Comparison of fermentation of selected fructooligosaccharides and other fiber substrates by canine colonic microflora

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Objective—To compare fermentation characteristics of fructooligosaccharides (FOS) and other fiber substrates that are commonly found in canine diets.

Sample Population—Fecal samples from 3 adult dogs.

Procedure—The ability of fiber substrates to be used in microbial fermentation reactions was assessed by use of an in vitro fermentation system. Dogs were fed a commercially available food, and feces were collected for use as the microbial inoculum. Substrates used were beet pulp, cellulose, soy fiber, mannanoligosaccharides (MOS), FOS, and 4 inulin products (inulin 1, 2, 3, and 4). Each substrate was incubated anaerobically with fecal inoculum and growth media for 6, 12, and 24 hours, and production of short-chain fatty acids (SCFA) was measured.

Results—Total production of SCFA was higher for fermentation of the 4 inulin products and FOS, whereas fermentation of beet pulp, MOS, and soy fiber resulted in moderate concentrations of SCFA. Fermentation of cellulose produced the lowest concentrations of total SCFA without detection of butyrate or lactate. Butyrate production was greatest for fermentation of the 4 inulin products and FOS. Total lactate production was greatest for FOS and inulin 4. As expected, production of SCFA increased for all substrates as fermentation time increased.

Conclusions and Clinical Relevance—Canine fecal microflora ferment FOS-containing substrates in a similar manner, with little fermentation of cellulose-based carbohydrates. Furthermore, results of an in vitro fermentation system indicate that fiber type affects the metabolic activity of microorganisms, thus influencing the amount and nature of the end products of fermentation. (*Am J Vet Res* 2001;62: 609–615)

Fructooligosaccharides (FOS) are sucrose oligomers naturally found in a number of plants (eg, garlic, chicory, onion, and tomatoes).[1,2] Inulin, a natural storage oligomer of fructose, also is contained in these same plants.[3] These substances are indigestible by mammalian enzymes but can be metabolized by enzymes secreted by normal colonic microflora.

Consumption of FOS promotes beneficial microflora such as *Bifidobacteria* spp, which aid in suppression of some potentially pathogenic bacteria.[4,5] Additionally, a decrease in the amount of toxic metabolites and detrimental enzymes that result from colonic fermentation has been documented with FOS supplementation of diets in humans.[6] Although physiologic effects of FOS fiber are studied most commonly in humans, they also play a beneficial role in diets formulated for other animals. In 1 study,[7] feeding healthy dogs a diet containing FOS resulted in a significant increase in *Lactobacillus* organisms and slightly increased Bacteroidaceae. These purportedly beneficial microflora produce **short-chain fatty acids (SFCA)**, and an increase in their numbers is believed to be indicative of a healthy intestinal environment. In another study,[8] bacterial populations in dogs with small intestinal bacterial overgrowth decreased when fed a diet containing FOS. A decrease in bacterial populations may aid in alleviating poor motility, retention of food, decreased gastric acidity, diarrhea, and weight loss, all of which have been attributed to bacterial overgrowth. These reports offer the promise of benefits; however, few studies have tested the effects of various oligosaccharides on production of SCFA by the **gastrointestinal (GI)** tract of dogs.

Short-chain fatty acids, predominately acetate, propionate, butyrate, and lactate, are produced during microbial fermentation of fiber. Concentrations of SCFA increase as the proportion of fiber reaching the large intestine increases,[9] and concentrations of SCFA are a direct function of the bacterial population and proportional to the time or extent to which digesta are retained.[10] Another study in animals revealed that SCFA[11] are found in the GI tract of all herbivores, most omnivores, and, depending on the diet, many carnivores. Furthermore, specific SCFA have distinctive physiologic roles within these animals.[11] It has been reported that SCFA act as the primary energy source for the intestinal mass, contributing up to 70% of its required maintenance needs.[12] Other studies have documented that SCFA contribute between 5 and 28% of the total maintenance energy requirement in pigs[13,14] and 70 to 80% of the energy requirements in ruminants.[15] In addition, it has been reported that SCFA are conducive to the growth of commensal microflora while concurrently hindering the growth of some potentially pathogenic species.[16-18] Short-chain fatty acids are potent stimulators of insulin secretion, cholesterol metabolism, blood flow to the GI tract, and proliferation of epithelial cells.[11] Although little research has been conducted in dogs or cats, it is reasonable that SCFA also would be beneficial in these species.

