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Dietary Fiber for Dogs: IV. In Vitro Fermentation of Selected Fiber Sources by Dog Fecal Inoculum and In Vivo Digestion and Metabolism of Fiber-Supplemented Diets

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ABSTRACT: Two experiments were conducted to evaluate single sources and blends of dietary fiber in dog food. In Exp. 1, 14 fibrous substrates were fermented in vitro using dog feces as the source of inoculum. Organic matter disappearance was lowest ($P < .05$; < 10%) for Solka Floc® and oat fiber and greatest ($P < .05$; > 80%) for fructooligosaccharides (FOS) and lactulose. Solka Floc, oat fiber, gum karaya, and xanthan gum produced the least ($P < .05$; < 1 mmol/g of substrate OM) total short-chain fatty acids (SCFA). Lactulose, citrus pectin, and guar gum produced the greatest ($P < .05$; > 6.8 mmol/g of substrate OM) total SCFA. In Exp. 2, six diets were formulated based on results obtained in Exp. 1. Treatments included 1) beet pulp (BP), 2) Solka Floc (SF), 3) citrus pulp (CP), 4) stool blend (SB), 5) SCFA blend (SC), and 6) combination blend (CB). Digestibility of DM and total dietary fiber (TDF) was greatest ($P < .05$; 87.3 and 60.8%, respectively) for dogs consuming the SC diet. Feces from dogs fed SC were scored as more unformed and liquid in consistency than feces from dogs fed the other diets. Dogs consuming the SF and SB diets had the lowest ($P < .05$; 11.0 and 4.1%, respectively) TDF digestibilities. Organic matter disappearance values derived from substrates fermented in vitro reasonably predicted the fiber digestibility of diets fed to dogs. Moderately fermentable dietary fiber sources, such as BP, promote excellent stool characteristics without compromising nutrient digestibility, and may promote gastrointestinal tract health by optimizing SCFA production.

Key Words: Fiber, Fermentation, Dogs, Intake, Digestibility, Short-Chain Fatty Acids

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Introduction

Previous research with dogs (Fahey et al., 1990a,b, 1992) showed that different sources of dietary fiber, when added at approximately 7.5% of diet DM, have dramatic effects on wet stool output and fiber digestibility and lesser effects on lipid digestibility, energy metabolism, and digesta retention time. Although certain effects are dependent on physical properties of the fiber (e.g., viscosity properties resulting in decreased nutrient digestion and[or] absorption), other effects of fiber may result from its fermentation. Fiber fermentation results in the production of short-chain fatty acids (**SCFA**), which have been implicated in several important physiological processes. Some of these include a trophic effect on colonic mucosa of rats given total parenteral nutrition (Friedel and Levine, 1992), utilization of butyrate by rat colonic epithelial cells as the preferred energy substrate (Roediger, 1982), and prevention of colon cancer in humans (Young and Gibson, 1991).

Titgemeyer et al. (1991) used an in vitro technique to assess fermentation of fiber sources by humans. This technique could be used to predict fiber fermentability by dogs as well. The objective of Exp. 1 was to determine the fermentability of selected fibrous substrates by dog fecal microflora. Results of Exp. 1 then were used to formulate three diets containing blends of fibrous substrates with different fermentation patterns; these diets were used in Exp. 2. The objectives of Exp. 2 were to determine how selected fibrous substrates, when added to dog diets as single

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Table 1. Chemical composition of fibrous substrates used in Exp. 1

Substrate	DM, %	% of DM		
		OM	TDF[a]	Crude protein
Beet pulp	91.4	90.4	68.4	9.1
Carob bean gum	87.9	96.0	36.1	5.0
Citrus pectin	93.2	97.9	66.3	2.1
Fructooligosaccharides[b]	95.9	99.5	0	0
Gum arabic	90.8	95.7	87.6	2.4
Gum karaya	89.8	94.1	83.1	.7
Gum talha	92.6	95.9	86.0	1.1
Guar gum	89.3	99.0	93.4	4.4
Lactulose[b]	99.7	98.9	0	0
Locust bean gum	90.9	98.7	92.9	5.9
Oat fiber	96.5	96.8	85.7	.9
Rice bran	87.9	90.1	22.9	16.2
Solka Floc®	96.6	99.4	91.6	.2
Xanthan gum	92.9	89.9	82.9	5.4

[a]Total dietary fiber.
[b]These substrates are both greater than 95% pure. However, because they are extremely soluble, they were not recovered by the TDF assay.

sources and as blends, affected nutrient intake, digestibility, energy metabolism, digesta retention time, and stool consistency.

Experimental Procedures

Experiments 1 and 2 were conducted using guidelines set forth by the University of Illinois Laboratory Animal Care Committee.

Experiment 1

Substrates. Substrates used were Solka Floc® (Fiber Sales and Development Corp., St. Louis, MO), oat fiber (Canadian Harvest USA, Cambridge, MN), beet pulp (Michigan Sugar, Saginaw, MI), rice bran (ADM-Krause Milling Company, Shawnee Mission, KS), fructooligosaccharides (**FOS**; Coors Biotech, Golden, CO), lactulose (Sigma Chemical, St. Louis, MO), gum karaya, xanthan gum, gum talha, carob bean gum, locust bean gum, guar gum, citrus pectin (HM rapid), and gum arabic (all from TIC Gums, Belcamp, MD). Chemical composition of substrates is presented in Table 1.

Donors. Three female English Pointers were given ad libitum access twice daily to Eukanuba® Original (Iams Co., Lewisburg, OH), a commercially available dog food containing beet pulp as a source of fiber, for 14 d before collection of a single fecal sample. Dogs were housed individually in cages (approximately 1.25 m × 1.25 m × 1.25 m) that were equipped with a slatted floor. Dogs had access to water at all times and were housed in a climate-controlled room (20°C).

