Gastrointestinal passage in cheetahs fed a natural diet

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Promoters: Prof. dr. Geert Janssens
Annelies De Cuyper

Literature Review as part of the Master's Dissertation

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PREFACE

Before starting this research report I want to thank the people that made it possible for me to do this research.

I first want to thank my promoter, Geert Janssens. He presented me the research subject on which I could work during my internship at the Cheetah Conservation Fund. I also want to thank the rest of the staff at the Laboratory of Animal Nutrition at Ghent University for the analysis of the faecal samples and especially Annelies De Cuyper for always being willing to help me with any questions I had.

I am grateful to Laurie Marker, founder and executive director of the Cheetah Conservation Fund for giving me the chance to carry out this research on five cheetahs housed at CCF. A special thank you to Anne Schmidt-Küntzel, research geneticist and assistant director for animal health and research and Juliette Erdtsiek, head curator.

The rest of the CCF staff (Brian Badger, Eli Walker, Kate Vannelli, Ryan Marcel Sucaet, Mari-Ann Da Silva and Janny Cornwell) and other interns (Zack David, Mackenzie Mossing, David Hejna, Brianna Treme, James Judelson, Catie Mong and Caleb French) helped me with the logistics of the research.

I also want to thank Marcus Clauss for the calculations used in this study and all the advice along the way.

And finally I want to thank my friends, family and husband for their support.
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It has been speculated that whole carcass feeding could have a beneficial effect on cheetahs, also by slowing gastric emptying and thereby reducing the production of putrefactive compounds from protein fermentation. This study was set up to identify the typical total tract passage time of cheetahs fed a natural diet, as a first step in understanding the cheetah’s digestive strategy.

The five female cheetahs monitored during this study were aged 9 to 14 (body mass $33 \pm 3$ kg) and housed at the Cheetah Conservation Fund in Namibia. They were fed pieces of a donkey carcass including skin, bones, cartilage with most of the fat removed and supplemented with vitamins and minerals. The pieces averaged at 2 kg per cheetah. They were additionally fed organs including heart, kidney, spleen and liver with an average of 220 g per cheetah almost every day. The animals were adjusted to this diet over a period of 11 days. Three grams of titanium dioxide were added to their food at 10.20 h in the morning of day 12 and the cheetahs were monitored in the subsequent 72 h, during which time faeces were collected on an individual basis. The samples were dried and stored frozen until analysis for marker concentration.

The maximum concentrations of the marker in the faeces were between 5 and 20 h after marker ingestion. From 36 h onwards the concentrations were close to basal level. One cheetah had diarrhoea during the period of faeces collection but showed no deviant marker excretion profile. The calculated mean retention times were 22 h, 26 h and 28 h for three of the five cheetahs. Additionally, it was observed that cheetahs mainly defecated in mornings and evenings. This might fit in their natural behaviour, but it implies that the observed total tract mean retention times might be affected by the time of feeding.

**Keywords:** acinonyx – carcass - diet - retention time - titanium dioxide
SAMENVATTING

Tijdens de afgelopen eeuw zijn de aantallen van jachtluipaarden in het wild gedaald met 90%. De soort is ook opgenomen in appendix 1 van CITES, deze bevat de diersoorten die met uitsterven bedreigd zijn. Het is daarom belangrijk ervoor te zorgen dat wilde populaties beschermd worden en dat de diersoort het goed doet in gevangenschap.

Uit gegevens verzameld van observaties in 6 landen over een tijdspanne van ongeveer 50 jaar blijkt dat enkele diersoorten frequenter worden gedood door jachtluipaarden. Dit impliceert dat jachtluipaarden de voorkeur geven aan deze soorten: Blesbok, impala, springbok, Thomson's gazelles en Grat's gazellen.

Een studie van 2013 geeft het dieet weer van 12% van de jachtluipaarden wereldwijd gehouden in gevangenschap. Het meest voorkomende dieet was rauw vlees (37%), gevolgd door commercieel bereide diëten (20%) en karkassen (8%). 35% van de jachtluipaarden krijgt een mengeling van deze diëten.

Het blijkt dat jachtluipaarden in gevangenschap een hogere prevalentie kennen van enkele degeneratieve ziekten in vergelijking met wilde jachtluipaarden van ongeveer dezelfde leeftijd. De populaties in gevangenschap hadden een hoge prevalentie van gastritis, glomerulosclerose, systemische amyloïdose en veno-occlusieve ziekte.

In de eerste plaats heeft stress een algemene negatieve invloed op de werking van het immuunsysteem en kan dus een rol spelen bij elke ziekte. Een onvoldoende gevoel van voldoening, honger of te weinig stimulatie van de dieren door hun voeding kunnen oorzaken zijn van stress gekoppeld aan het gekozen dieet.

Een overmatige aanvoer van eiwit verhoogt de productie van verscheidene schadelijke verbindingen met een negatief effect in het bijzonder op de nieren plus, eenmaal de aminozuren geabsorbeerd zijn, induceren ze renale hypertensie en sclerose.

Gastritis komt ook vaker voor bij dieren die paardenvlees of een hoge inname van ruw eiwit kennen. Gastritis komt minder vaak voor bij dieren die minimaal één keer per week gevoed worden met kip, huiden, ingewanden of ribben.

Door voederstrategieën te verbeteren kan de prevalentie van deze aandoeningen in gevangenschap teruggedrongen worden.

Uit fermentatie van eiwitten en koolhydraten kunnen voedzame componenten gevormd worden maar ook schadelijke. Uit recent onderzoek blijkt dat bij jachtluipaarden die karkassen eten de concentraties van indoxyl sulfaat in het bloed dalen. De aanwezigheid van minder goed verteerbare vezels in het dieet heeft blijkbaar een positieve invloed.
Hieruit blijkt dat het dieet van deze dieren een grote invloed kan hebben op hun gezondheidstoestand. Voor het behoud van de soort is het belangrijk een voldoende grote, genetisch diverse en gezonde populatie in gevangenschap te onderhouden. Het is daarom belangrijk hun dieet en de manier waarom het gepresenteerd wordt te optimaliseren.


