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ABSTRACT: The natural diet of felids contains highly digestible animal tissues but also fractions resistant to small intestinal digestion**,** which enter the large intestine where they may be fermented by the resident microbial population. Little information exists on the microbial degradability of animal tissues in the large intestine of felids consuming a natural diet. This study aimed to rank animal substrates in their microbial degradability by means of an in vitro study using captive cheetahs fed a strict carnivorous diet as fecal donors. Fresh cheetah fecal samples were collected, pooled, and incubated with various raw animal substrates (chicken cartilage, collagen, glucosamine-chondroitin, glucosamine, rabbit bone, rabbit hair, and rabbit skin; 4 replicates per substrate) for cumulative gas production measurement in a batch culture technique. Negative (cellulose) and positive (casein and fructo-oligosaccharides; FOS) controls were incorporated in the study. Additionally, after 72 h of incubation, short-chain fatty acids (SCFA), including branched-chain fatty acids (BCFA), and ammonia concentrations were determined for each substrate. Glucosamine and glucosamine-chondroitin yielded the greatest OM cumulative gas volume (OMCV) among animal substrates ($P < 0.05$), whereas total SCFA production was greatest for collagen ($P < 0.05$). Collagen induced an acetate production comparable to FOS and a markedly high acetate-to-propionate ratio (8.41:1) compared to all other substrates (1.67:1 to 2.97:1). Chicken cartilage was rapidly fermentable, indicated by a greater maximal rate of gas production (R_{max}) compared with all other substrates ($P < 0.05$). In general, animal substrates showed an earlier occurrence for maximal gas production rate compared to FOS. Rabbit hair, skin, and bone were poorly fermentable substrates, indicated by the least amount of OMCV and total SCFA among animal substrates ($P < 0.05$). The greatest amount of ammonia production among animal substrates was measured after incubation of collagen and rabbit bone ($P < 0.05$). This study provides the first insight into the potential of animal tissues to influence large intestinal fermentation in a strict carnivore, and indicates that animal tissues have potentially similar functions as soluble or insoluble plant fibers in vitro. Further research is warranted to assess the impact of fermentation of each type of animal tissue on gastro-intestinal function and health in the cheetah and other felid species.

Key words: animal tissue, *Acinonyx jubatus*, felids, fermentation characteristics, in vitro, microbial degradation

INTRODUCTION

The use of plant-derived dietary fiber in commercially-prepared feline foods has increased in recent years, primarily because of its desirable effects on food intake, appetite, digestion, and intestinal function (Fahey et al., 2004). Plant-derived fermentable fiber also stimulates the production of short-chain fatty acids (**SCFA**), which influence colonic function and health, as well as metabolism in the domestic cat (Groeneveld et al., 2001; Hesta et al., 2001, Hesta et al., 2005; Terada A. et al., 1993; Verbrugghe et al., 2009, Verbrugghe et al., 2010). However, when considering the natural feeding ecology of strict carnivores like the cat, the intake of these plant-derived carbohydrates is negligible (Plantinga et al., 2011). On the other hand, the ration of wild felids contains considerable amounts of animal tissue with indigestible components, such as bones, tendons, skin, hair, and feathers that will enter the hindgut and are potential substrates for fermentation.

Although there is a plethora of data showing the effects of plant-derived fiber sources on the fermentation in the large intestine of animals, there is a dearth of information on the effect of animal derived components. A recent studies in cheetahs (*Acinonyx jubatus*; Depauw et al., 2011) showed differences in intestinal fermentation patterns when animals were fed meat-only diets vs. whole prey, indicating a possible impact of enzymatically-undigested animal tissue on hindgut fermentation. This study was conducted to evaluate the in vitro fermentation kinetics and end-product profiles achieved by different animal substrates that are assumed to be poorly digested by a strict carnivore, the cheetah.

MATERIALS AND METHODS

All animal procedures were approved by the Safari Park Beekse Bergen Institutional Animal Care and Use Committee.

Fermentation Substrates

Unprocessed hair, skin, and bone were obtained from the carcasses of 3 adult female rabbits (2.5 to 3.0 kg) raised for human consumption and obtained from a local slaughterhouse (BVBA Van Assche, Drongen,

Belgium). Rabbits were skinned and hair was obtained by shaving the skin using an electric clipper and washing with demineralized water. Subcutaneous fat was manually removed from the skin using a scalpel. All organs and the majority of muscle were manually removed. The remaining carcass was cut into pieces and placed in plastic containers containing flesh-eating maggots **(**Megafish, Ghent, Belgium**)** for 1 wk at ambient temperature. Thereafter, the remaining pieces of muscle were manually removed and bones were cleaned using a wire-brush. Cartilage was collected from sternums of broiler hens raised for human consumption and obtained from a local wholesale business (De Parelhoen Wild en Gevogelte, Merelbeke, Belgium). The aforementioned animal substrates are termed "self-prepared" and were lyophilized and ground in a Wiley mill to pass through a 1-mm sieve.