Recently, it was documented that results of an in vitro fermentation technique correlated well ($R^2 > 0.90$; $P < 0.05$) with results for in vivo fiber digestion by dogs and cats.[19,20] The study reported here was designed

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to use an in vitro fermentation technique to assess the potential influence of selected oligosaccharides and other commonly used fiber substrates on colonic fermentation in dogs.

Materials and Methods

Animals—Three healthy adult dogs (a male Pointer, a mixed-breed male, and a mixed-breed female) were used in the study. Dogs were maintained as part of the research colony at our animal care facility for ≥ 2 years prior to inclusion in the study. Dogs were group-housed in a climate-controlled facility (20 C) with daily interaction among multiple groups of dogs in a common area. Dogs were fed a commercial diet, and feces were collected for use in fermentation studies. Analysis of the commercial food was performed (**Appendix 1**). The commercial diet met all nutrient requirements for dogs.[21]

All dogs had ad libitum access to water and were fed a commercial food[a] for ≥ 14 days prior to collection of feces. One day prior to collection of feces, dogs were separated and housed individually to enable proper collection of feces.

Substrates—The study compared 4 inulin products, which are all sources of fructooligosaccharides, one additional FOS source, a source of mannanoligosaccharides, soy fiber, beet pulp (positive-control sample), and wood cellulose (negative-control sample). These substrates were selected because of their use or potential use in foods formulated for dogs.

Inulin is a storage polysaccharide consisting of a molecule of sucrose joined by a β(2-1) linkage to a number of fructose molecules; the number of fructose molecules commonly ranges from 30 to 35 but may be as many as 60. Inulin has a chain-length distribution known as the **degree of polymerization (DP)**, which is calculated as follows: DP = No. of fructose molecules + 1. Of the 4 inulin products, 2 (inulin 1[b]and 2[c]) were commercially purified products of chicory root. Both had an average DP of 9; however, inulin 2 was a product that was further processed to optimize solubility. The 2 additional inulin extracts were mixtures of oligo- and polysaccharides comprising fructose joined together by β(2-1) linkages. These polymers contain a molecule of sucrose, most of which are terminated by a glucose unit. The DP of one of the products (inulin 3)[d] was > 12, whereas the other (inulin 4)[e] had a DP that ranged between 2 and 8.

A FOS product[f] contained mixtures of glucose and fructose with a DP that ranged from 3 to 5. It was enzymatically synthesized from sucrose by action of an enzyme from the fungus *Aspergillus niger*. **Mannanoligosaccharides (MOS)**,[g] which are used as a supplement for diets of animals, were derived from the yeast cell wall of *Saccharomyces cerevisiae* var *boulardii*. A soy fiber product[h] was derived from the cell wall material of soybean cotyledon. Finally, a fiber product[i] was used that was almost exclusively composed of cellulose.

Medium composition—Substrate fermentation was conducted as described by Sunvold et al.[20] A specific medium was used to culture the microflora (**Appendix 2**). All components, except for the vitamin mixes, were added before the medium was autoclaved. Vitamin mixes were aseptically added to the medium in an anaerobic chamber after they were sterilized by use of a 0.2-µm filter.

Procedure—Dogs were monitored continuously, and immediately after a dog defecated, fecal material was collected and transported in a sealed container to our laboratory for use in the in vitro fermentation procedure. Fecal material from each dog was collected and immediately placed in separate plastic bags that were sealed after expelling excess air. Fecal collection bags were not sterile; the clean bags were stored in a dry sanitary environment prior to use. Fecal material from each dog served as a separate microbial source for the in vitro fermentation procedure. Each of the 9 substrates evaluated in the study was tested separately in each of the 3 microbial sources.

Within 15 minutes after collection, each fecal sample was diluted 1:10 (wt:vol) in previously warmed (39 C) anaerobic dilution solution[22]; the mixture was blended in a sterile blender for 15 seconds, using an anaerobic glove box.[j] After blending, each fecal solution was filtered through 4 layers of cheesecloth. Appropriate sample and blank 50-ml plastic centrifuge tubes containing 30 ml of medium and 310 mg of substrate were aseptically inoculated with 1 ml of diluted fecal material. The solution provided a 1:100 dilution of the substrate. Tubes were capped with stoppers equipped with 1-way gas release valves. Duplicate tubes were incubated at 39 C for 6, 12, and 24 hours. After incubation, 4-ml aliquots of fluid were collected from each tube and prepared for SCFA analysis.

