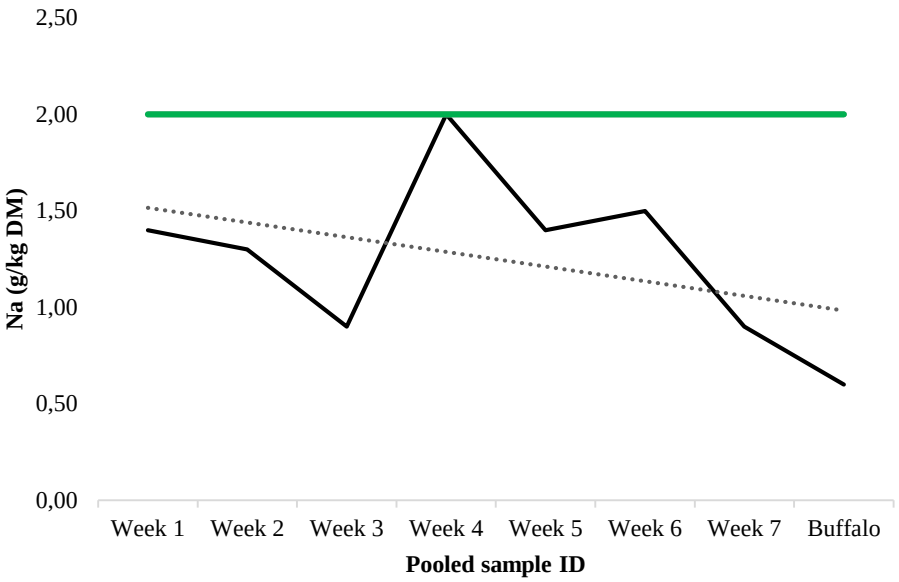


Graph 4.14 K (g/kg DM) level in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.15 Sodium (Na)

Most of the Na is present in the body fluids and soft tissues of animals. Like K, Na is involved in the osmotic regulation of the body fluids and acid-base equilibrium of the animal body, together with Cl (McDonald *et al*., 2002). The author further reports that Na is the main cation of the blood and other extracellular fluids in the animal body. Na plays an important role in the absorption of sugars and amino acids in the digestive tract, as well as playing a role in the transmission of nerve impulses (McDonald *et al*., 2002). Na is added to the diet of the cheetah at HESC in the form of NaCl by means of the CVM-supplement. The Na contributed to the diet in this way is ca. 39.3% Na m/m.

No recommendations for Na levels have been established for cheetah in captivity. AAFCO suggests a value of 0.20g/kg (green line on Graph 4.15) for cats of all physiological stages and ages. The levels of Na were variable for the beef samples over the experimental period, with a mean of 5.60g/kg DM (Graph 4.15). The buffalo sample had a Na level of 2.50g/kg DM. The variability between the samples can be attributed to sampling error or wrong application or uneven application of the CVM-supplement. Looking at the analyses done during this trial, the amount of Na supplied to the cheetah was not sufficient to meet the 0.20g/kg Na requirement for cats in any physiological status, including growth, pregnancy and maintenance in adult cats. Table 4.2 contains specific Na values of all samples. The significance of the differences is unknown as no statistical analyses were possible.

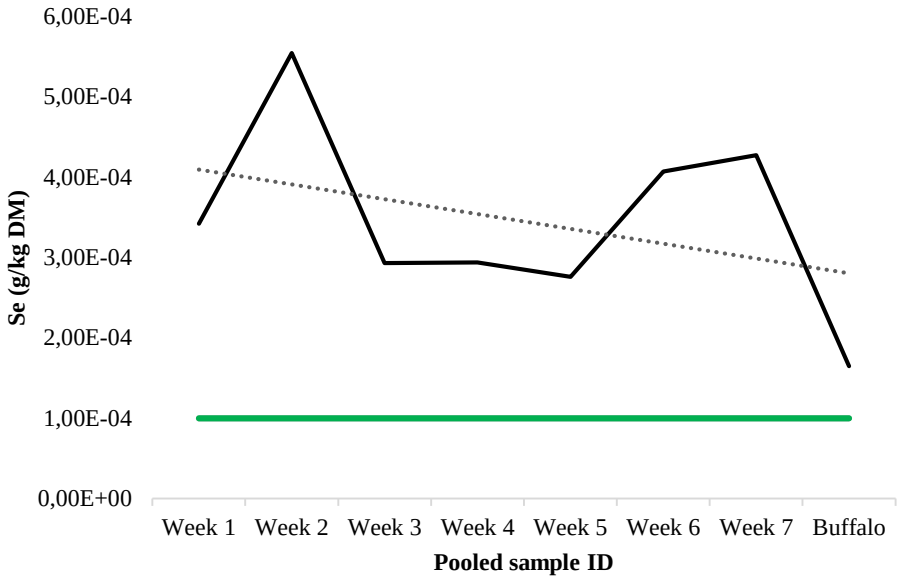


Graph 4.15 Na (g/kg DM) level in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.16 Selenium (Se)

Kleyn (2012) reports that Se is required in small amounts in the diet and is closely associated with vitamin E (Vit E). Se plays a key role in the protection of cell membranes from oxidative damage by hydrogen peroxide (H_2O_2), as Se is part of the structure of the enzyme that removes H_2O_2 after Vit E (McDonald *et al*., 2002). Other functions reported by the author includes a sparing effect on Vit E, playing a role in the immune system, protection against heavy metal poisoning, forms a part of the enzyme converting T_4 to the physiologically active T_3.

No feed guidelines for Se has been given for cheetah in captivity. AAFCO suggests a level of $1.00x10^{-4}$ g/kg Se in the diet of growing, pregnant and adult cats. The levels of Se were variable for the beef samples over the experimental period, with a mean of $3.70x10^{-4}$g/kg DM (Graph 4.16). The buffalo sample had a Se level of $1.65x10^{-4}$g/kg, much lower than the mean of the beef samples. Both the beef and the buffalo diet sampled during this trial supplied more than the required Se for cats in all physiological statuses. Table 4.2 contains specific Se values of all analysed samples of supplemented meat. The significance of the differences is unknown as no statistical analyses were possible.



Graph 4.16 Se (g/kg DM) level in supplemented meat fed at HESC over the entire experimental period measured from pooled samples per week.

4.2.17 Summary of feed analyses

In this section all the feed analyses results are given (Table 4.2) and discussed. The results of the feed analyses showed variations of the minerals in the feed over the experimental period. High variations were especially observed in the specific minerals that is included in the CVM-supplement and this might imply poor dosing of the supplement and/or sampling errors. This supports the theory that either the supplement was not added to the meat chunks as recommended or that the amount of supplement included per analysed sample was not representative of the supplemented meat provided per individual animal.

In order to address the above mentioned problems it is recommended to ensure that all feed samples are representative for the entire feeding crate for future sampling. Sampling should take into account what ratio of supplement to meat chunk is applied for each animal and the sample should contain a representative amount of CVM-supplement. Sample size can also be increased in order to allow all the required nutrient analyses of multiple samples per week. If enough samples can be analysed per week, more degrees of freedom become available and enable the use of statistical analyses. A further recommendation that can be made is that the CVM-supplement should be presented to the animals in a way to ensure that the animals receive their total daily requirement of the supplement. This might be in the form of a separate meatball given to each individual per feeding, in addition to the meat chunk feeding.

4.3 Blood parameters

In this section the results of the blood parameters determined during this trial, by on site analyses (including IDEXX VetTest® 8008) and blood samples analysed by IDEXX and AMPATH laboratories, will be presented and discussed. The raw data of analyses done by IDEXX and AMPATH laboratories, as well as the on-site analyses done using the IDEXX VetTest® 8008, are added in Appendix D. Statistical analyses were done for the raw data in Appendix D and will be presented and discussed in this section. Blood sample values from tables added in Appendix D were statistically analysed.

Table 4.3 shows the results for the statistical analyses of all the blood parameters measured in blood serum and plasma (Mean±SD) during this trial. Table 4.3 will also be included into the discussion below.

Table 4.3 Results for statistical analyses of blood parameters measured in blood serum and plasma (Mean±SD)

Blood parameter	Unit	Week 1			Week 3			Week 5			Week 7			x		
		C	L	$\bar{x}$	C	L	$\bar{x}$	C	L	$\bar{x}$	C	L	$\bar{x}$	C	L	x
Albumin (Alb)	g/l	35.72 ±2.588[12]	34.82 ±1.483[12]	35.27 ±2.058[ab]	37.12 ±2.168[1]	34.82 ±1.483[2]	35.97 ±2.160[ab]	37.21 ±1.826[12]	36.42 ±1.517[12]	36.81 ±1.581[a]	35.52 ±3.782[12]	34.82 ±0.837[12]	35.17 ±2.616[b]	36.39 ±2.608[*]	35.38 ±1.436[#]	35.77 ±2.146
Ca	mmol/l	2.58 ±0.052	2.57 ±0.058	2.57 ±0.052	2.57 ±0.071	2.55 ±0.039	2.56 ±0.055	2.58 ±0.159	2.55 ±0.091	2.57 ±0.118	2.65 ±0.106	2.53 ±0.066	2.59 ±0.104	2.59 ±0.097	2.55 ±0.062	2.57 ±0.083
Ca:P ratio		0.94 ±0.184	0.99 ±0.277	0.96 ±0.224	0.97 ±0.279	0.88 ±0.182	0.93 ±0.226	0.92 ±0.194	0.98 ±0.159	0.95 ±0.165	0.94 ±0.176	0.93 ±0.147	0.93 ±0.153	0.94 ±0.196	0.94 ±0.187	0.94 ±0.189
Cortisol (Cort)	nmol/l	89.75 ±69.261	71.98 ±38.619	80.86 ±53.689	104.93 ±89.378	78.26 ±50.965	91.59 ±70.871	64.24 ±21.831	74.67 ±46.837	69.46 ±36.133	75.65 ±95.200	68.86 ±22.397	72.25 ±28.584	83.65 ±58.099	73.44 ±37.841	78.91 ±48.735
Creatinine (Creat) Vet Test	µmol/l	167.16 ±34.040	175.97 ±48.216	171.57 ±39.510[ab]	213.36 ±43.999	174.57 ±34.953	193.97 ±43.182[a]	212.02 ±83.396	179.17 ±60.491	195.60 ±68.341[a]	170.36 ±46.163	141.97 ±29.467	156.17 ±39.871[b]	190.73 ±52.710	167.93 ±44.034	178.49 ±49.140
Creatinine (Creat) IDEXX	µmol/l	233.37 ±57.130	245.11 ±76.206	239.24 ±63.672[ab]	291.17 ±42.105	267.11 ±71.535	279.14 ±57.112[a]	291.82 ±68.911	263.91 ±67.763	277.87 ±64.950[ab]	241.57 ±70.776	220.71 ±39.884	231.14 ±50.022[b]	264.48 ±61.412	249.21 ±62.935	255.95 ±61.755
Free Triiodothyronine (Free T_3)	pmol/l	4.56 ±0.495	4.33 ±0.200	4.30 ±0.286[a]	3.82 ±0.378	3.77 ±0.466	3.79 ±0.401[bc]	3.63 ±0.265	3.63 ±0.265	3.63 ±0.237[c]	4.40 ±0.390	4.39 ±0.409	4.39 ±0.377[a]	4.03 ±0.475	4.03 ±0.490	4.04 ±0.475
Free Tyroxine (Free T_4)	pmol/l	7.73 ±4.095[12]	14.39 ±7.863[12]	11.06 ±6.866	12.20 ±4.044[12]	16.00 ±3.344[12]	14.10 ±4.025	11.62 ±7.442[2]	23.42 ±14.222[1]	17.52 ±12.687	16.78 ±4.479[12]	21.92 ±4.936[12]	19.35 ±4.848	12.08 ±6.056[*]	18.94 ±8.856[#]	15.61 ±8.199
Globulin (Glob)	g/l	35.98 ±3.421	34.22 ±2.363	35.10 ±3.100	37.58 ±4.919	35.99 ±4.183	36.78 ±4.408	36.15 ±4.193	37.19 ±3.271	36.67 ±3.539	35.98 ±4.550	34.99 ±3.464	35.48 ±3.864	36.42 ±4.019	35.60 ±3.388	36.05 ±3.698
HDL	mmol/l	2.64 ±0.378	2.56 ±0.251	2.60 ±0.306[bc]	2.70 ±0.224	2.78 ±0.228	2.74 ±0.217[ab]	2.90 ±0.392	3.00 ±0.406	2.95 ±0.378[a]	2.38 ±0.114	2.40 ±0.255	2.39 ±0.187[c]	2.65 ±0.330	2.69 ±0.356	2.66 ±0.340
Lactate (Lac) Vet Test	mmol/l	4.74 ±1.762	4.84 ±1.994	4.79 ±1.775	5.78 ±2.989	3.49 ±2.330	4.63 ±2.799	5.28 ±2.458	5.53 ±4.041	5.40 ±3.232	5.28 ±2.458	5.53 ±4.041	5.40 ±3.232	5.48 ±2.781	3.16 ±1.334	4.32 ±2.390
Lactate (Lac) IDEXX	mmol/l	4.29 ±0.820	3.35 ±1.778	3.82 ±1.389	4.19 ±3.136	3.83 ±3.618	4.01 ±3.197	4.43 ±1.992	4.46 ±4.200	4.44 ±2.675	4.89 ±2.749	3.17 ±1.768	4.03 ±2.281	4.45 ±2.167	3.70 ±2.593	4.07 ±2.385
LDL	mmol/l	0.58 ±0.351	0.50 ±0.283	0.54 ±0.306	0.60 ±0.224	0.56 ±0.228	0.58 ±0.217	0.53 ±0.392	0.58 ±0.406	0.56 ±0.378	0.52 ±0.114	0.34 ±0.255	0.43 ±0.187	0.56 ±0.330	0.50 ±0.356	0.53 ±0.340
Packed cell volume (PCV)	%	53.09 ±3.620	57.33 ±4.126	55.21 ±4.251	55.09 ±2.782	53.56 ±4.130	54.32 ±3.433	56.27 ±4.649	58.58 ±5.097	57.43 ±4.788	58.56 ±10.448	55.19 ±5.196	56.87 ±7.995	55.75 ±6.063	56.16 ±4.718	55.95 ±5.346
Phosphorous (P)	mmol/l	2.42 ±0.454	2.55 ±0.724	2.48 ±0.576	2.50 ±0.701	2.26 ±0.465	2.37 ±0.571	2.34 ±0.373	2.50 ±0.454	2.42 ±0.397	2.47 ±0.385	2.35 ±0.401	2.42 ±0.373	2.43 ±0.460	2.41 ±0.497	2.42 ±0.473
Total Cholesterol (TChol)	mmol/l	3.65 ±0.207	3.46 ±0.513	3.55 ±0.398[ab]	3.45 ±0.422	3.66 ±0.439	3.55 ±0.422[ab]	3.79 ±0.695	3.96 ±0.808	3.87 ±0.716[a]	3.57 ±0.084	3.42 ±0.358	3.49 ±0.246[b]	3.62 ±0.400	3.62 ±0.554	3.62 ±0.479
Total Protein (TP)	g/l	71.34 ±5.814	70.66 ±5.273	71.00 ±5.259	74.34 ±6.731	70.86 ±4.764	72.60 ±5.851	73.18 ±5.802	73.26 ±4.147	73.22 ±4.622	71.34 ±8.204	69.66 ±4.219	70.50 ±6.240	55.75 ±6.310	56.16 ±4.454	71.79 ±5.420
Triglycerides (TG)	mmol/l	0.91 ±0.589	0.92 ±0.228	0.92 ±0.421	0.73 ±0.462	0.78 ±0.471	0.76 ±0.440	1.60 ±2.092	1.10 ±0.608	1.35 ±1.374	0.78 ±0.612	1.22 ±0.716	1.01 ±0.666	1.01 ±1.021	1.01 ±0.523	0.99 ±0.794
Urea (BUN) Vet Test	mmol/l	11.72 ±1.419	11.79 ±1.119	11.75 ±1.205	11.02 ±0.999	11.45 ±1.744	11.23 ±1.357	12.43 ±1.132	12.32 ±1.907	12.38 ±1.517	11.46 ±1.539	11.81 ±1.631	11.63 ±1.504	11.66 ±1.280	11.84 ±1.436	11.73 ±1.398
Urea (BUN) IDEXX	mmol/l	11.52 ±1.376	11.46 ±1.335	11.49 ±1.279	10.96 ±1.092	11.34 ±1.837	11.15 ±1.437	12.35 ±0.987	12.26 ±2.065	12.31 ±1.580	11.66 ±1.651	11.76 ±1.540	11.71 ±1.508	11.62 ±1.292	11.71 ±1.617	11.65 ±1.450
Vitamin D_3 (Vit D_3)	ng/ml	26.85 ±9.367	25.81 ±7.035	26.33 ±7.190	24.13 ±8.384	24.61 ±7.120	24.37 ±7.333	26.85 ±6.519	25.25 ±5.198	26.05 ±4.550	27.67 ±6.017	29.07 ±5.656	28.37 ±5.646	26.38 ±7.165	26.18 ±5.920	26.26 ±6.469

