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ORIGINAL ARTICLE

Animal fibre: The forgotten nutrient in strict carnivores? First insights in the cheetah

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Summary

As wild felids are obligate carnivores, it is likely that poorly enzymatically digestible animal tissues determine hindgut fermentation, instead of plant fibre. Therefore, faecal concentrations of short-chain fatty acids (SCFA, including branched-chain fatty acids, BCFA), indole and phenol were evaluated in 14 captive cheetahs, fed two different diets differing in proportion of poorly enzymatically digestible animal tissue. Using a cross-over design, the cheetahs were fed exclusively whole rabbit or supplemented beef for 1 month each. Feeding whole rabbit decreased faecal propionic ($p < 0.001$) and butyric ($p = 0.013$) acid concentrations, yet total SCFA was unaltered ($p = 0.146$). Also, a remarkably higher acetic acid to propionic acid ratio ($p = 0.013$) was present when fed whole rabbit. Total BCFA ($p = 0.011$) and putrefactive indole ($p = 0.004$) and phenol ($p = 0.002$) were lower when fed whole rabbit. Additionally, serum indoxyl sulphate, a toxic metabolite of indole, was analysed and showed a quadratic decrease ($p = 0.050$) when fed whole rabbit. The divergent SCFA ratios and the decrease in putrefaction when fed whole rabbit could be caused by the presence of undigested tissue, such as skin, bone and cartilage, that might have fibre-like functions. The concept of *animal fibre* is an unexplored area of interest relevant to gastrointestinal health of captive cheetahs and likely other felids.

Introduction

It is widely recognized that the intestinal microbiota ecology and its associated fermentation processes have important implications for gut health and as a consequence, health of the host (Salminen et al., 1998; Guarner and Malagelada, 2003; Guarner, 2006). Dietary fibre has become a subject of increasing interest in both human and animal (including cats) nutrition. Depending on the source and amounts used, fibre can function as a pre-biotic, weight loss product or therapeutic medium for gastrointestinal disorders in cats (reviewed in Fahey et al., 2004). Fibre can also be an important substrate for the production of short-chain fatty acids (SCFA) in the colon. The latter are an indispensible energy source for the colonocytes, stimulate colonic blood flow and motility and decrease the growth of pathogenic microbiota (Cummings, 1981; Montagne et al., 2003; Rondeau et al., 2003; Wong et al., 2006).

Felids possess a well developed and active gut microbial population (Johnston et al., 1993; Sparkes et al., 1998; Vester et al., 2010) despite the obligate carnivory of the felid taxon. Moreover, beneficial effects of plant fibre on the metabolism and intestinal function in domestic cats have been described (Sunvold et al., 1995; Hesta et al., 2001; Fahey et al., 2004; Verbrugghe et al., 2009, 2010). In the wild, however, consumption of plant fibre can be

expected to be very low to negligible in most felids. Moreover, a natural feline diet consists of substantial amounts of low to non-digestible (glyco)protein-rich matter, such as raw bones, tendons, cartilage, skin, hair or feathers, that are potential substrates for large intestinal microbes. Therefore, it is likely that protein-rich constituents are determining the fermentation pattern in the felid intestine instead of plant fibre. However, until now, the role of dietary (glyco)protein in hindgut fermentation has received little attention in these carnivores.

Fermentation of protein sources is generally regarded as detrimental for gut health because many of its by-products, such as ammonia, indolic and phenolic compounds, are toxic and have been linked to intestinal disease (Weber et al., 1987; Matsui et al., 1995; Pedersen et al., 2002; Tuohy et al., 2006). Although wild felids consume bones, tendons, cartilage, etc. on a regularly basis, in captivity the intake of these animal tissues varies greatly depending on the given diet (commercial, meat-only, and carcass). Hence, it is important to investigate whether the presence or absence of these animal tissues elicits changes in intestinal fermentation, thereby influencing gastrointestinal health or risk to disease.