Medium Composition and Substrate Fermentation. The composition of the medium used to culture the fecal microflora is listed in Table 2. All medium components except the vitamin mixes were added before autoclaving. The vitamin mixes were aseptically added after they were filter-sterilized. The

Table 2. Composition of medium used in Exp. 1

Component	Concentration in medium
	mL/L
Solution A[a]	330.0
Solution B[b]	330.0
Trace mineral solution[c]	10.0
Water-soluble vitamin mix[d]	20.0
Folate:biotin solution[e]	5.0
Riboflavin solution[f]	5.0
Hemin solution[g]	2.5
Short-chain fatty acid mix[h]	.4
Resazurin[i]	1.0
Distilled H_2O	296.0
	g/L
Yeast extract	.5
Trypticase	.5
Na_2CO_3	4.0
Cysteine·HCl·H_2O	.5

[a]Composition (grams/liter): NaCl, 5.4; KH_2PO_4, 2.7; $CaCl_2·H_2O$, .16; $MgCl_2·6H_2O$, .12; $MnCl_2·4H_2O$, .06; $CoCl_2·6H_2O$, .06; $(NH_4)_2SO_4$, 5.4.
[b]Composition: K_2HPO_4, 2.7 g/L.
[c]Composition (milligrams/liter): EDTA (disodium salt), 500; $FeSO_4·7H_2O$, 200; $ZnSO_4·7H_2O$, 10; $MnCl_2·4H_2O$, 3; H_3PO_4, 30; $CoCl_2·6H_2O$, 20; $CuCl_2·2H_2O$, 1; $NiCl_2·6H_2O$, 2; $Na_2MoO_4·2H_2O$, 3.
[d]Composition (milligrams/liter): thiamin·HCl, 100; d-pantothenic acid, 100; niacin, 100; pyridoxine, 100; *p*-aminobenzoic acid, 5; vitamin B_{12}, .25.
[e]Composition (milligrams/liter): folic acid, 10; d-biotin, 2; NH_4HCO_3, 100.
[f]Composition: riboflavin, 10 mg/L in 5 mmol/L of HEPES.
[g]Hemin, 500 mg/L in 10 mmol/L of NaOH.
[h]250 mL/L each of *n*-valerate, isovalerate, isobutyrate, and DL-α-methyl butyrate.
[i]Resazurin, 1 g/L in distilled H_2O.

freshly voided fecal sample from each dog was immediately placed in a plastic bag that was sealed after expressing excess air. The sample was diluted 1:10 (wt/vol) in a 39°C anaerobic dilution solution (Bryant and Burkey, 1953) and blended for 15 s in a Waring blender (Dynamics Corporation of America, New Hartford, CT). Blended, diluted feces were filtered through four layers of cheesecloth and the filtrate was sealed in a 250-mL Erlenmeyer flask under CO_2. Plastic centrifuge tubes (50-mL) containing 30 mL of medium and 310 mg of substrate or blank were aseptically inoculated with 1 mL of diluted feces, resulting in a substrate concentration of 10 mg/mL. Tubes were flushed with CO_2 and capped with stoppers equipped with one-way gas release valves.

Duplicate tubes were incubated at 39°C for 6, 12, or 24 h. After this end point time, 4-mL aliquots of fluid were sampled from each tube and prepared for SCFA analyses. The remaining 27 mL was combined with four volumes of 95% ethanol to precipitate the soluble polysaccharide fractions. After 1 h, samples were filtered through Whatman 541 filter paper and residues were washed sequentially with 78% ethanol, 95% ethanol, and acetone. The precipitation and filtration step is similar to the one used to measure total dietary fiber by the Prosky et al. (1985) method. Samples then were dried at 105°C, weighed, ashed (500°C), and weighed again to determine OM disappearance (**OMD**). In vitro OMD (percentage) was calculated as follows:

$$\{1-[(\text{OM residue} - \text{OM blank})/\text{initial OM}]\} \times 100$$

where OM residue is the OM recovered after 6, 12, or 24 h of fermentation, OM blank is the OM recovered in the corresponding blank (tubes containing medium, diluted feces, but no substrate) after the same fermentation time and same dog, and initial OM is that placed in the tube before fermentation.

The 4-mL aliquot of fluid removed from the tubes for SCFA analysis was immediately mixed with 1 mL of 25% (wt/vol) metaphosphoric acid, precipitated for 20 min, and centrifuged at 25,000 × *g* for 20 min. Samples of the supernates were collected after centrifugation and were further diluted 1:5 by adding 5% metaphosphoric acid. Concentrations of SCFA in the diluted supernates were determined with a Hewlett-Packard (Hewlett-Packard, Avondale, PA) Model 5890A gas chromatograph equipped with a hydrogen flame-ionization detector. A GP 60/80 Carbopack C column (Supelco, Bellefonte, PA; 76 cm × 4 mm i.d.) with .3% Carbowax 20M and .1% H_3PO_4 was used. Nitrogen was used as the carrier gas with a flow rate of 50 mL/min. Oven temperature was 120°C, and detector and injector temperatures were 200°C. The SCFA concentration in blank tubes was subtracted from the SCFA concentration in tubes containing substrate prior to calculation of SCFA production.

Table 3. Ingredient composition of basal diet fed in Exp. 2

Ingredient	% DM basis
Pre-gelled cornstarch	to 100
Chicken protein[a]	40.28
Chicken fat	13.23
Egg	2.50
Vitamin/mineral premix[b]	2.10
Chicken liver meal	1.00
Brewer's dried yeast	1.00
DL-Methionine	.60
Fiber source	—[c]

[a]Combination of chicken and chicken by-product meal.
[b]Provided per kilogram of diet: vitamin A, 26,400 IU; vitamin E, 136 IU; vitamin D_3, 1,760 IU; thiamine, 16.5 mg; riboflavin, 29.0 mg; niacin, 58.0 mg; d-pantothenic acid, 35.3 mg; biotin, .52 mg; folic acid, 1.4 mg; choline, 2,295 mg; vitamin B_{12}, .25 mg; monosodium phosphate, 6 g; potassium chloride, 5 g; choline chloride, 3 g; Mn, 42.2 mg; Zn, 202.9 mg; Cu, 29.2 mg; Co, .5 mg; and Se, .26 mg.
[c] Beet pulp (BP) = 12.48%; Solka Floc® (SF) = 8.03%; citrus pulp (CP) = 14.45%; stool blend (SB) = Solka Floc (6.25%) + gum arabic (2.08%); short-chain fatty acid blend (SC) = citrus pectin (4.49%) + carob bean gum (2.23%) + locust bean gum (2.23%) + gum talha (2.26%); combination blend (CB) = beet pulp (9.51%) + citrus pectin (1.20%) + guar gum (1.20%).

