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Adaptation of healthy adult cats to select dietary fibers in vivo affects gas and short-chain fatty acid production from fiber fermentation in vitro

K. A. Barry, B. J. Wojcicki, L. L. Bauer, I. S. Middelbos, B. M. Vester Boler, K. S. Swanson, and G. C. Fahey Jr.[1]

Department of Animal Sciences, University of Illinois, Urbana 61801

ABSTRACT: Nine young adult (1.73 ± 0.03 yr) male cats were used to determine the effects of microbial adaptation to select dietary fiber sources on changes in pH in vitro and on total and hydrogen gas, short-chain fatty acid (SCFA), and branched-chain fatty acid (BCFA) production. Cats were adapted to diets containing 4% cellulose, fructooligosaccharides (FOS), or pectin for 30 d before fecal sampling. Each cat was used as a single donor, and fecal inoculum was reacted with each of the aforementioned fiber substrates. Adaptation to dietary FOS resulted in a greater change in pH when exposed to FOS than pectin (adaptation × substrate, $P < 0.001$). When exposed to the FOS substrate, adaptation to dietary FOS or pectin increased hydrogen gas production (adaptation × substrate, $P = 0.021$). Adaptation to dietary FOS increased acetate and total SCFA production when exposed to FOS substrate in vitro (adaptation × substrate, $P = 0.001$). When exposed to the FOS substrate, propionate production tended to increase with adaptation to dietary cellulose (adaptation × substrate, $P = 0.060$). The BCFA + valerate tended to decrease with adaptation to dietary FOS when exposed to FOS substrate in vitro (adaptation × substrate, $P = 0.092$). Fructooligosaccharides resulted in the greatest change in pH and production of total gas ($P < 0.001$), hydrogen gas ($P < 0.001$), acetate ($P < 0.001$), propionate ($P < 0.001$), butyrate ($P < 0.001$), total SCFA ($P < 0.001$), and total BCFA + valerate production ($P < 0.001$). Adaptation to the FOS or pectin diet increased production of hydrogen gas with FOS and pectin substrates. Adaptation to pectin increased ($P = 0.033$) total gas production with FOS and pectin substrates. Overall, adaptation to either FOS or pectin led to greater SCFA and gas production, but adaptation to FOS resulted in the greatest effect overall.

Key words: adaptation, cat, fermentation, fiber, fructan, microbiota

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INTRODUCTION

Currently, in vitro fermentation analyses are used to estimate the fermentability of substrates within the gastrointestinal tract of animals by measuring production of gas and short- and branched-chain fatty acids (**SCFA** and **BCFA**, respectively). In vitro analysis of fibrous substrates is particularly useful when investigating a novel fiber source for a diet or investigating how a fiber source is utilized by the microbiota of an animal in vivo. In vitro fermentation with cat inoculum showed variability in ability to predict in vivo fermentability of fibrous substrates (Sunvold et al., 1995a). Generally, single fiber sources and more simplistic fiber blends were more accurately predicted than a complex, highly fermentable blend. Research related to fiber supplementation is lacking for cats, likely because the cat is a true carnivore and dietary fiber does not make up a large portion of its typical diet. However, cats can benefit from dietary fiber supplementation, especially when suffering from metabolic and gastrointestinal disorders that are affected by fiber (Nelson et al., 2000; Verbrugghe et al., 2009).

A concern related to investigating in vitro fermentation is the extent to which microbial adaptation to a dietary fiber source affects the outcomes measured. The diet consumed by the donor animal may greatly influence the results of any in vitro analysis performed. Sunvold et al. (1995b) observed that OM disappearance and SCFA production were greater when cats were

[1]Corresponding author: gcfahey@illinois.edu
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adapted to a diet containing 12.5% beet pulp compared with no supplemental fiber. However, this study did not investigate the effects of adapting the large bowel microbiota of the cat to the fiber source being investigated in vitro, which could result in even greater responses in vitro. The objective of this research was to determine the effects of microbial adaptation in vivo to fiber source on changes in pH and total and hydrogen gas, SCFA, and BCFA production in vitro.

