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Fruit and vegetable fiber fermentation by gut microflora from canines

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ABSTRACT: The objective of this study was to assess fermentability by canine gut microflora to include short-chain fatty acid (SCFA) production, organic matter (OM) disappearance, and gas production of vegetable and fruit fiber sources compared to fiber standards (psyllium, citrus pectin, and Solka Floc). Fiber sources included apple pomace, carrot pomace, flaxseed, fruit blend (mixture of peach, almond, nectarine, and plum), grape pomace, pea hulls, pistachio, and tomato pomace. Substrates were fermented in vitro for 4, 12, and 24 h with fecal flora obtained from three healthy dogs. Citrus pectin had the highest OM disappearance, SCFA production, and gas production at all times of fermentation; psyllium was intermediate and Solka Floc was lowest. A wide variation in fermentability was noted among the vegetable and fruit fiber sources. Apple pomace, carrot pomace, and flaxseed had the greatest fermentability as assessed by OM disappearance. Pea

hulls and tomato pomace had intermediate OM disappearances, and fruit blend, grape pomace, and pistachio were poorly fermented. Carrot pomace produced the largest amounts of gas and SCFA. Apple pomace produced high concentrations of gas but intermediate concentrations of SCFA. Pea hulls and tomato pomace produced intermediate concentrations of gas and SCFA, whereas flaxseed, fruit blend, grape pomace, and pistachio produced low amounts of these fermentation products. For all substrates collectively, OM disappearance was highly correlated with both gas production ($r^2 = 0.782$ and 0.723 for 12- and 24-h values, respectively) and SCFA production ($r^2 = 0.737$ and 0.738 for 12- and 24-h values, respectively). In general, OM disappearance, gas production, and SCFA production were related to the insoluble:soluble fiber ratio in the samples; as the insoluble:soluble ratio decreased (increased soluble fiber), the OM disappearance, gas production, and SCFA production increased.

Key Words: Canidae, Fermentation, Fiber, Fruit, Vegetables

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Introduction

In the past, all types of dietary fiber were assumed to have a similar effect in the gastrointestinal tract. However, the classification of fiber according to solubility and fermentability has resulted in a change of opinion on this topic. Soluble fiber has high water-holding capacity, readily forms gels, increases luminal viscosity, and is easily degraded by microflora in the large bowel, resulting in significant concentrations of short-chain fatty acids (SCFA). In contrast, insoluble fiber has little water-holding capacity, decreases transit time, is only partially degraded by microflora, and in-

creases fecal bulk (Davidson and McDonald, 1998). Because fiber sources contain different insoluble:soluble fiber ratios, their rate and extent of fermentation may be substantially different.

Due to the expense and difficulties associated with in vivo systems for estimating fiber fermentation, in vitro systems using dog fecal inoculum have been used (Sunvold et al., 1995a,b). Although several in vitro and in vivo studies using dogs have examined the fermentative characteristics of beet pulp, cellulose, citrus pectin, and guar gum (Sunvold et al., 1995a,b,c), very little data exist on fruit and vegetable fibers. Fruit and vegetable by-products from the human food industry may offer an inexpensive alternative. Fibers from fruits and vegetables contain several bioactive compounds (i.e., flavonoids and carotenoids), which increases their nutritional value in dog foods. Fruit and vegetable fibers also contain a good balance of soluble and insoluble fiber, which promotes gastrointestinal health (Sauracalixto and Larrauri, 1996).

The objective of this study was to assess fermentability, including SCFA production, extent of substrate dis-

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Table 1. Composition of fibrous substrates used in the in vitro experiment

Item	DM, %	OM	Fat	CP	TDF ^a	Insol ^b	Sol ^c	I:S ^d
% DM								
Test substrate								
Apple pomace	98.4	98.8	13.7	6.4	79.3	68.1	11.2	6.1:1
Carrot pomace	91.6	82.3	3.8	11.3	55.2	35.9	19.3	1.9:1
Flaxseed	94.6	96.5	43.4	20.7	32.6	23.7	8.9	2.7:1
Fruit blend ^e	78.0	84.5	3.3	11.3	65.3	57.3	8.0	7.2:1
Grape pomace	86.8	88.3	7.7	15.9	54.7	50.2	4.5	11.1:1
Pea hulls	89.1	96.9	3.1	16.2	69.7	66.7	3.0	22.2:1
Pistachio	92.1	97.3	3.9	5.3	85.9	83.4	2.5	33.3:1
Tomato pomace	88.0	94.8	16.6	18.9	56.9	52.7	4.2	12.6:1
Reference substrate								
Citrus pectin	92.6	97.9	0.0	0.8	65.4	0.0	65.4	—
Psyllium	92.6	97.4	0.0	2.4	90.0	82.4	7.6	10.8:1
Solka Flocc	95.6	100.0	0.0	0.3	99.3	97.0	2.3	42.2:1