Statistical Analyses. The GLM procedures of SAS (1988) were used to analyze data from this experiment. The experimental design was a randomized complete block with a 14 × 3 factorial arrangement of substrates and fermentation times as treatments. Inocula source (dog) was used as the blocking factor. Therefore, substrate, time, substrate × time, and dog were used in the statistical model. Arithmetic means are reported along with the SEM for all treatments. When significant ($P < .05$) treatment effects were detected, means were compared by the least significant difference method (Cochran and Cox, 1957). Responses to the effect of increasing fermentation time within individual substrates were characterized by conducting linear and quadratic contrasts.

Experiment 2

Animals and Diets. Thirty adult English Pointers (19.9 ± .6 kg) were used in this experiment. Ten neutered male and 20 spayed female dogs were grouped by weight and then randomly assigned to one of six diets. Each dog was housed individually in a cage (approximately 1.25 m × 1.25 m × 1.25 m) equipped with a wire-mesh floor and urine drip pan to allow separation of feces and urine. Dogs had free access to fresh water at all times and were housed in a climate-controlled building with the temperature kept between 21 and 26°C.

Ingredient composition of the basal diet is presented in Table 3. Each diet met NRC (1985) nutrient requirements. Fiber was added to the diets to provide approximately 7.5% supplemental total dietary fiber (**TDF**). Fiber sources used were beet pulp (**BP**), Solka Floc (**SF**), citrus pulp (**CP**), a blend of

Table 4. Chemical composition of diets fed in Exp. 2

Diet[a]	DM, %	% of DM						GE, kcal/g of DM
		OM	Crude protein	TDF[b]	Lipid	Ca	P	
BP	93.3	93.2	32.0	8.9	21.9	1.9	1.1	5.5
SF	94.4	94.1	31.0	9.6	23.2	2.0	1.1	5.6
CP	92.8	93.3	32.9	11.7	24.2	2.1	1.1	5.6
SB	94.9	94.0	30.9	9.5	22.4	1.9	1.1	5.5
SC	92.3	93.7	32.0	9.0	23.3	2.0	1.0	5.6
CB	91.4	93.4	32.8	9.9	22.8	2.1	1.0	5.5

[a]BP = beet pulp, SF = Solka Floc®, CP = citrus pulp, SB = stool blend (75% Solka Floc, 25% gum arabic), SC = short-chain fatty acid blend (40% citrus pectin, 20% gum talha, 20% carob bean gum, 20% locust bean gum), CB = combination blend (80% beet pulp, 10% citrus pectin, 10% guar gum).
[b]Total dietary fiber.

fibers selected to minimize fermentation and maximize stool quality (stool blend [**SB**]: 75% Solka Floc, 25% gum arabic), a blend of fibers selected to maximize SCFA production (SCFA blend [**SC**]: 40% citrus pectin, 20% gum talha, 20% carob bean gum, 20% locust bean gum), and a blend of fibers selected to optimize both SCFA production and stool quality (combination blend [**CB**]: 80% beet pulp, 10% citrus pectin, 10% guar gum). Citrus pulp consists of the pulp residue from juice extraction whereas citrus pectin consists of a further refined product from citrus peels. Citrus pulp was obtained from Freeman Industries (Tuckahoe, NY), whereas the other fiber sources used in the diets were from the same sources cited in Exp. 1. The fiber blends were formulated from results of Exp. 1 based on the 24-h total SCFA production of each fibrous substrate. For example, after 24 h of fermentation, the total amount of SCFA produced from the individual fiber sources for the SCFA blend would theoretically be 5.2 mmol/g of substrate OM with a 63.5% OMD. Likewise, the CB and SB would result in 3.8 mmol/g of substrate OM with a 46.6% OMD and .5 mmol/g of substrate OM with a 9.4% OMD, respectively, after 24 h of fermentation. All experimental diets were formulated with pre-gelled cornstarch to reduce the likelihood of starch being fermented. The chemical composition of diets fed is presented in Table 4.

Sample Collection. All dogs had ad libitum access to Eukanuba Original twice daily for 5 d before consuming treatment diets. Twelve days were used for adjustment to treatment diets. Five days of total urine and fecal collection followed the adjustment period. Diets were sampled daily beginning 1 d before the initiation of urine and fecal collection. Dry matter intakes were recorded during the collection period. To ensure total urine collection from males, the lower part of the sides of the woven wire cages were covered with plastic. Urine was collected daily into vessels containing 10 mL of 6 *N* HCl. Urine was weighed, volume measured, subsampled (200 mL), refrigerated (4°C) until centrifuged (25,000 × *g* for 20 min), and refrigerated again until analyzed. Feces were collected daily, weighed, consistency scored[5], frozen (−20°C), freeze-dried (FTS Triphilizer, FTS Systems, Stone Ridge, NY), ground through a Wiley mill with a 1-mm screen, and stored for future analyses. The day following termination of urine and fecal collection, all dogs were orally dosed with four capsules, each containing .25 g of Cr-mordanted NDF isolated from beet pulp. The isolate contained 33.8 mg of Cr/g of NDF. The Cr mordanting procedure used is described by Uden et al. (1980). Dosing occurred within 30 min after the morning feeding. Fecal collection began 4 h after dosing and occurred every 4 h until 60 h after dosing. Feces were weighed, the time recorded, and the feces frozen (−20°C) for future analyses. Feces were dried later at 55°C and ground through a Wiley mill with a 1-mm screen. The procedure of Thielemans et al. (1978), as described by Warner (1981), was used to calculate the digesta mean retention time in the dogs. This method calculates mean retention time from concentration data for individual fecal samples collected following pulse-dose marker administration. The equation used was as follows:

$$\text{Mean retention time} = \sum_{i=1}^{n} t_i c_i \Delta t_i \; / \; \sum_{i=1}^{n} c_i \Delta t_i$$

where c_i is the concentration of marker in the i^{th} sample, collected at time t_i over a time interval Dt_i. Use of this approach requires the assumption that inflow and outflow of the system are continuous, constant, and equal.