Hydrolyzed bovine collagen (Peptan B; Rousselot Healthy Choice, Angoulême, France), glucosamine (1,500 mg formula, Lucovitaal; PK Benelux/Pharma Care BV, Uden, the Netherlands) and a glucosamine-chondroitin mixture (Tri-Gluco Support; New Care Supplements BV, s-Hertogenbosch, the Netherlands) were sourced commercially. Fructo-oligosaccharide (**FOS;** Orafti P95; Beneo-Group, Tienen, Belgium) was used as a positive control (Sunvold et al., 1995a), cellulose (Arbocel, BWW 40, J. Rettenmaier & Söhne, Rosenberg, Germany) served as a negative control (Sunvold et al., 1995b), and lactic casein (Casein; VWR, Leuven, Belgium) was included as a representative of highly fermentable protein source (Macfarlane et al., 1992).

Donors and Feces Collection

Fresh feces were obtained from 8 adult female cheetahs (aged 2 to 10 yr) housed at Safari Park Beekse Bergen (Hilvarenbeek, the Netherlands). As diet type affects large intestinal microbial population, 2 realisticly opposing dietary extremes (beef muscle meat and whole rabbit) for the natural diet of cheetahs were used**.** Consequently, after pooling all samples from both diet groups, a broad microbial spectrum was present, which allowed the ranking of fermentability of the substrates. Variation among animals was not part of the study objectives.

The cheetahs were randomly divided into 2 groups and assigned to 1 of 2 diets. The diets consisted of either beef muscle meat (1.5 kg/d) supplemented with 30 g/kg vitamin-mineral mix (Carmix Special,

Kiezebrink Putten BV, The Netherlands) or whole rabbit (2 to 2.5 kg/d; Kiezebrink Putten BV, The Netherlands). Cheetahs were adapted to 1 of the 2 test diets for 3 wk before feces were collected for in vitro fermentation. The animals were fasted 1 d a week.

On the collection day, the animals were individually housed indoors and a voluntarily voided fecal sample was collected from each animal. Immediately after voiding, samples were collected from the concrete floor into sterile plastic containers filled with carbon dioxide and stored on ice. Collection and transportation to the laboratory of the Animal Nutrition Group (Wageningen University, Wageningen, the Netherlands) were completed within a 4-h time span. Thereafter, samples were pooled by weight across the 2 dietary groups to provide a representative sample.

Incubation for Gas Production Measurement

Pooled fresh feces were diluted in an anaerobic buffer/mineral solution (Becker et al., 2003) and homogenized using a hand held blender. The mixture was filtered through sterile nylon gauze with pores approximately 0.4 mm in diameter. The filtrate was then mixed with additional anaerobic buffer/mineral solution to obtain a 5% fecal solution (w/v). Thereafter, 60 mL buffered fecal suspension was added to each fermentation bottle containing a precisely weighed amount (approximately 0.5 g) of substrate. All procedures were carried out under strict anaerobic conditions by using a constant stream of carbon dioxide during preparation of the substrate-feces suspension.

Fermentation bottles (250-mL serum bottles, Scott, Mainz, Germany**)**, were agitated in 39°C shaking water baths attached to an electronic pressure transducer, as described by Cone et al. (1996). Each substrate was incubated in 4 replicates and 4 blank bottles were incubated containing only 60 mL buffered fecal suspension. Over the following 72 h, cumulative gas production was continuously monitored and recorded (Cone et al. , 1996). After 72 h, 0.75 mL of fermentation liquid was added to a 2 mL safe-lock tube (Eppendorf AG, Hamburg, Germany) containing 0.75 mL of 0.033 H3PO4 for SCFA (including BCFA) analysis or 0.75 mL of 10% $C_2HCl_3O_2$ for NH_3 analysis prior to storage at -20° C until analysis.

Chemical Analyses

Proximate analysis of each substrate was performed in duplicate. Dry matter and crude ash were determined by drying to a constant weight at 103°C and combustion at 550°C, respectively. Organic matter was calculated by subtracting ash from 100. Crude protein (N × 6.25) was determined using the Kjeldahl method (ISO, 2005). Crude fat (**CF**) was analyzed according to the Berntrop method (ISO, 1999) and crude fiber by acid-alkali digestion (ISO, 1981). Nitrogen free extract (**NFE**) was calculated by subtracting ash, CP, CF, and crude fiber from 100. Acid detergent fiber was analyzed according to Van Soest (1973), while total dietary fiber (**TDF**) was determined in duplicate as described by Prosky et al. (1985). Fatty acids and ammonia were determined as described by Bosch et al. (2008).