^aTotal dietary fiber.^bInsoluble fiber.^cSoluble fiber.^dInsoluble:soluble fiber ratio.^eFruit blend is a mixture of peach, almond, nectarine, and plum.

appearance during fermentation, and gas production of fruit and vegetable fibers compared to dietary fiber standards. The desired outcome was to identify a superior fiber source or combination of sources to be used in premium dog foods.

Materials and Methods

Substrates and Donors

Fruit and vegetable fiber sources were tested along with three fiber standards. The standards included psyllium original texture sized husk (Proctor & Gamble Co., Cincinnati, OH), citrus pectin (TIC Gums, Belcamp, MD), and Solka Flocc (Fiber Sales and Development Corp., St. Louis, MO). The fruit and vegetable fiber sources included apple pomace, carrot pomace, flaxseed, fruit blend (a mixture of peach, almond, nectarine, and plum), grape pomace, pea hulls, ground pistachio, and tomato pomace. The pulpy material remaining after the juice has been pressed from a fruit or vegetable is commonly referred to as a "pomace." All fruit and vegetable fiber sources were obtained by Heinz Pet Products. Fiber standards were used in each assay to validate the efficacy of the experimental conditions imposed (a database containing information on the key response criteria measured in this experiment is available for these substrates, and any deviation in results obtained with these substrates resulted in invalidation of the data for the entire set of samples). Substrate chemical composition data are presented in Table 1.

Three healthy dogs (average age = 2.5 yr; average BW = 23.1 kg) served as sources of feces from which the inoculum was prepared. The donors consumed normal diets and no antibiotics in the 3 mo preceding the experiment. The main ingredients in the diet included various soy protein sources, potato starch, brewer's rice,

chicken bone residue, beet pulp, flaxseed, and fancy bleachable tallow. The diets contained between 6 and 12% TDF, 23 to 25% fat, and 30 to 32% CP.

Fermentation Procedures

Substrates were fermented in vitro for 4, 12, or 24 h with the fecal microflora obtained from each of the three dogs. Two sets of fermentations were conducted with inoculum prepared from each subject. One set was carried out to determine organic matter disappearance (OMD) and SCFA production. Freshly voided feces from each of the three donors were used to inoculate all substrate × time combinations in triplicate. Triplicate tubes containing no substrate also were fermented with each inoculum source and time point to enable appropriate corrections for OMD and SCFA production not arising from the substrates.

The composition of the semidefined medium used for the fermentation is presented in Table 2. All components except for the vitamin solutions were mixed prior to autoclave sterilization of the medium. Filter-sterilized vitamin solutions were added just before dispensing the medium, which was maintained under anaerobic conditions at all times after preparation. Aliquots (26 mL) of a semidefined medium, added to maintain microbial viability, were aseptically transferred into 50-mL tubes containing 300 mg of substrate. Anaerobic conditions were maintained by sealing the tubes with rubber stoppers equipped with one-way gas release valves.

Gas production was measured using Balch tubes. Ten-milliliter aliquots of medium were transferred to Balch tubes containing 115 mg of substrate and capped with butyl rubber stoppers. These tubes then were sealed with aluminum caps. All tubes were stored at 4°C for approximately 12 h prior to inoculation to enable

hydration of the substrates before initiating fermentation. Tubes were placed in a 37°C water bath approximately 30 min before inoculation.

Fresh feces from the three donors were collected in plastic bags that were sealed after expressing excess air and maintained at 37°C until inoculum was prepared (within 5 min). Each fecal sample was diluted 1:10 (wt/vol) in anaerobic dilution solution by blending it for 15 s in a Waring blender (Waring Products, New Hartford, CT) under a stream of CO₂. Blended, diluted feces were filtered through four layers of cheesecloth and sealed in 125-mL serum bottles under CO₂.