Binnen de observatieperiode van 72 uur werden 26 meststalen verzameld waarvan telkens twee monsters genomen werden om de concentratie van de marker te bepalen. De meeste stalen hebben we kunnen koppelen aan de correcte jachtluipaard. Bij vijf stalen lukte dit niet omdat deze defecatie momenten 's nachts plaatsvonden en ik tijdens de nacht afhankelijk was van bewegingsgevoelige nachtcamera's. Wanneer we deze concentraties uitplotten tegen de tijd zien we dat de maximale merkerconcentraties plaatsvinden tussen de 5 en 20 uur na merker ingestie. Vanaf 36 uur na merker ingestie zijn de concentraties ongeveer gelijk aan het basale niveau.

De mean retention time (MRT) werd berekend met de resultaten van marker concentratie volgens de procedure van Thielemans et al. 1987. Bij twee van de vijf jachtluipaarden bleven de maximale merker concentraties onder 0.6 g/100 g droge stof. Deze waarden benaderen het basale niveau en er werd daarom bij de berekening van de MRT geen rekening gehouden met deze dieren. Voor de andere drie jachtluipaarden werd een MRT berekend van 28.298 u, 21.893 u en 26.106 u. De gemiddelde MRT voor deze drie jachtluipaarden is 25 ± 3 uur.
1. INTRODUCTION

The cheetah was first classified as *Felis jubatus* but when realizing that the cheetah was unique in different ways from other cats the genus was changed into *Acinonyx*. The cheetah is the only surviving species in that genus. (Marker, 2002)

Over the past century, cheetah numbers in the wild have declined with 90%. In protected areas, numbers of competitive predators such as leopards and lions can become high whereby cheetahs immigrate into non-protected areas. Furthermore, the habitat of the cheetah is lost to human population and prey numbers decline. (Marker, 2002)

Today, wild cheetahs can be found in two continents. In Asia, in Iran and possibly Pakistan, approximately 100 cheetahs inhabit a small region. In Africa, less than 15000 cheetahs exist dispersed over an area covering 29 countries. (Marker, 2002)

The cheetah is listed on Appendix 1 by CITES. Cites, the Convention on International Trade in Endangered Species of wild fauna and flora, wants to ensure that harvesting and international trade does not threaten the survival of species. Over different appendices they classify species depending on the level of protection they need. Appendix 1 catalogues species that are the most endangered among CITES-listed animals and plants. The cheetah is also listed as vulnerable on the IUCN red list.

Another factor to consider is that the cheetah is a genetically uniform species. The genetic monomorphism at examined loci in the cheetah is almost non-existent in other wild species. Such genetic uniformity might have resulted from a population bottleneck followed by inbreeding. (O’Brien et al, 1985) The bottleneck is placed at the end of the Pleistocene, when major extinction of large vertebrates occurred, about 10000 years ago. This was determined by back calculation of the divergence of mtDNA in Felidae and mutation rates of VNTR loci in other species. (Menotti-Raymond et al, 1992) The lack of genetic variation has several consequences. This could be the cause of reproductive abnormalities seen in this species. Spermatozoa concentration, percent mobility and normal morphology are less than that observed in domestic cats. (Wildt et al, 1983) There is a correlation between the reduction in genetic diversity with skeletal and congenital abnormalities (Marker-Kraus, 1997). This may also influence the immune system. For example, a much higher percentage of the cheetah population has succumbed to FIPV than in the domestic cat population. This may be due in part to an abnormality in their immune response. (Evermann et al, 1989) Because of the inability to express deleterious recessive alleles the cheetah is more susceptible to environmental and ecological changes (O’Brien et al, 1985).

It is therefore important to take adequate conservation measure to sustain wild cheetah populations. Different cheetah conservation parties have seen the light of day over the past decades such as the cheetah conservation fund in Namibia, cheetah conservation Botswana, cheetah conservation project Zimbabwe, Mara cheetah project, Iranian cheetah society,… The ultimate goal is to stop the decline in free-ranging cheetah numbers in Africa and Asia in order for the cheetah to survive as a species in the wild. Part of species conservation is the presence of a sustainable captive population. These animals in captivity can create awareness about the problem with people around the world. They can serve a significant role in
species research. They can even provide offspring that could potentially be released into the wild. It is therefore important to have a sufficiently large and genetically diverse captive population of healthy cheetahs.

But the health of cheetahs in captivity is a matter of concern: renal failure is an important cause of death in captive cheetahs, which is often caused by systemic AA amyloidosis associated with chronic inflammation such as gastritis. The prevalence of gastritis is ten times higher in a captive setting than in free-ranging cheetahs (Munson et al, 2004). Previous work in captive cheetahs has demonstrated that animal fibres such as hair, bone and cartilage protect against the negative effects of protein fermentation, hence avoiding the absorption of toxic metabolites and the evolution towards intestinal inflammation. It was hypothesized that this protective effect was caused by slowing down passage rate and the concomitant reduction of the production of putrefactive compounds from protein fermentation (Depauw et al, 2011).

The present study was set up to identify the typical total tract passage time of cheetahs fed a natural diet, as a first step in understanding the cheetah’s digestive strategy.