MATERIALS AND METHODS

All animal care procedures were approved by the University of Illinois Institutional Animal Care and Use Committee before initiation of the experiment.

Substrates

Substrates used were α-cellulose (Solka Floc, International Fiber Corp., North Tonawanda, NY), fructooligosaccharides (**FOS**; SynergyC, BENEO-Group, Tienen, Belgium), and pectin (HM Pectin, TIC Gums, White Marsh, MD). These substrates were selected for their variable fermentability and solubility properties. Alpha-cellulose and FOS are currently used as sources of dietary fiber in feline diets, and pectin was investigated as a potential dietary fiber source.

Donors

Three male domestic short-hair cats (age = 1.73 ± 0.03 yr; BW = 5.7 ± 1.3 kg) were fed a diet containing α-cellulose as the source of fiber; 3 cats (age = 1.68 ± 0.11 yr; BW = 5.8 ± 1.4 kg) were fed a diet containing pectin as the source of fiber; and 3 cats (age = 1.73 ± 0.03 yr; BW = 6.4 ± 0.2 kg) were fed a diet containing FOS as the source of fiber for 31 d before collection of a single fecal sample from each cat. The diets were formulated to contain 4% supplemental total dietary fiber from each fiber source. The cellulose diet was chosen to represent a diet containing a nonfermentable, insoluble fiber source; the pectin diet was chosen to represent a diet containing a highly fermentable, soluble fiber source; and the FOS diet was chosen to represent a diet containing a highly fermentable, completely soluble, prebiotic fiber source. A detailed description of the diets and animal housing conditions is presented in Barry et al. (2010).

Medium Composition and Substrate Fermentation

For each time point (0 and 12 h fermentation), 115 mg of substrate was weighed in triplicate into 16-mL Balch tubes that were used in a model that simulated large bowel fermentation (Bourquin et al., 1993). The composition of the medium used to conduct the in vitro fermentation experiment is presented in Table 1.

Table 1. Composition of microbiological medium used in the in vitro experiment

Component	Amount
Liquid solutions, mL in medium	
Solution A[1]	330.0
Solution B[2]	330.0
Distilled water	296.0
Water-soluble vitamin mix[3]	20.0
Trace mineral solution[4]	10.0
Folate:biotin solution[5]	5.0
Hemin solution[6]	5.0
Riboflavin solution[7]	5.0
Resazurin[8]	1.0
Short-chain fatty acid mix[9]	0.4
Solid chemicals, g in medium	
Yeast extract	0.5
Trypticase	0.5
Na_2CO_3	4.0
$Cys{\cdot}HCl{\cdot}H_2O$	0.5

[1]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; and $(NH_4)_2SO_4$, 5.4.

[2]Composition: K_2HPO_4, 2.7 g/L.

[3]Composition (mg/L): thiamine·HCl, 100; D-pantothenic acid, 100; niacin, 100; pyridoxine, 100; *p*-aminobenzoic acid, 5; and vitamin B_{12}, 0.25.

[4]Composition (mg/L): EDTA (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; $CuCl_2{\cdot}2H_2O$, 1; $NiCl_2{\cdot}6H_2O$, 2; and $NaMoO_4{\cdot}2H_2O$, 3.

[5]Composition (mg/L): folic acid, 10; D-biotin, 2; and NH_4HCO_3, 100.

[6]Hemin, 500 mg/L in 10 mmol/L of NaOH.

[7]Riboflavin, 10 mg/L in 5 mmol/L of 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES).

[8]Resazurin, 1 g/L in distilled H_2O.

[9]Contained 250 μL each of *n*-valerate, isovalerate, isobutyrate, and DL-α-methyl butyrate per milliliter.