To the best of our knowledge, hindgut fermentation in felids on a strictly carnivorous diet has not yet been investigated. Unlike domestic cats, captive exotic felids are typically fed an exclusively carnivorous diet. Moreover, the feeding of whole prey is encouraged in zoos, because this has been shown to improve the oral health and behavioural needs of the animals (Bond and Lindburg, 1990; Mcphee, 2002). Therefore, the current study was designed to evaluate faecal characteristics and concentrations of fermentation end products in the cheetah (*Acinonyx jubatus*) as affected by two types of strictly carnivorous diets (whole prey versus meat-only).

Materials and methods

Experimental design

Four male and ten female cheetahs, housed at Ree Park - Ebeltoft Safari (Ebeltoft, Denmark), entered this study. The animals were aged between 22 months and 7 years (mean 3.0 ± 1.6 years), and no medical or health problems were reported or apparent on remote examination. All cheetahs were treated prophylactically for internal parasites (0.2 mg/kg ivermectin per os, Ecomectin®; Scanimal-Health ApS, Pandrup, Denmark) 1 week before the onset of the trial. Four cheetahs were housed individually, whereas four females and one mother with four subadult cubs (22 months of age) were group housed in outdoor enclosures (natural grass and soil terrain), with free access to indoor enclosures (concrete flooring).

The animals were divided into two groups, with each group randomly assigned to one of two test diets. The test diets consisted of exclusively (i) 2.5–3 kg/day/animal unsupplemented whole rabbit (Kiezebrink Putten B.V., Hoge Eng Oost, the Netherlands), including skin, fur and viscera, and (ii) 1.2–1.3 kg or 1.5–1.6 kg (for females and males respectively) chunk beef (Kiezebrink Putten B.V.), supplemented with 10 g/kg (as is) of a vitamin and mineral premix (Carnizoo®; Twilmij B.V., Stroe, the Netherlands) based on the feeding protocol of this zoo. Before the onset of the trial, the cheetahs were mainly fed on supplemented beef. A cross-over study design was used, whereby all cheetahs were acclimated to their diet for 3 weeks before sampling was performed in the fourth week of both testing periods. Outdoor enclosures were cleaned of all food scraps and faeces, and indoor floor beddings were removed prior to the initiation of sampling in the final week of each test period.

The cheetahs were fed separately, and the amount of food offered and refused was weighed on a daily basis during each sample collection period. Feed refusals were not analysed. Therefore, calculated nutrient intakes were based on the amount of consumed food x analysed nutrition composition of the provided diet. All faeces from each enclosure were collected on a daily basis during the final 5 days. In cases where cheetahs were group housed, the mean value of faecal weight was recorded and divided by the number of cheetahs in the enclosure. All faeces were scored by the same person as follows: 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 (adapted from Vester et al., 2008). Faeces were weighed and stored at −20° until further analysis of dry matter (DM).

Additionally, one individual fresh faecal sample from each cheetah was obtained during each collection period. Therefore, cheetahs were separately housed indoors for a maximum of 6 h and monitored. Fresh faecal samples were collected within 15 min of defecation. The fresh faeces were immediately scored and weighed, and pH was determined using a portable pH meter (HI 99141, pH electrode probe HI 72911; Hanna Instruments, Temse, Belgium). A separate aliquot of faeces was collected for analysis of SCFA [including branched-chain fatty

acids (BCFA)] and a second aliquot for the determination of indole, phenol and p-cresol. All samples were stored at −20 °C.

During each collection period, 10 cheetahs (six females and four males) were anaesthetized with intramuscularly administered Ketamin (2.5 mg/kg, Ketaminol®; Intervet/Schering-Plough Animal Health, Ballerup, Denmark) and Medetomidin (0.05 mg/kg, Sedator®; Novartis Healthcare A/S, Copenhagen, Denmark) as part of the zoo's routine veterinary plan. Cheetahs were weighed and had an average weight of 42 kg (±5 kg). As part of the health examination whilst under anaesthesia, blood was drawn from the saphenous vein of each animal, and a subsample of serum was donated to this study for analysis of serum indoxyl sulphate (a metabolite of intestinal indol production, Evenepoel et al., 2009). As a result, serum samples were available from four cheetahs fed the beef diet and six cheetahs fed whole rabbit. Serum samples were centrifuged and immediately stored at −20 °C until analysis.