Data Analysis

Cumulative gas production curves were fitted to the data using a monophasic model as described by Groot et al. (1996). Organic matter cumulative volume (**OMCV**, mL gas/g OM in fermentation bottle) was calculated as follows: $OMCV = [A/(C:t)^{B}]$, in which A = asymptotic gas production (mL gas/g OM); B = switching characteristics of the curve; C = time at which half of the asymptotic had been reached (h); and t = time (h). Maximum gas production rate ($\mathbf{R_{max}}$, mL/h) and time of occurrence (h) of R_{max} ($\mathbf{T_{max}}$) were calculated as described by (Bauer et al., 2004): $R_{max} = \{A \times C^{B} \times B \times T_{max}^{(-B-1)}\}/\{1 + [C^{B} \times T_{max}^{(-B)}]\}^{2}$ and $T_{max} = C \times \{[(B-1)/(B+1)]^{[1/B]}\}$, respectively.

Effect of substrates on fermentation kinetics and fermentation products on 72 h of incubation was tested for significance ($P < 0.05$) using a 1- way ANOVA test. For each variable, differences among means of substrates were detected by ANOVA using the Tukey multiple range test. Individual means were considered different when they exceeded the minimum significant difference calculated by Tukey multiple range test.

Linear regression analysis was performed to investigate the relationship between selected chemical composition variables (independent variables) and SCFA concentration at 72 h incubation (dependent variable) for the animal substrates. Cellulose, casein, and FOS were excluded from regression analysis because these

substrates cannot be regarded as dietary components in a strictly carnivorous diet. All analyses were performed using SPSS 17.0 (SPSS, Chicago, IL).

RESULTS

The proximate composition of the substrates is shown in Table 1. Rabit bone was low in OM compared to other substrates. Crude protein was highest for collagen, followed by rabbit hair, casein, rabbit skin, and chicken cartilage. Moderate amounts of CP were found in glucosamine-chondroitin, glucosamine, and rabbit bone, whereas CP was not detected in the FOS and cellulose substrates. All self-prepared animal substrates were higher in fat content, with rabbit skin having the highest fat content compared to the other substrates. Nitrogen free extract was highest in FOS, high in glucosamine-chondroitin and glucosamine, moderate in cartilage, and low in all other substrates. The ADF concentration was high in rabbit hair and cellulose and moderate in glucosamine-chondroitin. Total dietary fiber was highest for cellulose, moderate for glucosamine-chondroitin and collagen, low in rabbit bone, hair, and skin, and negligible in the other substrates.

Fermentation kinetic parameters and end-products were expressed on an OM basis. Incubation with glucosamine and glucosamine-chondroitin yielded high OMCV (271 and 211 mL/g OM, respectively; $P < 0.05$) compared to other substrates, whereas OMCV with the positive control substrate or FOS was 365 mL/g OM (Figure 1 and Table 2). Chicken cartilage and casein fermentation resulted in similar OMCV, which was moderate compared to FOS ($P < 0.05$). Incubations of inoculum with collagen resulted in low OMCV (80 mL/g OM; $P < 0.05$). Organic matter cumulative gas volume from incubated cellulose, rabbit hair, bone, and skin were similar and yielded the least OMVC compared with others (< 30 mL/g OM; $P < 0.05$). Because of missing model parameters, the statistical program could not estimate R_{max} and T_{max} of casein, cellulose, and rabbit bone and hair. The greatest maximal rate of gas production was recorded for chicken cartilage, which was greater ($P < 0.05$) than FOS. Time of occurrence of R_{max} or T_{max} was later ($P < 0.05$) for FOS compared to chicken cartilage and collagen.

End-product profile per unit of OM differed among substrates (Table 3). The greatest total SCFA production was recorded for FOS ($P < 0.05$), followed by collagen, casein and glucosamine ($P < 0.05$). The FOS and collagen showed comparable acetate production. Collagen not only had a high production of total SCFA but also resulted in a high acetate to propionate ratio relative to all other substrates (8.41:1 for collagen and 1.67:1- 2.97:1 for other substrates). Chicken cartilage and glucosamine-chondroitin produced similar total SCFA production, which was moderate compared to FOS ($P < 0.05$). Total SCFA production from incubated rabbit bone and skin was low ($P < 0.05$), whereas total SCFA production from rabbit hair was negligible and comparable to the negative control cellulose. Butyrate production was greatest for casein and glucosamine ($P < 0.05$). Incubation with casein resulted in the greatest total BCFA production ($P < 0.05$), which was more than double compared to all other substrates that had similar total BCFA production. Considerable variation in BCFA ratios was observed among substrates. In all animal substrates, isovalerate was the main BCFA, whereas fermentation of FOS, glucosamine, and glucosamine-chondroitin led to valerate as the main BCFA. The greatest amount of ammonia production was observed for casein, collagen, and rabbit bone ($P < 0.05$), whereas the least ammonia production was detected for FOS, cellulose, and rabbit hair ($P < 0.05$).