Understanding the link between diet and pathology, form and function of the gastro-intestinal tract and its microorganisms have been addressed in the literature study. The goal of this study is taking the first steps into improving feeding strategies and to address diseases that may result from poor nutrition to benefit the health of captive cheetahs. Further research will be necessary to fine-tune the nutrition of these felines in captivity.
2. LITERATURE STUDY

2.1. THE CHEETAH’S DIGESTIVE SYSTEM

2.1.1 Form and function

Felines have well developed non-gustatory papillae on the dorsal surface of their tongues. These sharp, hard, backwardly directed papillae help removing meat from bones while feeding. The oesophagus in carnivores has striated muscle extends all the way to the stomach which makes it possible for them to regurgitate easily. (Ewer, 1973)

Felidae usually have 30 teeth in their permanent dentition: 6 incisors, 2 canines, 6 premolars and 2 molars in the maxilla; 6 incisors, 2 canines, 4 premolars and 2 molars in the mandible. P2 is regularly absent in cheetahs as in a number of other short-faced species such as the caracal and the lynx. In cheetahs the cheek teeth are very narrow and together with the small roots incapable of dealing with large bones. (Ewer, 1973)

Carnivores and especially felines have a simple stomach, a predominant small intestinal tract and diminished large intestinal tract with a reduction in the distinction between colon and rectum (Fig. 1). Felines have a relatively small colon, its length is only about 20% of the total length of their digestive tract. (Ewer, 1973; Kerr et al, 2013)

Most components are absorbed in the small intestine (Stevens et al, 2004). The wall of the small intestine has villi to enlarge its surface and maximise absorption. In the small intestine, the duodenum is a mixing site and absorption mainly takes place in the jejunum. Monosaccharides, amino acids and peptides are actively absorbed. Carrier proteins transfer these nutrients across the intestinal wall. Fats are absorbed by passive diffusion after micelle formation. Short-chain fatty aids (SCFA) are absorbed in the bloodstream without the need for bile salts. Minerals and water-soluble vitamins are absorbed by diffusion and carrier-
mediated transport. Fat-soluble vitamins (vitamin A, D, E and K) are transferred across the brush border by passive diffusion. (McDonald et al, 2011)

Digesta have higher oxygen content and move at a higher velocity in the proximal part of the digestive tract. When the content reaches the large intestine, there is no more oxygen present and bacteria commence fermentation. (Buddington et al, 1998)

### 2.1.2 intestinal microbiota

Cats, unlike other mammals, have a high number of bacteria in the proximal part of their small intestine (Papasouliotis et al, 1998). The presence of microbiota in the intestines has several advantages. They compete with pathogenic microorganisms for nutrients and adhesion sites. The normal microbiota can break down food components so the host can use them. This also lets the host adapt more easily to dietary changes. They can produce new components, which can serve as a source of energy or influence the host's gastrointestinal function. (Hooper et al, 2002) The latter shows that bacteria itself can influence function of the host's digestive tract. In dogs, glucagon-like peptides 1 and 2 and possibly other hormones are released when bacteria ferment indigestible fibres. This stimulates mucosal proliferation and transport in the small intestine (Massimino et al, 1998).

The mammalian digestive tract can contain several hundred species of microorganisms. They vary depending on a whole range of factors. The faecal flora is different from the one in the body. In the gastrointestinal system, the microorganism species in the lumen are different from those associated with the intestinal wall. Different segments of the intestine harbour different species. Aside from these differences within the individual animal, microbial populations vary between individuals. This is caused by differences in diet, climatic conditions and even genetics: faecal microbial profiles are more similar in monozygotic twins than in unrelated individuals. (Hooper et al, 2002; Kelly et al, 1994) It is important to emphasize that an animal’s diet has a major impact on gut microbiota and intestinal bacterial fermentation (Backus et al, 2002; Carey et al, 2013). The addition of fibre to a diet changes digestibility and faecal metabolites (Kerr et al, 2013). Several studies show positive effects on the health of animals by influencing the fermentation process. Verbrugghe et al, 2010, for example, demonstrates a greater retention of N when fermentable substances are added to the food of domestic cats. This results in the conserving of amino acids so they can be used in other processes such as immunity and tissue preservation. This offers opportunities to improve the health of cheetahs in captivity by modifying their diet (Kerr et al, 2013).

A 3-year study about the intestinal microbiota in cheetahs in captivity showed about 24% change between consecutive samples in healthy cheetahs. The microbiota is mainly dominated by members of *Clostridium clusters I, XI and XIVa and Lactobacillaceae*. (Becker et al, 2015)
2.1.3 Microbial fermentation

Fermentation is a process by which microorganisms in the gastro-intestinal tract obtain energy through breaking down carbohydrates and nitrogen-containing compounds such as mucus, enzymes or urea without requiring oxygen. Bacterial fermentation produces short-chain fatty acids, ammonia and bacterial protein. In non-ruminants, the primary SCFA are acetate, butyrate and propionate. Glycolysis in bacterial cells converts monosaccharides to pyruvate originating the production of ATP (adenosine triphosphate). Pyruvate can be converted in other SCFA resulting in additional ATP production. These SCFA are absorbed through the intestinal wall by passive diffusion. (Hooper et al, 2002) The presence of SCFA creates a more acidic environment in the intestine, which decreases the number of pathogens (Valenzuela et al, 2013).

In sheep, the colonic epithelium uses most of the butyrate for energy production. It also induces epithelial proliferation and differentiation (Pennington, 1951). Acetate enters the bloodstream and is utilized primarily by skeletal and cardiac muscle. Propionate is largely converted to glucose in the liver. SCFA in general influence blood flow and absorptive functions of the intestines. (Bergman, 1990)

Some of the produced ammonia is incorporated in bacterial protein and other ammonia is or absorbed or excreted with the faeces. In felines, bacterial protein is not absorbed and is lost in the faeces. (Stevens et al, 2004) Other species have developed specific mechanisms to utilise some of the bacterial protein and vitamins. For example, many rodents practice coprophagy, where they re-ingest their faeces. Rabbits and hares can produce caecotrophes during certain parts of the day. These softer faecal samples are re-ingested. (Hörnicke, 1981) Foregut fermenters can also use some of the bacterial protein. Ruminants have developed a four-compartment stomach. Non-ruminant foregut fermenters such as the koala or proboscis monkeys use a mechanism of regurgitation and remastication. (Matsuda et al, 2011)

Aside from these beneficial metabolites, fermentation also produces a wide number of toxic metabolites. The most common are ammonia, amines, nitrosamines, phenols, cresols and indole (Samanta et al, 2013).