Chemical analyses

Diet analyses

Three representative samples of both diets were ground using a meat mincer and homogenized, before subsamples were lyophilized. For the whole rabbit, stomach and intestines were removed, because this was left uneaten by all cheetahs. The dry subsamples were subsequently ground in a Whiley mill to pass a 1-mm sieve. Dry matter and ash were determined by drying to a constant weight at 103 °C and combusting at 550 °C respectively. Crude protein (6.25 × N) was determined using the Kjeldahl method (ISO, 2005), and crude fat was analysed according to the Berntrop method (ISO, 1999). Crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF) and total dietary fibre (TDF) were determined using the Official Analytical Chemists methods (Van Soest, 1973; ISO, 1981; Prosky et al., 1985; Van Soest et al., 1991). Total collagen content was determined using the draft I.S.O. standard method for hydroxyproline (1980: ISO/DIS 3496.2). Metabolizable energy (ME) of the test diets was calculated using NRC (2006) estimation procedures (Atwater factors) for domestic dogs, as recommended by Clauss et al. (2010). Proximate analysis of the diets is shown in Table 1.

Fermentation end products

For the analysis of SCFA and BCFA, samples were thawed and 1 g of faecal material was transferred into a centrifuge tube together with 5 ml of water/phosphoric acid/formic acid (550:10:1, v/v/v). Samples were shaken manually before centrifugation (15 min at 22 000 *g* and at 4 °C, Beckman J2-HS, Palo Alto, CA, USA), after which the supernatant was filtered through glass wool prior to GC analysis according to Van Nevel and Demeyer (1977).

Table 1 Analysed nutrient composition of the test diets (%) on dry matter basis (mean ± SD)

	Whole rabbit (*n* = 3*)	Supplemented beef (*n* = 3†)
Dry matter	31.9 ± 1.8	50.1 ± 6.4
Crude protein	61 ± 4	86 ± 9
Collagen (% of crude protein)	18.9 ± 0.9	11.2 ± 3.3
Crude fat	26 ± 3	10 ± 10
Crude ash	11.1 ± 0.9	5.5 ± 0.3
Crude fibre	1.1 ± 0.5	0.9 ± 0.8
ADF	6.1 ± 1.5	2.0 ± 0.2
NDF	8.0 ± 1.2	3.1 ± 1.9
TDF	3.4 ± 0.6	1.2 ± 0.2
GE (kJ/100 g)‡	2510 ± 10	2360 ± 20
ME (kJ/100 g)§	2013 ± 74	1769 ± 223

DM, dry matter; ADF, acid detergent fibre; NDF, neutral detergent fibre; TDF, total dietary fibre; GE, gross energy; ME, metabolizable energy.
*Stomach and intestines were removed, because this was left uneaten by all cheetahs.
†Beef analyses: beef shoulder, beef back and beef upper leg.
‡Determined by bomb calorimetry.
§Calculated: 16.7* crude protein + 37.7* crude fat + 16.7* NfE (domestic dogs, NRC, 2006); NfE, nitrogen-free extract = 100 − crude protein − crude fat − crude fibre − crude ash.

Faecal samples for indole, phenol and p-cresol analysis (1 g) were ground, homogenized and spiked with 100 μl of internal standard (100 μg/ml 5-methylindole). Extraction was performed by adding 15 ml of hexane followed by 10 min of ultrasonic vibration, 10 min of rotation (130 tpm) and further ultrasonic vibration for 10 min. After clarification at 4 °C, the supernatant was decanted and the faecal matter was extracted second time with 15 ml of hexane and subsequent ultrasonic vibration and rotation. Supernatants were pooled and centrifuged at 14 000 *g* for 10 min at 4 °C, and 200 μl of this combined supernatant was transferred to a GC vial for analysis. All chromatographic analyses were performed with a trace gas chromatograph coupled to a PolarisQ quadrupole ion trap mass spectrometer and a Finnigan MAT A200S autosampler (Thermo-Finnigan, Austin, TX, USA).

Bacterial nitrogen was estimated according to the method described by Hesta et al. (2003) adapted from Mason (1969). In brief, bacterial N is described

as the difference between the N concentration in the residue of NDF analysis with and without sodium dodecyl sulphate (SDS) treatment.