All medium components, except for the vitamin mixes, were added before autoclaving. The vitamin mixes were aseptically added after they were filter sterilized. An aliquot (10 mL) of medium was aseptically transferred into the appropriate Balch tubes, capped with butyl rubber stoppers, and sealed with aluminum caps. All tubes were stored at 4°C for approximately 12 h to enable hydration of the substrates before initiating fermentation. Tubes were placed in a 37°C water bath approximately 30 min before inoculation.

Freshly voided fecal samples from each cat were immediately placed into sterile sampling bags (Whirlpac, Fisher Scientific, Pittsburgh, PA) that were sealed after expressing excess air. Each sample then was diluted 1:10 (wt/vol) in previously warmed (39°C) anaerobic diluting solution (Bryant and Burkey, 1953) by blending it for 15 s in a Waring blender under a stream of CO_2. Blended, diluted feces were filtered through 4 layers of cheesecloth and sealed in 100-mL serum bottles under CO_2.

Appropriate sample and blank tubes were aseptically inoculated with 1.5 mL of diluted feces, which provided a 1:100 dilution of the substrate. Tubes were incubated

at 39°C with periodic mixing for 0 or 12 h, after which time they were stored in a cold room for at least 24 h to halt microbial growth. Tubes were stored because not all fecal samples could be collected at the same time from the cats, which did not allow for immediate sample processing. First, a 1-mL aliquot was taken with a gastight syringe for gas composition (H_2 and CH_4) with a gas analyzer (Quintron Instrument Co., Milwaukee, WI). Gas production then 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 headspace contained in the Balch tube before the initiation of fermentation. Gas production (mL) was calculated as gas produced from the substrate minus gas produced from the blank divided by original sample weight expressed on a DM basis. The pH of tube contents was measured using a standard pH meter (Denver Instrument Co., Arvada, CO). Finally, a 2-mL aliquot was taken from each tube for SCFA and BCFA analyses.

SCFA and BCFA Analyses

The 2-mL aliquot of fluid removed from the sample tubes for SCFA and BCFA analyses was immediately added to 0.5 mL of 25% metaphosphoric acid, precipitated for 30 min, and centrifuged at 20,000 × g at 4°C for 20 min. The supernatant was decanted and frozen at −20°C overnight in microfuge tubes. After freezing, the supernatant was thawed and centrifuged in microfuge tubes at 10,000 × g for 10 min at room temperature. Concentrations of SCFA and BCFA in the supernatants were determined using a gas chromatograph (5890A series II, Hewlett-Packard, Palo Alto, CA) and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Oven, detector, and injector temperatures were 125, 175, and 180°C, respectively. Short-chain fatty acid and BCFA concentration values were corrected for blank tube production of SCFA and BCFA, then also corrected for production of SCFA and BCFA at the 0-h time point. All samples were run in duplicate, and an error term of ≤5% was considered acceptable.

Statistical Analyses

Data were analyzed as a split-plot design using the Proc Mixed procedure (SAS Inst. Inc., Cary, NC). The whole plot consisted of a 3 × 3 factorial arrangement of treatments with dietary adaptation as the treatment effect. The subplot effects were substrate and the interaction between diet and substrate. Therefore, dietary adaptation, substrate, and the interaction of dietary adaptation × substrate were used in the statistical model. The Tukey adjustment was added to protect against experiment-wise error, and the Satterthwaite procedure was used to determine the denominator degrees of freedom. Least squares means were reported along with the pooled SEM for all response criteria. When significant ($P < 0.05$) differences and interactions, or significant trends ($P < 0.10$), were detected, individual means were compared using the LSD method of SAS (Milliken and Johnson, 1984).

RESULTS

Dry matter and OM concentrations of substrates were similar. Dry matter was 94.8, 95.6, and 91.4% for cellulose, FOS, and pectin, respectively. Corresponding OM values were 99.7, 99.9, and 98.1%, respectively.