Presence of water in the digesta has an influence on the degree of fermentation. In vitro experiments show that fermentation is accelerated in a watery environment. The intestinal content in cheetahs is rather dry compared to other species. This can be a limiting factor for fermentation in carnivores (Kienzle, 1994). Nevertheless fermentation occurs in the gastrointestinal tract in cats and more specific also in cheetahs (Depauw et al, 2011; Sunvold et al, 1995).

A consequence of the presence of carbohydrates in the diet is a decrease of protein digestibility. This is caused by increased passage rate, endogenous nitrogen excretion, microbial growth and N-fixation. (Kienzle, 1994; Morris et al, 1976)

In cheetahs, animal-derived components can be used by microorganisms for the production of SCFA. Different substrates result in differences in fermentation rate and in the proportions of SCFA produced.
(Williams et al, 2001) Cartilage ferments quickly and collagen renders a high acetate to propionate ratio. The fermentation of protein also produces putrefactive compounds. The diet of felines has a high concentration of protein but the prevalence of diseases associated with these putrefactive compounds is low in free-ranging cheetahs. The discovery of the presence of a microorganism that uses indoles and phenols for growth in the intestines in domestic cats suggests that felines are adapted to the high ratio of protein fermentation. (Lubbs et al, 2009) The fermentation of meat generates more putrefactive compounds (Depauw, 2012).

Recent research has shown a decrease in serum indoxyl sulphate in cheetahs fed whole rabbits compared to cheetahs fed supplemented beef. This implies that beneficial fermentation has a positive effect on the animal’s health (Depauw et al, 2011).

2.1.4 Adaptations to their carnivorous diet

The cheetah is classified in the family of the Felidae, in the Order of the Carnivora (Hunter et al, 2003). As a feline, cheetahs are strict carnivores. Cats cannot adjust the activities of aminotransferases or urea cycle enzymes probably because of the high protein content of their diet (Rogers et al, 1977). Cats have essential nutrients that are not essential in any other mammalian family. It is plausible that, because cats consume high levels of protein, some enzymes have been deleted or altered. (Morris, 2002)

Two amino acids are typically essential in cats. Taurine is one of the most abundant free amino acids in mammalian tissue (Markwell et al, 1994). The enzymes responsible for the synthesis of taurine from cysteine or cysteinesulphinic acid, cysteine dioxygenase and cysteinesulphinic acid decarboxylase are present but the activities of the enzymes are low (Knopf et al, 1978). Arginine is synthesized from citrulline by the sequential action of enzymes. As in taurine, the activity of enzymes, pyrroline-5-carboxylate synthase and ornithine aminotransferase is low, which results in low levels of citrulline. (Morris, 2002; Morris et al, 1978)

Aside from these amino acids, three vitamins are typically essential in the diet of cats. Vitamin A is found only in animal tissue while plants contain carotene, its precursor. In cats, the enzyme for cleavage of the carotene molecule has been deleted, making them unable to convert carotene in retinol. (Morris, 2002) A deficiency in vitamin A can result in a range of symptoms but in cats especially it can lead to reproductive disorders (McDonald et al, 2011). This is important in cheetahs because they are difficult to breed in captivity (Marker et al, 1989).

Vitamin B3 is synthesized from tryptophan and cats possess all the necessary enzymes. They also have a high activity of picolinic carboxylase. This enzyme metabolizes tryptophan so that it cannot be used for Vitamin B3 synthesis. (Morris, 2002) Cats are unable to produce vitamin D through dermal photosynthesis because of a low concentration in 7-dehydrocholesterol (How et al, 1994; Morris, 1998).
Although the most prominent dietary components are protein, cats are able to digest carbohydrates such as glucose, sucrose, lactose, dextrin and starch (Morris et al, 1976). Still some adaptations to the low levels of carbohydrates are present in cats. They lack salivary amylase and have low activities of intestinal and pancreatic amylase. Furthermore, there is a reduced activity of intestinal disaccharidases that break down carbohydrates in the intestines. (Zoran, 2002) Finally, they have a low activity of glucokinase and glycogen synthetase in the liver and the glucokinase is not adaptive (Kienzle, 1994).

These differences in enzymes from other mammals normally do not lead to deficiencies in healthy cats on a natural diet. All essential nutritional compounds are present in animal tissue in sufficient quantities. Therefore, it is believed that these enzyme changes are evolutionary adaptations to the feline diet.

2.2. THE DIET

2.2.1 The diet of free-ranging cheetahs

Free-ranging cheetahs will bring down prey ranging from 23 to 56 kg. (Hayward et al, 2006) Male coalitions can bring down slightly bigger prey because they work together while bringing the animal down (Mills et al, 2004). Data compiled from six countries: Kenya, Namibia, South-Africa, Tanzania, Zambia and Zimbabwe over a time span starting from 1956 till 2005 shows five prey species, blesbok, impala, springbok, Thomson’s gazelles and Grat’s gazelles that are killed more often than expected based on their abundance. This implicates that cheetahs prefer these species. (Hayward et al, 2006)

Small prey might be underrepresented because most of these studies use data from observations of a kill or finding carcass remains. Kills of small prey are often unobserved because of quick consumption and lack of remains (Randall, 1970).