Linear regression resulted in following equation: SCFA (mmol/g OM) = 5.16 (± 0.57) + 0.06 (± 0.02) (TDF% - ADF%) ($R^2 = 0.576$, $P = 0.048$). Other equations were not statistically significant.

DISCUSSION

In vitro techniques have been widely used to assess the fermentability of potential food ingredients intended for animal consumption, including domestic cats (Barry et al., 2011; Sunvold et al., 1995b). Despite the known variation in the microbial community along the gastro-intestinal tract, the use of fresh feces as inocula has been shown to be suitable for in vitro screening of large intestinal fermentation characteristics in dogs (Bosch et al., 2008) and pigs (Williams et al. 2005). Such in vitro techniques enable the evaluation and ranking of a broad range of feed ingredients for their fermentability (Becker et al., 2003; Cone and van Gelder, 1999; Williams et al., 2005). As far as we know, the present study showed for the first time that specific

components in animal tissues have the ability to contribute to intestinal microbial fermentation in the cheetah, and most probably in other felids as well**.** The fermentation of plant fiber sources in the hindgut of carnivores, such as the domestic cat and dog, has previously been reported to have potential consequences for gut function (Bueno et al., 1981), digestibility (Fekete et al., 2000), stool consistency (Hesta et al., 2001), microbiota (Bueno et al., 2000), satiety (Bosch et al., 2009), and metabolism (Hesta et al., 2005; Verbrugghe et al., 2009; Verbrugghe et al., 2010). As such, our findings indicated the need to account for fermentable compounds from animal origin in the diet of carnivores and may provide new insights into promoting intestinal health.

Type 1 collagen is present in bone tissues, tendons, ligaments, and skin, and is the most abundant collagen in the body (Asghar and Henrickson, 1982). Collagen differs from most proteins as it is a complex mesh of fibrils, which are essentially compact molecular clumps. Although collagen is generally regarded as a protein, it is known that glucose and galactose are covalently bound and form an integral part of the collagen protein structure in vertebrate tissues (Asghar and Henrickson, 1982). Moreover, connective tissue contains substantial concentrations of glycosaminoglycans, especially cartilage. Connective tissue may, therefore, make an important contribution to the carbohydrate fraction of a carnivore's diet besides hepatic glycogen. If not digested enzymatically in the small intestine, these compounds will provide protein and carbohydrate substrates to the large intestinal microbiota.

When interpreting the cumulative gas production curves, fermentation of NFE-rich supplements, such as FOS, glucosamine, and glucosamine-chondroitin, was substantially greater than substrates, which were low in NFE, indicating that the saccharide components of the substrates induce fermentation. Additionally, gas production rates (R_{max}) were substantially greater in NFE-rich substrates, with the exception of chicken cartilage. However, the maximum gas production rate for these NFE-rich substrates was reached markedly later (T_{max}) than the self-prepared animal substrates (chicken cartilage and rabbit skin). Although only moderate in its NFE content, chicken cartilage resulted in the highest R_{max} value, which was achieved in a relatively short period of time (T_{max} = 1.2 h). This indicates that specific bacteria, capable of utilizing cartilage components in a more efficient manner than other substrates**,** were present in the inoculum in sufficient concentration to elicit

almost immediate fermentation. In contrast, it seems that more time was necessary before gas production could be detected when incubating vegetable carbohydrates (FOS) and non-pure sources of glycosaminoglycans (i.e., glucosamine-chondroitin and its precursor, glucosamine). The latter is indicative of a requirement for adaptation and proliferation of selective microbiota using these substrates.

Animal derived substrates low in carbohydrates and OMCV also showed considerable SCFA production. Despite the absence of NFE, concentrations of total SCFA for collagen were second highest of all tested substrates, indicating that substantial fermentation took place. The disparity between gas and SCFA production observed can be explained by the underestimation of gas production in the presence of protein when using a bicarbonate-buffered in vitro system (Cone and van Gelder, 1999). In such a system, the gas produced is typically of dual origin; referred to as 'direct' and 'indirect' gas production (Blummel et al., 1997). Direct gas production is a result of fermentation, whereas indirect gas is also produced after buffering of released SCFA. However, in the presence of protein, produced ammonia acts as an additional buffer and, therefore, buffering with bicarbonate decreases, followed by less indirect gas production. Hence, when evaluating the in vitro fermentability of substrates, not only gas production but also SCFA production should be evaluated. Taking gas production kinetics as well as SCFA production into account, glucosamine and chondroitin sulfate were highly fermentable, whereas casein, and chicken cartilage and collagen were only moderately fermentable compared to the positive control (FOS). Rabbit hair, skin, and bone were poorly fermentable substrates.