Chemical Analyses. The DM, OM, and crude protein contents of diets and feces were determined by AOAC (1984) methods. Total dietary fiber content of diets and feces was determined by the Prosky method (1985). The total lipid content of diets and feces was

[5]1 = hard, dry, pellets: small, hard mass; 2 = hard, formed, dry stool: remains firm and soft; 3 = soft, formed, moist: softer stool that retains shape; 4 = soft, unformed: stool assumes shape of container, pudding-like; 5 = watery: liquid that can be poured.

determined by the AACC (1983) method. Bomb calorimetry (Model 1261, Parr Instrument, Moline, IL) was used to determine the GE content of feed, urine, and feces, and these values were used to calculate the DE and ME contents of the diets. To determine the GE of the urine, 1 mL of urine was added to a 1-g cellulose pellet. Pellets were dried (55°C) and the GE determined with the bomb calorimeter. The GE of the blank pellets (cellulose only) was then subtracted from the GE of the pellet and the urine. Diets were prepared for Ca determination by the AOAC (1975) method. Feces were prepared for Cr determination by the method of Williams et al. (1962). Calcium content of diets and Cr content of feces collected for retention time measurements were determined using atomic absorption spectrophotometry (Model 306, Perkin-Elmer, Norwalk, CT). The P content of the diets was determined using the AOAC (1984) method.

Statistical Analyses. The data from this experiment were analyzed as a randomized complete block design with weight used as the blocking factor. The GLM procedures of SAS (1988) included diet and weight in the model statement. Arithmetic means are reported along with the SEM for all treatments. Treatment means were compared using the LSD procedure protected by a significant ($P < .05$) F-test (Cochran and Cox, 1957).

Results

Experiment 1

In general, as length of fermentation time increased, OMD and SCFA production from substrates also increased. This increase over time usually occurred in a linear manner for OMD and acetate, propionate, and total SCFA production. This increase over time usually occurred in a quadratic manner for butyrate production. All substrate × time interactions resulted in an overall F-test that was significant at $P < .05$. The following results are for 24-h fermentation times unless otherwise noted. The OMD was lowest ($P < .05$) for Solka Floc, oat fiber, gum karaya, xanthan gum, and gum arabic (Table 5). Citrus pectin, FOS, lactulose, and guar gum had the highest ($P < .05$) OMD; the remaining substrates were intermediate. The greatest increase in OMD between 6 and 24 h of fermentation occurred for locust bean gum and guar gum.

Fermentation of Solka Floc, oat fiber, gum karaya, xanthan gum, gum arabic, rice bran, and gum talha resulted in the lowest ($P < .05$) acetate productions. Acetate production was greatest ($P < .05$) for citrus pectin. Locust bean gum, guar gum, and lactulose each had at least a fourfold increase in acetate production between 6 and 24 h of fermentation. Because blank values for acetate, propionate, butyrate, and total SCFA production were sometimes greater than values for the poorly fermentable substrates (Solka Floc, gum karaya, xanthan gum, and gum arabic), blank-corrected values were negative in some cases. These negative values were not significantly different from zero and thus were due to random error.

Propionate productions generally were lowest ($P < .05$) for Solka Floc, oat fiber, gum karaya, and xanthan gum. Propionate productions from locust bean gum, guar gum, FOS, and lactulose were greatest ($P < .05$); the remaining substrates were intermediate. Locust bean gum, guar gum, and lactulose had the greatest increases (at least eightfold) in propionate production between 6 and 24 h of fermentation.

Butyrate productions were lowest ($P < .05$) for Solka Floc, oat fiber, gum karaya, and xanthan gum. Beet pulp, gum talha, locust bean gum, citrus pectin, and carob bean gum had the greatest ($P < .05$) butyrate productions; the remaining substrates produced intermediate amounts of butyrate.

Solka Floc, oat fiber, gum karaya, and xanthan gum had the lowest ($P < .05$) total SCFA productions. Locust bean gum, guar gum, citrus pectin, FOS, and lactulose had the greatest ($P < .05$) total SCFA production; the remaining substrates were intermediate. Total SCFA productions during fermentation of gum arabic, gum talha, locust bean gum, guar gum, and lactulose all increased at least fivefold between 6 and 24 h of fermentation.

Experiment 2

The intakes of DM, N, and lipid did not differ ($P > .05$) among treatments (Table 6). However, TDF intake was lowest ($P < .05$) for dogs consuming the BP, SB, and SC diets. This was due to the combined effect of numerical differences in total intake and diet TDF concentration (Table 4). Possible reasons for differences in TDF concentrations among diets include slight variation in TDF values for different lots of beet pulp or potential changes in fiber structure as affected by the extrusion process.

Digestibilities of DM by dogs consuming the BP, SF, CP, and SB diets were lowest ($P < .05$; 82.2, 83.8, 82.3, and 82.7%, respectively), whereas dogs consuming the SC diet had the highest ($P < .05$) DM digestibility (87.3%). The greater DM digestibility by dogs consuming the SC treatment may be reflective of the higher TDF digestibility (60.8%). Dogs consuming the SF and SB treatment diets had the lowest ($P < .05$) TDF digestibilities (11.0 and 41.1%, respectively). In contrast to TDF digestibility, N and lipid digestibilities were greatest ($P < .05$) for dogs consuming the SF and SB diets. The lowest ($P < .05$) lipid digestibility occurred for dogs consuming the CP and CB diets.