[ab] Values with different superscript differ significantly (P<0.05) using the Fisher test.
[12] Values with different superscript differ significantly (P<0.05) using the Fisher test.
[*#] Values with different superscript differ significantly (P<0.05) using the Fisher test.

C refers to control group.
L refers to lure group.

4.3.1 Albumin (Alb)

A reference value range for the Alb level given by the IDEXX VetTest® 8008 for adult domestic cats is 22.00g/l to 40.00g/l. The results from the statistical analyses are shown in Table 4.4. The mean values for two groups over time were 35.77g/l. When referring to the type III sum of squares (type III SS), both the treatment (p-value of 0.0415) and weight (p-value of 0.0002) had an effect on the Alb levels in the animal blood, according to the Fisher test with $p<0.05$. Bechert *et al*. (2002) reported a mean of 37.80g/l and 43.30g/l for their two experimental periods. The total variation of the Alb value for the two experimental periods of Bechert *et al*. (2002) were between 35.00g/l and 47.00g/l. Depauw *et al*. (2012) found a mean Alb level of 40.00g/l in a study on six cheetah fed a supplemented beef diet. All the values from the trial were within the normal range of the VetTest®, as well as the overall range found by Bechert *et al*. (2002). The R^2 for the Alb levels was 0.146, which means that the statistical model explains 14.6% of the variation in the Alb levels over time, in this case the treatment and the weight of the animal. The low R^2 value shows that the model used was not a good fit for the data analysed ($p=0.739$). The rest of the variation can be attributed to multiple different variables, including weather and age.

Cunningham (2002) describes Alb as one of the plasma proteins produced by the liver and deposited into the blood while blood passes through the liver. In the total serum protein of a healthy animal, Alb is the most abundant (IDEXX VetTest® Manual). Alb, together with Glob, are one of the important components of the immune response (Cunningham, 2002). Alb is further described as a transporter of many molecules, including cortisol, fatty acids, steroid transport and thyroid hormones. Cunningham (2002) reports that Alb also plays a role in the glomerular filtration system, as it cannot pass through the selective barrier and molecules bound to Alb are also retained within the blood stream. Bechert *et al*. (2002) reports that a negative nitrogen balance is the main reason for protein deficiency and it can lead to hypoalbuminaemia. Alb levels are tested to give an indication of the hepatic and renal function, and also the degree of hydration in the tested individual (IDEXX VetTest® Manual). The manual further reports that it is important to do complementary tests with Alb tests. Alb and Glob are used to calculate the total protein (TP). This calculation is automatically done by the VetTest® analyser.

Although there is a statistically significant difference between the mean Alb levels of the lure and control group, the difference is not numerical significant. The low numerical difference between the two groups might imply that exercise did not have an added benefit to the lure group. The Alb results indicated no significant deviation from the reference values, which might suggest that the animals from both experimental groups were in good health, pertaining to hepatic and renal function, as well as fluid balance throughout the experimental period.

Table 4.4 Control vs lure over time for Alb levels (g/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	35.72 (± 2.588)^{12}	34.82 (± 1.483)^{12}	35.27 (± 2.058)^{ab}
3	37.12 (± 2.168)^{1}	34.82 (± 1.483)^{2}	35.97 (± 2.160)^{ab}
5	37.21 (± 1.826)^{12}	36.42 (± 1.517)^{12}	36.81 (± 1.581)^{a}
7	35.52 (± 3.782)^{12}	34.82 (± 0.837)^{12}	35.17 (± 2.616)^{b}
$\bar{x}$	36.39 (± 2.608)*	35.38 (± 1.436)$^{\#}$	35.77 (± 2.146)

^{ab} Row means with different superscript differ significantly ($P<0.05$) using the Fisher test.
^{12} Values with different superscript differ significantly ($P<0.05$) using the Fisher test.
$^{*\#}$ Column means with different superscript differ significantly ($P<0.05$) using the Fisher test.

4.3.2 Globulin (Glob)

A reference value range for the Glob level given by the IDEXX VetTest® 8008 for adult domestic cats is 28.00-51.00g/l. The results from the statistical analyses are shown in Table 4.5. The mean value for both treatments over the entire experimental period was 36.05g/l. Bechert *et al*. (2002) only measured Glob level in one of their two experimental periods. The range of Glob values that they reported was between 24.00g/l and 33.00g/l, with a mean value of 28.50g/l. Our mean falls outside what Bechert *et al*. (2002) found, but falls within the range provided by the VetTest® software. When referring to the type III sum of squares (type III SS), animal weight ($p<0.0001$) had a significant effect on the Glob levels in the animal blood, according to the Fisher test with $p<0.05$. The R^2 for Glob levels was 0.600, which means that the statistical model explains 60%

of the variation in the Glob levels over time, in this case the treatment and the weight of the animal. The R^2 value shows that the model used was a good fit for the data analysed (p=0.0003). The rest of the variation can be attributed to multiple different variables, including weather and age.

Cunningham (2002) reports that Glob is one of the primary plasma proteins. The author further describes Glob, together with Alb, as one of the most important components of the immune response. A specific corticosteroid-binding Glob, also known as transcortin, has a high affinity for cortisol. It carries 75% of the total cortisol in the blood plasma (Cunningham, 2002). Glob is also used, in combination with Alb to calculate the total protein (TP), automatically done by the VetTest® analyser.

There was, however, no significant difference of the Glob levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals. There was also no significant statistical or numerical difference in the cortisol (Cort) levels in the serum over time between the two experimental groups or for animal body size. This might also support that there was no significant difference between the two groups over the entire experimental period, or within any group over time. The Glob results indicated no significant deviation from the reference values, which might suggest that the animals from both the control and lure group was, pertaining to hepatic function, renal function and fluid balance, in good health, throughout the experimental period.

Table 4.5 Control vs lure over time for Glob levels (g/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	35.98 (± 3.421)	34.22 (± 2.363)	35.10 (± 3.100)
3	37.58 (± 4.919)	35.99 (± 4.183)	36.78 (± 4.408)
5	36.15 (± 4.193)	37.19 (± 3.271)	36.67 (± 3.539)
7	35.98 (± 4.550)	34.99 (± 3.464)	35.48 (± 3.864)
$\bar{x}$	36.42 (± 4.019)	35.60 (± 3.388)	36.05 (± 3.698)

[ab] Row means with different superscript differ significantly (P<0.05) using the Fisher test.
[12] Values with different superscript differ significantly (P<0.05) using the Fisher test.
[*#] Column means with different superscript differ significantly (P<0.05) using the Fisher test.

4.3.3 Total Protein (TP)

The IDEXX VetTest® 8008 for standard range for TP levels in adult domestic cats is 57.00-89.00g/l. Results from the statistical analyses are shown in Table 4.6. The mean value for the duration of the experimental period for both treatments is 71.79g/l. Bechert *et al*. (2002) had a two phase trial and the mean TP values for the two periods were 66.30g/l and 66.00g/l respectively. The authors had an overall range for the TP values of 60-76g/l. The mean value for this trial falls in what Bechert *et al*. (2002) found, and falls within the range provided by the VetTest® software. When referring to the type III sum of squares (type III SS) it is seen that animal weight (p<0.0001) had an effect on the TP levels in the animal blood, according to the Fisher test with p<0.05. The R^2 for TP levels was 0.601, which means that the statistical model explains 60.10% of the variation in the TP levels over time, in this case the treatment and the weight of the animal. The R^2 value shows that the model used was a good fit for the data analysed (p=0.0002). The rest of the variation can be attributed to multiple different variables, including weather and age.

The TP of serum refers to all the proteins found in the aqueous phase of the blood (IDEXX VetTest® Manual). The manual further reports that Alb is the major part of the TP and the rest of the TP is composed of α-, β- and γ-globulins, and the Glob concentration can be determined by simply subtracting the Alb concentration from the TP concentration. Cunningham (2008) reports that Plasma proteins or TP are synthesized in the liver. TP concentration can be used to evaluate hepatic and renal function, degree of hydration and it might also indicate gastrointestinal lesions (IDEXX VetTest® Manual). The manual recommends measuring the Alb concentration with TP to evaluate hepatic and renal function.

Considering the TP and Alb levels that was measured, no abnormality from reference values for cats could be found, which might be an indication that the renal and hepatic health of these animals were within recommendation and they had a good fluid balance throughout the experimental period. It might be suggested that exercise had no significant influence on either group of animals since no significant difference could be found between the control and lure group over time.

Table 4.6 Control vs lure over time for TP levels (g/l) measured in blood serum (Mean±SD)

Week	Control	Lure	$\bar{x}$
1	71.34 (± 5.814)	70.66 (± 5.273)	71.00 (± 5.259)
3	74.34 (± 6.731)	70.86 (± 4.764)	72.60 (± 5.851)
5	73.18 (± 5.802)	73.26 (± 4.147)	73.22 (± 4.622)
7	71.34 (± 8.204)	69.66 (± 4.219)	70.50 (± 6.240)
$\bar{x}$	55.75 (± 6.310)	56.16 (± 4.454)	71.79 (± 5.420)

4.3.4 Packed Cell Volume (PCV)

PCV were measured in the serum (vacutainer containing blood and no extra additives) as the anti-coagulant factors can influence the PCV (Cunningham, 2002). The mean PCV (Table 4.7) for both groups over the two weeks were 55.95%. Looking at other studies a PCV of 38.84% and 40.36% were found over two experimental periods, with a maximum over the two periods of 58.70%, by Bechert *et al*. (2002). Reference PCV values for cheetah in captivity are not available to date, but values for the domestic cat are reported as 35-40%, with a minimum of ≥30% as still acceptable (Ford & Mazzafero, 2012). The R^2 value of 0.233 indicates that only 23% of the variation was described by the statistical model used and 77% of the variation is due to random effects. These effects may include temperature, humidity and time of feeding.

Numerically the overall mean PCV value for the lure group was higher, but the numerical difference is not consistent throughout the seven-week period, as shown in the higher control mean values in weeks three and seven. A similar numerical difference between the control and lure group, throughout the data, could have been an indication that there was splenic contraction in the lure group, resulting in the higher PCV post exercise (lure group) as seen in weeks one and five. No clear pattern in numerical differences can be deducted from the results from this trial, which might suggest that exercise had no significant impact on animals from either treatment group.