One thing noticed was the very high ratio of acetic-to-propionic acid for collagen, in contrast to all other substrates. An in vivo study has also reported a very high acetic to propionic acid ratio (6 to 1) in feces from cheetahs fed whole rabbit, compared to supplemented beef (Depauw et al., 2011). In the present study, the rating of substrates was achieved by combining the inoculum of cheetahs fed the 2 relative extreme diets for carnivores (whole prey and muscle meat) to ensure that a representative fecal inoculum was obtained. The distinct difference in fermentation profile in the study by Depauw et al. (2011**)** showed the role of diet type (meat-only vs. whole prey) in fermentation kinetics in the large intestine as a result of the type and amount of enzymatically-indigested animal tissue present in the hindgut.

In vegetable substrates, TDF is a valuable analytical measure for fiber content and, thus, valuable in predicting fermentability of a substrate. Moreover, as ADF in plants is regarded as the insoluble fiber fraction (cellulose + lignin), the subtraction of ADF from TDF will be highly indicative of the fermentation capacity of a substrate. In animal substrates, however, it is important to realize that these analytical variables should not be interpreted in exactly the same manner as they would in herbivore diets; rabbit hair analysis resulted in 86% CP and 70% ADF, clearly indicating that a substrate high in ADF can contain substantial amounts of CP. Regression analysis indicated that also for animal substrates, the variable TDF% - ADF% is the most important determining factor to influence fermentation processes in the cheetah. As for herbivores, ADF seems to represent indigestible fiber, thus reducing fermentation rate. Despite the discrepancies when using these analytical variables for animal components, they seem to fit well as predictors. Because of the protein rich ration of strict carnivores, it is unlikely that nitrogen supply will be the limiting factor in microbial degradation processes. Fitting a similar regression equation for protein as for TDF-ADF did not result in a significant relationship, indicating that protein is not the driving force in differences between fermentability of animal components.

Microbial degradation of carbohydrates generates mainly acetate, butyrate, and propionate, along with gases (e.g., H_2, CH_4, and CO_2). Although protein fermentation also produces acetate, butyrate, and to a lesser extent, propionate (Macfarlane et al., 1992), the branched-chain fatty acids, as part of the intestinal SCFA, mainly originate from protein fermentation (Rasmussen et al., 1988). A study by Macfarlane et al. (1992) indicate that casein fermentation resulted in BCFA being approximately 20% of the produced SCFA, which is consistent with our findings. However, it is important to note, that the animal compounds resulted in substantially lower BCFA production compared to casein. In the current study, an expected low concentration of BCFA was found from the carbohydrate-rich fermentable substrates such as FOS, glucosamine, and glucosamine-chondroitin. Likewise, animal substrates low in NFE content showed a marked reduction in propionate production.

While the fermentation of carbohydrates and protein generates SCFA, protein fermentation also leads to the production of several putrefactive compounds such as ammonia, indole, phenol, p-cresol, and biogenic amines (Cummings and Macfarlane, 1991; Williams et al., 2001). Because putrefactive compounds have been related to chronic intestinal inflammatory diseases in humans (Matsui et al., 1995; Pedersen et al., 2002; Tuohy et al., 2006; Weber et al., 1987), future research should include an extensive determination of putrefactive compounds. In the current study, ammonia production was greatest after incubation of casein, collagen, and rabbit bone, while low concentrations of ammonia were produced from the carbohydrate-rich fermentable substrates.

Considering the high protein content of the natural prey of felids (Plantinga et al., 2011), protein fermentation can be expected to occur in the hindgut. However, despite the high protein intake, in contrast to the captive situation, there is a low incidence of GI disease in free-ranging cheetahs (Munson et al., 2005). It is, therefore, possible that strict carnivores, such as the cheetah, have developed specific adaptive mechanisms that enable them to deal with the potentially negative consequences of protein fermentation. This hypothesis is supported by recent findings where specific microorganisms, Novospingobium sp., were identified in the domestic cat hindgut that can utilize indoles and phenols as a substrate for growth (Lubbs et al., 2009), indicating a possible species specific adaptation. The use of mainly meat only diets for captive cheetahs (Bell, 2010; Dierenfeld, 1993) is in contradiction to the regular intake of raw bones, cartilage, fur, organs, and connective tissues by wild cheetahs (Marker et al., 2003). In vivo research on cheetahs introduced the importance of animal derived components with fiber like effects on minimizing putrefaction (Depauw et al., 2011). Hence, it is important to investigate whether the presence or absence of particular animal tissues elicits changes in digestion and intestinal fermentation, thereby influencing gastrointestinal health or risk to disease. Rabbit hair, skin, and bone were poor fermentation substrates. The possibility exists that these non-fermentable animal tissues may have similar effects to other fibers, which include delaying gastric empting, influencing transit time, motility and absorption, and serving as colonic fill.