The effects of dietary adaptation, substrate, and dietary adaptation × substrate interaction are presented in Table 2. A smaller change in pH was observed with the pectin substrate when microbiota were adapted to the FOS diet than to the cellulose or pectin diets (adaptation × substrate, $P < 0.001$). Regarding the FOS substrate, greater amounts of hydrogen gas were produced with microbial adaptation to the FOS and pectin diets than to the cellulose diet (adaptation × substrate, $P = 0.021$). Production of acetate and total SCFA was greater when microbiota were adapted to the FOS diet and exposed to the FOS substrate (adaptation × substrate, $P < 0.001$). When fecal inoculum was obtained from cats adapted to the cellulose diet and exposed to the FOS substrate, propionate production values tended to be greater than for values when cats were adapted to the pectin diet (adaptation × substrate, $P = 0.060$). When fecal inoculum was obtained from cats adapted to the FOS diet and exposed to FOS substrate, total BCFA + valerate production values tended to be smaller compared with the inoculum obtained from those adapted to cellulose (adaptation × substrate, $P = 0.092$). No interactions were observed for total gas or butyrate production.

Adaptation to diet had no effect on change in pH or acetate, butyrate, or total SCFA production. Total gas production was greater ($P = 0.033$) with adaptation to the pectin diet and hydrogen gas production was greater with adaptation to the FOS and pectin diets than the cellulose diet. Propionate production tended to be greater with dietary adaptation to cellulose. When adapted to the FOS diet, BCFA + valerate concentration was less ($P = 0.092$) than the cellulose diet. Methane gas was undetected in all samples.

With regard to all outcome variables measured, a similar pattern was observed for all substrates. The cellulose substrate generated the smallest ($P < 0.001$) values for change in pH and production of total gas, hydrogen gas, acetate, propionate, butyrate, total SCFA, and BCFA + valerate. The pectin substrate resulted in greater ($P < 0.001$) values than cellulose, and the FOS substrate resulted in the greatest ($P < 0.001$) values of the 3 substrates tested in all of the aforementioned variables.

Table 2. Change in pH, gas produced, and short- (SCFA) and branched-chain fatty acids (BCFA) after a 12-h in vitro fermentation of select substrates with feline fecal inoculum from 3 donors adapted to 1 of 3 fiber sources[1]

	Cellulose[2]			FOS[2]			Pectin[2]				*P*-value		
Item	Cellulose[4]	FOS[4]	Pectin[4]	Cellulose[4]	FOS[4]	Pectin[4]	Cellulose[4]	FOS[4]	Pectin[4]	SEM[3]	Adap	Subst	Adap × Subst
pH	0.0	0.0	0.0	−1.4	−1.6	−1.4	−1.0[a]	−0.9[b]	−1.2[a]	0.1	0.270	<0.001	<0.001
Total gas, mL/g of DM	0.0	0.0	0.0	82.6	90.5	103.9	54.6	58.6	61.1	4.0	0.033	<0.001	0.141
Hydrogen gas, mmol/g of DM	0.0	0.0	0.0	604.8[a]	2,046.4[b]	2,329.6[b]	186.6	794.7	659.5	240.0	0.025	<0.001	0.021
SCFA, mmol/g of DM													
Acetate	0.03	0.02	0.15	1.89[a]	3.61[b]	2.17[a]	1.58	1.14	1.81	0.24	0.327	<0.001	0.001
Propionate	0.02	0.02	0.05	1.79[c]	1.40[cd]	1.09[d]	0.96	0.31	0.71	0.15	0.076	<0.001	0.060
Butyrate	0.00	0.00	0.05	0.86	1.02	1.28	0.41	0.72	0.69	0.12	0.176	<0.001	0.373
Total	0.06	0.04	0.25	4.54[a]	6.04[b]	4.54[a]	2.94	2.17	3.21	0.26	0.694	<0.001	0.001
BCFA, mmol/g of DM													
Total BCFA + valerate	0.00	0.00	0.05	0.48[c]	0.23[d]	0.41[cd]	0.16	0.12	0.21	0.04	0.018	<0.001	0.092

[a,b]Within a substrate, means without a common superscript differ ($P < 0.05$).