The packed cell volume indicates the amount of blood cells in relation to the overall blood volume. A contraction (Cunningham, 2002) of the spleen during exercise can increase the PCV by 35-50%. According to Voss *et al*. (2002), PCV enables the blood to carry more oxygen to the muscle, resulting in higher levels of aerobic respiration. Cunningham (2002) reports blood PCV influences the blood viscosity, and with an increase with PCV the viscosity increases because of the increase in the number of cells. The authors further report that an abnormal high PCV is called polycythaemia (relatively too many cells). The blood of an animal can carry more than the normal oxygen level in the blood (permitting the Hb levels are high enough), which might be to the animal's benefit. A problem with this increase in blood viscosity, is that the workload on the heart increases in order to move the 'thicker' blood.

According to the statistical analyses discussed above, there was no statistically significant difference over time within the animals or between the control and lure group of animals for PCV, which might suggest that exercise had no significant influence on either group of animals.

Table 4.7 Control vs lure over time for PCV (%) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	53.09 (± 3.620)	57.33 (± 4.126)	55.21 (± 4.251)
3	55.09 (± 2.782)	53.56 (± 4.130)	54.32 (± 3.433)
5	56.27 (± 4.649)	58.58 (± 5.097)	57.43 (± 4.788)
7	58.56 (± 10.448)	55.19 (± 5.196)	56.87 (± 7.995)
$\bar{x}$	55.75 (± 6.063)	56.16 (± 4.718)	55.95 (± 5.346)

4.3.5 Blood Urea Nitrogen (BUN)

Standard range values given for BUN by the VetTest® analyser is between 5.7-12.9mmol/l. Bechert *et al*. (2002) found a range of values from 5.21mmol/l to 23.21mmol/l and means for the two experimental periods of 12.71mmol/l and 14.46mmol/l respectively. In another study by Depauw *et al*. (2012) animals on a supplemented beef diet had a mean BUN value of 16.1mmol/l. The overall mean for the BUN values are 11.73mmol/l, and no significant difference existed between the two treatment groups. The values that was found during our trial was well within the range given by the VetTest® analyser, as well as findings by other authors. The statistical analyses results for these values are given in Table 4.8. The R^2 for the BUN values are

0.146, which means that the statistical model explains 14.6% of the variation in the BUN values over time, in this case the treatment and the weight of the animal. The rest of the variation can be attributed to multiple different variables, including weather and age.

During the use of protein, as a source of energy, the nitrogen (or amine) group is removed from the amino acids during a process of deamination and released nitrogen, which is converted to ammonia (Thomson *et al*., 2008). BUN is described by the IDEXX VetTest® Manual as a highly toxic metabolite. Campbell (2008) reports that BUN is a very expensive (bioenergy wise) molecule to produce from ammonia and CO_2. The ammonia is transported to the liver, where it is combined with CO_2 to produce BUN (Thomson *et al*., 2008). The authors further report that the BUN is released into the bloodstream and transported to the kidneys for excretion. The BUN level can be used as an indicator of renal disease (IDEXX VetTest® Manual).

Table 4.8 Control vs lure over time for BUN levels (mmol/l) measured in blood serum (Mean±SD) with the VetTest® analyser.

Week	Control	Lure	$\bar{x}$
1	11.72 (± 1.419)	11.79 (± 1.119)	11.75 (± 1.205)
3	11.02 (± 0.999)	11.45 (± 1.744)	11.23 (± 1.357)
5	12.43 (± 1.132)	12.32 (± 1.907)	12.38 (± 1.517)
7	11.46 (± 1.539)	11.81 (± 1.631)	11.63 (± 1.504)
$\bar{x}$	11.66 (± 1.280)	11.84 (± 1.436)	11.73 (± 1.398)

A portion of the remaining volume of each sample was sent to IDEXX laboratories to do BUN analyses on. The range given by IDEXX for this method of BUN determination was 5.83-13.85mmol/l. The statistical analyses for these values are given in Table 4.9. All means fell within the range provided for the entire experimental period and both treatments. The R^2 value of 0.121 shows that only 12.1% of the variation is explained by the statistical model. These values do not differ significantly (p=0.8348). The rest of the variation can be attributed to multiple different variables, including weather and age.

Table 4.9 Control vs lure over time for BUN levels (mmol/l) measured in blood serum (Mean±SD) by IDEXX laboratories.

Week	Control	Lure	$\bar{x}$
1	11.52 (± 1.376)	11.46 (± 1.335)	11.49 (± 1.279)
3	10.96 (± 1.092)	11.34 (± 1.837)	11.15 (± 1.437)
5	12.35 (± 0.987)	12.26 (± 2.065)	12.31 (± 1.580)
7	11.66 (± 1.651)	11.76 (± 1.540)	11.71 (± 1.508)
$\bar{x}$	11.62 (± 1.292)	11.71 (± 1.617)	11.65 (± 1.450)

No significant numerical difference exists between the BUN results received from the VetTest® analyses done on site and the BUN analyses done at IDEXX laboratories. This is a good indicator that the BUN results monitored at HESC using the VetTest® is up to the same standards as IDEXX laboratories. There was no significant difference in the BUN levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

4.3.6 Calcium (Ca)

The VetTest® reference values given by the software for Ca is 1.95-2.83mmol/l. Statistical analyses results for Ca are given in Table 4.10. The mean Ca content is 2.57mmol/l for both the treatment groups over the duration of the trial. In a study by Depauw *et al*. (2012), mean Ca values for a supplemented beef diet was 2.7mmol/l. The mean value of this trial falls well within the reference values provided, as well as close to the value reported by Depauw *et al*. (2012). Statistical analyses showed no significant treatment or weight effects on the measured Ca levels. The R^2 value of 0.1938 indicates that only 19.38% of the variation is explained by the statistical model used. This means that 80.62% of the variation can be attributed to multiple different variables, including weather and age.

Ca is the basic building block of the skeletal system (McDonald *et al*., 2002). Ca^{2+} functions listed by the IDEXX VetTest® manual includes Ca^{2+} as an enzyme activator, a key role player in muscle contraction, osmoregulation and blood clotting. Ca exists in many forms, including bound forms and as Ca^{2+}, its ionic form in the blood (IDEXX VetTest® Manual). The relationship between Ca and P is discussed in the Ca results from

the feed analyses section (4.2.8). Blood Ca levels will, however, always be within a narrow range of value (eg. Table 4.10), since blood Ca levels are well regulated. Whenever a drop of Ca in the blood occurs, bone is demineralised and Ca and P is released.

The absence of a statistically significant difference was found in the blood Ca levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

Table 4.10 Control vs lure over time for Ca levels (mmol/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	2.58 (± 0.052)	2.57 (± 0.058)	2.57 (± 0.052)
3	2.57 (± 0.071)	2.55 (± 0.039)	2.56 (± 0.055)
5	2.58 (± 0.159)	2.55 (± 0.091)	2.57 (± 0.118)
7	2.65 (± 0.106)	2.53 (± 0.066)	2.59 (± 0.104)
$\bar{x}$	2.59 (± 0.097)	2.55 (± 0.062)	2.57 (± 0.083)

4.3.7 Phosphorous (P)

The VetTest® reference values given by the software for P is 1.00-2.42mmol/l. Statistical analyses results for P are given in Table 4.11. The mean P content is 2.42mmol/l for both the treatment groups over the duration of the trial. In a study by Depauw *et al*. (2012) mean P values for a supplemented beef diet was 1.9mmol/l. The mean value of this trial on the upper border of, but still within, the reference values provided. The mean level of P reported by Depauw *et al*. (2012) was lower than the value in our trial. Statistical analyses showed a significant weight effect, but no treatment effect for the measured P levels. The R^2 value of 0.6868 indicates that 68.68% of the variation is explained by the statistical model used ($p<0.0001$). This means that only 31.32% of the variation are not accounted for by the statistical model used to evaluate the data. This 31.32% can be attributed to multiple different variables, including weather and age.

McDonald *et al*. (2002) names P the element with more functions in the animal body than any other element. The author and the IDEXX VetTest® Manual reports that P is part of the structure of nucleic acids, phospholipids and phosphoproteins. McDonald *et al*. (2002) reports that P play an important role many aspects of metabolism, including energy metabolism and fluid balance. It is mostly found in bone and teeth (Kleyn, 2012 & McDonald *et al*., 2002). A relationship between Ca and P exists and this relationship is discussed in detail in sections 4.2.6, 4.2.7 and 4.2.8. P analyses results can be used as an indicator of the severity of renal disease (IDEXX VetTest® Manual).

No statistically significant difference was found in the blood P levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

Table 4.11 Control vs lure over time for P levels (mmol/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	2.42 (± 0.454)	2.55 (± 0.724)	2.48 (± 0.576)
3	2.50 (± 0.701)	2.26 (± 0.465)	2.37 (± 0.571)
5	2.34 (± 0.373)	2.50 (± 0.454)	2.42 (± 0.397)
7	2.47 (± 0.385)	2.35 (± 0.401)	2.42 (± 0.373)
$\bar{x}$	2.43 (± 0.460)	2.41 (± 0.497)	2.42 (± 0.473)

4.3.8 Ca:P ratio

Table 4.12 shows the results of the statistical analyses. A mean Ca:P ratio for both treatment groups over the experimental time, as well as the overall mean, is 0.94:1. The Ca:P ratio in the blood is close to 1:1. Statistical analyses shows a significant weight effect, but no treatment effect for the measured Ca:P ratio. The R^2 value of 0.6979 indicates that 69.79% of the variation is explained by the statistical model used ($p<0.0001$). This means that only 30.21% of the variation are not accounted for by the statistical model used to evaluate the data. This 30.21% can be attributed to multiple different variables, including weather and age.

McDonald *et al*. (2002) reports that the Ca:P ratio is an important ratio to consider, especially when formulating a feed. The author further reports that excess P can easily be excreted, mainly through the kidneys. In the case of a shortage of blood Ca, parathyroid hormone activates the resorption of bone in order to correct the blood Ca concentrations. A problem with this system is that Ca and P are released together. Excess P in

the blood is excreted and lost by the kidneys and can result in a P deficiency (McDonald *et al*., 2002). The recommended ratio for Ca:P is 1:1 to 2:1 (as discussed in section 1). In this trial the ratio of Ca:P was not high enough to meet the minimum recommendation of 1:1, but the numerical difference was not significant enough to show that the animals was in poor health during the experimental period. This numerical difference is justifiable if Ca and P is considered separately, since the levels measured in blood during this trial was within recommendations for both minerals in all individual animals for the entire experimental period.

No statistically significant difference was found in the blood P levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

Table 4.12 Control vs lure over time for Ca:P ratio measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	0.94 (± 0.184)	0.99 (± 0.277)	0.96 (± 0.224)
3	0.97 (± 0.279)	0.88 (± 0.182)	0.93 (± 0.226)
5	0.92 (± 0.194)	0.98 (± 0.159)	0.95 (± 0.165)
7	0.94 (± 0.176)	0.93 (± 0.147)	0.93 (± 0.153)
$\bar{x}$	0.94 (± 0.196)	0.94 (± 0.187)	0.94 (± 0.189)

4.3.9 Creatinine (Creat)

The VetTest® reference values given by the software for Creat is 71-212µmol/l, and 102-299µmol/l from IDEXX laboratories. Statistical analyses results for Creat are presented in Table 4.13 for results from the VetTest® Chemical analyser. The mean Creat content is 178.49µmol/l (VetTest®) and 255.95µmol/l for both the treatment groups over the duration of the trial. In a study by Depauw *et al*. (2012) mean Creat values for a supplemented beef diet was 180µmol/l. The mean values of Creat for the trial fall within the set parameters for both sets of data and the value reported by Depauw *et al*. (2012). Statistical analyses showed a significant weight effect, but no treatment effect for the measured Creat levels of both analyses. The VetTest® analyses showed a p-value of 0.0009. Row means in the VetTest® analyses showed a significant difference over time (Table 4.13), which might suggest that the level of Creat that is produced, lowered over time. The R^2 value for the VetTest® analyses of 0.4442 indicates that only 44.42% of the variation is explained by the statistical model used. This means that 55.58% of the variation are not accounted for by the statistical model used to evaluate the data. This 55.58% can be attributed to multiple different variables, including weather and age.

Table 4.13 Control vs lure over time for Creat levels (µmol/l) measured in blood serum (Mean±SD) with the VetTest® analyser.

Week	Control	Lure	$\bar{x}$
1	167.16 (± 34.040)	175.97 (± 48.216)	171.57 (± 39.510)^{ab}
3	213.36 (± 43.999)	174.57 (± 34.953)	193.97 (± 43.182)^{a}
5	212.02 (± 83.396)	179.17 (± 60.491)	195.60 (± 68.341)^{a}
7	170.36 (± 46.163)	141.97 (± 29.467)	156.17 (± 39.871)^{b}
$\bar{x}$	190.73 (± 52.710)	167.93 (± 44.034)	178.49 (± 49.140)

^{ab} Row means with different superscript differ significantly (P<0.05) using the Fisher test.

Row means in results from IDEXX laboratories Table 4.14 showed a significant difference over time, which might suggest that the level of Creat that is broken down lowered over time. The IDEXX laboratories test showed a p-value of 0.0004. The R^2 value for the IDEXX analyses of 0.4442 indicates that only 44.45% of the variation is explained by the statistical model used. This means that 55.55% of the variation are not accounted for by the statistical model used to evaluate the data. This 55.55% can be attributed to multiple different variables, including weather and age.

Table 4.14 Control vs lure over time for Creat levels (µmol/l) measured in blood serum (Mean±SD) IDEXX laboratories.