Each 4-ml aliquot was immediately mixed with 1 ml of 25% metaphosphoric acid, allowed to precipitate for 20 minutes, and centrifuged (25,000 × g for 20 minutes). A sample of the supernatant was collected, and the pellet material was discarded. A 2-ml aliquot of the sample was filtered through a 0.45-µm nylon filter,[k] using centrifugation (8,000 × g for 3 minutes).

Concentrations of SCFA in the filtrate were determined by injecting 3 µl of filtrate into a gas chromatograph[l] equipped with a flame ionization detector and an autosampler.[m] A capillary column[n] (0.53 mm [ID] × 30 m) was used to separate the SCFA. Hydrogen was used as the carrier gas with a linear velocity of 50 cm/s. Temperature of the injector and detector ports was 225 and 250 C, respectively. Initial column temperature was 100 C, which was maintained for 1 minute, and then increased at a rate of 8 C/min to a temperature of 200 C, which was maintained for 2 minutes. Chromatographic data was collected, using a computer software program.[o]

Concentrations of D- and L-lactate were determined, using nicotinamide adenine dinucleotide and lactate dehydrogenase to generate the reduced form of nicotinamide adenine dinucleotide, which was measured at 340 nm, using a clinical chemistry analyzer.[p] A 500-µl sample was obtained from the 4-ml aliquot that had previously been treated with 25% metaphosphoric acid. Following calibration of the analyzer, each sample was vortexed for 10 seconds, centrifuged (1,400 × g for 5 minutes), and then assayed. Values for production of acetate, propionate, butyrate, and total SCFA were derived by determining the mean for duplicate replications of each substrate.

Statistical analysis—Data were analyzed by use of a model that consisted of a randomized complete block design with a 9 × 3-factorial arrangement of substrates and fermentation times as treatments. Dogs were considered to be the blocks. The model included the following terms: substrate, fermentation time, dog, and substrate × time interaction. Data were analyzed, using the general linear models procedure of a statistical program.[23] All data were reported as least-squares means ± SEM for all treatments. Significance was defined as $P \leq 0.05$.

Results

In general, production of SCFA from substrates increased as duration of fermentation increased. Total mean production of SCFA (pooled for all durations of

fermentation) was highest for fermentation of the 4 inulin products and FOS (3.1 to 3.6 mmol/g of organic matter; **Table 1**). Beet pulp, MOS, and soy fiber were significantly less fermentable (1.0 to 1.5 mmol/g of organic matter), and cellulose was least fermentable (0.05 mmol/g of organic matter). Similar observations were made for substrate-by-time interactions, because fermentation of the 4 inulin products and FOS produced significantly higher concentrations of total SCFA at 6, 12, and 24 hours after onset of fermentation, compared with concentrations for the remaining substrates (**Table 2**). Fermentation of the 4 inulin products, FOS, and MOS yielded the greatest increase (nearly 5-fold increase) in production between 6 and 24 hours after onset of fermentation.

Fermentation of the 4 inulin products and FOS produced significantly higher mean acetate concentrations (1.9 to 2.4 mmol/g of organic matter; Table 2) than did fermentation of the remaining substrates. Fermentation of beet pulp and MOS produced moderate acetate concentrations (mean, 1.1 and 0.8 mmol/g of organic matter, respectively). Fermentation of soy fiber yielded a small amount of acetate (0.6 mmol/g of organic matter), whereas fermentation of cellulose produced the least amount (0.03 mmol/g of organic matter; Table 1). Fermentation of the 4 inulin products, FOS, and MOS yielded the greatest increase (≥ 3-fold increase) in acetate production between 6 and 24 hours after onset of fermentation (Table 2). Fermentation of soy fiber and beet pulp yielded moderate increases in acetate production between 6 and 24 hours after onset of fermentation; however, acetate production did not increase significantly ($P = 0.10$) from the fermentation of cellulose.

Fermentation of inulin 3 and 4 and FOS resulted in significantly higher amounts of propionate (0.8 to 1.0 mmol/g of organic matter; Table 1), compared with production for other substrates. Fermentation of inulin 1 and 2 yielded the next-highest propionate production, whereas fermentation of beet pulp, MOS, and soy fiber all resulted in moderate concentrations (0.28 to 0.40 mmol/g of organic matter). Fermentation of cellulose resulted in the lowest mean propionate concentration among all substrates. Fermentation of the 4 inulin products and MOS resulted in a ≥ 7-fold increase in production between 6 and 24 hours after onset of fermentation; however, production from fermentation of MOS was significantly less than that for fermentation of the 4 inulin products. Propionate production resulting from fermentation of FOS increased significantly between 6 and 12 hours after onset of fermentation; however, concentrations at 12 hours were significantly (6-fold) higher than concentrations at 24 hours.