The target species are small because the cheetah's anatomy is adapted for reaching high speed. They can reach a speed of 103 km/h making it the fastest land mammal in the world (Sharp et al, 1996). As a result, the canines of a cheetah are small and thus the roots are small, creating space for an enlarged nasal passage. This way, more air can be sucked in during exercise or while holding the prey by the throat. (Ewer, 1973) The skull is small; the jaws are short and can therefore not support a large masseter muscle (Marker et al, 2003). The legs are slender and the most elongated of any large cat. (Ewer, 1973; Hunter et al, 2003) With these adaptations, the cheetah is limited in prey size but also vulnerable for kleptoparasites. Up to 12% of kills can be lost to kleptoparasites, spotted hyena and lion in particular. To minimize the chance of a confrontation with these predators, the cheetah is diurnal. (Hayward et al, 2006; Marker et al, 2003)
Once a kill has been made, the cheetah will start consuming his prize. They will first feed on the organs, except for the intestines. Aside from organs, muscle tissue is consumed but the appendicular musculature remains untouched at many kills. If the prey weighs less than 10 kg, most bones will probably be consumed. If the prey is larger, between 30-50 kg, only parts of the rib cage and vertebral column will be consumed. Cheetahs can consume up to 10 kg of food in 2 hours. In most cases they will leave their kill after 1 or 2 hours to avoid encounters with stronger predators. When the population of lions and hyenas in the area is low, they can stay at a kill for a longer period of time. (Phillips, 1993) Studies at Kruger National Park show that cheetahs in a coalition obtain about 1.4 kg/day/cheetah (Mills et al, 2004).

2.2.2 The diet of captive cheetahs

Adult male cheetahs averaging 40 kg require 9.21 MJ/day (maintenance energy) and females averaging 30 require 7.54 MJ/day (Marker et al, 1998). Most zoos in North America feed their cheetahs a commercially prepared horsemeat-based mixture with additional carcasses (portions or whole) once or twice per week (Boler et al, 2009). On average, the cheetahs eat about 1.4 kg daily and little more than half of the zoos utilize a fasting day every week. This feeding schedule results in an intake of approximately 7.52 MJ per cheetah per day (Dierenfeld, 1993). At the Cheetah Conservation Fund in Namibia, global leader in research and conservation of cheetahs, adult cheetahs are fed carcass portions covered with a powder
containing vitamins and minerals ranging from 1.4 to 1.6 kg per cheetah per day. From these pieces most fat and the skin were removed. They were fasted once a week and got additional organ, about 500 g per cheetah when available.

Captive cheetahs that are fed supplemented pieces of meat do not seem to get a well-balanced calcium:phosphorus ratio. Whole rabbit carcasses for example provide a more complete source of minerals. (Depauw et al, 2011)

In the past, zoos have based themselves on diets of domestic cats to compose those of captive wild cats (Vester et al, 2009). Domesticated cats might have adapted to a diet with a higher carbohydrate concentration in comparison to their wild ancestors (Plantinga et al, 2011). A similar example can be found in the domestication of dogs. Where wolves are carnivorous with very little plant consumption, dogs have a more omnivorous nature. This is linked with the domestication of dogs during the switch from nomadic hunter-gatherers to a sedentary lifestyle associated with agriculture. It is there that the wolf found a new food source in human-derived vegetal and animal food waste. During domestication, among other genes, three involved in starch digestion and glucose uptake were the target of selection. (Axelsson et al, 2013) (Bosch et al, 2014)

Phylogenetic analysis of faecal microbiota from captive cheetahs reveals underrepresentation of Bacteroidetes and Bifidobacteriaceae Which is in contrast to data previously reported in domestic cats where Bacteroidetes and Bifidobacteriaceae are common residents of the faecal microbiota (Becker et al, 2014). Therefore it is important that zoos try to mimic the cheetah’s natural diet and not rely on that of domestic cats.

A survey carried out in 2013 reports the diet of 12% of the captive cheetah population housed in 33% facilities known to have cheetahs. The most common diet type was raw meat (37%), followed by commercially prepared food (20%) and carcasses (8%). A mixture of these diets was fed to 35%. Of these diets, 53% were supplemented with vitamins and minerals. (Whitehouse-Tedd et al, 2015)

2.2.3 Link between diet in captivity and health issues

Some disorders are more common in cheetahs in captivity than in the wild. This suggests that properties of captivity are inducing factors in these disorders. Because of the genetic monomorphism in the cheetah and lack of heterogeneity at the HCM loci, infectious diseases were presumed. Yet, a study showed that the causes of mortality in captive cheetahs are mostly degenerative diseases and there is a correlation between severity of the disease and time spent in captivity. This emphasizes even more the importance of the properties of imprisonment in the pathogenesis of these disorders. (Terio et al, 2003)
A study compared disease prevalence in free-ranging Namibian cheetahs with those in two captive populations of similar ages. The captive populations had a high prevalence of gastritis, glomerulosclerosis, systemic amyloidosis and veno-occlusive disease. (Munson et al, 2004)

A first property of imprisonment to address is the presence of stress in captive animals. Stress results in the release of ACTH, which stimulates cells of the adrenal cortex. The long-term result is a hyperplasia of the cortex, which has been observed in captive but not in free-ranging cheetahs. (Terio et al, 2003) Stress has a general negative influence on the function of an animal's immune system and can therefore play a role in any kind of disease. More specific, it is thought that stress plays an important part in the occurrence of glomerulosclerosis. Renal lesions most closely resemble these of early diabetes mellitus. But since none of these animals had pancreatic lesions and no other symptoms of diabetes were present, the cause for this could be the exposure to chronic stress. 80% of the animals in the study had adrenocortical hyperplasia, which results in hyperglycemia and could explain the type of lesions in the kidneys. (Bolton et al, 1999)

The diet and the way in which it is offered can reduce the amount of stress experienced by the animal. Temporal and spatial variation in feeding reduces the occurrence of stereotypical behavior. Since the time spent pacing correlates with excreted levels of cortisol metabolites, the reduction of stereotypical behavior is an indication for stress reduction. By changing the feeding routine and making it less predictable, stress and its negative effects on health can be limited. (Quirke et al, 2011; Quirke et al, 2012) Not only time and place of food provision is important but also the form in which it is presented. Carcasses or portions of carcasses make cheetahs more possessive over their food, play is more often observed around the food and the animals take more time to smell. Longer time is spent on their food than cheetahs fed a formulated diet, making less time for boredom. (Bond et al, 1989; Lindburg, 1988)

A second property is the diet. In captivity, cheetahs can be fed commercial diets, raw meat, carcasses or a mixture of these. The choice of diet formulation can influence the prevalence of the degenerative diseases most frequently observed in this species.