Week	Control	Lure	$\bar{x}$
1	233.37 (± 57.130)	245.11 (± 76.206)	239.24 (± 63.672)[ab]
3	291.17 (± 42.105)	267.11 (± 71.535)	279.14 (± 57.112)[a]
5	291.82 (± 68.911)	263.91 (± 67.763)	277.87 (± 64.950)[ab]
7	241.57 (± 70.776)	220.71 (± 39.884)	231.14 (± 50.022)[b]
$\bar{x}$	264.48 (± 61.412)	249.21 (± 62.935)	255.95 (± 61.755)

[ab] Row means with different superscript differ significantly (P<0.05) using the Fisher test.

McDonald *et al*. (2002) & IDEXX VetTest® Manual reports that Creat is the product of the muscle protein Creat if metabolised for energy. McDonald *et al*. (2002) further reports that Creat is eventually excreted through the kidneys and it is a form of metabolisable energy that is lost. A constant value of Creat production can be changed by factors such as diet, exercise, age and catabolism (IDEXX VetTest® Manual). The manual further reports that Creat can be used as an indicator for renal disease, but should be run in combination with TP, P, ALB and BUN for more reliable results.

The numerical differences in the results from the on-site VetTest® analyser and IDEXX laboratories, VITROS 350 analyser, for Creat respectively can firstly be attributed to the calibration of the two different analysers used in the two different situations. Another contributing factor for the difference in the values can be the analytical method in which the two analysers operate. (analysers discussed in detail in sections 3.6.3.1 and 3.6.3.3)

A statistical significant difference was found in the blood Creat levels over time for the average of the two treatments, with a decrease in the numerical value from week one to seven seen in both the VetTest® and IDEXX results for the lure group, but not the control group. This numerical difference within and between the control and lure group over time, might suggest that exercise had a significant influence on the amount of Creat production in the lure group of animals, which was not seen in the control group. This might be an indication that the lure treatment can favour a lower level of muscle broken down and used in metabolism for energy over time.

4.3.10 Lactic acid or Lactate (Lac)

The VetTest® reference values given by the software for Lac is 0.60mmol/l to 2.50mmol/l, and no reference was provided by IDEXX laboratories. Statistical analyses results for Lac are given in Table 4.15 for analyses done on the VetTest® Chemical analyser and results from IDEXX laboratories are given inTable 4.16. The mean Lac content is 4.78mmol/l (VetTest®) and 4.07mmol/l (IDEXX) for the treatment groups over the duration of the trial. The mean values of Lac for the trial fall outside the set parameters for both sets of data and both treatments. Statistical analyses showed a significant weight effect for the plasma samples ($p<0.0001$), but no treatment effect for the measured Lac levels in the plasma (IDEXX laboratories). No treatment or weight effect is shown for the serum samples by the statistical analyses. The R^2 value for the VetTest® analyses on serum of 0.1373 indicates that only 13.73% of the variation is explained by the statistical model used. This means that 86.27% of the variation are not accounted for by the statistical model used to evaluate the data. This 86.27% can be attributed to multiple different variables, including weather and age.

Table 4.15 Control vs lure over time for Lac levels (mmol/l) measured in blood serum (Mean±SD) with the VetTest® analyser.

Week	Control	Lure	$\bar{x}$
1	4.74 (± 1.762)	4.84 (± 1.994)	4.79 (± 1.775)
3	5.78 (± 2.989)	3.49 (± 2.330)	4.63 (± 2.799)
5	5.28 (± 2.458)	5.53 (± 4.041)	5.40 (± 3.232)
7	5.48 (± 2.781)	3.16 (± 1.334)	4.32 (± 2.390)
$\bar{x}$	5.32 (± 2.358)	4.26 (± 2.604)	4.78 (± 2.513)

The R^2 value for the IDEXX analyses on plasma (Table 4.16) of 0.081 indicates that only 0.81% of the variation is explained by the statistical model used. This means that 99.19% of the variation are not accounted for by the statistical model used to evaluate the data. This 99.19% can be attributed to multiple different variables, including weather and age.

Table 4.16 Control vs lure over time for Lac levels (mmol/l) measured in blood plasma (Mean±SD) IDEXX laboratories.

Week	Control	Lure	$\bar{x}$
1	4.29 (± 0.820)	3.35 (± 1.778)	3.82 (± 1.389)
3	4.19 (± 3.136)	3.83 (± 3.618)	4.01 (± 3.197)
5	4.43 (± 1.992)	4.46 (± 4.200)	4.44 (± 2.675)
7	4.89 (± 2.749)	3.17 (± 1.768)	4.03 (± 2.281)
$\bar{x}$	4.45 (± 2.167)	3.70 (± 2.593)	4.07 (± 2.385)

Lac is produced by anaerobic metabolism of glucose in the muscle and RBC (IDEXX VetTest® Manual; Thompson *et al*., 2008). The Lac is then transported to the liver in the bloodstream, where it can be metabolised to glucose. The liver has an upper limit in the conversion rate of Lac back to glucose, and if the anaerobic breakdown of glucose by muscle and RBC exceed the liver's capacity, then the Lac level in the blood starts to increase. The raised Lac level can also be the effect of reduced metabolic activity in the liver (VetTest® Manual). The VetTest® Manual reports that Lac is not an indicator of a specific disease but more an indicator of critic metabolic imbalance. Thompson *et al*, (2008) reports that intense exercise causes Lac to build up in tissues, especially muscle tissue, and this contributes to the stiffness and soreness. The authors further report that this Lac is released into the blood and transported to the liver.

The results for the Lac measured on the VetTest® analyser and the results from IDEXX laboratories do not differ numerically significantly, which indicates that on-site serum analyses using the VetTest® analyser can be used instead of sending plasma to an off-site laboratory. This is to the benefit of HESC with regards to many aspects including saving in effort, cost and time.

No statistically significant difference was found in the blood Lac levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

4.3.11 Cortisol (Cort)

The reference range for cortisol in adult domestic felines given by Second Chance (www.2ndchance.info/normaldogandcatbloodvalues.htm) is 27.59nmol/l to 110.36nmol/l. Results of statistical analyses are given in Table 4.17. All mean values for Cort over entire experimental period for both the treatment groups within the set reference range for cats. No treatment or weight effect is shown for the Cort levels by the statistical analyses. The R^2 value for Cort analyses of 0.062 indicates that only 0.62% of the variation is explained by the statistical model used. This means that 99.38% of the variation are not accounted for by the statistical model used to evaluate the data. This 99.38% can be attributed to multiple different variables, including weather and age.

Table 4.17 Control vs lure over time for Cort levels (nmol/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	89.75 (± 69.261)	71.98 (± 38.619)	80.86 (± 53.689)
3	104.93 (± 89.378)	78.26 (± 50.965)	91.59 (± 70.871)
5	64.24 (± 21.831)	74.67 (± 46.837)	69.46 (± 36.133)
7	75.65 (± 95.200)	68.86 (± 22.397)	72.25 (± 28.584)
$\bar{x}$	83.65 (± 58.099)	73.44 (± 37.841)	78.91 (± 48.735)

Thompson *et al*. (2008) describes Cort as part of the hormone response that trigger the breakdown of for example glycogen for energy. The hormone is further defined as a hormone synthesised in the adrenal cortex. The authors further report that Cort levels increase when energy is exhausted during physical stress such as injury or exercise. Thompson *et al*. (2008) reports that Cort is a trademark for the primitive "fight or flight" response, but accumulated Cort can be present in cases where long term psychological stress occurred.

It might be suggested that exercise had no significant influence on either group of animals since no significant difference could be found in the serum Cort levels between the control and lure group over time.

4.3.12 Free Triiodothyronine (Free T_3)

Cunningham (2002) reports that the mean value for T_3 in domestic feline is 9.94pmol/l and the range in domestic cats is 5.99pmol/l to 17.20pmol/l. AMPATH laboratories give a range for humans at 2.70pmol/l to 7.30pmol/l. Statistical analyses results for T_3 are given in Table 4.18. The mean T_3 content is 4.04pmol/l. The mean values of T_3 for the trial fall inside the range given for domestic feline by Cunningham (2002) and AMPATH laboratories for humans. Statistical analyses showed a significant effect of week (time) for the plasma samples (p=0.0012), but no treatment effect for the measured T_3 levels in the plasma. Significant differences were seen in the means of weeks, although over time no significant change occurred. The R^2 value for T_3 is 0.5334, which indicates that 53.34% of the variation is explained by the statistical model used (p=0.0157). This means that only 46.66% of the variation are not accounted for by the statistical model used to evaluate the data and can be attributed to multiple different variables, including weather and age.

Free T_3 is described by McDonald *et al*. (2002) as the physiologically active form of thyroid hormones. The majority of T_3 is produced outside the thyroid gland through the process of deiodination of T_4 (Cunningham, 2002). The author further reports that, on the base of relative size, the highest amount of T_3 is produced in the muscle tissue, although the most deiodinating enzymes are found in the liver and kidneys. Almost all T_3 that circulates in the blood is bound to plasma proteins as the majority of the transport of T_3 in blood occurs by means of binding to plasma proteins (Cunningham, 2002). The author further reports that very little free T_3, that can interact with receptors on target cells, are found in the blood and in humans this level is as low as 0.3% free T_3. In dogs this value is slightly more than 1.0%, as the affinity of the plasma proteins for T_3 binding is lower (Cunningham, 2002). The half-life of both T_3 and T_4 are long relative to other hormones in the body. T_3 is reported to have a half-life of 24 hours in humans, where other hormone half-lives are a few seconds to minutes. Thyroid hormones accelerate the basal metabolic rate, accelerating growth, and the overall oxygen consumption by the whole organism (McDonald *et al*., 2002). The author further reports that the hormones are involved in digestion, the immune response, muscle functions, seasonality of reproduction and controls the development of the foetus.

No statistically significant difference was found in the blood Free T_3 levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

Table 4.18 Control vs lure over time for Free T_3 levels (pmol/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	4.56 (± 0.495)	4.33 (± 0.200)	4.30 (± 0.286)^{a}
3	3.82 (± 0.378)	3.77 (± 0.466)	3.79 (± 0.401)^{bc}
5	3.63 (± 0.265)	3.63 (± 0.265)	3.63 (± 0.237)^{c}
7	4.40 (± 0.390)	4.39 (± 0.409)	4.39 (± 0.377)^{a}
$\bar{x}$	4.03 (± 0.475)	4.03 (± 0.490)	4.04 (± 0.475)

^{ab} Row means with different superscript differ significantly (P<0.05) using the Fisher test.

4.3.13 Free Tetraiodothyronine or Free Tyroxine (Free T_4)

A reference range given by IDEXX (2010) for adult domestic cats is 9.00pmol/l to 33.50pmol/l and a range of 7.70pmol/l to 38.60pmol/l for adult domestic dogs. Statistical analyses results for T_4 are given in Table 4.19. The mean T_4 content is 15.61pmol/l. The mean values of T_4 for the trial fall inside the range given for domestic cats and dogs by IDEXX (2010). Statistical analyses show a significant effect of treatment for the plasma samples (p=0.0054), but no significant effect of time or weight. Significant differences were seen in the means of the two treatments which means that the treatment caused the animals that had lure as a treatment to have a higher mean T_4 level over the experimental period. The R^2 value for T_4 is 0.3855, which indicates that 38.55% of the variation is explained by the statistical model used (p=0.0428). This means that only 61.45% of the variation are not accounted for by the statistical model used to evaluate the data and can be attributed to multiple different variables, including weather and age.

Two diiodotyrosine molecules (produced in the thyroid gland) couples to form tetraiodothyronine. The majority of T_4 is produced inside the thyroid gland (Cunningham, 2002). The author further reports that almost all T_4 that circulates in the blood is bound to proteins as the majority of the transport of T_4 in blood occurs by means of binding to tyroxine-binding globulin (TBG). Cunningham (2002) also report that very little free T_4, that can interact with receptors on target cells, are found in the blood and in humans this level is as low as 0.03% free T_4. In dogs this value is slightly less than 1.0%, as the affinity of the plasma proteins for T_4 binding

is lower (Cunningham, 2002). The half-life of both T_3 and T_4 are long relative to other hormones in the body. T_4 is reported to have a half-life of six to seven days in humans, where other hormone half-lives are a few seconds to minutes. Thyroid hormones accelerate the basal metabolic rate, accelerating growth, and the overall oxygen consumption by the whole organism (McDonald *et al*., 2002). The author further reports that the hormones are involved in digestion, the immune response, muscle functions, seasonality of reproduction and controls the development of the foetus.

The difference in the blood Free T_4 levels between the means for the control and lure group over time was statistically and numerically significant, which might suggest that exercise had an influence on the lure group. The higher amount of free T_4 in the blood provides more substrate to form the active free T_3. When considering the free T_3 in the two groups it was found that no statistical significant difference existed between the T_3 of the two treatments. This might be an indication that the rate at which the free T_3 was removed from the bloodstream was higher in the lure group than the control group. If this assumption of the rate of T_3 lowering in the bloodstream is correct, it might indicate that the metabolic rate of the animals from the lure group was higher during the experimental period than that of the control group.

Table 4.19 Control vs lure over time for Free T_4 levels (pmol/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	7.73 (± 4.095)[12]	14.39 (± 7.863)[12]	11.06 (± 6.866)
3	12.20 (± 4.044)[12]	16.00 (± 3.344)[12]	14.10 (± 4.025)
5	11.62 (± 7.442)[2]	23.42 (± 14.222)[1]	17.52 (± 12.687)
7	16.78 (± 4.479)[12]	21.92 (± 4.936)[12]	19.35 (± 4.848)
$\bar{x}$	12.08 (± 6.056)[*]	18.94 (± 8.856)[#]	15.61 (± 8.199)

[12] Values with different superscript differ significantly (P<0.05) using the Fisher test.
[*#] Column means with different superscript differ significantly (P<0.05) using the Fisher test.

4.3.14 Lipogram

A lipogram or lipid profile is a test that is done for the four key markers to assess the animal blood cholesterol levels. The lipogram gives a reasonably accurate reading of the levels of total cholesterol (TChol), HDL cholesterols (HDL), LDL cholesterols (LDL) and triglycerides (TG).