Butyrate production was significantly higher for fermentation of the 4 inulin products and FOS (0.27 to 0.35 mmol/g of organic matter Table 1); however, fermentation of inulin products yielded a higher, but not significantly so ($P = 0.11$), mean butyrate concentration than fermentation of FOS. Fermentation of MOS, beet pulp, and soy fiber yielded intermediate amounts (0.19, 0.11, and 0.06 mmol/g of organic matter, respectively), whereas fermentation of cellulose resulted in undetectable amounts of butyrate. With the exception of cellulose, butyrate concentra-

Table 1—Amount of short-chain fatty acids* produced during fermentation of various fiber products by canine intestinal icroflora

Substrate	ACE	PRO	BUT	LAC	TSCFA
Beet pulp	1.08	0.28	0.11	0.07	1.47
Cellulose	0.03	0.02	0.00	0.00	0.05
FOS	1.94	0.85	0.27	0.49	3.06
Inulin					
1	2.26	0.72	0.33	0.30	3.31
2	2.25	0.71	0.35	0.28	3.31
3	2.41	0.81	0.34	0.25	3.56
4	2.24	0.98	0.30	0.41	3.53
MOS	0.85	0.41	0.19	0.03	1.44
Soy fiber	0.65	0.28	0.06	0.02	1.00
SEM	0.13	0.08	0.03	0.04	0.21
LSD	0.36	0.22	0.08	0.11	0.58

*Values represent least-squares means (mmol of fatty acid/g of organic matter [OM]) for pooled data obtained for all fermentation times (ie 6, 12, and 24 hours after onset of fermentation).

ACE = Acetate. PRO = Propionate. BUT = Butyrate. LAC = Lactate. TSCFA = Total short-chain fatty acids. LSD = Least-squared difference. FOS = Fructooligosaccharides. MOS = Mannanoligosaccharides.

Inulin 1 = Commercially purified product of chicory root with a degree of polymerization (DP) of 9. Inulin 2 = commercially purified product of chicory root with DP of 9 that was further processed to optimize solubility. Inulin 3 = Mixture of oligo– and polysaccharides with DP > 12. Inulin 4 = Mixture of oligo– and polysaccharides with DP between 2 and 8.

Table 2—Values for short-chain fatty acid production 6, 12, and 24 hours after onset of fermentation of various fiber products by canine intestinal microflora

	ACE (mmol/g of OM)			PRO (mmol/g of OM)			BUT (mmol/g of OM)			ACE:PRO			TSCFA (mmol/g of OM)		
Substrate	6	12	24	6	12	24	6	12	24	6	12	24	6	12	24
Beet pulp	0.64	0.69	1.90	0.14	0.16	0.54	0.07	0.07	0.20	4.6:1	6.0:1	4.0:1	0.85	0.92	2.60
Cellulose	0.04	0.00	0.04	0.21	0.17	0.30	0.00	0.00	0.00	1.6:1	7.8:1	1.4:1	0.06	0.01	0.07
FOS	0.71	2.34	2.78	0.15	0.92	0.15	0.12	0.32	0.38	5.0:1	2.5:1	1.9:1	0.97	3.60	4.60
Inulin															
1	0.79	2.77	3.22	0.15	0.64	1.38	0.14	0.40	0.46	5.6:1	5.3:1	2.6:1	1.07	3.81	5.06
2	0.86	2.66	3.24	0.16	0.62	1.35	0.15	0.38	0.51	5.7:1	5.6:1	2.4:1	1.17	3.66	5.10
3	0.99	3.07	3.18	0.18	0.87	1.39	0.13	0.40	0.47	5.6:1	4.4:1	2.3:1	1.30	4.34	5.04
4	0.90	2.65	3.18	0.14	1.10	1.72	0.13	0.37	0.40	6.3:1	2.4:1	1.9:1	1.17	4.11	5.29
MOS	0.33	0.89	1.34	0.10	0.34	0.79	0.06	0.22	0.28	1.9:1	2.6:1	3.1:1	0.49	1.45	2.40
Soy fiber	0.40	0.56	1.00	0.13	0.22	0.48	0.03	0.05	0.10	3.2:1	2.3:1	2.0:1	0.56	0.84	1.56
SEM	—	0.23	—	—	0.14	—	—	0.05	—	—	1.4:1	—	—	0.37	—
LSD	—	0.64	—	—	0.39	—	—	0.14	—	—	3.9:1	—	—	1.02	—

— = Not applicable.
See Table for remainder of key.