When fed carcasses, the animals need to exercise their masticatory apparatus. Insufficient wear and atrophy from disuse of the masticatory apparatus contributes to the development of focal palatine erosion. This is a self-inflicted injury of the palate caused by the lower first molars. Food particles can be lodged in

<table>
<thead>
<tr>
<th>Disease</th>
<th>free-ranging Namibia</th>
<th>captive North America</th>
<th>captive South Africa</th>
</tr>
</thead>
<tbody>
<tr>
<td>gastritis</td>
<td>11%</td>
<td>99%</td>
<td>99%</td>
</tr>
<tr>
<td>glomerulosclerosis</td>
<td>13%</td>
<td>67%</td>
<td>81%</td>
</tr>
<tr>
<td>renal amyloidosis</td>
<td>4%</td>
<td>35%</td>
<td>37%</td>
</tr>
<tr>
<td>veno-occlusive disease</td>
<td>9%</td>
<td>56%</td>
<td>93%</td>
</tr>
<tr>
<td>adrenal cortex hyperplasia</td>
<td>8%</td>
<td>63%</td>
<td>43%</td>
</tr>
</tbody>
</table>
the defect and cause inflammation. This disorder has only been described in captive cheetahs fed a soft, commercial diet. (Fitch et al, 1982) Soft food also tends to produce more bacterial plaque than firm food (Egelberg, 1965). Firm food has a stimulating effect on the capillaries in the gingival tissue, which improves vitality of all of the supporting structures (Burwasser et al, 1939; Pelzer, 1940).

An excessive supply of protein can have several detrimental effects on the feline’s health. Feeding too frequently or only providing skeletal meat can cause this excessive supply. When this surplus of protein reaches the large intestine microbial fermentation increases. Fermentation of protein leads among other things to the production of several putrefactive compounds such as ammonia, indole, phenol, p-cresol, and biogenic amines. (Depauw et al, 2012) Tyrosine and tryptophan are metabolized by colonic bacteria to respectively p-cresol and indole. Indole is metabolized in the liver to indoxyl sulphate and p-cresol is metabolized to p-cresyl sulphate in the intestinal membrane. Transformed in the bloodstream, these protein metabolites have a negative effect on kidney function, inducing for example glomerular sclerosis, which could explain the high prevalence of glomerulosclerosis in captive cheetahs. Other organ systems are affected as well resulting for example in aortic calcification and aortic wall thickening. (Niwa, 2010; Rossi et al, 2013) An additional effect of excessive protein absorption on the kidneys is the induction of increased renal blood flow and renal hypertension resulting in sclerosis (Bosch et al, 1983; Friedman, 2004).

56% of North American and 93% of South African captive cheetahs suffer from veno-occlusive disease (VOD) in comparison to 8% in a free-ranging population. In 70% of livers with these lesions, a proliferation of Ito cells was established. Hypervitaminosis A may be responsible since Ito cells are vitamin A storing cells. These cells are found in the perisinusoidal space resulting in a perisinusoidal fibrosis that could possibly evolve into VOD. (Gosselin et al, 1988) Food with a high content of vitamin A, liver for example or too much vitamin supplementation could be an inducer of this disease in a captive setting.

A survey conducted in 2013 including 184 captive cheetahs in 86 international facilities compared the prevalence of certain diseases to type of diet. This survey demonstrated that animals suffer less from diarrhea when their diet includes carcasses or ribs at least once per week. Animals would vomit less frequent when fed long bones at least once every week and would vomit more frequent when fed goat meat. Gastritis was also more common in animals fed horsemeat or had a high intake of crude protein. Both goat and horse meat have a high protein content. This protein excess can modify microbiota composition and increase the production of putrefactive compounds. Gastritis was less common in animals fed chicken or hides, viscera, ribs, muscle meat at least once per week. (Whitehouse-Tedd et al, 2015)

An explanation for the high prevalence of systemic AA amyloidosis and renal amyloidosis can be the high prevalence of gastritis in the cheetahs. In 100% of the cheetahs with renal amyloidosis, an inflammation disease was identified, most commonly gastritis. (Papendick et al, 1997) Serum amyloid A protein, a serum precursor of AA-protein is produced in a variety of conditions such as inflammation. The amyloid deposits in organs and impairs normal organ function. (Kumon et al., 1993)
2.2.4 Dietary considerations

Within most zoos worldwide there are three types of diet, carcasses, prepared meat and commercial diets, that are commonly offered to felids.

As previously mentioned, carcass feeding has several beneficial effects on the animal’s health but there are some disadvantages. When presented with a large carcass the animal may eat selectively from it. This could result in an improperly balanced diet. In some regions it might be difficult to find a continuous supply of carcasses. The meat is raw so there is a chance of spreading diseases as is with feeding prepared meat. After feeding a carcass the enclosures require more cleaning. Finally, zoo visitors can object to seeing carnivores feeding on whole carcasses. Because zoos are financially dependent on their visitors, some zoos decided to switch from whole carcasses to pieces of meat or a commercial diet. (Young, 1997)

A survey taken in the United Kingdom in 1997 showed that almost all zoo visitors approved the feeding of insects to reptiles, both on- and off-exhibit. Less, but still a large number of zoo visitors approves the feeding of live rabbits to carnivores. (Ings et al, 1997) This shows that the public, although it is only representative for the United Kingdom, has an understanding for feeding the correct diet to zoo species. It also indicates that there is a hierarchy in what people can accept concerning the type of species to be fed. The public has fewer problems when insects are fed than when rabbits are fed. The zoo community might therefore need to invest more in educating the visitor's on the necessity of suitable diets for large carnivores.