Serum indoxyl sulphate

A standard of indoxyl sulphate was weighed and dissolved in acetonitrile, and a series of standard solutions were prepared. Each standard solution of indoxyl sulphate (100 μl; Sigma, Bornem, Belgium) was spiked in blank serum (200 μl) (guinea pig serum, Sera Laboratories International, West Sussex, UK) to prepare calibrators in the concentration range of 0.01–5.00 μg/ml. The calibrators were deproteinized with 100 μl acetonitrile, and 100 μl of internal standard was added (1.0 μg/ml methylparaben in acetonitrile). Calibration curves were plotted by linear regression of the area ratios (compound peak areas relative to the internal standard peak areas) against the concentration of indoxyl sulphate. Serum samples (200 μl) were extracted with 100 μl acetonitrile and spiked with 100 μl of internal standard (1.0 μg methylparaben/ml acetonitrile) in microcentrifuge tubes. Standard solutions and samples were vigorously vortexed and stored at −20 °C overnight. After thawing, the tubes were vortexed again and centrifuged (14 000 *g*) for 30 min at 4 °C. Subsequently, 50 μl of each supernatant was combined with 950 μl aqueous formic acid (0.1%) in HPLC vials for U-HPLC-MS/MS analysis. U-HPLC-MS/MS analysis was performed on a triple quadrupole mass analyser (TSQ Vantage, Thermo Electron, San Jose, CA, USA).

Statistical analyses

Statistical analyses were performed using Superior Performing Software Systems version 17 (SPSS, Chicago, IL, USA). A paired sample *t*-test was performed to evaluate the effects of test diet on food intake, faecal pH and fermentation end products. Consistency scores (based upon mean values of the 5-day total collection) and faecal concentrations of BCFA and p-cresol were not normally distributed; therefore, the non-parametric Wilcoxon signed ranks test was used.

Serum concentrations of indoxyl sulphate, obtained from four cheetahs fed the beef diet and six cheetahs fed whole rabbit, were analysed by the independent two-sample *t*-test. Statistical significance was accepted at $p \leq 0.05$.

Results

Daily food intake was higher ($p = 0.005$) when cheetahs were fed whole rabbit, whereas ME intake was lower ($p < 0.010$) for this diet group, compared with supplemented beef (Table 2). Crude protein intake was lower ($p < 0.001$) when fed whole rabbit, but collagen intake did not differ amongst diet groups ($p = 0.063$). Total crude fat intake and TDF intake were higher ($p < 0.001$) for cheetahs fed the rabbit diet, compared with the supplemented beef diet. On the contrary, crude fibre intake was higher ($p = 0.019$) when fed supplemented beef.

Based upon the values of the 5-day total collection, faecal output (as is) was higher ($p = 0.032$) when fed whole rabbit (304 ± 28 g/day) compared with supplemented beef (192 ± 72 g/day). Faeces from individuals over a 5-day period tended ($p = 0.068$) to firmer consistency when fed whole rabbit (2.1 ± 0.2) compared with supplemented beef (3.1 ± 1.2).

Fresh faecal samples showed comparable ($p = 0.474$) percentages of faecal moisture, and faecal pH did also not differ ($p = 0.721$) amongst diet type (Table 3). Moreover, bacterial nitrogen was unaffected by diet ($p = 0.093$).

Excretion of SCFA resulted in higher concentrations of propionic acid ($p < 0.001$) and butyric acid ($p = 0.013$) for cheetahs fed supplemented beef, compared with when fed whole rabbit (Table 3). In contrast, concentrations of acetic acid ($p = 0.117$) and total SCFA ($p = 0.146$) did not differ amongst diets. Ratios of acetic to propionic acid were lower ($p = 0.007$) for cheetahs fed supplemented beef. The same was observed for the acetic to butyric ratio ($p = 0.032$). Cheetahs fed supplemented beef had higher concentrations of isobutyric acid ($p = 0.028$), isovaleric acid ($p = 0.008$) and total BCFA ($p = 0.011$) than cheetahs fed whole rabbit.