Table 3—Percentage of the total amount of short-chain fatty acids produced 6, 12, and 24 hours after onset of fermentation of various fiber products by canine intestinal microflora

	ACE (%)			BUT (%)			TLAC (mmol/g of OM)			L-LAC (%)		
Substrate	**6**	**12**	**24**	**6**	**12**	**24**	**6**	**12**	**24**	**6**	**12**	**24**
Beet pulp	75.2	64.8	72.4	8.3	22.0	7.8	0.07	0.10	0.04	79.2	75.9	60.3
Cellulose	20.0	58.7	56.9	0.0	8.3	2.2	0.00	0.00	0.00	0.0	0.0	0.0
FOS	73.4	64.8	59.9	11.6	8.7	8.1	0.16	0.65	0.67	84.3	70.6	76.6
Inulin												
1	74.0	73.1	64.2	12.5	10.7	9.2	0.12	0.38	0.43	77.3	73.2	76.2
2	74.3	73.5	63.6	12.2	10.4	10.0	0.10	0.34	0.40	79.8	74.4	74.3
3	75.3	71.4	63.2	10.8	9.3	9.3	0.11	0.35	0.29	77.3	71.5	71.6
4	76.0	64.0	60.1	11.5	8.9	7.4	0.12	0.60	0.51	79.5	69.3	77.6
MOS	66.9	60.1	56.5	12.3	12.7	12.0	0.00	0.05	0.04	52.4	62.4	57.9
Soy fiber	70.9	67.8	64.7	5.7	6.1	5.7	0.00	0.03	0.02	59.2	41.6	36.0
SEM	—	9.0	—	—	6.3	—	—	0.07	—	—	25.6	—
LSD	—	24.9	—	—	17.4	—	—	0.19	—	—	70.9	—

TLAC = Total lactate concentration, calculated as amount of L-lactate + amount of D-lactate. *See* Tables 1 and 2 for remainder of key.

tions increased significantly between 6 and 24 hours after onset of fermentation for all substrates examined (Table 2).

Mean total lactate production was significantly higher for fermentation of inulin 4 and FOS, compared with that for fermentation of other substrates (Table 1). Fermentation of the other 3 inulin products produced the next-highest amount of lactate. Total lactate yield from fermentation of soy fiber, MOS, and beet pulp (0.02 to 0.07 mmol/g of organic matter) was minimal, whereas fermentation of cellulose resulted in undetectable concentrations. Fermentation of soy fiber and MOS yielded approximately equivalent amounts of D- and L-lactate, whereas fermentation of beet pulp, the 4 inulin products, and FOS yielded substantially less D-lactate than L-lactate **(Table 3)**.

Discussion

The fermentative capacity in dogs generally is believed to be limited because of anatomic features of the GI tract (eg, a small cecum, unsacculated large intestine) and the carnivorous nature of their diet in the wild. However, domesticated canids often consume diets containing > 35% starch; thus, the composition of diet has become more omnivorous in nature. Additionally, studies have revealed that dogs can utilize dietary fiber, depending on its source.[24-27] Furthermore, it has been indicated that FOS fiber can alter intestinal bacteria populations in clinically normal dogs.[7] Because all species of bacteria ferment some component of the digesta and produce various SCFA,[11] alterations in microflora may, in turn, modify these fermentative end products.

Investigations have revealed that SCFA play several important roles in maintaining health of animals. It has been reported that SCFA, mainly acetate, propionate, and butyrate, act as primary energy sources for the intestinal mass in rabbits[12] and contribute up to 28% of the total maintenance energy requirement in pigs.[14] In addition, SCFA promote gastric emptying and growth of commensal microorganisms, increase insulin secretion, and alter cholesterol metabolism. Research in these areas in dogs is limited; however, on the basis of studies in other animals, factors that affect production and utilization of SCFA may be important in maintaining health of the GI tract and the entire dog.