Wet fecal output was greatest ($P < .05$) for dogs consuming the BP, CP, and CB diets, whereas dogs

Table 5. Organic matter disappearance (OMD) and acetate (ACE), propionate (PRO), butyrate (BUTY), and total short-chain fatty acid (SCFA) production after various times of in vitro fermentation of fibrous substrates with dog fecal microflora

Substrate[a]	OMD, %[b]			ACE, mmol/g of OM[b]			PRO, mmol/g of OM[b]			BUTY, mmol/g of OM[b]			Total SCFA, mmol/g of OM[b]		
	6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h
Beet pulp	19.8	21.7	38.2	.53	.87	2.03	.16	.22	.80	.08	.22	.70	.76	1.19	3.01
Carob bean gum	50.9	48.1	49.8	1.28	1.90	2.10	.56	.94	1.44	.10	.31	.65	1.94	3.15	4.19
Citrus pectin	60.4	73.0	84.9	1.87	3.68	4.54	.64	1.33	1.76	.17	.33	.54	2.68	5.35	6.84
Fructooligosaccharides	88.3	81.4	84.5	1.60	2.68	2.86	.63	1.49	2.52	.18	.27	.30	2.41	4.43	5.67
Gum arabic	18.8	16.1	24.6	.10	.12	.62	.06	−.01	.47	.01	.00	.40	.17	.12	1.49
Gum karaya	12.8	15.9	18.5	.24	.21	.61	.02	−.08	.01	.03	.01	.02	.29	.14	.64
Gum talha	34.0	31.8	36.3	.24	.40	.71	.12	.60	.97	.03	.04	.60	.39	1.03	2.28
Guar gum	18.5	41.6	75.3	.58	1.70	3.07	.43	1.72	3.79	.06	.20	.41	1.07	3.62	7.26
Lactulose	99.7	89.0	87.8	.37	1.88	3.47	.18	2.35	4.52	.06	.29	.35	.61	4.52	8.34
Locust bean gum	21.1	49.3	61.7	.60	1.81	2.60	.40	1.72	2.70	.06	.23	.52	1.05	3.75	5.81
Oat fiber	8.2	5.0	7.8	.16	.10	.19	.10	.02	.14	.03	.00	.03	.28	.12	.35
Rice bran	35.5	34.8	44.2	.41	.52	.66	.28	.35	.76	.20	.26	.42	.88	1.14	1.84
Solka Floc®	5.0	1.8	4.3	.03	−.03	.09	.02	−.05	.05	.01	−.01	.00	.06	−.10	.14
Xanthan gum	11.6	13.1	28.0	.44	.52	.80	.06	−.04	.10	.04	.02	.05	.53	.50	.95
Pooled SEM		3.8			.20			.20			.08			.38	
LSD		10.5			.55			.55			.22			1.05	

[a]The interaction of substrate × time was significant ($P < .05$) for OMD, ACE, PRO, BUTY, and total SCFA production.

[b]The significant ($P < .05$) linear (L) and quadratic (Q) effects of OMD, ACE, PRO, BUTY, and total SCFA over time for each substrate were as follows: beet pulp, L for OMD, ACE, PRO, total SCFA; carob bean gum, L for PRO, BUTY, Q for ACE, total SCFA; citrus pectin, L for ACE, PRO, total SCFA; fructooligosaccharides, L for ACE, PRO, total SCFA; guar gum, L for OMD, ACE, PRO, BUTY, total SCFA; gum arabic, L for ACE, PRO, total SCFA, Q for BUTY; gum karaya, L for total SCFA, Q for ACE, BUTY; gum talha, L for ACE, PRO, total SCFA, Q for BUTY; lactulose, L for ACE, PRO, total SCFA, Q for OMD, BUTY; locust bean gum, L for ACE, total SCFA, Q for OMD, PRO; oat fiber, Q for BUTY; rice bran, L for PRO, total SCFA; Solka Floc, Q for BUTY; xanthan gum, L for total SCFA.

Table 6. Dry matter (DM), nitrogen (N), lipid, and total dietary fiber (TDF) intakes and digestibilities by dogs consuming experimental diets

Item	Diet[a]						SEM	Treatment effect[b]
	BP	SF	CP	SB	SC	CB		
DM intake, g/d	314.0	353.6	359.6	302.8	310.8	391.0	26.6	.18
N intake, g/d	16.1	17.6	18.9	15.0	15.9	20.5	1.4	.08
Lipid intake, g/d	68.7	81.9	86.9	67.7	72.5	89.1	6.1	.08
TDF intake, g/d	28.0[c]	34.0[c,d]	42.0[e]	28.8[c]	28.0[c]	38.6[d,e]	2.8	< .01
DM digestibility, %	82.2[c]	83.8[c,d]	82.3[c,d]	82.7[c,d]	87.3[e]	84.3[d]	.7	< .01
N digestibility, %	83.6[c]	89.8[d]	83.9[c]	89.6[d]	86.2[e]	82.8[c]	.7	< .01
Lipid digestibility, %	94.1[c,d]	96.1[e]	93.9[c,f]	95.2[d,e]	94.1[c,d]	92.8[f]	.4	< .01
TDF digestibility, %	29.0[c]	11.0[d]	43.0[e]	4.1[d]	60.8[f]	51.3[e,f]	3.6	< .01

[a]BP = beet pulp, SF = Solka Floc®, CP = citrus pulp, SB = stool blend (75% Solka Floc, 25% gum arabic), SC = short-chain fatty acid blend (40% citrus pectin, 20% gum talha, 20% carob bean gum, 20% locust bean gum), CB = combination blend (80% beet pulp, 10% citrus pectin, 10% guar gum).
[b]The overall treatment effect *P*-value.
[c,d,e,f]Means within a row lacking a common superscript letter differ ($P < .05$).

consuming the SF, SC, and SB diets excreted smaller amounts of wet feces (Table 7). The lowest ($P < .05$) fecal DM percentage occurred for dogs consuming the BP, CP, and CB diets, whereas the greatest ($P < .05$) fecal DM percentage occurred for dogs consuming the SF and SB diets. Because TDF intake greatly affects wet fecal output, wet fecal output also is expressed per gram of TDF intake. In this case, dogs fed the CP and CB diets excreted a moderate amount of feces (6.6 and 6.7 g of feces/g of TDF intake, respectively) relative to their fiber intake, whereas dogs fed the BP diet produced the greatest ($P < .05$) amount of feces/gram of TDF intake (8.2); dogs fed the SF and SB diets excreted the least ($P < .05$) amount of feces/gram of TDF intake (3.6 and 3.9, respectively).