The same trend was observed for the measured putrefactive compounds (Table 3). Indole ($p = 0.004$)

Table 2 Energy (MJ/d), food and nutrient intake (g/d) of 14 cheetahs fed whole rabbit and supplemented beef for 1 month (mean ± SD)

	Whole rabbit	Supplemented beef	p
ME intake	12.6 ± 1.0	14.5 ± 1.4	0.010
Daily food intake	1935 ± 142	1633 ± 155	0.005
Crude protein intake*	375 ± 28	696 ± 66	<0.001
Collagen intake	70 ± 5	78 ± 7	0.063
Crude fat intake*	160 ± 12	79 ± 7	<0.001
Crude fibre intake*	5.9 ± 0.6	7.1 ± 0.8	0.019
TDF intake*	20.6 ± 1.5	8.1 ± 0.8	<0.001

ME, metabolizable energy; TDF, total dietary fibre.

*Food refusals were weighed but not analysed, nutrient intakes are based on daily food intake × analysed nutrient composition of the provided diet.

Table 3 Moisture, pH, bacterial nitrogen, and SCFA, indole, phenol and p-cresol concentrations of fresh faecal samples from 14 cheetahs fed whole rabbit and supplemented beef for 1 month (mean ± SD)

	Whole rabbit (n = 14)	Supplemented beef (n = 14)	p
Moisture (%)	61 ± 8	62 ± 7	0.474
pH	7.0 ± 0.6	7.1 ± 0.5	0.721
N-bacterial (%)	0.8 ± 0.5	0.5 ± 0.2	0.093
μmol/g, as is			
Acetic acid	67 ± 16	77 ± 23	0.117
Propionic acid	14 ± 8	28 ± 9	0.001
Butyric acid	13 ± 6	18 ± 9	0.013
Acetic: propionic acid	6.0 ± 3.2	2.9 ± 0.6	0.007
Acetic: butyric acid	5.7 ± 1.5	4.9 ± 1.7	0.032
Isobutyrate	0.4 ± 0.9	2.5 ± 3.2	0.028
Isovalerate	1.3 ± 2.9	5.3 ± 4.2	0.008
Total BCFA	1.7 ± 3.7	7.8 ± 7.2	0.011
Total SCFA	127 ± 16	97 ± 9	0.146
μg/g, as is			
Indole	56 ± 51	145 ± 79	0.004
Phenol	51 ± 74	148 ± 77	0.002
p-Cresol	ND	7 ± 11	0.068

BCFA, branched-chain fatty acids; SCFA, short-chain fatty acids; ND, not detected.
Total SCFA = acetic acid + propionic acid + butyric acid + isovaleric acid + isobutyric acid.

as well as phenol (p = 0.002) concentrations were significantly higher in cheetahs fed supplemented beef. The concentration of p-cresol was below the detection limit (15 μg/g) in all faecal samples of cheetahs fed whole rabbit and was detectable in four of 14 cheetahs fed supplemented beef. No statistical difference in faecal p-cresol concentration (p = 0.068) was present.

Serum indoxyl sulphate values were four times higher (p = 0.050) in cheetahs fed supplemented beef (0.12 ± 0.07 mg/dl) than when fed whole rabbit (0.03 ± 0.02 mg/dl).

Discussion

Felids (including cheetahs) on commercially prepared diets containing plant-derived fermentable carbohydrates have been shown to produce considerable concentrations of SCFA (Hesta et al., 2001; Vester et al., 2008, 2010; Verbrugghe et al., 2010). Although no plant-derived fermentable carbohydrates were added to the test diets in the present study, the level of SCFA production detected in faeces from cheetahs fed either whole rabbit or supplemented beef was comparable to previously reported levels in domestic cats fed commercial diets (Hesta et al., 2001; Verbrugghe et al., 2010). Yet, concentrations of SCFA were lower than those reported for cheetahs fed a raw beef-based diet (Nebraska Brand®, North Platte, NE, USA) (Vester et al., 2008). However, this commercial diet contained beet pulp, which is a moderately fermentable fibre in cats (Sunvold et al., 1995) and might have caused increasing SCFA production. Because the gastrointestinal tract of whole rabbit was left uneaten by all cheetahs, both test diets can be regarded as strict carnivorous. Therefore, our findings indicate that the undigested part of an animal-based diet can be a source of SCFA production in the cheetah and potentially other carnivores.