Use of the in vitro technique in the study reported here revealed that certain fiber sources could be extensively fermented by bacteria typically found in the GI tract of dogs, resulting in differing molar proportions of SCFA. For example, the 4 inulin products and FOS were all highly fermentable. Little variation in fermentation patterns would be expected for these substrates, because their chemical compositions are extremely similar. Hartemink et al[28] concluded that in an in vitro system, inulin 4 and FOS preparations were readily fermented by human enterobacteria, with fermentation of inulin 4 being most rapid. In addition, FOS utilization by canine fecal microflora resulted in high total SCFA production (5.67 mmol/g of organic matter) 24 hours after onset of fermentation.[27] This value is slightly higher than concentrations (Table 2) detected in the study reported here (mean, 5.02 mmol/g of organic matter); however, the difference could be attributable to variations in the number of organisms in the 2 inoculum sources. When an inoculum contains a lower concentration of viable organisms, less fermentation of substrate may result. Nevertheless, bacterial counts were not conducted in either study.

Similar to other studies,[19,27] results of the study reported here indicate that fermentation of beet pulp produced moderate amounts of SCFA. However, Titgemeyer et al[29] reported that in vitro fermentation of beet pulp by human fecal microflora produced minimal amounts of SCFA, which again indicates differences in fermentative activity among species. In addition, composition of beet pulp products is somewhat variable, which may influence SCFA production. Unlike the other substrates, cellulose typically results in low **organic matter disappearance (OMD)** values (< 10%), which yields limited amounts of SCFA (< 1 mmol/g of substrate [dry-matter basis]).[27,29-30]

Generally, as retention time of digesta increases, in vivo fermentation increases, which in turn results in higher SCFA production. Therefore, it is reasonable to expect that as duration of in vitro fermentation increases, there will be a resulting increase in SCFA production. Results indicated that, with the exception of cellulose, fermentation of all substrates increased between 6 and 24 hours, as evidenced by

higher concentrations of total SCFA. Fermentation of the 4 inulin products, FOS, and the product that contained MOS yielded nearly a 5-fold increase in production between 6 and 24 hours after onset of fermentation. Sunvold et al[19] reported similar results when studying in vitro fermentation of selected fiber sources by canine fecal inoculum.

In the GI tract of monogastrics, fermentation begins when food enters the stomach and continually increases as digesta are moved through the tract, with maximum fermentation taking place in the large intestine.[10,31] Sunvold et al[27] illustrated that when values for total digestible fiber of diets formulated with supplemental fiber were matched with the 24-hour in vitro OMD values for each respective fiber source, the resulting correlation was high ($R^2 = 0.93$, $P < 0.01$). In that study, mean retention time ranged from 21.0 to 32.3 hours and was calculated in dogs that were orally dosed with chromium-mordanted nondetergent fiber within 30 minutes after feeding of diets containing various fiber sources or fiber blends. Total digestible fiber in the diet then was compared to OMD values after 6, 12, and 24 hours of fermentation. Results of additional studies support these findings and indicate that in vitro assessment of fiber utilization by dogs, cats, and humans is a rapid noninvasive way to predict in vivo fermentation of fiber.[19,20,29] The strong correlation between in vivo values for total digestible fiber and in vitro OMD values indicates that values obtained after 24 hours of in vitro fermentation would likely represent profiles in the large intestine. Assuming there is less residence time for fiber in the small intestine, in vitro production of SCFA at 6 hours would probably be more indicative of events in the small intestine.

Altering the components of a diet can change the metabolic activity of microorganisms, thus influencing the amount and nature of fermentation end products.[32] Because varying roles have been established for each SCFA, it is important to investigate alterations in fermentation profiles. The **acetate-to-propionate ratio (ACE:PRO)** was calculated to determine such shifts. For the sample obtained 6 hours after onset of fermentation, ACE:PRO decreased for the 4 inulin products, FOS, and soy fiber (Table 2), whereas the ACE:PRO increased for beet pulp, cellulose, and MOS, thus indicating variations in fermentation patterns among substrates. In ruminants, rapid fermentation of substrate leads to production of less acetate and more propionate.[33] In the study reported here, acetate production continued to increase as fermentation time increased from 6 to 24 hours; however, the percentage increase in propionate production was greater, thus decreasing the ACE:PRO. On the basis of the findings of Van Soest,[33] a lower ACE:PRO would indicate that as duration of fermentation increases, these substrates are degraded more rapidly.