Diet consumed resulted in a trend ($P = .08$) for dogs consuming the SF and SB diets to defecate least often (2.2 and 1.5 defecations/d, respectively), whereas dogs consuming the CP and CB diets defecated most often (2.9 and 3.0 defecations/d, respectively). Digesta mean retention time was numerically longest for dogs consuming the SB diet (32.3 h); dogs consuming the BP, CP, and CB diets had the shortest digesta mean retention times (21.0, 21.4, and 22.6 h, respectively). Fecal consistency scores were highest ($P < .05$) for dogs consuming the SC diet (3.7), whereas dogs fed the SF and SB diets had the lowest ($P < .05$) fecal consistency scores (2.4 and 2.5, respectively).

Digestible energy (kilocalories/gram of DM intake) was greatest ($P < .05$) for dogs consuming the SC diet, which is reflective of the increased TDF digestibility of this diet (Table 8). Differences in ME existed only when expressed as a percentage of DE. In this case, dogs consuming the SC and SB diets had the lowest ($P < .05$) conversion of DE to ME (93.6 and 94.4%, respectively), and dogs fed the SF diet had the highest ($P < .05$) conversion of DE to ME (97.3%).

Table 7. Fecal characteristics of dogs consuming experimental diets

Item[b]	Diet[a]						SEM	Treatment effect[c]
	BP	SF	CP	SB	SC	CB		
Wet fecal output, g/d	230.8[d]	124.0[e]	274.6[d]	109.0[e]	138.8[e]	257.0[d]	18.0	< .01
Fecal DM, %	24.7[d]	46.6[e]	23.1[d]	47.2[e]	29.8[f]	23.9[d]	1.5	< .01
g of Wet feces/g of TDF intake	8.2[d]	3.6[e]	6.6[f]	3.9[e,g]	4.9[g]	6.7[f]	.4	< .01
No. of defecations/d	2.6	2.2	2.9	1.5	2.7	3.0	.4	.08
Mean retention time, h	21.0	25.2	21.4	32.3	26.3	22.6	3.3	.19
Fecal consistency score[h]	2.8[d,e]	2.4[f]	2.9[d]	2.5[e,f]	3.7[g]	3.1[d]	.1	< .01

[a]BP = beet pulp, SF = Solka Floc®, CP = citrus pulp, SB = stool blend (75% Solka Floc, 25% gum arabic), SC = short-chain fatty acid blend (40% citrus pectin, 20% gum talha, 20% carob bean gum, 20% locust bean gum), CB = combination blend (80% beet pulp, 10% citrus pectin, 10% guar gum).
[b]Total dietary fiber.
[c]The overall treatment effect *P*-value.
[d,e,f,g]Means within a row lacking a common superscript letter differ ($P < .05$).
[h]1 = hard, dry, pellets: small, hard mass; 2 = hard, formed, dry stool: remains firm and soft; 3 = soft, formed, moist: softer stool that retains shape; 4 = soft, unformed: stool assumes shape of container, pudding-like; 5 = watery: liquid that can be poured.

Table 8. Gross, digestible, and metabolizable energy values of experimental diets fed to dogs

Item	Diet[a]						SEM	Treatment effect[b]
	BP	SF	CP	SB	SC	CB		
GE intake, kcal/d	1,735	1,974	2,023	1,670	1,754	2,168	149	.17
DE								
kcal/d	1,513	1,745	1,760	1,472	1,585	1,908	134	.21
kcal/g DM intake	4.81[c]	4.94[d]	4.89[c,d]	4.85[c,d]	5.10[e]	4.88[c,d]	.03	< .01
% of GE	87.1[c]	88.5[c]	86.9[c]	88.0[c]	90.4[d]	88.1[c]	.6	< .01
ME								
kcal/d	1,462	1,697	1,688	1,394	1,484	1,847	134	.18
kcal/g DM intake	4.64	4.80	4.68	4.58	4.77	4.74	.06	.11
% of GE	84.0	86.1	83.2	83.1	84.6	85.4	1.0	.30
% of DE	96.3[c,d]	97.3[d]	95.7[c,d,e]	94.4[c,e]	93.6[e]	96.9[d]	.8	.02

[a]BP = beet pulp, SF = Solka Floc®, CP = citrus pulp, SB = stool blend (75% Solka Floc, 25% gum arabic), SC = short-chain fatty acid blend (40% citrus pectin, 20% gum talha, 20% carob bean gum, 20% locust bean gum), CB = combination blend (80% beet pulp, 10% citrus pectin, 10% guar gum).
[b]The overall treatment effect *P*-value.
[c,d,e]Means within a row lacking a common superscript letter differ ($P < .05$).

Discussion

Experiment 1

The following discussion on substrate fermentation cites research using other species because, to our knowledge, no quantification of SCFA production by microflora indigenous to the gastrointestinal tract of the dog has been published.

Several experiments have used in vitro techniques for evaluating the fermentation characteristics of fibrous substrates in humans (McBurney et al., 1990; Bourquin et al., 1992; Mortensen et al., 1992). Although concern exists regarding the usefulness of information from in vitro techniques, results of these studies generally agree with in vivo results. For example, Titgemeyer et al. (1991) noted that pectin and oat fiber fermented in vitro by human fecal inoculum were degraded to approximately the same extent as similar substrates utilized by rats in vivo (Nyman and Asp, 1982, 1988). Bourquin et al. (1993b) reported wide variation in in vitro fermentation characteristics among fecal inocula from individual donors. This is in agreement with Nyman et al. (1986), who found wide variation in fiber utilization by humans in vivo. McBurney and Sauer (1993) attempted to validate a technique to quantify the energy derived by colonic fermentation of intestinal contents at the terminal ileum of humans. To accomplish this, the ME of ileal contents was determined indirectly by an in vitro fermentation technique and directly by using ileal-cannulated pigs. A strong correlation ($r = .94$; $P < .0001$) was found between in vitro and in vivo results.