Over the last decades, zoos have been developing more naturalistic enclosures. This shift brought forward two advantages. The animals seem to be more active, which stimulates the public's interest and empathy in that species. It can also have in informal educational impact. Visitors get an insight in how wild animals live and behave. (Reale et al, 1996) The diet fed to the zoo animals should also be part of that informal learning experience.

A survey conducted in 2014 reveals that, of the 86 participating facilities, only zoos in North American give their cheetahs a commercial diet, whereas none of the zoos in Europe or Africa do so (Whitehouse-Tedd et al., 2015). There is room for improvement, especially in Northern American zoos to inform their visitors about the normal diet for all species, especially carnivores. When the general public accepts the feeding of carcasses they will have a better understanding of the species and the entire animal kingdom. Furthermore, zoos will be able to feed their animals the best possible diet without concern for the public opinion.
3. MATERIALS AND METHODS

3.1 THE CHEETAHS

The five female cheetahs used for this research have all been living together in a 2 ha large enclosure for a while without problems at the Cheetah Conservation Fund (CCF). Amani arrived at CCF in 2005 when she was about one year old. At the time of the research she was 9 years old and weighed 36 kg. Blondi and her sister Dusty arrived at CCF in 2000 when they were about 3 months old. At the time of the research they were 14 years old, Blondi weighed 36 kg and her sister 32 kg. Samantha arrived at CCF in 2003 as a cub. At the time of the research she was 10 years old and weighed 30 kg. Sandy arrived at CCF in 2000 when she was about 8 weeks old and at the time of the research she weighed 30.5 kg.

3.2 THEIR NORMAL DIET

Adult cheetahs were fed carcass portions, usually horse or donkey preferably ranging from 1.4 to 1.6 kg per cheetah per day. From these pieces most fat and the skin was removed. They were fasted once a week and get additional organ when available. The carcass pieces were covered with a powder containing vitamins and minerals. Each 100 grams of that powder contained: moisture 1.5 g; calcium 35 g; phosphorus 2.9 g; vit A 27500 iu; vit D 2450 iu; vit E 245 mg; vit C 500 mg; vit B1 12 mg; vit B2 21 mg; vit B6 12 mg; vit B12 100 µg; biotin 750 µg; folic acid 1750 µg; phantothenic acid 50 µg; niacin 200 mg.

3.3 STUDY DESIGN

The diet of the cheetahs used in this study was slightly adjusted 11 days before adding the titanium dioxide marker to their food to give the intestinal microbiota time to adjust to the changed diet. Normally, the skin is removed from the pieces of carcass. In this study, skin and hairs were not removed. The carcass pieces – during the study this was donkey – were covered in vitamin and mineral powder (compositions see above) and they were fed organ on a regular basis. When collecting the leftovers, some complete pieces of skin were found. After a couple of days the large skin flaps on the meat were partially removed so that only small pieces of skin remained on the carcass pieces. After that adjustment, no more leftover pieces of skin were found; presumably most of them were ingested by the animals (Fig. 3).
During nights, it was not possible to stay with the animals because of logistic limitations. Camera traps were used to monitor the animals at night. The cameras couldn’t easily cover the whole enclosure. Therefore we tried to keep them in a smaller enclosure during nights. After the first night we found Blondi with a wound on her toe probably because of the time spend in the small enclosure during the night. The decision was made to keep them in a slightly bigger enclosure during nights. During daytime they were kept in their normal enclosure of 2 ha. Because their normal enclosure of 2 ha is too big to watch all the animals at once, the animals were kept in that smaller enclosure day and night during the 72h after marker ingestion (Fig. 4). Because of the wound on Blondi’s toe she got antibiotics (amoxicilline and clavulanic acid) once on day 2, two times on day 3 till 6 and once on day 7.

Fig. 4: piece of carcass with patches of skin and hair remaining, CCF Namibia, foto by Dhana Leemans
3.4 COURSE OF THE STUDY

On day 12 in the morning, after giving the cheetahs 11 days to adjust to their slightly different diet, all cheetahs got a portion of organ (kidney and liver) with 3 grams of titanium dioxide. All five cheetahs ingested their potion of organ (weight of the carcass pieces and organs in table 1). From that time onwards the cheetahs were observed during daytime. They had access to enclosure 2A and 2B (see plan of enclosure Fig. 1). Two persons were always present at the enclosure during days to observe the cheetahs. The time of defecation and the identity of the cheetahs were registered and afterwards the keepers collected the faeces. At sunset the keepers went in the enclosure and put up camera traps. The camera traps took 3 pictures after detecting movement and covered almost the whole enclosure. During nights all cheetahs were kept in enclosure 2A. In the morning, the keepers checked the enclosure for faeces, if present, the samples were collected and location was registered. Than camera trap pictures were checked for cheetahs defecating at that location. The collected samples were dried in the sun and frozen pending to be transported to Belgium for analysis. Marker concentration was determined following the procedure developed by Myers et al. (2004).
Table 1: outline of the study; weight of the carcass pieces and organs before feeding (skull: the carcass pieces included a skull which was divided in a mandibular- and maxillary-part; fasting day: the animals were not fed that day).