[c,d]Within a substrate, means without a common superscript differ ($P < 0.10$).

[1]FOS = fructooligosaccharide; Adap = dietary adaptation; and Subst = substrate.

[2]Top row indicates substrate.

[3]Pooled SEM.

[4]Second row indicates dietary adaptation.

DISCUSSION

The objective of this research was to determine if microbial adaptation to a specific dietary fiber source by the cat affects the results of an in vitro analysis of fiber fermentability. Several challenges are presented when attempting to study the fermentability of fibers and nondigestible carbohydrates in the large intestine, the greatest of which is gaining access to this area without disrupting normal intestinal function and microflora. Thus, in vitro fermentation analyses conducted with fecal microbiota are used to simulate fermentation in the large intestine. The question is whether cats adapted to select dietary fibers will produce a microbial inoculum in the large bowel that will subsequently affect fermentation patterns of pure fiber substrates upon in vitro incubation. Few data of this type are available with cats.

Regardless of microbial adaptation, cellulose remained refractory to fermentation and resulted in minimal SCFA and BCFA production, and no changes in pH, total gas, or hydrogen gas were observed. These results were anticipated because cellulose is unfermentable by microbiota from all nonruminant species studied to date, and these results are similar to those of Sunvold et al. (1995a,b). The production values for total gas, hydrogen gas, and SCFA by cellulose were even less than those observed by Campbell and Fahey (1997) using human fecal inoculum. In regard to the effect of microbial adaptation, the SCFA response to cellulose is similar to that observed in rats adapted to a diet containing cellulose (Stark and Madar, 1993; Goñi et al., 2001).

Microbial adaptation to dietary treatments affected several outcome variables in the present study. Adaptation to pectin increased total and hydrogen gas production. Because pectin is rapidly fermented in the colon (Anderson and Chen, 1979), these results were not surprising. Adaptation to FOS increased hydrogen gas production, but not total gas production. Similar to pectin, FOS is rapidly fermented as it enters the colon. However, FOS has a simpler structure (straighter chain) and lesser degree of polymerization than pectin, so the gastrointestinal microbiota may have been able to adapt to the FOS substrate more readily than to the pectin substrate, thereby reducing the quantity of total gas produced. Furthermore, adaptation to FOS decreased BCFA + valerate production. Research in dogs has observed that concentrations of SCFA increase and BCFA decrease when animals are supplemented with FOS (Swanson et al., 2002; Propst et al., 2003). This indicates a shift away from protein fermentation; BCFA are generated from the fermentation of AA in the colon (Macfarlane and Cummings, 1991). Finally, adaptation to cellulose increased propionate production. As stated previously, cellulose is largely unaffected by fermentation in the colon of the cat, and the observed increase in propionate production was not expected. The observed changes in concentrations of propionate appear to be associated with microbial adaptation to pectin and are likely due to some unfermented pectin substrate remaining in the fecal sample used to generate the inoculum.

The FOS substrate, regardless of microbial adaptation to dietary fiber source, exerted the greatest impact on all outcome variables when compared with the cellulose or pectin substrates. Although FOS has not been investigated in vitro using the cat, the results for acetate production in the present study are similar to those with dogs (Bosch et al., 2008). Propionate, butyrate, and total SCFA concentrations were greater using dog fecal inoculum (Bosch et al., 2008). Propionate production was greater, and pH, butyrate, total SCFA, and total gas production were less, in the present study compared with in vitro studies utilizing human fecal inoculum (Hernot et al., 2009; Vester Boler et al., 2009). However, acetate and hydrogen gas values were equivalent to or less than those measured using human fecal inoculum (Hernot et al., 2009; Vester Boler et al., 2009).