Appropriate sample and blank tubes were aseptically inoculated with diluted feces. Four milliliters of inoculum was used for the 50-mL tubes, whereas 1.5 mL was used for the Balch tubes. Tubes were incubated at 37°C with periodic mixing for the respective fermentation times. At the appropriate time, tubes were removed from the 37°C incubation and processed immediately for analysis. A 2-mL subsample was taken from each 50-mL tube for SCFA analysis, and the remaining 28 mL was combined with 112 mL of 95% ethanol and left to precipitate for 1 h. To recover unfermented residues, samples were filtered and washed with ethanol and acetone. Samples then were dried at 105°C for 24 h.

Table 2. Composition of medium used for in vitro fermentation

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	0.4
Resazurin ⁱ	1.0
Distilled H ₂ O	296.0
	g/L
Yeast extract	0.5
Trypticase	0.5
Na ₂ CO ₃	4.0
Cysteine HCl·H ₂ O	0.5

^aComposition (g/L): NaCl, 5.4; KH₂PO₄, 2.7; CaCl₂·H₂O, 0.16; MgCl₂·6H₂O, 0.12; MnCl₂·4H₂O, 0.06; CoCl₂·6H₂O, 0.06; (NH₄)₂SO₄, 5.4.

^bComposition: K₂HPO₄, 2.7 g/L.

^cComposition (mg/L): ethylenediaminetetraacetic acid (disodium salt), 500; FeSO₄·7H₂O, 200; ZnSO₄·7H₂O, 10; MnCl₂·4H₂O, 3; H₃PO₄, 30; CoCl₂·6H₂O, 20; CuCl₂·2H₂O, 1; NiCl₂·6H₂O, 2; Na₂MoO₄·2H₂O, 3.

^dComposition (mg/L): thiamin·HCl, 100; d-pantothenic acid, 100; niacin, 100; pyridoxine, 100; *p*-aminobenzoic acid, 5; vitamin B₁₂, 0.25.

^eComposition (mg/L): folic acid, 10; d-biotin, 2; NH₄HCO₃, 100.

^fComposition: riboflavin, 10 mg/L in 5 mmol/L of Hepes.

^gHemin, 500 mg/L in 10 mmol/L NaOH.

^h250 mL/L each of *n*-valerate, isovalerate, isobutyrate, and DL- α -methylbutyrate.

ⁱResazurin, 1 g/L in distilled H₂O.

When dry, samples were weighed, transferred to tared aluminum pans, and ashed at 450°C to determine OMD. In vitro OMD after fermentation was calculated as 1 minus [(dry residue weight minus blank residue weight) minus (the organic residue weight minus the organic residue weight from the appropriate blank) divided by the original dry sample weight on an OM basis].

Chemical Analyses

Dry matter and OM were determined by AOAC (1984) methods. Total lipid content was determined by acid hydrolysis followed by ether extraction according to Budde (1952). Crude protein was calculated from Kjeldahl N values (AOAC, 1984). Total dietary fiber concentration was determined using methods of Prosky et al. (1984, 1992). Samples to be analyzed for SCFA (2 mL) were mixed with 0.5 mL of 250 g/L *m*-phosphoric acid, precipitated for 30 min, and then centrifuged at 25,900 $\times g$ for 20 min. The supernate was decanted and frozen at -20°C in microfuge tubes. After freezing, the supernatant was thawed and centrifuged in microfuge tubes at 13,000 $\times g$ for 10 min. Concentrations of SCFA were determined via gas-liquid chromatography. Briefly, concentrations of acetate, propionate, and butyrate were determined in the supernate of the tubes using a Hewlett-Packard 5890A Series II gas chromatograph (Palo Alto, CA) and a glass column (180 cm \times 4 mm i.d.) packed with 10% SP-1200/1% H₃PO₄ on 80/100 mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier gas with a flow rate of 75 mL/min. Oven temperature, detector temperature, and injector temperature were 125, 175, and 180°C, respectively. Short-chain fatty acid concentrations were corrected for by the quantities of SCFA produced in blank tubes.

Gas Production

Gas production was determined by fluid displacement (water with 5% HCl and resazurin) at equal pressure using a manometer (Campbell and Fahey, 1997). Corrections were made for temperature, pressure, and head space contained in the Balch tube prior to initiation of fermentation. Gas production (mL) was calculated as gas production from the sample minus gas production from the blank divided by original sample OM weight.