Proteins entering the hindgut of carnivores may originate from meat, as well as connective tissues (e.g. collagen), bones, hair or feathers. Whilst meat protein is considered to be highly enzymatically digestible, connective tissue is not (Asghar and Henrickson, 1982) and will therefore enter the hindgut in a relatively unmodified state, where it may be fermented by the bacterial population (Macfarlane and Allison, 1986). Putrefaction in the hindgut is expected to increase in association with a higher dietary connective tissue content. This was confirmed in a study by Vester et al. (2010), in which an increase in dietary collagen resulted systematically in lower apparent protein digestibility and higher fermentation end products concentrations (SCFA, BCFA, indol, phenol, p-cresol and ammonia) in the domestic cat as well as exotic felids (including cheetahs). Therefore, it could be hypothesized that when cheetahs were fed whole rabbit, which contains a relatively large amount of connective tissue, higher putrefaction would be expected than when feeding exclusively meat.

However, the opposite was observed and both the faecal propionic acid, butyric acid and BCFA concentrations, as the concentrations of putrefactive compounds (indole, phenol and p-cresol), were higher for cheetahs fed supplemented beef in comparison with cheetahs fed whole rabbit. Compared with the whole rabbit diet used in the current study, the commercial diet used by Vester et al. (2010) contained similar collagen amounts, but no raw bone, hair or skin was provided, as was also the case for the supplemented beef diet used here. Additionally, although we expected that collagen intake would be consistently higher in cheetahs fed whole rabbit, no significant difference in collagen intake could be shown between the two diet groups in the current study. This indicates that when feeding whole prey, bacterial fermentation is influenced not only by the presence of connective tissue, but apparently also by

the presence of other indigestible animal tissue, such as bone, cartilage and hair. These tissues could have a modifying effect on hindgut fermentation, which may explain the divergent faecal end product concentrations found in cheetahs fed whole rabbit.

However, crude protein intake was significantly higher in beef-fed cheetahs than rabbit-fed cheetahs, and more meat protein might have escaped enzymatic digestion and might have been partially fermented upon beef consumption, resulting in higher fermentation rates. Moreover, feeding whole rabbit resulted in a higher fat intake, which might have been a confounding factor, influencing gastric emptying (Covasa and Ritter, 2000) and digestibility (Swanson et al., 2004). Nonetheless, this does not explain the marked contrast in acetic acid to propionic acid (A/P) and acetic acid to butyric acid ratio between the diet groups. The A/P ratio seen in cheetahs fed supplemented beef was comparable to values previously reported in the domestic cat fed commercial diets (Sunvold et al., 1995; Hesta et al., 2001; Verbrugghe et al., 2010) and cheetahs fed commercial raw-meat-based diets (Vester et al., 2008, 2010). However, the significantly higher A/P in cheetahs fed whole rabbit in the current study has not previously been reported in any felid. This high A/P ratio suggests that the differences in fermentation pattern between test diets were not exclusively influenced by a difference in protein or fat intake.

In domestic cats, an increase in A/P is associated with slower fermentation rates caused by dietary insoluble fibre, such as cellulose (Sunvold et al., 1995). The finding that faeces from cheetahs fed whole rabbit exhibited a higher A/P but comparable acetic acid concentration, suggests that fermentation occurred at a slower rate because of the presence of hair, skin and bones. Total dietary fibre, a valuable analytical measure for fibre content in commercial cat diets, was three times higher in whole rabbit compared with supplemented beef. Moreover, ADF, generally regarded as insoluble fibre, was also three times higher in whole rabbit. Although it should be taken into account that fibre content in these strict carnivorous diets cannot be interpreted the same way as plant fibre and likely contains (glyco)protein-rich material, the high TDF and ADF content analysed in whole rabbit is of interest. Possibly, the poorly digestible animal tissue included in whole rabbit has similar functions as insoluble fibre that might explain the divergent A/P ratio.