A moderate amount of fermentation occurred for gum arabic, beet pulp, gum talha, and carob bean gum. Several researchers (Adiotomre et al., 1990; Titgemeyer et al., 1991; Bourquin et al., 1993b) found that fermentation of gum arabic resulted in greater than 6.5 mmol total SCFA/g of substrate after 24 h of fermentation by human fecal microflora. This is in contrast to the current results indicating less than 1.5 mmol total SCFA produced/g of substrate OM and is indicative of a major difference in fermentative activity between human and dog fecal microflora. Fermentation of beet pulp by human fecal microflora produced 1.15 mmol total SCFA/g of substrate (Titgemeyer et al., 1991), whereas in this experiment, dog fecal microflora produced approximately 3 mmol total SCFA/g of substrate. Besides differences in fermentative activity between species, beet pulp is extremely variable in composition and may produce variable amounts of SCFA depending on its source.

Other substrates (locust bean gum, guar gum, citrus pectin, FOS, and lactulose) resulted in high (> 5 mmol/g of substrate OM) total SCFA production after 24 h of fermentation, corroborating results from other studies. For example, citrus pectin produced greater than 6 mmol of SCFA/g of substrate OM when fermented for 24 h by human fecal microflora (Titgemeyer et al., 1991). Moreover, the fibrous component of a diet containing 10% guar gum fed to rats was almost completely digested (Nyman and Asp, 1982).

Although some research has been reported on the usefulness (e.g., promotion of growth of favorable intestinal bacteria, blood cholesterol reduction, decreased constipation) of FOS in the diet (Tokunaga et al., 1986; Hidaka et al., 1990, 1991), limited research exists that attempts to quantify SCFA production from this material. Hosoya et al. (1988) found that human fecal microflora produced .29 g of acetate, .2 g of propionate, and .1 g of butyrate/g of FOS after 8 h of fermentation. This is equivalent to approximately 4.8 mmol of acetate, 2.7 mmol of propionate, and 1.1 mmol of butyrate per gram of substrate, indicating a greater amount of fermentation occurring with human vs dog fecal microflora.

Because a molecule of FOS is very small (≤ 4 fructose moieties + 1 glucose moiety), it was soluble

during ethanol precipitation and, consequently, was unrecoverable in our filtration step. Increased bacterial mass recovered after 24 h of fermentation may have caused OMD values of FOS and lactulose to be slightly lower than after 6 h of fermentation. Thus, the OMD value of FOS reported here is not a good indicator of actual fermentation.

Lactulose, a disaccharide of galactose and fructose, is resistant to mammalian enzyme degradation and, consequently, reaches the colon. As with FOS, lactulose also was unrecoverable in the filtration step of the in vitro procedure. For this reason, the OMD data pertaining to this substrate are of little value, but like FOS, lactulose was extremely fermentable. Vince et al. (1990) reported that lactulose fermented for 48 h in vitro by human fecal microflora produced 6.8 mmol of SCFA/g of substrate.

Citrus pectin fermentation produced nearly a 3:1 ratio of acetate:propionate for all fermentation times, whereas lactulose fermentation produced less than 1 mmol of acetate/mmol of propionate after 12 and 24 h of fermentation. Furthermore, substrates such as citrus pectin and FOS were fermented quite rapidly, whereas SCFA production from lactulose greatly increased only after 12 h of fermentation. These data indicate that fermentation of different fibrous substrates not only produces different amounts of SCFA, but also produces different proportions of the major SCFA and at different rates. Other research indicates that the proportions of individual SCFA produced in vitro is dependent on the substrate being fermented (McBurney and Thompson, 1989a; Titgemeyer et al., 1991; Mortensen et al., 1992). Recent evidence (Sunvold et al., 1995) indicates that the source of fiber in the diet of donor animals also influences in vitro SCFA production.

Chemical composition is generally indicative of substrate fermentation potential. For example, substrates that contain large amounts of cellulose, such as Solka Floc and oat fiber, typically have low OMD values (< 10%) and produce few SCFA (< 1 mmol/g of substrate DM) (Titgemeyer et al., 1991; Bourquin et al., 1993b) after 24 h of fermentation using human fecal inoculum. These substrates also are composed primarily of insoluble fiber, whereas substrates such as citrus pectin, guar gum, locust bean gum, FOS, and lactulose are composed primarily of soluble fiber. Higher OMD and SCFA production values for these substrates confirm the observation that soluble fibers usually are more fermentable than insoluble fibers (Vinik and Jenkins, 1988).

Citrus pectin is composed primarily of uronic acids (Titgemeyer et al., 1991). The composition of pectin has been proposed to play a role in production of a high amount of acetate relative to propionate, as we and others (McBurney and Thompson, 1989b; Titgemeyer et al., 1991) found, but this also may be dependent on other factors (e.g., polysaccharide structure) as well (Titgemeyer et al., 1991).

Because different fibrous substrates may result in different fermentation profiles, it is important to recognize that individual SCFA (acetate, propionate, and butyrate) have different effects on the host animal. Roediger (1982) noted that butyrate was the preferential energy source of colonocytes from rats. Cummings (1991) suggested that, in hindgut-fermenting (nonruminant) species, acetate is largely used by peripheral tissues as a fuel source but also may alter glucose metabolism in muscle tissue and spare fatty acid oxidation. Propionate is the major glucose precursor in ruminants (Cummings, 1991). Furthermore, propionate may spare amino acids that would be needed for gluconeogenesis in the post-absorptive state (Demigne and Remesy, 1991). With continued research in this area, an optimal SCFA profile might be determined to be beneficial to the host. A diet then could be formulated with a certain type of fiber or blend of fibers that would provide this desired SCFA profile.