Statistical Analysis

Data were analyzed as a randomized complete block with fecal donor serving as block. Treatments, which were factorially arranged, included substrate and length of fermentation. Therefore, donor, substrate, time, and substrate \times time were used in the statistical model. All analyses were performed according to the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Least squares means were reported along with the

Table 3. Organic matter disappearance (OMD) after various times of in vitro fermentation of fibrous substrates with dog fecal microflora^a

Item	OMD, %			
	0 h	4 h	12 h	24 h
Test substrate				
Apple pomace	10.2	15.3	20.6	34.9
Carrot pomace	19.4	28.1	34.3	41.3
Flaxseed	20.8	25.7	41.1	51.7
Fruit blend ^b	3.7	10.2	13.8	13.8
Grape pomace	16.0	12.4	16.4	16.5
Pea hulls	10.0	13.4	14.8	24.7
Pistachio	9.5	6.3	9.5	9.3
Tomato pomace	24.4	24.2	26.5	35.0
Reference substrate				
Citrus pectin	45.1	56.7	76.1	81.9
Psyllium	0.8	5.3	10.2	35.4
Solka Floc	0.5	-0.9	0.8	3.5
Pooled SEM	2.9			
LSD ^c	8.6			

^aAll substrate × time interactions were significant ($P < 0.05$).

^bFruit blend is a mixture of peach, almond, nectarine, and plum.

^cLeast significant difference between any two mean values in the same column ($P < 0.05$).

pooled SEM for all treatments. When treatment differences were detected ($P < 0.05$), means were compared by the least significant difference method. The strength of relationship between OMD vs SCFA and OMD vs gas production was determined by performing Pearson correlation tests using SAS.

Results and Discussion

In general, OMD, SCFA production, and gas production increased over time. All substrate × time interactions were significant ($P < 0.05$) for OMD, gas production, acetate, propionate, and total SCFA production. A trend ($P = 0.17$) was detected for butyrate production.

Organic matter disappearance values are presented in Table 3. Organic matter disappearance at 0 h does not reflect microbial fermentation but is most likely due to the disappearance of soluble carbohydrates that are present in the substrates and not retained during filtering. To estimate substrate disappearance as a result of fermentation, the 0-h value may be subtracted from the values at 4, 12, and 24 h. Due to random error, OMD values at 0 h were sometimes greater than 4-, 12-, or 24-h values from poorly fermented substrates (grape pomace, pistachio, Solka Floc, and tomato pomace). Although the soluble carbohydrates present in the sample are available for fermentation, it is unknown what proportion is used by the microbes as an energy source. Therefore, the measurement of OMD is not as accurate as the measurement of the fermentation products (i.e., SCFA and gas), which is a direct measurement of fermentation.

In agreement with other studies, citrus pectin was highly fermentable and had the highest OMD for all

times of fermentation. Psyllium and Solka Floc had intermediate and low OMD values, respectively. The OMD values of citrus pectin and Solka Floc in the current study are similar to those reported in past studies using dog fecal bacteria (Sunvold et al., 1995a,b,c) and human fecal bacteria (Campbell and Fahey, 1997).

Of the fruit and vegetable fibers, flaxseed and pistachio had the greatest and lowest fermentabilities, respectively, as measured by OMD. After 24 h of fermentation, apple pomace, carrot pomace, flaxseed, and tomato pomace seemed to be moderately fermented, whereas fruit blend, grape pomace, pea hulls, and pistachio were poorly fermented. Soluble fibers are more easily fermented than insoluble fibers (Cummings, 1984), which agrees with our data. In general, as the insoluble:soluble fiber ratio of the substrates increased, OMD values decreased.

The TDF digestibility of dog diets containing different sources of fiber has been shown to range from 4.1 to 60.8% (Sunvold et al., 1993). Reasons for the diversity among substrates include differences in chemical composition and polysaccharide structure (Titgemeyer et al., 1991; Sunvold et al., 1995b). Therefore, it was not surprising to observe large differences in OMD.

Total gas production, measured as milliliters of gas/gram of original substrate OM, increased over time for all substrates (Table 4). Gas production and OMD values for all substrates were highly correlated after 12 h ($r^2 = 0.78$) and 24 h ($r^2 = 0.72$) of fermentation. Therefore, with a few exceptions, substrates with high OMD values also produced large amounts of gas.