Total cholesterol (TChol), HDL cholesterol (HDL), LDL cholesterol (LDL) and triglycerides (TG)

Cholesterol is present in all animal cells and is an important component of various biological membranes (McDonald *et al*., 2002). Cholesterol also acts as a precursor for steroid hormones. The authors further report that ca. 30% of all cholesterol are in the free report, which leave 70% bound to lipoproteins (complexes of protein and lipids held together by non-covalent bonds). Lipoproteins exist as sphere-shaped structures in plasma, with a triacylglycerol and ester core. The shell surrounding the core contains unesterfied cholesterol and phosphatidyl cholines. The HDLP fraction has about 45% protein and 55% lipid and the VLDLP fraction has about 10% protein and 90% lipids. Prolonged high levels of cholesterol in the blood results in the deposition of cholesterol on the walls of blood vessels. The deposition occurs as a result of the low solubility of cholesterol. The deposit hardens over time and artherosclerotic plaque forms, a leading cause for clot formation and even a heart attack. McDonald *et al*. (2002) reports that there is evidence to support that the risk of coronary heart disease increases with high plasma concentrations of LDL-cholesterol and an inverse relationship between the risk for the disease and HDL-cholesterol concentrations. The level of polyunsaturated (PUFA) and saturated (SFA) fatty acids are also important as SFA causes cholesterol levels to increase and PUFA cause it to decrease, except trans-PUFA (acts similar to SFA).

According to McDonald *et al*. (2002) normal values for TChol range from 3.10mmol/l to 5.69mmol/l. A reference range of 2.00mmol/l to 4.60mmol/l, given on the report by AMPATH laboratories, is the recommended range for TChol levels in humans. In a study by Crissey *et al*. (2003) a mean value of 4.90mmol/l was found in a study of the lipid and vitamin concentrations in different species of felids. The results for the statistical analyses are given in Table 4.20. The overall mean for both treatments over the duration of the trial is 3.62mmol/l. This mean value for the entire experimental period falls well within the two ranges and close to the mean value (Crissey *et al*., 2003) provided above. All other means from the trial also fall within the two ranges given by McDonald *et al*. (2002) and AMPATH laboratories. The means over time did differ, and they showed an overall decrease over time. When referring to the type III SS, weight had an effect (p=0.0142) on

the TChol levels in the animal blood, according to the Fisher test with $p<0.05$. The R^2 for the TChol levels was 0.3009, which means that the statistical model explains 30.09% of the variation in the TChol levels over time. The R^2 value shows that the model used was not a good fit for the data analysed ($p=0.1623$). The rest of the variation can be attributed to multiple different variables, including weather and age. The absence of a statistically significant difference was found in the blood TChol levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

Table 4.20 Control vs lure over time for TChol levels (mmol/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	3.65 (± 0.207)	3.46 (± 0.513)	3.55 (± 0.398)^{ab}
3	3.45 (± 0.422)	3.66 (± 0.439)	3.55 (± 0.422)^{ab}
5	3.79 (± 0.695)	3.96 (± 0.808)	3.87 (± 0.716)^{a}
7	3.57 (± 0.084)	3.42 (± 0.358)	3.49 (± 0.246)^{b}
$\bar{x}$	3.62 (± 0.400)	3.62 (± 0.554)	3.62 (± 0.479)

^{ab} Row means with different superscript differ significantly ($P<0.05$) using the Fisher test.

As discussed above, HDL is the cholesterol type with a higher density, and it also have an effect of reducing the risk of coronary heart disease when the HDL level in the blood plasma is high.

AMPATH provided a reference range of 1.0mmol/l to1.6mmol/l for the HDL levels in humans. A mean value of 3.6mmol/l was found in a study by Crissey *et al*. (2003). The results for the statistical analyses are given in Table 4.21. The overall mean for both treatments over the duration of the trial is 2.66mmol/l. This mean value for the entire experimental period is above the range given by AMPATH laboratories but close to the mean value provided by Crissey *et al*. (2003) above. The means of both the treatments over time did differ, and they showed an overall decrease over time. When referring to the type III SS, time (week) had an effect ($p<0.0001$) on the HDL levels in the animal blood, according to the Fisher test with $p<0.05$. The R^2 for the HDL levels was 0.3878, which means that the statistical model explains 38.78% of the variation in the HDL levels over time. The R^2 value shows that the model used was a reasonably good fit for the data analysed ($p=0.0410$). The rest of the variation can be attributed to multiple different variables, including weather, age, etc. No statistically significant difference was found in the blood HDL levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

Table 4.21 Control vs lure over time for HDL levels (mmol/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	2.64 (± 0.378)	2.56 (± 0.251)	2.60 (± 0.306)^{bc}
3	2.70 (± 0.224)	2.78 (± 0.228)	2.74 (± 0.217)^{ab}
5	2.90 (± 0.392)	3.00 (± 0.406)	2.95 (± 0.378)^{a}
7	2.38 (± 0.114)	2.40 (± 0.255)	2.39 (± 0.187)^{c}
$\bar{x}$	2.65 (± 0.330)	2.69 (± 0.356)	2.66 (± 0.340)

^{ab} Row means with different superscript differ significantly ($P<0.05$) using the Fisher test.

As mentioned above, with an increased level of LDL cholesterol in the blood, the risk for a heart attack increases. LDL is a low density type of cholesterol and is considered the bad cholesterol.
Crissey *et al*. (2003) reports a LDL value of 0.90mmol/l in domestic cats. Hansen (2012) reports that a LDL level of <2mmol/l is acceptable in humans. A mean value of 1.2mmol/l was found for cheetah in captivity by Crissey *et al*. (2003). The results for the statistical analyses are given in Table 4.22. The overall mean for both treatments over the duration of the trial is 0.53mmol/l. This mean value for the entire experimental period is below the value for domestic cats, the mean value of the study by Crissey *et al*. (2003) and the upper level provided by Hansen (2012). All other means also had roughly the same values than that of the overall mean. When referring to the type III SS, weight had an effect ($p<0.0001$) on the LDL levels in the animal blood, according to the Fisher test with $p<0.05$. The R^2 for the LDL levels was 0.5267, which means that the statistical model explains 52.67% of the variation in the LDL levels over time. The R^2 value shows that the model used was a good fit for the data analysed ($p=0.0025$) as 52.67% of the variation are accounted for in the model. The rest of the variation can be attributed to multiple different variables, including weather and age. The absence of a statistically significant difference was found in the blood LDL levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

Table 4.22 Control vs lure over time for LDL levels (mmol/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	0.58 (± 0.351)	0.50 (± 0.283)	0.54 (± 0.306)
3	0.60 (± 0.224)	0.56 (± 0.228)	0.58 (± 0.217)
5	0.53 (± 0.392)	0.58 (± 0.406)	0.56 (± 0.378)
7	0.52 (± 0.114)	0.34 (± 0.255)	0.43 (± 0.187)
$\bar{x}$	0.56 (± 0.330)	0.50 (± 0.356)	0.53 (± 0.340)

A TG consist out of a three-carbon glycerol with three fatty acid chains attached to it (Thompson *et al*., 2008). TG's are found in the core of lipoproteins (McDonald *et al*., 2002) as discussed above.

A reference range given for TG by Backeus *et al*. (1997), as reported by Depauw *et al*. (2012), is 0.113mmol/l to 0.430mmol/l. TG given by Crissey *et al*. (2003) for domestic cats is 0.630mmol/l (as taken from Fox *et al*. (1993)). The average TG level that Depauw *et al*, (2012) found in the trial where cheetah were fed supplemented beef, is 0.588mmol/l. A reference range given for TG by AMPATH laboratories for humans is 0.30mmol/l to 1.10mmol/l. The results for the statistical analyses are given in Table 4.23. The overall mean for both treatments over the duration of the trial is 0.99mmol/l. This mean value for the entire experimental period is within the range referenced by Depauw *et al*. (2012) for cheetah, above the value that Fox *et al*. (1993) reported for domestic cats, above values reported for captive cheetah on a supplemented beef diet by Depauw *et al*. (2012) and within the values given by AMPATH laboratories for humans. All other means in Table 4.23 also had roughly the same relationship with the referenced data values above. When referring to the type III SS, there was no effect by treatment, time, or weight on the TG levels in the animal blood, according to the Fisher test with $p<0.05$. The R^2 for the TG levels was 0.1409, which means that the statistical model explains only 14.09% of the variation in the TG levels over time. The R^2 value shows that the model used was not a good fit for the data analysed ($p=0.7583$) as 85.91% of the variation not accounted for in the model. The 85.91% of the variation can be attributed to multiple different variables, including weather and age. The absence of a statistically significant difference was found in the blood TG levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

Table 4.23 Control vs lure over time for TG levels (mmol/l) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	0.91 (± 0.589)	0.92 (± 0.228)	0.92 (± 0.421)
3	0.73 (± 0.462)	0.78 (± 0.471)	0.76 (± 0.440)
5	1.60 (± 2.092)	1.10 (± 0.608)	1.35 (± 1.374)
7	0.78 (± 0.612)	1.22 (± 0.716)	1.01 (± 0.666)
$\bar{x}$	1.01 (± 1.021)	1.01 (± 0.523)	0.99 (± 0.794)

4.3.15 25-hydroxy-vitamin D or Vitamin D_3 (Vit D_3)

A mean reference value given for Vit D_3 by Crissey *et al*. (2003) for cheetah is 33.00ng/ml. The reference range given by AMPATH laboratories on the blood analyses report is 32.00ng/ml to 80ng/ml for humans. The results for the statistical analyses are given in Table 4.24. The overall mean for both treatments over the duration of the trial is 26.26ng/ml. This mean value for the entire experimental period is close to the mean value of the study by Crissey *et al*. (2003) and within the range given by AMPATH laboratories for humans. All other means also had roughly the same values than that of the overall mean. When referring to the type III SS, weight had an effect ($p<0.0001$) on the vit D_3 levels in the animal blood, according to the Fisher test with $p<0.05$. The R^2 for the vit D_3 levels was 0.5013, which means that the statistical model explains 50.13% of the variation in the vit D_3 levels over time. The R^2 value shows that the model used was a good fit for the data analysed ($p=0.0036$) as 50.13% of the variation are accounted for in the model. The rest of the variation can be attributed to multiple different variables, including weather and age.

Vit D_3 is absorbed in the small intestine and transported to the liver by blood (McDonald *et al*., 2002). The authors further report that vit D_3 is metabolised to 1,25-hydroxycholecalciferol in the liver and then transported to the kidneys where it is converted to 1,25-dihydroxycholecalciferol (biologically more active than 1,25-hydroxycholecalciferol). McDonald *et al*. (2002) further describes that 1,25-dihydroxycholecalciferol is transported to various target tissues where it regulates DNA transcription in the intestinal microvilli, including the synthesis of specific m-RNA responsible for Ca-binding protein production. Ca-binding protein

is involved in the Ca absorption form the intestinal lumen. The amount of 1,25-dihydroxycholecalciferol produced in the kidneys is controlled by parathyroid hormone (PTH). PTH secretion is increased by the parathyroid gland, in reaction to lowered Ca levels in the blood, and PTH stimulates the kidneys to produce increased levels of 1,25-dihydroxycholecalciferol, which increases the Ca absorption from the intestinal lumen (McDonald *et al*., 2002). In addition to the above mentioned functions, McDonald *et al*. (2002) also report that 1,25-dihydroxycholecalciferol increases the reabsorption of Ca and P from bone and by the kidneys, as well as increasing the absorption of P from the intestine. No statistically significant difference was found in the blood Vit D_3 levels between the control and lure group over time, which might suggest that exercise had no significant influence on either group of animals.

Table 4.24 Control vs lure over time for Vit D_3 levels (ng/ml) measured in blood serum (Mean±SD).

Week	Control	Lure	$\bar{x}$
1	26.85 (± 9.367)	25.81 (± 7.035)	26.33 (± 7.190)
3	24.13 (± 8.384)	24.61 (± 7.120)	24.37 (± 7.333)
5	26.85 (± 6.519)	25.25 (± 5.198)	26.05 (± 4.550)
7	27.67 (± 6.017)	29.07 (± 5.656)	28.37 (± 5.646)
$\bar{x}$	26.38 (± 7.165)	26.18 (± 5.920)	26.26 (± 6.469)

4.3.16 Summary of blood parameters

The plasma protein analyses (sections 4.3.1, 4.3.2 & 4.3.3) shows no abnormality from reference values for cats and it can be concluded on these results that the animals in this trial were in good health, pertaining to hepatic and renal function, as well as fluid balance throughout the experimental period. Both treatment and weight showed a significant effect on the difference in the Alb levels between the individuals of the two groups over time. Weight also showed a significant effect on the Glob levels for the control and lure group over time. PCV values of this trial was higher than both the reference values given by Ford & Mazzaferro (2012) and those reported by Bechert *et al*. (2002). This may be an indicator of more cells in the blood after the running, but statistical analyses did not show any significant treatment effect. The values for BUN falls within the ranges provided by both the VetTest®software and IDEXX laboratories. BUN did fall within the same maximum and minimum values found by other authors. A statistical analysis of the results shows that no treatment or weight effect was seen in the experimental period. BUN values also indicate good renal health.

When evaluating the results of Ca and P, we need to consider the two in a ratio of Ca:P. A statistically significant effect of weight on the ratio was found, as well as on P itself. The effect might be an indicator that the animals are more at risk of renal disease or damage, and at risk of P deficiencies developing. With the analyses of feed, Ca levels was highly variable and the P levels were constant. It is highly likely that Ca is released remobilised from bone, and P is lost through urine. A recommendation is to strive to meet an ideal Ca:P ratio of 2:1.