The importance of SCFA, particularly butyrate, as a metabolic fuel for large intestinal cells of rats has been documented.[34] However, until recently, this phenomenon had not been evaluated in dogs. Drackley et al[35] reported that canine enterocytes and colonocytes readily oxidize butyrate, propionate, glucose, and glutamine. Analysis of results also suggests that luminal butyrate is a potential energy source for enterocytes and colonocytes, because butyrate oxidization is not affected by glutamine, β-hydroxybutyrate, acetate, or propionate.[35] However, when substrates are combined (5 m*M* glucose, 5 m*M* butyrate, 5 m*M* propionate, and 1 m*M* glutamine), oxidation of glucose and propionate by canine enterocytes and oxidation of butyrate, glutamine, and propionate by canine colonocytes is decreased. These findings suggest that feeding a diet that promotes availability of butyrate in the small intestine would be advantageous to dogs. Banta et al[36] found that dogs fed meat-based diets had substantial concentrations of SCFA in the jejunum, whereas dogs fed cereal-based diets did not. Because dogs have evolved primarily as carnivores, the findings of Banta et al[36] and Drackley et al[35] support the physiologic importance of butyrate production in the small intestine and, to some extent, the large intestine of dogs. Analysis of results of our study indicated that fermentation of FOS-containing substrates yielded increased amounts of butyrate, with values from inulin products slightly higher than those from FOS. Similar observations were reported by Sunvold et al,[q] who indicated that in vitro production of butyrate was substantially greater for fermentation of beet pulp and FOS than for acemannan or cellulose. Because the possibility exists that butyrate has a potential role in maintenance of the health of the GI tract in dogs, it may be of great benefit to determine alternative fiber sources that enhance in vivo production of butyrate.

In the past, it was believed that lactate was an unimportant intermediate of microbial fermentation in many species; however, recent studies have indicated otherwise. Hinton et al[37] observed that in vitro production of lactate by commensal bacteria decreased pH of the media such that the growth of pathogenic *Salmonella* Typhimurium and *Escherichia coli* 0157:H7 was inhibited. In addition, it has been documented that lactate is metabolized by *Veillonella* spp to produce acetate, propionate, carbon dioxide, and hydrogen, which appear to be important components in reducing colonization by pathogenic bacteria.[38,39] The proportion of lactate produced from fermentation of inulin 3 and FOS was significantly higher than that produced from fermentation of other substrates used in the study. In addition, lactate production from fermentation of FOS constituted approximately 39% of the total SCFA in another study.[q] That value is considerably higher than the value reported here, but it could be explained on the basis of variation in microbial numbers as well as differences in species composition between inoculum sources. Nevertheless, fermentation of dietary fiber that results in higher lactate concentrations may be important for providing dogs with an increased resistance to colonization of enteropathogenic organisms.

Dietary fiber is believed to be among the more prominent colonic stimuli for cellular proliferation or differentiation, resulting in morphologic and metabolic changes within the GI tract. In another study,[40] weight of the canine large intestine (per kg of body

weight) was increased when beet pulp or pectin-gum arabic was fed, relative to that when cellulose was fed as the principle fiber source. Additional studies revealed that addition of a blend of fermentable fiber to the diet of dogs increased colonic blood flow,[r] increased excretion of bacterial nitrogen, and increased overall fecal nitrogen excretion.[s]

Analysis of data generated from a study conducted on dogs indicates that use of fermentable fiber blends decreases urinary excretion of nitrogen while increasing fecal excretion of nitrogen without compromising delivery of essential nutrients to the host and without adverse effects such as diarrhea or excessive flatulence.[41] This repartitioning of nitrogen excretion reduces the reliance on the kidneys for nitrogen disposal, allowing for feeding of increased amounts of dietary protein to dogs that have chronic renal failure.[42] It also was reported that ingestion of fermentable fiber by healthy dogs increased secretion of insulin and increased intestinal capacity for glucose transport, resulting in improved glucose homeostasis.[43] Thus, fermentation of dietary fiber has important physiologic and anatomic effects on the nutritional status of dogs. The importance of fiber digestion in the overall well being of dogs deserves further attention.