Fermentation of citrus pectin produced the highest amounts of gas over all times of fermentation, and Solka

Table 4. Total gas production after various times of in vitro fermentation of fibrous substrates with dog fecal microflora^a

Item	Total gas production, mL/g original substrate OM		
	4 h	12 h	24 h
Test substrate			
Apple pomace	7.3	42.9	79.1
Carrot pomace	40.0	75.1	98.8
Flaxseed	3.2	14.2	29.6
Fruit blend ^b	1.5	9.0	19.7
Grape pomace	9.2	15.4	27.6
Pea hulls	3.8	23.0	46.1
Pistachio	3.0	3.7	12.4
Tomato pomace	10.2	23.4	44.8
Reference substrate			
Citrus pectin	72.4	156.3	170.2
Psyllium	0.1	32.1	82.9
Solka Floc	0.0	0.0	2.7
Pooled SEM	3.2		
LSD ^c	9.4		

^aAll substrate × time interactions were significant ($P < 0.05$).

^bFruit blend is a mixture of peach, almond, nectarine, and plum.

^cLeast significant difference between any two mean values in the same column ($P < 0.05$).

Floc produced the lowest. After 12 and 24 h of fermentation, apple pomace, carrot pomace, and psyllium produced large amounts of gas, whereas flaxseed, grape pomace, pea hulls, and tomato pomace produced intermediate levels. Fruit blend and pistachio fiber consistently produced low concentrations of gas over all times of fermentation.

The production of the three major SCFA (acetate, propionate, and butyrate) after 4, 12, and 24 h of fermentation (mmol/g original substrate OM) and SCFA molar proportions after 12 h of fermentation are presented in Table 5. As with OMD and gas production, citrus pectin produced the largest amounts of total SCFA over all times of fermentation and psyllium and Solka Floc produced intermediate and low amounts of SCFA, respectively.

After 12 h of fermentation, total SCFA production from carrot pomace was three times greater than for any other fruit and vegetable fibers. Pistachio produced the lowest amounts of total SCFA after 12 h. The remaining fruit and vegetable fibers produced moderate amounts of total SCFA at this time point. After 24 h of fermentation, carrot pomace produced approximately twice as much total SCFA as any other substrates. Apple pomace, flaxseed, pea hulls, and tomato pomace produced moderate amounts of total SCFA, and fruit blend, grape pomace, and pistachio produced low levels after 24 h of fermentation.

Although the amount of total SCFA produced can be used as a measure of fermentability, molar proportions of SCFA can provide additional information about the quality of substrate. Butyrate is the preferred energy substrate of colonic epithelium (Roediger, 1982) and has been estimated to account for 70% of its total energy consumption (Roediger, 1980). There is also evidence that butyrate impedes the development of cancer of the colon. Therefore, a substrate producing a high molar ratio of butyrate would be desirable for inclusion in canine diets and may be considered of higher quality than one producing low butyrate concentrations.

The molar proportions of SCFA (acetate:propionate:butyrate) were similar for most substrates after 12 h of fermentation. Apple pomace produced the highest proportion of acetate (81.6% of total SCFA) and psyllium produced the lowest after 12 h of fermentation (51.4%). The high acetate (81.6% of total SCFA) produced from the fermentation of apple pomace was probably due to its pectin content. Apple pectin is high in uronic acids, which leads to high acetate production (Salvador et al., 1993). The high level of acetate produced by apple pomace and pea hulls subsequently led to a lower proportion of propionate. The opposite occurred with psyllium, which had the largest proportion of propionate (49.0% of total SCFA). Molar proportions of butyrate were low for all substrates in the current experiment, indicating that none of the fiber sources tested in this experiment was effective in modifying the proportion of this important SCFA. This is fairly consistent among fiber sources, with the exception of

resistant starch (Mathers and Dawson, 1991; Schepach et al., 1988).

The main products of microbial fermentation are gas and SCFA. Because SCFA production is desired and gas production is not, a low ratio of gas:SCFA is favorable. The ratios of total gas formation (mL/g original substrate OM):total SCFA production (mmol/g original substrate OM) for all substrates after 12 and 24 h of fermentation are presented in Table 6.