Creat levels fall within the set ranges for both sets of data. The means for the weeks changed significantly over time, which might be an indicator that less Creat is produced over time. This might be an indication that the lure animals had a benefit of improvement in efficiency of energy metabolism, and perhaps becoming more fit than the control group, over time. In this trial the Lac levels for both serum and plasma samples, analysed by two different methods, had values far above the reference values. No treatment effect is shown in the statistical analyses. This might be an indication that the influence of Lac cannot be seen one hour postprandial. The Lac needs to be released from the muscle tissue into the bloodstream before the serum or plasma analyses of Lac level will show it. The rate at which Lac moves from the muscle tissue into the bloodstream is unknown, and one hour postprandial might be either too long or to short. Further investigation is needed in the rate of Lac transport in cheetah tissue specifically. Another reasoning behind the Lac levels might be that cheetah have a natural slower metabolism of Lac in the liver, which might lead to higher Lac levels in the blood circulation.

Cort values fall well within the given range for domestic adult cat at rest. Cort can be an indicator of stress on animals over a period of time. Due to the values not falling outside the given range, it can be deducted that little or no long term stress was induced by the treatments on the animals.

T_3 and T_4 values are between the given ranges. T_3 changed varied over time, although the change from the beginning to the end of the experimental period was not significant. The T_4 increased over time for both the control and the experimental group, although the difference between the two treatment groups are

significant over time. The increased T_4 indicates that the activity of the thyroid gland in the two groups, especially the experimental lure group, increased over time. The increase in the thyroid gland activity indicates that the metabolic rate of the animals increased over time.

The results of the lipogram are overall favourable. TChol values ranged within the given values, and week (time) had an effect, although not significant. With the HDL levels, it is seen that the values are above those of humans, and close to the reported value of Crissey *et al*. (2003). There is a non-significant effect of week on the HDL levels. Higher levels of HDL in the blood is preferred, as it is the 'good' cholesterol molecule and promotes cardiovascular health. LDL levels are below reference values. It is preferred that LDL levels are as low as possible. TG levels are within or close to the reference values given. Although the TG values falls above the reference for some of the data, the TG is not critically high.

When considering the vit D_3 results, we have to look at the results in relation to the lipogram, Ca and P results. The overall vit D_3 results were favourable, in correlation to the overall lipogram, Ca and P results.

Based on the blood parameters analysed in the two groups, no significant difference was found over the seven-week experimental period. Possible reasons for the lack of significant differences between the two groups can include one or more of the following: the frequency of exercise the lure group was subjected to might have been too short or not frequent enough, the experimental period was too short, or the sample size of the two groups of animals was too small. One or more of these factors could have resulted in the fact that specific blood parameters were largely unaffected by the treatments and no treatment effect was observed.

CHAPTER 5: CONCLUSION

5. Introduction

The aim of this research study was to evaluate the effects of physical exercise on the overall physiological status of cheetah in captivity. The physical exercise treatments included feeding of cheetah either in their enclosures with no exercise, or feeding in combination with an exercise programme (by means of a lure).

The conclusions and recommendations are based on the aim given in section 4.

5.1 Conclusions and recommendations

5.1.1 Conclusions and recommendations based on physical health evaluation

Based on the nine points that were used to evaluate the physical health, no animals had a detrimental health report as a result of either of the treatments. Based on these results, all animals stayed in their respective treatment groups for the duration of the trial. For collection of data on faecal quality, it is recommended that animals should be monitored more closely over time.

5.1.2 Conclusions and recommendations based on feed analyses

Feed analyses mainly showed that the use of the CVM-supplement with the feed presented to the animals during the experimental period was poorly managed. It is recommended that the CVM-supplement should be presented to the animals by a more controllable means, such as the specific CVM-supplement, according to requirement, mixed into a ball of minced meat for each animal. Logistically it can be different and specific balls can be prepared according to specific body types, sex and age. It is also recommended that the meat from game species should be supplemented, because analyses showed that the buffalo samples did not meet the Ca:P and CP:EE ratios. The buffalo meat was inferior to the means of beef samples for most of the analyses done on the feed samples.

The amount of fat removed from the feed had an influence on the CP:EE ratio. This can be detrimental to the health of the animals and induce disorders, as a specific amount of CP:EE need to be included in the feed presented to cheetah. It is recommended that no fat is removed from meat presented to the cheetah, as fat plays a vital role in the energy density of the diet.

The Ca:P ratio shows low levels of Ca. These low levels can cause reduced skeletal integrity over time, which will be detrimental to the health of the cheetah. It is also important to strive for the ideal 2:1 Ca:P ratio in the feed presented to the cheetah. Ca is one of the first limiting minerals in meat. The high level of variation in Ca levels of supplemented meat samples analysed during the trial supports the theories of either incorrect use of the CVM-supplement in the meat or sampling error (either from feeding crates or in Nutrilab). An optimal ratio will allow for optimal digestion, absorption and metabolism of Ca and P. No feed guidelines are available for Mg, Cu, Fe, Mn, Zn, K, Na or Se for captive cheetah. Values were evaluated against the cat food standards as stipulated by AAFCO (Table 2.1).

A new method for feed sampling is recommended, where the overall contents of the feeding crate should be taken into consideration. A possible solution to the CVM-supplement not representing the same ratio to meat in the samples taken might sampling before supplementation and the addition of the correct ratio of supplement after sampling. This can, however, prevent detection of poor management of the CVM-supplementation.

5.1.3 Conclusions and recommendations based on blood parameter analyses

It was not possible to do a haemogram due to the location of HESC, as samples need to be analysed at the laboratories within 24 hours of sampling. The values for Hb, RBC, white blood cells (WBC) and platelet count. These analyses would have shown whether an increase in the oxygen carrying ability occurred in the lure group pertaining to Hb and RBC levels.

Cheetah are very susceptible to developing Ca deficiencies. Based on plasma protein, Ca, P and thyroid gland analyses the renal and hepatic function of the animals in both the control and lure group was in physiological health. Based on the results for Ca:P and P respectively, animals are more at risk for renal disease

or damage, and at risk of Ca deficiencies developing. Using the CVM-supplement according to the guidelines should rectify the problem.

PCV values of this trial was higher than reference values. This may be an indicator of more blood cells in the blood after the running, but statistical analyses did not show any significant treatment effect. It is recommended that measures should be taken to reduce the amount of haemolysis that occurs in samples, because haemolysis can influence the PCV values.

Creat levels falls within the set ranges for both sets of data. The means for the weeks changed significantly over time, which might be an indicator that less muscle is broken down for extra energy reserves in the animals from the lure group than those of the control group over time. An evaluation of the Creat levels over a longer time or a bigger sampling group might give a more definite result.

The Lac needs to be released from the muscle tissue into the bloodstream before the serum or plasma analyses of Lac level will show it. The rate at which Lac moves from the muscle tissue into the bloodstream in cheetah is unknown, and one hour postprandial might be either too long or to short. Further investigation is needed in the rate of Lac transport in cheetah tissue specifically. Another reasoning behind the Lac levels might be that cheetah have a natural slower metabolism of Lac in the liver, which might lead to higher Lac levels in the blood circulation. Data can also be collected at 3 hours or 5 hours postprandial as a possible means to taking the metabolic rate into account.

Cort values falls well within the given range for domestic adult cat at rest. Cort can be an indicator of stress on animals over a period of time. Due to the values not falling outside the given range, it might indicate that little or no long term stress was induced by the treatments on the animals.

The increased T_4 indicates that the activity of the thyroid gland in the two groups, especially the experimental lure group, increased over time. The increase in the thyroid gland activity indicates that the metabolic rate of the animals increased over time.

The results of the lipogram are overall favourable. TChol values ranged within the given values, and week (time) had an effect, although not significant. With the HDL levels, it is seen that the values are above those of humans, and close to the reported value of Crissey *et al*. (2003). No significant effect of time was found on the HDL levels. Higher levels of HDL in the blood is preferred, as it is the 'good' cholesterol molecule and promotes cardiovascular health. LDL levels are below reference values. It is preferred that LDL levels are as low as possible. TG levels are within or close to the reference values given. It is, however, known that cheetah require an energy dense diet. Fat is a good source of energy that can be taken in through the diet. The relationship between the lipogram and fed fat in cheetah is not known, thus no definite conclusion or recommendations can be made on the base of the results from this trial.

When considering the vit D_3 results, we have to look at the results in relation to the lipogram, Ca and P results. The overall vit D_3 results were favourable, in correlation to the overall lipogram, Ca and P results.

It can be concluded that the animals were in good health over the entire experimental period, based on the blood parameters analysed during this trial. Overall conclusion based on the feeding method showed that neither group was in better health than the other.

5.1.4 Overall recommendation

After considering all parameters evaluated during this trial, it appears that there is no conclusive evidence supporting either a recommendation for or against physical exercise, by means of a lure, as a method of promoting the health and wellbeing of cheetah in captivity. No negative effect on the physiological, psychological or physical health of the animals were found, and the feeding method (running with lure before presenting feed) can safely be used as part of daily feeding routine of captive cheetah. From human studies it is known that individuals that exercise for the correct duration and at an adequate intensity increases circulating β-endorphin levels (Goldfarb & Jamurtas, 1997). In the wild, cheetah travel long distances, and this level of physical activity is not possible in captivity, due to restricted enclosure sizes (as discussed in 2.9). β-endorphin levels are associated with psychological wellbeing. In humans, individuals with sufficient β-endorphin levels, usually also have better cardiac, as well as other aspects of health. During this trial, animals showed increased levels of interest and excitation while the lure was set up in their enclosures, in relation to other activities, such as cleaning of facilities. With regard to the psychological wellbeing of the animal it is recommended that the lure should be used as an environmental enrichment at HESC.

5.1.5 Limitations

For the evaluated parameters in both blood and feed samples, little or no recommended levels are available for cheetah, especially in captivity.

No baseline was done for the measured parameters before the onset of the trial. This should be done, especially pertaining to the lure group, to enable a true evaluation of physical activity on the physiological wellbeing of the animals before, during and after the trial.

A limited number of subjects were made available for this trial, and this caused a limited amount of data available for evaluation and can influence the accuracy of the data. The reason for the limited number of animals are both because of cost, risk of loss and numbers due to the endangered status of cheetah. It would have been better to use animals from different locations in order to reduce the influence of random effects on the variability of data.

Other reasons for the lack of significant differences between the two groups can include one or more of the following: the frequency of exercise the lure group was subjected to might have been too short or not frequent enough or the experimental period was too short. One or more of these factors could have resulted in the fact that specific blood parameters were largely unaffected by the treatments and no treatment effect was observed during this trial.

5.1.6 Important questions to consider in further research

- Should the frequency of physical exercise be increased to better match free roaming cheetah activity levels?
- Should the experimental period be extended?
- What is the nutrient requirements for cheetah in captivity?
- How do age, sex, physiological stage and environment influence the nutrient requirements for cheetah in captivity?
- What feeding method will be the best for the cheetah in captivity?
- What form of feed (supplemented meat, minced meat, commercially prepared diet, etc.) would be best for cheetah?

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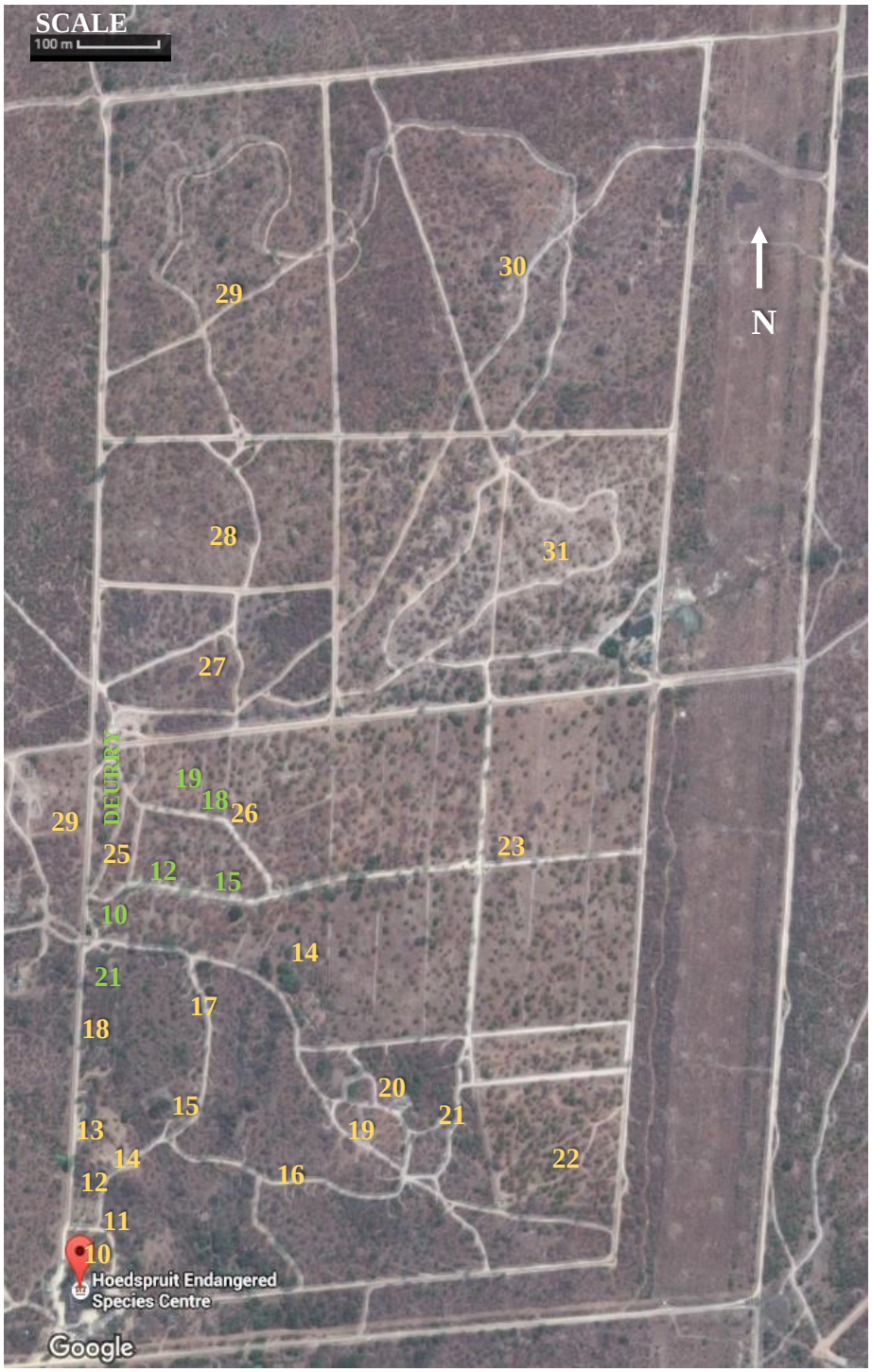
APPENDIX