Although information in the literature on the digestibility of raw collagen is conflicting (Asghar and Henrickson, 1982; Reutersward et al., 1985a; Reutersward, 1985b; Vaughn et al., 1979), future in vitro research involving predigested animal substrates would be of value to better reflect the starting condition of each substrate in vivo prior to entering the hindgut. This could be of particular importance for the glycosaminoglycans present in connective tissue because the amount of this substrate that actually enters the hindgut in vivo may be negligible due to prior enzymatic digestion. This scenario would have important consequences for the in vivo relevance of in vitro fermentation studies of specific animal substrates such as cartilage. On the other hand, when using predigested substrates, it is important to take into account that highly digestible animal tissues may sometimes escape enzymatic breakdown in the small intestine and enter the hindgut relatively unchanged.

In conclusion, the present data indicated that animal derived components can be an important source of SCFA in the cheetah, with possible implications for gut health and metabolism. The fermentation kinetics of cartilage was unique among the substrates studied because it showed a very rapid onset of fermentation, whereas collagen seemed to induce an exceptionally high acetate to propionate ratio. Given the lack of unprocessed, indigestible (or poorly digestible) animal components in either meat only or commercially prepared diets for captive cheetahs, investigation of the impact of these undigested animal components on intestinal fermentation in felids should be investigated.

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Table 1. Dry matter content (%) and chemical composition[1] of the animal- and plant-derived substrates fermented in vitro using cheetah fecal inoculums (%, DM basis)[1]

Substrate[2]	DM	OM	CP	CF	Cfiber	NFE[3]	ADF	TDF
Casein	90.3	98.6	80.3	0.0	0.1	18.1	0.4	1.1
Cellulose	93.2	100	0.0	0.1	93.9	6.1	55.7	93.5
Chicken cartilage[4]	95.6	90.8	61.3	5.1	0.1	24.3	1.1	<0.1
Collagen	90.6	99.8	99.6	0.2	0.0	0.0	0.7	13.1
Fructo-oligosaccharides	98.1	100	0.0	0.1	0.0	99.8	0.4	<0.1
Glucosamine-chondroitin	95.8	78.4	20.0	0.8	0.1	57.5	11.2	17.5
Glucosamine	99.8	99.9	38.7	0.8	9.9	50.7	0.7	<0.1
Rabbit bone[4]	90.6	47.3	27.5	4.6	11.4	3.8	4.7	2.8
Rabbit hair[4]	89.7	98.4	86.0	2.8	0.3	9.2	70.7	2.7
Rabbit skin[4]	93.7	94.9	70.9	19.0	0.2	4.8	7.8	2.6

[1]CF = crude fat; Cfiber = crude fiber; NFE = nitrogen-free extract; and TDF = total dietary fiber.

[2]Casein (VWR, Leuven, Belgium); cellulose (Arbocel, BWW 40; J. Rettenmaier & Söhne, Rosenberg, Germany); collagen (bovine, Peptan B; Rousselot Healthy Choice, Angoulême, France); fructo-oligosaccharides (Orafti P95; Beneo-Group, Tienen, Belgium); glucosamine (1,500 mg formula Lucovitaal; PK Benelux/Pharma Care BV, AS Uden, the Netherlands); glucosamine-chondroitin (Tri-Gluco Support; New Care Supplements BV, s-Hertogenbosch, the Netherlands).

[3]NFE = OM - CP - CF – Cfiber.

[4]Self-prepared substrates.

Table 2. Fermentation kinetic parameters of animal- and plant-derived substrates incubated 72 h with cheetah fecal inoculums[1]

Item	R_{max}, mL/h	T_{max}, h	OMCV, mL/g OM
Substrate[2]			
Casein	MP[5]	MP	111.0[c]
Cellulose	MP	MP	12.7[a]
Chicken cartilage[3]	71.4[e]	1.2[a]	119.0[c]
Collagen	6.7[a]	0.6[a]	80.0[b]
Fructo-oligosaccharides	59.1[d]	7.4[b]	365.0[f]
Glucosamine	26.0[b]	4.7[ab]	271.0[e]
Glucosamine-Chondroitin	36.1[c]	4.5[ab]	211.0[d]
Rabbit bone[3]	MP	MP	29.7[a]
Rabbit hair[3]	MP	MP	17.0[a]
Rabbit skin[3]	6.7[a]	0.5[a]	28.2[a]
SEM	5.5	0.8	19.4
MSD[4]	10.2	6.2	31.6

[a-f]Means lacking a common superscript letter within each column are different ($P \leq 0.05$).