[1]Eukanuba Premium Performance, The Iams Co, Lewisburg, Ohio.
[b]Inulin HD, Imperial Suiker Unie, Sugar Land, Tex.
[c]Inulin IQ, Imperial Suiker Unie, Sugar Land, Tex.
[d]Raftiline, Raffinerie Tirlemontoise Inc, Tienen, Belgium.
[e]Raftilose, Raffinerie Tirlemontoise Inc, Tienen, Belgium.
[f]Nutraflora FOSGTC, Golden Technologies, Golden, Colo.
[g]Sabomos, Alltech Inc, Nicholasville, Ky.
[h]Fibrim145, Protein Technologies International, St Louis, Mo.
[i]Solka Floc, Fiber Sales and Development Corp, St Louis, Mo.
[j]Protector glove box, LabConco, St Louis, Mo.
[k]Microspin, Whatman Lab Sales, Hillsboro, Ore.
[l]Varian 3400 gas chromatograph, Varian Associates, Walnut Creek, Calif.
[m]Varian model 8200CX, Varian Associates, Walnut Creek, Calif.
[n]Restek Stabilwax DA, Restek Corporation, Bellefonte, Pa.
[o]HP General Chemstation software, Hewlett-Packard, Avondale, Pa.
[p]Cobas Mira Roche, Somerville, NJ.
[q]Sunvold GD, Reinhart GA, Adams SM, et al. Fermentability of several potential dietary fiber sources for canines (abstr). *J Vet Intern Med* 1996;10:A158.
[r]Howard MD, Kerley MS, Mann FA, et al. Dietary fiber sources alter colonic blood flow and epithelial cell proliferation in dogs (abstr). *J Anim Sci* 1997;75(suppl 1):A170.
[s]Howard MD, Sunvold GD, Reinhart GA, et al. Effect of fermentable fiber consumption by the dog on nitrogen balance and fecal microbial nitrogen excretion (abstr). *FASEB J* 1996;10:A257.

Appendix 1

Analysis of a commercially available food formulated for dogs[a]

Ingredients
Chicken, chicken byproduct meal, rice flour, ground corn, ground grain sorghum, fish meal, chicken fat (preserved with mixed tocopherols and citric acid), dried beet pulp, chicken digest, dried egg product, brewers dried yeast.

Calories (metabolizable energy)
4,332 kcal/kg, dry-matter basis.

Guaranteed analysis
Crude protein (minimum), 25.0%; crude fat (minimum), 16.0%; crude fiber (maximum), 5.0%; moisture (maximum), 10.0%; omega-6 fatty acids (minimum), 2.53%; omega-3 fatty acids (minimum), 0.46%.

Appendix 2

Composition of medium used for in vitro fermentation

Component	Concentration in medium
Solution A (ml/L)*	330.0
Solution B (ml/L)†	330.0
Trace mineral solution (ml/L)‡	10.0
Water-soluble vitamin mix (ml/L)§	20.0
Folate:biotin solution (ml/L)‖	5.0
Riboflavin solution (ml/L)¶	5.0
Hemin solution (ml/L)#	2.5
Short-chain fatty acid mix (ml/L)**	0.4
Resazurin (ml/L)††	1.0
Distilled H_2O (ml/L)	296.0
Yeast extract (g/L)	0.5
Trypticase (g/L)	0.5
Cysteine HCl • H_2O (g/L)	0.5
Na_2CO_3 (g/L)	4.0

Composition (g/L):*NaCl, 5.4; KH_2PO_4, 2.7; $CaCl_2 \cdot H_2O$, 0.16; $MgCl_2 \cdot 6H_2O$, 0.12; $MnCl_2 \cdot 4H_2O$, 0.06; $CoCl_2 \cdot 6H_2O$, 0.06; $(NH_4)_2SO_4$, 5.4 . Composition (g/L):†K_2HPO_4, 2.7. Composition (mg/L):‡EDTA (disodium salt), 500; $FeSO_4 \cdot 7H_2O$, 200; $ZnSO_4 \cdot 7H_2O$, 10; $MnCl_2 \cdot 4H_2O$, 3; H_3PO_4, 30; $CoCl_2 \cdot 6H_2O$, 20; $CuCl_2 \cdot H_2O$, 1; $NiCl_2 \cdot 6H_2O$, 2; $Na_2MoO_4 \cdot 4H_2O$, 3. Composition (mg/L):§Thiamin HCl, 100; D-pantothenic acid, 100; niacin, 100; pyridoxine, 100; *p*-aminobenzoic acid, 5; Vitamin B_{12}, 0.25. Composition (mg/L):‖Folic acid, 10; D-biotin, 2; NH_4HCO_3, 100. Composition:riboflavin, 10 mg/L in 5 m*M* HEPES.\ #Hemin, 500 mg/L in 10 m*M* NaOH. **250 ml/L of each of n-valerate, isovalerate, isobutyrate, and D-L-α-methyl butyrate. ††Resazurin, 1 g/L of distilled H_2O.

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