Experiment 2

Results of Exp. 1 were used to formulate the fiber blends incorporated into three of the treatment diets used in Exp. 2. Solka Floc and beet pulp were chosen as individual sources of fiber that vary in fermentability and are currently used in dog foods. Although not evaluated in Exp. 1, citrus pulp was chosen as the fiber source for another experimental diet because of its commercial availability.

Data indicate that DM digestibility is affected, at least in part, by increasing the fermentability of the dietary fiber component. For example, dogs fed the SC diet had a more than fivefold increase in fiber digestibility, resulting in a 4.5 percentage unit increase in DM digestibility compared to dogs fed the SF diet. The reduced fermentation of SF and SB relative to the other diets probably resulted in less microbial cell mass in feces and, consequently, improvements in apparent lipid and protein digestibilities by dogs fed these diets. Indeed, diets resulting in the lowest TDF digestibility also resulted in the lowest fecal excretion of N (data not shown). Thus, the improvement in apparent crude protein digestibility by dogs consuming the SF and SB diets may not actually reflect differences among diets in small intestinal absorption of protein but may reflect the decreased microbial protein present in their feces. The observation that the SC diet, relative to the other diets, had a greater DE content when expressed as a percentage of GE is another indication of increased fiber digestibility by dogs fed the SC diet.

Measurement of wet fecal output may reflect the water-holding capacity of dietary fiber. Wet fecal output also may be influenced by the amount of microbial cells and SCFA produced as a result of fiber fermentation. Because fiber intake greatly affects wet fecal output, we expressed our data as grams of wet

feces/gram of TDF intake. Dogs fed BP, CP, and CB diets generally had the highest wet fecal output.

Stool quality is important to the animal; diarrhea and constipation are undesirable conditions. Stool quality is important to the owner because it is often used as an indication of animal health. Thus, the influence of dietary fiber on stool quality is important. Optimal stool quality results when feces are of adequate firmness to prevent diarrhea but soft enough to prevent constipation. Two ways to measure stool quality are fecal DM percentage and stool consistency. In this experiment, fecal DM percentage increased when poorly fermentable fibers (SF and SB) were consumed. These results correspond to the decrease in stool consistency scores when dogs consumed these fibers, which indicates that a harder, firmer stool was excreted. However, fecal DM percentage values were not indicative of stool consistency values for all diets. For example, fecal DM percentage for dogs consuming the SC treatment was intermediate and their stool consistency scores were greatest. In addition, stool consistency values correlated poorly with fecal DM percentage ($R^2 = .40$; $P < .05$). These results indicate that fecal DM percentage did not entirely correspond with visual observation of the obviously liquid stool excreted by dogs consuming the SC diet. Thus, fecal consistency scores were more indicative of stool characteristics than was fecal DM percentage.

The number of defecations and digesta mean retention times generally followed wet fecal output. This also was the case in the experiment of Fahey et al. (1990a), who used diverse sources of fiber (beet pulp, tomato pomace, peanut hulls, wheat bran, and alkaline hydrogen peroxide-treated wheat straw), which were added to provide approximately 12.5% TDF. Although only trends existed for diet to influence the number of defecations per day and digesta mean retention time, results imply that diets containing Solka Floc (SF and SB) slowed the rate of digesta passage through the digestive tract.

Energy data (Table 8) indicate that the most fermentable diet (SC) had the highest DE value when fed to dogs. This agrees with the DM digestibility data. Alteration in the DE and ME of diets based on the source of dietary fiber has been demonstrated previously in this laboratory (Fahey et al., 1990a, 1992). Differences among diets occurred when ME was expressed as a percentage of DE. The SC diet resulted in the lowest ME value, which indicates that a greater amount of energy was excreted in the urine of dogs consuming the SC diet compared to those consuming the other diets. Because increased TDF digestibility occurred with this diet, increased ammonia production may have occurred from the increased intestinal fermentation. This may have led to increased ammonia absorption and, consequently, more urea excreted by the kidneys.

Results of Exp. 1 could be used to predict fiber utilization in vivo (Exp. 2). For example, the SC diet resulted in a TDF digestibility of approximately 61%. Calculation of the theoretical in vitro OMD after 24 h of fermentation of these fibers equals 63.5% [(4.49/11.21 × 84.9) + (2.23/11.21 × 49.8) + (2.23/11.21 × 61.7) + (2.26/11.21 × 36.3)]. Beet pulp in vitro OMD (38.2%) less accurately predicted in vivo TDF digestibility (29.0%). This may be due to the lower concentration of TDF in beet pulp than in other fiber sources. When the TDF digestibility value for each diet (the value for the CP diet was not used because citrus pulp was not used in Exp. 1) was matched with the 24-h in vitro OMD value for its respective fiber source(s), the resulting correlation was high ($R^2 = .93$; $P < .01$).

Bourquin et al. (1993a) stated that the in vitro system allows one to rank the fermentation of different substrates relative to one another. This information then could be used to formulate diets to be fed to animals for the purpose of evaluating in vivo physiological responses. Good agreement between the in vitro and in vivo results was evident from our experiments. Therefore, we view in vitro fiber evaluation as a means of predicting in vivo fiber fermentation responses. The in vitro technique is a relatively rapid, inexpensive, and non-invasive method that is performed under controlled laboratory conditions. Whereas in vivo methods only allow determination of fiber digestibility, the in vitro technique provides this information as well as information regarding fermentation end-products. Diets ostensibly could be formulated to provide an optimal fermentation profile when the relative importance of each SCFA in the gastrointestinal tract of the dog is known.

Implications

Results of fiber fermentation as evaluated in vitro can be used to reasonably predict in vivo fiber utilization responses by dogs. Sources of fiber that are moderately fermentable, such as beet pulp, promote gastrointestinal tract health while maintaining excellent stool characteristics and nutrient digestibility. Highly fermentable fibers, such as citrus pectin, locust bean gum, and carob bean gum, may cause undesirable stool characteristics. The challenge is to formulate diets containing the correct amount of fermentable and nonfermentable fibers that will result in optimal gastrointestinal tract health.

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