[1]Rmax = maximal rate of gas production; Tmax = time of occurrence of Rmax; OMCV = measured OM cumulative volume; and OM = 100 - ash. Measurements are based on 4 replicates per substrate.

[2]Casein (VWR, Leuven, Belgium); cellulose (Arbocel, BWW 40; J. Rettenmaier & Söhne, Rosenberg, Germany); collagen (bovine, Peptan B; Rousselot Healthy Choice, Angoulême, France); fructo-oligosaccharides (Orafti P95; Beneo-Group, Tienen, Belgium); glucosamine (1,500 mg formula Lucovitaal; PK Benelux/Pharma Care BV, AS Uden, the Netherlands); glucosamine-chondroitin (Tri-Gluco Support; New Care Supplements BV, s-Hertogenbosch, the Netherlands).

[3]Self-prepared substrates.

[4]MSD = minimum significant difference between any 2 mean values in the same column ($P < 0.05$).

[5]MP = unable to calculate for more than 2 replicates because of missing model parameters or B < 1 (Bauer et al., 2004).

Table 3. Concentration of short-chain and branched-chain fatty acids and ammonia of animal- and plant-derived substrates incubated 72 h with cheetah fecal inoculum[1]

	Fermentation end-product, mmol/g OM								
Item	Acetate	Propionate	Butyrate	isobutyrate	isovalerate	Valerate	Total BCFA	Total SCFA	Ammonia
Substrate[2]									
Casein	3.75^{d}	1.26^{de}	0.93^{g}	0.49^{e}	0.66^{g}	0.33	1.48^{b}	7.42^{gf}	14.50^{d}
Cellulose	0.89^{a}	0.41^{a}	0.09^{a}	0.05^{a}	0.06^{ab}	0.03	0.15^{a}	1.53^{a}	5.00^{ab}
Chicken cartilage[3]	3.22^{cd}	1.42^{e}	0.47^{cd}	0.14^{c}	0.19^{d}	0.06	0.39^{a}	5.50^{de}	8.37^{bc}
Collagen	5.92^{e}	0.70^{abc}	0.65^{def}	0.22^{d}	0.33^{f}	0.14	0.70^{a}	7.96^{g}	14.20^{d}
Fructo-oligosaccharides	5.88^{e}	3.53^{g}	0.70^{ef}	0.10^{b}	0.06^{ab}	0.10	0.26^{a}	10.37^{h}	3.36^{a}
Glucosamine	4.32^{d}	1.73^{f}	0.85^{fg}	0.06^{a}	0.06^{a}	0.09	0.20^{a}	7.11^{efg}	7.36^{b}
Glucosamine-chondroitin	3.55^{d}	1.09^{e}	0.43^{cde}	0.08^{ab}	0.09^{bc}	0.12	0.30^{a}	5.36^{ef}	8.07^{bc}
Rabbit bone[3]	2.26^{bc}	0.98^{cd}	0.35^{bc}	0.19^{d}	0.25^{e}	0.11	0.56^{a}	4.14^{cd}	11.70^{cd}
Rabbit hair[3]	1.18^{ab}	0.59^{ab}	0.16^{ab}	0.08^{ab}	0.12^{c}	0.05	0.25^{a}	2.18^{ab}	5.88^{ab}
Rabbit skin[3]	1.84^{ab}	0.78^{bc}	0.35^{bc}	0.15^{c}	0.21^{d}	0.03	0.39^{a}	3.36^{bc}	7.90^{bc}
SEM	0.31	0.15	0.05	0.02	0.03	0.03	0.07	0.48	0.62
MSD[4]	1.37	0.29	0.21	0.04	0.04	-	0.55	1.76	3.84

[a-h]Means lacking a common superscript letter within each column are different ($P \leq 0.05$).

[1]OM = 100 - ash; BCFA = branched-chain fatty acids; SCFA = short-chain fatty acids; FOS = fructo-oligosaccharide; total BCFA = iso valerate + valerate + iso butyrate; and total SCFA = acetate + propionate + butyrate + iso valerate + valerate + iso butyrate. Measurements are based on 4 replicates per substrate.