<table>
<thead>
<tr>
<th>day</th>
<th>carcass pieces (kg)</th>
<th>organ (kg)</th>
<th>type of organ</th>
<th>remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>not weighed</td>
<td>0.9</td>
<td>liver</td>
<td>Blondi 1 X anti-biotics in 100g of meat</td>
</tr>
<tr>
<td>2</td>
<td>9.3</td>
<td>0.7</td>
<td>spleen</td>
<td>Blondi 2 X anti-biotics in 100g of meat</td>
</tr>
<tr>
<td>3</td>
<td>skull</td>
<td>0.7</td>
<td>spleen</td>
<td>Blondi 2 X anti-biotics in 100g of meat</td>
</tr>
<tr>
<td>4</td>
<td>11.7</td>
<td>1.1</td>
<td>liver</td>
<td>Blondi 2 X anti-biotics in 100g of meat</td>
</tr>
<tr>
<td>5</td>
<td>8.1</td>
<td>1.1</td>
<td>kidney and heart</td>
<td>Blondi 1 X anti-biotics in 100g of meat</td>
</tr>
<tr>
<td>6</td>
<td>fasting day</td>
<td>1.4</td>
<td>kidney and heart</td>
<td>Blondi 1 X anti-biotics in 100g of meat</td>
</tr>
<tr>
<td>7</td>
<td>8.2</td>
<td>1.1</td>
<td>liver</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>11.25</td>
<td>1.7</td>
<td>kidney and liver</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10.6</td>
<td>1.0</td>
<td>kidney and liver</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.5</td>
<td>1.15</td>
<td>heart</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>13.15</td>
<td>0.5</td>
<td>liver</td>
<td>3 grams Ti O2 per cheetah</td>
</tr>
<tr>
<td>12</td>
<td>11.85</td>
<td>0.5</td>
<td>liver</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>skull</td>
<td>1.15</td>
<td>heart</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>12.15</td>
<td>1.4</td>
<td>liver</td>
<td></td>
</tr>
</tbody>
</table>

Table: 4. RESULTS

Over a period of 72 hours, 26 scat specimens were collected. From each specimen, two samples were taken and marker concentration was determined following the procedure developed by Myers et al. The mean value of these two concentrations is presented in table 2 with the corresponding time after ingestion. For most of the faeces specimen we were able to identify the cheetah. Five specimen were labelled unknown because these defecations were made during nights when we only had camera traps in the enclosure and a positive identification was difficult. In four of these five defecations (table 2, marked with *) the camera trap images could not help us establish when exactly the cheetahs defecated. To use the concentrations in the graph, they all were given the mean time of the time period where we relied on camera traps during that night.
Table 2: timeline of the study; each scat sample with its time of defecation, mean marker concentrations and cheetah identification.

In a graph that uses all the samples plotted out over time we can also use the unknown samples. The maximum concentrations of the marker in the faeces were between 5 and 20 hours after marker ingestion. From 36 hours onwards the concentrations were close to basal level. Another remark that can be made when we plot these defecations over time is that most of them are made during mornings and evenings.
Two of the cheetahs had a maximum mean marker concentration under 0.6 g/100 g dry matter (Blondi 0.442 g/100 g and Dusty 0.598 g/100 g). These values are similar to the base line of marker concentration that is present from about 30 hours after ingestion and stays present throughout the study in all collected samples. The maximum mean marker concentrations of the three other cheetahs (Samantha, Sandy and Amani) are about four times higher. That is why we will only continue with the data collected from Samantha, Sandy and Dusty.
With this information the mean retention time (MRT) can be determined. MRT is the integrated average time between marker ingestion and excretion. Following the procedure of Thielemans et al. 1987, the MRT for Samantha is 28.298 hours, Sandy 21.893 hours and Amani 26.106 hours. The average MRT in the cheetahs fed this natural diet thus was 25 ± 3 hours (mean ± standard deviation).
5. DISCUSSION

In the graph below (graph 5), the MRT in cats, dogs and cheetahs is presented in function of their body weight. A study on 6 senior cats by Peachey et al. in 2000 shows a mean MRT of 35.71 hours. Octanic acid was used here as a marker. Another study on 50 females in 13 breeds of dogs by Hernot et al. 2000 shows differences in MRT depending on the breed of dog and on the body weight. As a marker they used plastic beads. There is a somewhat linear relationship between MRT and body weight in dogs. In comparison to these species, the cheetah has a short MRT considering their body mass.

Graph 5: MRT in dogs, cats and cheetahs in function of their body mass.

At the Cheetah Conservation Fund in Namibia and in most captive settings cheetahs get fed daily, sometimes with one fasting day per week. Since the MRT in cheetahs fed a natural diet of carcass pieces averaging at 2kg/piece is 25 ± 3 hours (mean ± standard deviation) satiety can continue till the next feeding time except for the fasting day. Cheetahs in captivity are not routinely fed carcass. The most common diet type is raw meat followed by commercially prepared food (out of a survey carried out in 2013 reporting the diet of 12% of the captive cheetah population). These diets lack fibre components. Studies in other animals support that diets with a high insoluble fibre content may prolong the gastrointestinal transit time. A study in 2000 by Jewell D.E. et all. reports a voluntary decreased energy consumption in dogs fed a high-fibre diet. A different study presented at the International Nutritional Sciences Symposium 2013 by Pedreira at al. shows a delay in gastric emptying time and colonic filling time when 10% insoluble fibre was added to the kibble diet of dogs. These findings suggest that the feeling of saturation on diets low in fibre content can be shorter than 25 ± 3 hours. Which implements that the animals experience hunger for a longer period
without being in the capacity to resolve this feeling. This can be a source of stress. Stress has a general negative influence on the function of an animal’s immune system and can therefore play a role in any kind of disease. More specific, it is thought that stress plays an important part in the occurrence of glomerulosclerosis. This may explain the high prevalence of renal disease in captive cheetahs.

It would be interesting to determine MRT in cheetahs fed a raw meat and commercial diet. This could confirm the assumption of the dependence of MRT on insoluble fibre content. With that information, diets could be optimized and the general health of captive cheetahs could improve with hopefully a decline in mortality and better reproduction results.
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