After 12 h of fermentation, apple pomace had the highest gas:SCFA ratio (49.9), which was twice as high as that of all other substrates, excluding psyllium (28.2) and citrus pectin (25.3). Solka Floc produced no gas after 12 h. The remaining substrates had values between 12.3 and 20.4. After 24 h, Solka Floc had the highest value (54.0), followed by apple pomace (37.0) and grape pomace (33.3). The remaining substrates resulted in values between 20.6 and 27.0. From these data, it seems that apple pomace has the potential to produce high concentrations of gas in relation to SCFA, which may limit its use in pet foods.

In general, OMD, total SCFA production, and gas production data were in agreement. Our results suggest that carrot pomace is most highly fermentable by microflora that inhabit the dog's colon, followed by apple pomace, pea hulls, and tomato pomace, which were moderately fermentable. Fruit blend, grape pomace, and pistachio seem to be poorly fermented by canine microflora.

Pea hulls are rich in cellulose (Leterme et al., 1996). In fact, Ralet et al. (1993) estimated that 98% of the glucose residues in pea hulls are of cellulosic origin. In addition to cellulose, pea hulls are also composed of xylose- and arabinose-rich hemicelluloses and pectin, most of which is in the soluble fiber fraction (Leterme et al., 1996). In the current experiment, pea hulls were moderately fermentable, which was probably due to their high cellulose content in combination with their low concentrations of pectin and hemicelluloses.

Although flaxseed seemed to be highly fermentable as measured by OMD, the SCFA data suggested that it was not. In vivo studies with this ingredient suggest that it is indeed readily fermentable based on fecal moisture (T. Belay and R. G. Shields, unpublished data). At equal weight it contributed less TDF to the incubation system, which may have limited potential SCFA production. The high fat content of the flaxseed samples perhaps resulted in an overestimation of the OMD value. Similar to soluble carbohydrates, fat not retained during filtering inflates the OMD value. Due to its high fat and protein concentrations, the majority of flaxseed would likely be digested in vivo via hydrolytic and enzymatic digestion prior to the large intestine and contribute little to the microbial population. However, flaxseed meal, a by-product of oil extraction, may produce different results.

Fruit blend, grape pomace, and pistachio fiber were poorly fermented. The low fermentability of these substrates may have been due to the presence of lignin,

Table 5. Acetate (ACE), propionate (PRO), butyrate (BUTY), and total short-chain fatty acid (SCFA) production after various times of in vitro fermentation and molar proportions of SCFA after 12 h of fermentation of fibrous substrates with dog fecal microflora^a

Item	ACE, mmol/g original substrate OM			PRO, mmol/g original substrate OM			BUTY, mmol/g original substrate OM ^b			Total SCFA ^c , mmol/g original substrate OM			Molar proportions ^d			
	4 h	12 h	24 h	4 h	12 h	24 h	4 h	12 h	24 h	4 h	12 h	24 h	ACE	PRO	BUTY	
Test substrate																
Apple pomace	0.22	0.71	1.57	0.05	0.16	0.52	0.00	0.00 ^w	0.04 ^{wx}	0.27	0.86	2.14	81.6	18.9	1.1	
Carrot pomace	0.89	2.18	2.58	0.35	1.43	1.46	0.01	0.11 ^{xy}	0.18 ^{yz}	1.25	3.72	4.22	58.0	39.4	2.8	
Flaxseed	0.11	0.50	0.75	0.07	0.35	0.64	-0.01	0.02 ^{wxy}	0.05 ^{wxy}	0.16	0.86	1.44	56.6	41.7	2.5	
Fruit blend ^e	0.17	0.38	0.54	0.06	0.26	0.32	-0.01	0.00 ^w	0.02 ^{wx}	0.21	0.63	0.87	63.1	35.7	1.9	
Grape pomace	0.12	0.57	0.56	0.06	0.24	0.25	-0.01	0.01 ^w	0.03 ^{wx}	0.17	0.82	0.83	68.0	31.4	2.7	
Pea hulls	0.36	0.75	1.14	0.15	0.34	0.76	0.01	0.03 ^{wxy}	0.10 ^{xy}	0.52	1.13	2.00	68.7	29.7	2.9	
Pistachio	0.09	0.17	0.30	0.05	0.13	0.15	-0.01	0.00 ^w	0.01 ^w	0.13	0.30	0.46	60.3	36.9	3.5	
Tomato pomace	0.31	0.78	0.99	0.12	0.35	0.58	0.00	0.05 ^{wxy}	0.14 ^y	0.43	1.17	1.71	65.6	30.6	3.8	
Reference substrate																
Citrus pectin	1.20	3.69	4.17	0.64	2.37	2.27	0.02	0.12 ^y	0.23 ^z	1.86	6.18	6.66	59.4	38.8	2.0	
Psyllium	0.31	0.60	1.49	0.09	0.55	1.79	0.00	-0.01 ^w	0.02 ^{wx}	0.39	1.14	3.29	51.4	49.0	0.0	
Solka Floc	0.03	-0.01	0.03	0.01	0.01	0.01	0.00	0.00 ^w	0.00 ^w	0.04	-0.01	0.05	61.5	35.1	2.0	
Pooled SEM		0.21			0.13			0.03			0.30		4.6	4.4	0.9	
LSD ^f		0.62			0.38			0.09			0.89		13.6	13.0	2.7	