[2]Casein (VWR, Leuven, Belgium); cellulose (Arbocel, BWW 40; J. Rettenmaier & Söhne, Rosenberg, Germany); collagen (bovine, Peptan B; Rousselot Healthy Choice, Angoulême, France); fructo-oligosaccharides (Orafti P95; Beneo-Group, Tienen, Belgium); glucosamine (1,500 mg formula Lucovitaal; PK Benelux/Pharma Care BV, AS Uden, the Netherlands); glucosamine-chondroitin (Tri-Gluco Support; New Care Supplements BV, s-Hertogenbosch, the Netherlands).

[3]Self-prepared substrates.

[4]MSD = minimum significant difference between any 2 mean values in the same column ($P < 0.05$).

Manuscript E-2011-4377 Figure 1.

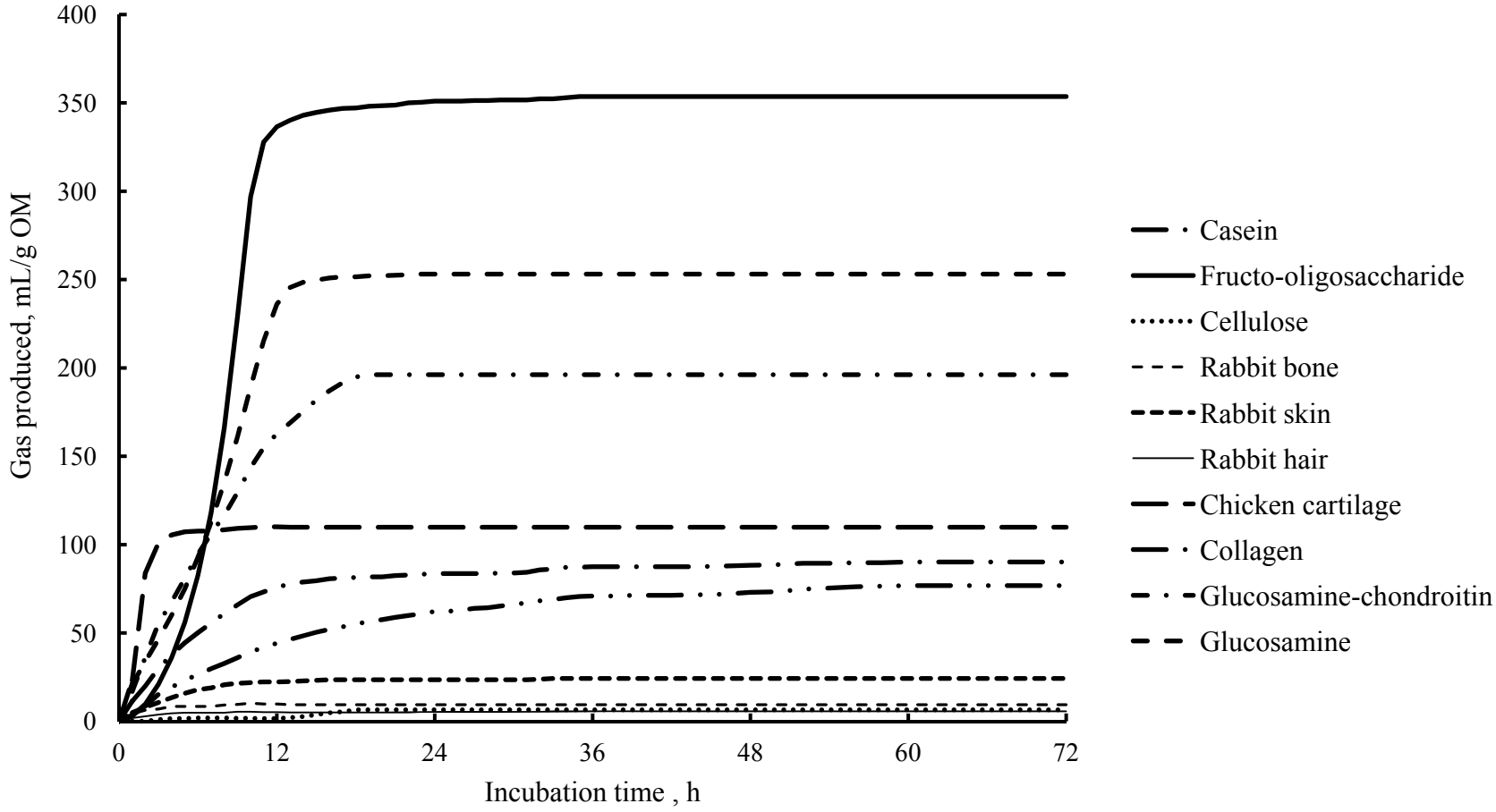

Figure 1. Cumulative gas production of animal- and plant-derived substrates incubated 72 h with cheetah fecal inoculum.