Appendix A



LEDGEND:

YELLOW NUMBERS

10 Cheetah Camps
11 Crèche
12 Multi-purpose camps
13 Black-footed cats
14 Rhinoceros
15 Teen camps
16 African wild cats
17 Bat eared fox
18 Reserve camp
19 Vulture restaurant
20 Ground hornbill
21 Standby cages (birds)
22 African wild dog camp
23 Cheetah male camps
24 Maternity camps
25 Cheetah female camps
25a Additional cheetah camp
26 African wild dog camp
27 African wild dog camp
28 Tsessebes
29 Secure camp (Lions)
30 Sable antelope and tsessebe
31 Buffalo and rhino

GREEN NUMBERS

***Camp numbers part of experiment

Amended from an aerial photograph supplied by HESC indicating the different holding camps and cages. Some of the species in the different enclosures may vary according to the needs of the centre. Map from Google maps. Imagery ©2016 DigitalGlobe, Map data ©2016 AfriGIS (Pty) Ltd, Google

Appendix B

Animal Monitoring Sheet (a)

Animal Identification: _____ **Dates: _____**

Evaluation and description of scores	Score	Date:		Date:		Date:		Date:		Date:		Date:	
		Time:		Time:		Time:		Time:		Time:		Time:	
		Weight:		Weight:		Weight:		Weight:		Weight:		Weight:	
Response to lure													
Normal (Following lure)	1												
Lowered interest, but still follow	2												
No interest, do not follow	3												
Gait													
Co-ordinated	1												
Non co-ordinated	2												
Lameness													
No lameness	1												
Moderately lame	2												
Not weight bearing on a limb	3												
Appetite													
Good	1												
Moderate	2												
Poor	3												
No feed intake	4												
Vomit													
Absent	1												
Present	2												

Animal Monitoring Sheet (b)

Animal Identification: _____ **Dates:** _____

Evaluation and description of scores	**Score**	**Date:**		**Date:**		**Date:**		**Date:**		**Date:**		**Date:**	
		Time:		**Time:**		**Time:**		**Time:**		**Time:**		**Time:**	
		Weight:		**Weight:**		**Weight:**		**Weight:**		**Weight:**		**Weight:**	
Coat Quality													
Shiny	1												
Dull	2												
Coat Condition													
Groomed	1												
Not groomed/ poor condition	2												
Eye Colour													
Light orange	1												
Dark Orange	2												
Eye Brightness													
Bright	1												
Dull	2												
Faecal Score													
Hard, dry pellets	1												
Dry, well-formed stool	2												
Soft, moist formed stool	3												
Soft, unformed stool	4												
Watery, liquid that can be poured	5												

Appendix C

Animal identification Sheet

Group	Number	HESC Identification	Sex	Approximate date of birth	Camp number	Microchip number	Family grouping details
Test Group	**1**	*Shelly*	♀	04/2011	18	4B6 80C 5338	Housed with Irma
	2	*Marlou*	♀	21/02/2013	Deurry*	4C4 859 2546	Housed with male 1 and another male of the same age that did not form part of the experiment
	3	*Irma*	♀	08/04/2011	18	4B6 840 6E00	Housed with Shelly
	4	*Male 1*	♂	12/04/2013	Deurry*	900006000191834	Housed with Marlou and another male of the same age that did not form part of the experiment
	5	*Shilla*	♀	13/09/2010	10	4C2 F03 1563	Alone
Control Group	**6**	*Smartie*	♀	03/09/2010	15	4C3 86E 2D08	Alone
	7	*Heidi*	♀	21/02/2013	12	4C4 965 4A33	With Male 2
	8	*Rosy*	♀	02/09/2011	21	4C2 870 5005	With 2 Males. Males not part of experiment
	9	*Male 2*	♂	12/04/2013	12	900006000187897	With Heidi
	10	*Mary-Jane*	♀	05/05/2012	19	711 024 5557	With 3 Males. Males not part of experiment

*Deurry camp can be roughly translated to drive thru camp. This camp had vehicles entering it from various access points. The layout of the camp were similar to other camps except for vehicles transporting tourists, feed and equipment entering throughout the day.

Appendix D

A summary of units and laboratories used for analyses of all blood parameters.

Blood parameter	Units	Analyses Location
Albumin (Alb)	g/l	VetTest
Ca	mmol/l	VetTest
Ca:P ratio		HESC
Cortisol (Cort)	nmol/l	IDEXX
Creatinine (Creat)	µmol/l	VetTest
Creatinine (Creat)	µmol/l	IDEXX
Free Triiodothyronine (Free T_3)	pmol/l	AMPATH
Free Tyroxine (Free T_4)	pmol/l	IDEXX
Globulin (Glob)	g/l	VetTest
HDL	mmol/l	AMPATH
Lactate (Lac)	mmol/l	VetTest
Lactate (Lac)	mmol/l	IDEXX
LDL	mmol/l	AMPATH
Packed cell volume (PCV)	%	HESC
Phosphorous (P)	mmol/l	VetTest
Total Cholesterol (TChol)	mmol/l	AMPATH
Total Protein (TP)	g/l	VetTest
Triglycerides (TG)	mmol/l	AMPATH
Urea (BUN)	mmol/l	IDEXX
Urea (BUN)	mmol/l	VetTest
Vitamin D_3 (Vit D_3)	ng/ml	AMPATH

Raw data for blood parameter results of on-site (including IDEXX VetTest® 8008) analyses

Animal #	Treatment[1]	Week	ALB g/l	UREA mmol/l	Ca mmol/l	CREA µmol/l	LAC mmol/l	PHOS mmol/l	TP g/l	GLOB g/l	PCV %	Ca:P
1	L	1	33,00	11,60	2,51	164,00	1,73	1,47	66,00	32,00	53,57	0,59
2	L	1	35,00	13,00	2,54	177,00	4,14	3,34	69,00	34,00	54,72	1,31
3	L	1	37,00	12,70	2,63	241,00	5,38	2,42	74,00	37,00	57,14	0,92
4	L	1	34,00	10,20	2,63	107,00	6,48	3,08	66,00	32,00	56,90	1,17
5	L	1	35,00	11,40	2,53	189,00	6,50	2,45	78,00		64,18	0,97
6	C	1	39,00	14,00	2,56	218,00	6,74	1,75	77,00	38,00	53,45	0,68
7	C	1	34,00	10,30	2,54	174,00	2,06	2,48	71,00	37,00	58,82	0,98
8	C	1	38,00	11,30	2,62	175,00	4,47	2,30	77,00	40,00	50,00	0,88
9	C	1	33,00	11,00	2,53	126,00	4,60	3,02	63,00	31,00	53,70	1,19
10	C	1	35,00	12,10	2,65	151,00	5,81	2,41	70,00	35,00	50,00	0,91
1	L	3	37,00	9,30	2,61	160,00	2,53	1,89	76,00	40,00	56,90	0,72
2	L	3	35,00	12,10	2,52	169,00	1,61	2,49	67,00	32,00	49,09	0,99
3	L	3	33,00	14,00	2,53	177,00	1,36	1,77	68,00	34,00	50,00	0,70
4	L	3	34,00	11,00	2,58	135,00	6,43	2,92	67,00	33,00	53,19	1,13
5	L	3	35,00	10,80	2,53	230,00	5,51	2,24	76,00	41,00	58,49	0,88
6	C	3	39,00	12,50	2,61	275,00	3,92	1,71	80,00	41,00	59,62	0,66
7	C	3	36,00	9,90	2,51	166,00	4,21	2,68	70,00	34,00	53,23	1,07
8	C	3	40,00	10,50	2,50	181,00	6,74	2,34	83,00	44,00	52,83	0,94
9	C	3	35,00	11,50	2,58	214,00	10,60	3,55	67,00	32,00	54,17	1,38
10	C	3	36,00	10,80	2,67	239,00	3,40	2,07	73,00	38,00	56,14	0,78
1	L	5	39,00	12,10	2,41	137,00	4,49	2,20	80,00	42,00	58,14	0,91
2	L	5	36,00	14,80	2,62	148,00	2,39	2,47	72,00	36,00	52,63	0,94
3	L	5	35,00	13,20	2,60	281,00	2,09	2,21	70,00	35,00	58,49	0,85
4	L	5	36,00	9,60	2,62	141,00	11,94	3,29	70,00	34,00	56,86	1,26
5	L	5	36,00	11,90	2,51	187,00	6,74	2,34	74,00	39,00	66,67	0,93
6	C	5	39,00	13,90	2,79	326,00	8,53	2,01	81,00	42,00	62,75	0,72
7	C	5	36,00	12,40	2,46	204,00	2,55	2,39	70,00	34,00	52,27	0,97
8	C	5	[2]									
9	C	5	35,00	12,00	2,45	138,00	5,07	2,91	68,00	33,00	55,10	1,19
10	C	5	38,00	11,20	2,61	163,00	5,03	2,33	71,00	34,00	53,85	0,89
1	L	7	36,00	9,70	2,59	159,00	2,27	2,09	76,00	40,00	60,87	0,81
2	L	7	34,00	10,40	2,52	140,00	2,22	2,72	65,00	32,00	51,28	1,08
3	L	7	35,00	12,70	2,50	160,00	2,57	2,06	69,00	34,00	48,28	0,82
4	L	7	35,00	13,30	2,60	91,00	5,41	2,87	67,00	32,00	57,38	1,10
5	L	7	34,00	12,90	2,44	158,00	3,35	2,06	71,00	37,00	58,00	0,84
6	C	7	38,00	12,00	2,71	252,00	9,01	1,97	79,00	41,00	65,38	0,73
7	C	7	32,00	9,60	2,52	145,00	1,40	2,56	66,00	34,00	40,38	1,02
8	C	7	39,00	12,80	2,70	165,00	6,20	2,48	80,00	41,00	63,79	0,92
9	C	7	31,00	10,10	2,55	137,00	4,58	2,99	61,00	31,00	64,15	1,17
10	C	7	38,00	12,90	2,76	161,00	6,18	2,21	72,00	34,00	59,62	0,80

[1] **L** refers to animals that received the lure as a treatment and **C** refers to animals that was part of the control group.
[2] Empty spaces indicates missing values.

Raw data for blood parameter results from IDEXX laboratories

Animal #	Treatment[3]	Week	UREA mmol/l	CREAT µmol/l	LAC mmol/l	CORT nmol/l	FreeT_4 pmol/l
1	L	1	11,00	231,00	1,20	27,60	19,70
2	L	1	13,00	215,00	1,90	108,00	24,10
3	L	1	12,70	358,00	4,40	117,00	14,40
4	L	1	9,90	151,00	5,50	53,50	4,47
5	L	1	10,70	268,00	3,80	53,80	9,25
6	C	1	13,70	317,00	5,50	81,90	7,09
7	C	1	9,90	255,00	3,60	106,00	14,90
8	C	1	11,10	201,00	3,80	199,00	5,25
9	C	1	11,40	166,00	3,70	31,20	4,93
10	C	1	11,60	239,00	4,70	30,60	6,60
1	L	3	9,10	255,00	5,60	62,90	21,40
2	L	3	12,10	243,00	1,40	61,20	16,90
3	L	3	14,00	310,00	0,90	27,60	12,90
4	L	3	11,00	168,00	9,40	72,60	14,50
5	L	3	10,50	357,00	1,90	167,00	14,30
6	C	3	12,60	355,00	3,40	70,90	14,50
7	C	3	11,50	283,00	9,60	262,00	5,12
8	C	3	9,80	275,00	3,30	87,70	12,80
9	C	3	10,60	243,00	1,50	62,60	14,80
10	C	3	10,40	311,00	3,00	41,40	13,90
1	L	5	11,90	216,00	3,30	50,20	14,50
2	L	5	14,80	262,00	2,40	114,00	15,80
3	L	5	13,30	376,00	2,10	84,70	18,90
4	L	5	9,20	205,00	10,30	6,47	48,60
5	L	5	12,10	258,00	4,20	118,00	19,30
6	C	5	13,60	357,00	6,70	63,70	21,10
7	C	5	12,20	317,00	2,10	93,00	6,27
8	C	5	[4]				
9	C	5	12,20	196,00	3,80	60,40	5,07
10	C	5	11,20	274,00	5,40	40,00	13,80
1	L	7	9,80	254,00	2,20	59,90	27,70
2	L	7	10,40	230,00	2,30	90,20	20,80
3	L	7	12,60	235,00	2,20	95,20	24,70
4	L	7	13,10	151,00	6,30	45,80	14,50
5	L	7	12,90	231,00	2,90	53,20	21,90
6	C	7	12,80	345,00	8,00	112,00	21,10
7	C	7	9,70	247,00	1,20	115,00	15,40
8	C	7	13,00	218,00	5,40	82,50	14,20
9	C	7	9,90	148,00	5,50	32,30	15,80
10	C	7	13,00	261,00	4,20	36,40	17,50

[3] **L** refers to animals that received the lure as a treatment and **C** refers to animals that was part of the control group.
[4] Empty spaces indicates missing values.