^aAll substrate × time interactions were significant ($P < 0.05$) for acetate, propionate, and total SCFA.

^bValues in the same column not sharing a common superscript letter differ ($P < 0.05$).

^cTotal SCFA = acetate + propionate + butyrate.

^dPercentage of total SCFA.

^eFruit blend is a mixture of peach, almond, nectarine, and plum.

^fLeast significant difference between any two mean values in the same column ($P < 0.05$).

Table 6. The ratio of total gas formation and total short-chain fatty acid (SCFA) production after 12 and 24 h of in vitro fermentation of fibrous substrates with dog fecal microflora

Item	Total gas (mL/g OM)/total SCFA (mmol/g OM)	
	12 h	24 h
Test substrate		
Apple pomace	49.9	37.0
Carrot pomace	20.2	23.4
Flaxseed	16.5	20.6
Fruit blend ^a	14.3	22.6
Grape pomace	18.8	33.3
Pea hulls	20.4	23.1
Pistachio	12.3	27.0
Tomato pomace	20.0	26.2
Reference substrate		
Citrus pectin	25.3	25.6
Psyllium	28.2	25.2
Solka Flocc	0.0	54.0

^aFruit blend is a mixture of peach, almond, nectarine, and plum.

condensed tannins, or resistant protein. Grape pomaces contain a small proportion of pectic substances (5 to 10%) and often have reduced protein digestibility due to formation of insoluble tannin-protein complexes (Bravo and Saura-Calixto, 1998). The cell solubles of grape pomace also may be less digestible because of covalent interaction of the pectins with lignin present in high amounts in grape cell walls (Wardrop and Bland, 1958). Previous in vivo work with dogs has confirmed that grape pomace negatively affects DM and CP digestibility (Allen et al., 1981).

In addition to providing the gut with a balance of soluble and insoluble fiber, many of the fruit and vegetable fiber sources examined in this study are excellent sources of bioactive compounds that have been associated with enhanced health. Flavonoids and other polyphenols have been reported to have several biological effects such as antioxidant activity, inhibition of platelet aggregation, and anti-microbial and anti-inflammatory action (Ho et al., 1992). Polyphenols that possess antioxidant activity are present in apple and grape pomaces (Saura-Calixto, 1998; Lu and Foo, 2000). Carotenoids such as β -carotene and lycopene, which have antioxidant activity and are believed to reduce the risk of certain cancers, are concentrated in carrot and tomato pomaces (Boileau et al., 1999).

In conclusion, a wide variation was noted in OMD, gas production, and SCFA production among the vegetable and fruit fiber sources. Of the vegetable and fruit fibers, apple pomace, carrot pomace, and flaxseed had the greatest fermentability measured by OMD. Pea hulls and tomato pomace had intermediate OMD values, and fruit blend, grape pomace, and pistachio were poorly fermented. Gas and SCFA production were highly correlated to OMD values after 12 and 24 h of fermentation. The one exception to this relationship

was that flaxseed had high OMD values but low gas and SCFA production values.

Implications

In vitro fermentation data with canine fecal microflora suggest that fruit and vegetable fibers have potential to be alternative fiber sources used in premium pet foods. In addition to containing desirable insoluble:soluble fiber ratios, these fibers contain several bioactive components that may enhance gastrointestinal health of the animal. Although it seems that fruit and vegetable fibers may be economical alternatives to beet pulp, in vivo studies must be conducted in order to more fully discern the nutritional value of these alternate fiber sources.

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