Pectin fermentation end product production values were intermediate to those for cellulose and FOS substrates, but were typically closer to the results observed for FOS. Total gas production was less and pH and hydrogen gas values similar to or less than those in the present study compared with studies utilizing human fecal inoculum (Hernot et al., 2009; Vester Boler et al., 2009). In previous research utilizing cat fecal inoculum, acetate, propionate, and total SCFA values were equivalent to or lower than those of the present study (Sunvold et al., 1995a,b). Propionate, butyrate, and total SCFA production were greater compared with results from a study using dog fecal inoculum (Bosch et al., 2008), but acetate concentrations were similar. Studies with human fecal inoculum showed less acetate, butyrate, and total SCFA production (Hernot et al., 2009; Vester Boler et al., 2009).

Microbial adaptation to a substrate should increase the efficiency with which a substrate would be used within a dietary matrix. Microbial adaptation to the FOS diet appeared to greatly affect the results observed when fecal microbiota from cats fed FOS were exposed to pure forms of this substrate in vitro. Acetate and total SCFA production were increased, and valerate concentrations decreased, when microbiota were adapted to the FOS diet and exposed to the FOS substrate when compared with the other treatment combinations. The observed numerical decrease in pH and increase in hydrogen gas production correlate well with the changes in SCFA production because these outcomes indicate increased fermentation and would be anticipated with increased SCFA. Production of acetate, propionate, butyrate, and total SCFA was greater in the present study compared with an in vitro study using fecal inoculum from dogs that were not adapted to a diet containing FOS (Bosch et al., 2008). Together, these observations

indicate that adaptation of microbiota to a given fiber source positively influences the production of SCFA.

When the impact of microbial adaptation on BCFA production is considered, a clear shift is observed in the impact of dietary FOS on the production of BCFA + valerate (values are least) when FOS is the in vitro substrate. It appears that these microbiota are primed to produce SCFA because the total SCFA concentration for this specific combination of dietary adaptation and substrate is greater than for any other combination studied. This observation is not static. When the microbiota are adapted to pectin, pectin-adapted microbiota produce numerically more valerate than cellulose- or FOS-adapted microbiota when exposed to the pectin substrate. In this instance, there may be more microbial mass associated with the fecal inoculum, or the microbiota may be interacting with other microbiota in the tubes to generate a more robust population that can ferment the pectin. Small amounts of fermentable substrate also may remain unfermented through the gastrointestinal tract, as evidenced by the response observed for pectin-adapted microbiota exposed to the cellulose substrate. Clearly, production of SCFA, and even some BCFA, is greater for this treatment and adaptation combination compared with other combinations, and the pattern of SCFA production follows a pattern similar to that observed for pectin fermentation in humans (Englyst et al., 1987).

When the effect of microbial adaptation to a dietary cellulose-containing treatment is investigated, the response varies depending on the type of fiber being fermented. If added to a cellulose substrate, microbes adapted to cellulose produce little gas, SCFA, or BCFA. However, if cellulose-adapted microbiota are exposed to a fermentable fiber such as FOS or pectin, the resulting fermentative end products are produced in high concentrations, sometimes greater than those produced by microbiota that are adapted to much more fermentable fibers. This is likely because the cellulose-adapted microbiota have the capacity to utilize these highly fermentable substrates for rapid growth but were, in a sense, deprived of energy because of the nonfermentable nature of the cellulose.

In conclusion, microbial adaptation to dietary fiber by the cat appears to affect the results of in vitro fermentation analyses, and this response is highly dependent on the source of dietary fiber. Although cellulose had little impact on gas and SCFA production, FOS exerted the largest impact on these outcomes. As evidenced by the results of the present study, the impact of microbial adaptation to a fiber varies by fiber source. Adaptation to FOS appears to influence the outcome variables measured in this experiment far more than adaptation to cellulose or pectin. These results address the need for consideration of the source of fiber in the diet of an animal selected as a fecal donor for in vitro studies. Careful consideration of the potential for microbial adaptation to a given fiber source will prevent unintentional bias and should be regarded as a factor critical to conducting a successful in vitro evaluation of fiber substrates.

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