Raw data for blood parameter results from AMPATH laboratories

Animal #	Treatment[5]	Week	TCHOL mmol/l	TG mmol/l	HDL mmol/l	LDL mmol/l	FreeT_3 pmol/l	VitD_3 ng/ml
1	L	1	3,10	0,80	2,50	0,30	[6]	32,10
2	L	1	3,60	1,00	2,40	0,80	4,30	20,40
3	L	1	3,10	1,20	2,40	0,40	4,10	34,10
4	L	1	4,30	1,00	3,00	0,80	4,50	18,30
5	L	1	3,20	0,60	2,50	0,20		23,80
6	C	1	3,90	0,60	3,30	0,30		38,00
7	C	1	3,80	0,40	2,40	1,10		18,40
8	C	1	3,20	1,40	2,50	0,20		34,60
9	C	1	3,70	0,50	2,60	0,60	3,90	17,10
10	C	1	3,60	1,70	2,40	0,60	4,60	27,60
1	L	3	3,50	1,50	3,00	0,20	4,40	30,10
2	L	3	3,80	0,40	2,80	0,80	3,40	18,40
3	L	3	3,10	0,40	2,40	0,40	3,70	28,10
4	L	3	4,30	1,00	2,90	0,90	3,30	15,40
5	L	3	3,60	0,60	2,80	0,50	4,10	30,70
6	C	3	3,10	0,30	3,00	0,20	4,00	29,80
7	C	3	3,70	0,50	2,70	0,90	3,20	15,30
8	C	3	3,90	0,80	2,60	1,00	3,80	15,20
9	C	3	2,90	1,50	2,40	0,30	4,10	31,10
10	C	3	3,60	0,60	2,80	0,50	4,10	30,70
1	L	5	3,80	1,80	3,10	0,20		32,40
2	L	5	4,80	1,60	3,30	1,20	3,30	20,20
3	L	5	3,10	0,40	2,40	0,40	3,80	26,60
4	L	5	4,80	0,60	3,40	0,90	3,70	20,00
5	L	5	3,30	1,10	2,80	0,20		26,70
6	C	5	4,20	4,70	3,30			32,60
7	C	5	3,10	0,40	2,40	0,40	3,80	26,60
8	C	5						
9	C	5	4,60	0,80	3,10	1,00	3,30	17,10
10	C	5	3,40	0,40	2,80	0,50	3,70	28,10
1	L	7	3,50	0,70	2,70	0,20	4,50	31,90
2	L	7	2,90	0,30	2,00	0,50	3,90	22,80
3	L	7	3,40	1,50	2,50	0,30	4,70	27,10
4	L	7	3,90	1,50	2,40	0,40	4,80	33,80
5	L	7	3,40	2,10	2,40	0,30	4,10	29,40
6	C	7	3,40	0,60	2,50	0,20	4,00	28,40
7	C	7	3,60	0,20	2,20	0,90	4,70	22,00
8	C	7	3,40	0,30	2,40	0,40	4,00	34,60
9	C	7	3,50	1,40	2,30	0,60	4,60	21,00
10	C	7	3,90	1,50	2,50	0,40	4,80	33,80

[5] **L** refers to animals that received the lure as a treatment and **C** refers to animals that was part of the control group.
[6] Empty spaces indicates missing values.

Appendix E

Summary of Animal Monitoring Sheet: Animal 1

Date	20 January 2014	22 January 2014	24 January 2014	27 January 2014	30 January 2014	01 February 2014	03 February 2014	05 February 2014	07 February 2014	10 February 2014	12 February 2014	14 February 2014	17 February 2014	19 February 2014	21 February 2014	24 February 2014	26 February 2014	28 February 2014	03 March 2014	05 March 2014	07 March 2014
Time	11:30	15:00	10:00	10:00	09:00	11:00	09:30	09:40	15:00	16:30	09:30	16:10	10:30	18:30	09:45	15:30	14:30	16:25	14:35	10:50	17:00
Response to lure	3	1	1	[7]		1	1	1	1	1	1	1	1	1		1	1	1	1	1	1
Gait	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lameness	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Appetite	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Vomit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Quality	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Brightness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Faecal Score						2															
Total	11	9	9	8	8	12	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9

[7] Cells with no value indicates a missing value

Summary of Animal Monitoring Sheet: Animal 2

Date	21 January 2014	23 January 2014	25 January 2014	29 January 2014	31 January 2014	04 February 2014	06 February 2014	08 February 2014	11 February 2014	13 February 2014	15 February 2014	18 February 2014	20 February 2014	22 February 2014	25 February 2014	27 February 2014	01 March 2014	04 March 2014	06 March 2014	08 March 2014
Time	17:00	10:00	16:00	16:00	09:00	09:40	09:00	17:00	16:40	15:25	08:15	18:30	09:10	08:35	17:10	16:30	09:00	14:42	15:15	14:37
Response to lure	1	1	1	1	1	2	1	[8]	1	1	1	1	1	1	[9]	1	1	1	1	1
Gait	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lameness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Appetite	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Vomit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Quality	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Brightness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Faecal Score	[10]					2														
Total	9	9	9	9	9	12	9	8	9	9	9	9	9	9	8	9	9	9	9	9

[8] No lure due to managers meeting
[9] No lure due to wet weather
[10] Cells with no value indicates a missing value

Summary of Animal Monitoring Sheet: Animal 3

Date	20 January 2014	22 January 2014	24 January 2014	27 January 2014	30 January 2014	01 February 2014	03 February 2014	05 February 2014	07 February 2014	10 February 2014	12 February 2014	14 February 2014	17 February 2014	19 February 2014	21 February 2014	24 February 2014	26 February 2014	28 February 2014	03 March 2014	05 March 2014	07 March 2014
Time	11:30	15:00	10:00	10:00	09:00	11:00	09:00	10:10	15:00	16:30	09:30	16:10	10:30	18:30	09:45	15:30	14:30	16:25	14:35	09:50	09:10
Response to lure	1	1	1	[11]		1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1
Gait	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lameness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Appetite	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Vomit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Quality	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Brightness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Faecal Score									1							4					
Total	9	9	9	8	8	9	9	9	10	9	9	9	9	9	9	13	9	10	9	9	9

[11] Cells with no value indicates a missing value

Summary of Animal Monitoring Sheet: Animal 4

Date	21 January 2014	23 January 2014	25 January 2014	29 January 2014	31 January 2014	04 February 2014	06 February 2014	08 February 2014	11 February 2010	13 February 2014	15 February 2014	18 February 2014	20 February 2014	22 February 2014	25 February 2014	27 February 2014	01 March 2014	04 March 2014	06 March 2014	08 March 2014	21 January 2014
Time	16:00	10:00	16:00	16:00	09:00	09:40	09:00	17:00	16:40	15:25	08:15	18:30	09:10	08:35	17:10	16:30	09:00	14:42	15:15	14:37	16:00
Response to lure	2	2	1	1	1	1	1	[12]	1	1	1	1	1	1	[13]	1	1	2	1	2	2
Gait	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lameness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Appetite	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Vomit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Quality	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Brightness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Faecal Score	[14]		3															2			
Total	10	10	12	9	9	9	9	8	9	9	9	9	9	9	8	9	9	12	9	10	10

[12] No lure due to managers meeting
[13] No lure due to wet weather
[14] Cells with no value indicates a missing value

Summary of Animal Monitoring Sheet: Animal 5

Date	21 January 2014	23 January 2014	25 January 2014	29 January 2014	31 January 2014	04 February 2014	06 February 2014	09 February 2014	11 February 2014	13 February 2014	15 February 2014	18 February 2014	20 February 2014	22 February 2014	25 February 2014	27 February 2014	01 March 2014	04 March 2014	06 March 2014	08 March 2014	21 January 2014
Time	16:00	10:00	09:15	16:00	09:00	09:20	08:40	15:50	15:50	15:20	08:20	18:33	09:00	10:15	17:00	16:45	09:15	14:55	15:20	14:48	16:00
Response to lure	3	1	1	1	1	1	1	1	1	1	1	1	1	1	[15]	1	1	1	1	1	3
Gait	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lameness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Appetite	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Vomit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Quality	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Brightness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Faecal Score	[16]																	1			
Total	11	9	9	9	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	11

[15] No lure due to wet weather
[16] Cells with no value indicates a missing value

Summary of Animal Monitoring Sheet: Animal 6

Date	20 January 2014	22 January 2014	24 January 2014	27 January 2014	30 January 2014	01 February 2014	03 February 2014	05 February 2014	07 February 2014	10 February 2014	12 February 2014	14 February 2014	17 February 2014	19 February 2014	21 February 2014	24 February 2014	26 February 2014	28 February 2014	03 March 2014	05 March 2014	07 March 2014
Time	09:30	10:00	10:00	10:00	09:00	11:00	10:00	11:30	15:00	11:35	14:30	11:00	10:40	18:00	09:40	09:45	09:25	11:15	14:40	12:10	11:35
Response to lure	CONTROL																				
Gait	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lameness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Appetite	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1
Vomit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Quality	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Brightness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Faecal Score	[17]					4															
Total	8	8	8	8	8	12	8	8	8	8	8	8	8	10	8	8	8	8	8	8	8

[17] Cells with no value indicates a missing value

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Summary of Animal Monitoring Sheet: Animal 7

Date	21 January 2014	23 January 2014	25 January 2014	29 January 2014	31 January 2014	04 February 2014	06 February 2014	08 February 2014	11 February 2014	13 February 2014	15 February 2014	18 February 2014	20 February 2014	22 February 2014	25 February 2014	27 February 2014	01 March 2014	04 March 2014	06 March 2014	08 March 2014	21 January 2014
Time	16:00	10:00	09:00	16:00	09:00	09:10	09:15	17:00	16:50	15:10	08:30	18:36	08:55	08:40	17:05	16:40	09:10	14:55	15:30	14:42	16:00
Response to lure	CONTROL																				
Gait	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lameness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Appetite	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Vomit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Quality	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Brightness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Faecal Score	[18]																				
Total	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

[18] Cells with no value indicates a missing value

Summary of Animal Monitoring Sheet: Animal 8

Date	20 January 2014	22 January 2014	24 January 2014	27 January 2014	30 January 2014	01 February 2014	03 February 2014	05 February 2014	07 February 2014	10 February 2014	12 February 2014	14 February 2014	17 February 2014	19 February 2014	21 February 2014	24 February 2014	26 February 2014	28 February 2014	03 March 2014	05 March 2014	07 March 2014
Time	10:00	10:00	10:00	10:00	09:00	11:00	09:45	11:30	15:00	11:35	10:00	11:00	10:50	18:10	09:52	09:30	09:15	11:25	08:50	10:00	11:20
Response to lure	CONTROL																				
Gait	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lameness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Appetite	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Vomit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Quality	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Brightness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Faecal Score	[19]	2																			
Total	8	10	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

[19] Cells with no value indicates a missing value

Summary of Animal Monitoring Sheet: Animal 9

Date	21 January 2014	23 January 2014	25 January 2014	29 January 2014	31 January 2014	04 February 2014	06 February 2014	08 February 2014	11 February 2014	13 February 2014	15 February 2014	18 February 2014	20 February 2014	22 February 2014	25 February 2014	27 February 2014	01 March 2014	06 March 2014	08 March 2014	21 January 2014	23 January 2014
Time	16:00	10:00	09:00	16:00	09:00	09:10	09:20	17:00	16:50	15:10	08:30	18:20	08:55	08:40	17:05	16:40	09:10	15:30	14:42	16:00	10:00
Response to lure	CONTROL																				
Gait	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lameness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Appetite	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Vomit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Quality	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Brightness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Faecal Score	[20]																				
Total	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

[20] Cells with no value indicates a missing value

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Summary of Animal Monitoring Sheet: Animal 10

Date	21 January 2014	23 January 2014	25 January 2014	29 January 2014	31 January 2014	04 February 2014	06 February 2014	09 February 2014	11 February 2014	13 February 2014	15 February 2014	18 February 2014	20 February 2014	22 February 2014	25 February 2014	27 February 2014	01 March 2014	06 March 2014	08 March 2014	21 January 2014	23 January 2014
Time	09:30	10:00	10:00	16:00	09:00	09:00	09:10	16:00	17:00	15:00	08:40	18:20	08:50	10:05	16:50	16:35	09:05	15:35	14:40	09:30	10:00
Response to lure	CONTROL																				
Gait	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lameness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Appetite	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Vomit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Quality	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coat Condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Colour	1	1	1	1	[21]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Brightness	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Faecal Score	2	[22]																			
Total	2	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

[21] Animal had some discharge from the eye, as well as swelling, because of a potential snake interaction.
[22] Cells with no value indicates a missing value

Appendix F

Animal Ethics Approval

UNIVERSITEIT VAN PRETORIA
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Animal Ethics Committee

PROJECT TITLE	**Effects of exercise and diet on physiological status of captive cheetah *(Acinonyx jubatus)***
PROJECT NUMBER	**EC119-13**
RESEARCHER/PRINCIPAL INVESTIGATOR	**M. Nickols**

STUDENT NUMBER (where applicable)	**290 87 555**
DISSERTATION/THESIS SUBMITTED FOR	**MSc (Agric) Animal Sciences: Production Physiology**

ANIMAL SPECIES	**Cheetah *(Acinonyx jubatus)***	
NUMBER OF ANIMALS	**10**	
Approval period to use animals for research/testing purposes		**January – August 2014**
SUPERVISOR	**Prof. EC Webb**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 27 January 2014
CHAIRMAN: UP Animal Ethics Committee	Signature