Nonetheless, total SCFA did not differ amongst test diets, which indicate a greater complexity. Until now, no information on the fermentation efficiency of poorly enzymatically digestible animal tissue has been available. However, it is likely that variability exists in the fermentation capacity of different animal tissues. It is assumed that collagen is fermentable in the cheetah hindgut, as it is in humans (Macfarlane and Allison, 1986), whereas hair presumably acts more like insoluble fibre. Alternatively, or in addition, the poorly digestible animal tissue included in whole rabbit may act as a bulking agent, filling the colon and providing a physical barrier between bacteria and substrate. This may be an effective mechanism to prevent or reduce spikes in protein fermentation. Furthermore, this colonic filling could have induced a change in microbial activity or species composition, which may explain the difference in fermentation pattern. Although faecal bacterial nitrogen was comparable amongst diet groups, specific microbiological research is needed to further evaluate the impact of poorly digestible animal tissue on the microbial ecology in this animal.

Our finding that putrefaction was significantly lower in cheetahs fed whole rabbit suggests that ingestion of poorly digestible animal tissue may yield a more beneficial fermentation pattern in the cheetah, and possibly other felids. Given the high incidence of gastrointestinal disease in captive cheetahs (Munson, 1993; Munson et al., 1999; Kotsch et al., 2002), in contrast to the wild (Munson et al., 2005), it would be of interest to further investigate the role of indigestible animal tissue in putrefaction and gut health in these strict carnivores. Also, more research is needed for a better understanding of the shift in SCFA ratios when fed whole rabbit, particularly in relation to gut health. It is possible that poorly enzymatically digestible animal tissues are an important component of a cheetah's diet and that these indigestible compounds influence fermentation processes and the microbial ecosystem of the gastrointestinal tract and can therefore be regarded as 'animal fibre'.

The significantly higher faecal output detected in cheetahs fed whole rabbit is consistent with many studies in dogs and cats fed a variety of fibre sources (reviewed in Fahey et al., 2004). In the present study, increased stool output is likely to have been caused by the presence of undigested hair and bones, as this was visually detectable. Also, the water-holding capacity of the stool may have differed between diet groups, resulting in divergent stool output. However, no distinction was made between total and bound faecal water in the present study, and therefore, water-holding capacity could not be evaluated. Additionally, feeding the whole rabbit diet resulted in firmer faeces, and the varia-

tion in faecal consistency was lower compared with the supplemented beef diet. Although the softer faecal consistency when fed supplemented beef does not necessarily include that gut health was compromised, the cheetahs fed supplemented beef were observed to have intermittent diarrhoea in contrast to cheetahs fed whole rabbit. These results further suggest that the intake of indigestible animal tissue may be beneficial to gut health of captive cheetahs. Although the mechanism by which whole rabbit improved faecal consistency remains to be determined, it is possible that the presence of animal fibre may also influence gastrointestinal processes such as gastric empting, satiety, passage rate, motility and absorption. Such effects, however, warrant further investigation.

Putrefactive compounds may not only damage the gut ecosystem, but also influence the general metabolism of the host as they can be converted into toxic metabolites. Recently, the importance of bacterial fermentation in the production of uraemic toxins has been highlighted in humans (Evenepoel et al., 2009; Schepers et al., 2010). Conversion of phenols and indoles leads to uraemic toxins such as p-cresol sulphate and indoxyl sulphate. The latter has been, inter alia, associated with kidney inflammation and the progression of chronic renal failure (Niwa et al., 1999; Kawamoto et al., 2003). Because chronic kidney failure is a major cause of death in captive cheetahs (Munson, 1993; Papendick et al., 1997; Bolton and Munson, 1999; Munson et al., 1999), it was relevant to investigate whether feeding whole rabbits, which induced a decrease of faecal indole, also reduced the concentration of serum indoxyl sulphate. The latter was confirmed because serum indoxyl sulphate of cheetahs fed whole rabbits, resulted in a quadratic decrease compared with cheetahs fed supplemented beef. These results indicate that the differences in fermentation pattern found in this study also affected metabolites present in the blood and thus stress the importance of promoting beneficial fermentation patterns for the overall health of the cheetah.

Our findings highlight the need to improve the understanding of the effects of whole prey diets on gastrointestinal function and health in the cheetah, and other exotic felids. Hence, further research on nutrient digestibility, satiety, passage rates, fermentation and the microbial ecosystem is warranted. Moreover, this study underscores the possible importance of fermentation processes on the overall health of the cheetah and introduces therefore a novel area of interest relevant to the health of captive cheetahs and most probably other feline species.

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