

Effects of short-chain fructooligosaccharides and galactooligosaccharides, individually and in combination, on nutrient digestibility, fecal fermentative metabolite concentrations, and large bowel microbial ecology of healthy adult cats

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ABSTRACT: Short-chain fructooligosaccharides (scFOS) and galactooligosaccharides (GOS) are nondigestible oligosaccharides that result in a prebiotic effect in some animal species; however, the cat has not been well studied in this regard. This experiment evaluated scFOS and GOS supplementation on nutrient digestibility, fermentative end product production, and fecal microbial ecology of cats. Eight healthy adult cats were fed diets containing no prebiotic, 0.5% scFOS, 0.5% GOS, or 0.5% scFOS + 0.5% GOS (scFOS + GOS) in a replicated 4 × 4 Latin square design. Apparent total tract CP digestibility was decreased ($P < 0.05$) when cats were fed a diet containing scFOS + GOS compared with the other treatments. Dry matter, OM, acid hydrolyzed fat, and GE digestibilities were not different ($P > 0.05$) among treatments. Cats fed scFOS-, GOS-, and scFOS + GOS-supplemented diets had greater ($P < 0.05$) fecal *Bifidobacterium* spp. populations com-

pared with cats fed the control diet. Fecal pH was less ($P < 0.05$) for cats fed the scFOS + GOS-supplemented diet compared with the control. Butyrate ($P = 0.05$) and valerate ($P < 0.05$) concentrations were greater when cats consumed the scFOS + GOS diet. Acetate tended ($P = 0.10$) to be greater when cats were fed the scFOS + GOS diet. Total short-chain fatty acid ($P = 0.06$) and total branched-chain fatty acid ($P = 0.06$) concentrations also tended to be greater when cats consumed the scFOS + GOS treatment. Fecal protein catabolites, including ammonia, 4-methylphenol, indole, and biogenic amines, blood lymphocytes, neutrophils, total white blood cell counts, or fecal DM concentration and output did not differ ($P > 0.05$) among treatments. Low level supplementation of scFOS, GOS, and their combination exert positive effects on select indices of gut health in cats.

Key words: cat, galactooligosaccharide, microbiota, nutrient digestibility, protein catabolite, short-chain fructooligosaccharide

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INTRODUCTION

Prebiotics are nondigestible food ingredients that modify the microbial ecology of the colon and improve indices of host health (Gibson and Roberfroid, 1995). The effect of prebiotic supplementation of cats has not been well studied. *Bifidobacterium* spp. and *Lactobacillus* spp. are desirable bacteria due to their beneficial effects, including inhibition of pathogenic bacteria and improving host immunity in humans. Previous research noted that cats supplemented with 175 mg of lactosucrose/d (Terada et al., 1993) or 0.75 (Sparkes et al., 1998) and 4% oligofructose in the diet (Barry et

al., 2010) had modified colonic microbial populations compared with an oligosaccharide-free control. Moderate-to-high inclusion quantities of fructans were noted to reduce total tract CP digestibility of cats (Hesta et al., 2001). In dogs, low inclusion of short-chain fructooligosaccharides (scFOS) or inulin (0.2 and 0.4% of diet) increased ileal nutrient digestibility with no effect on fecal quality or microbial ecology of the colon, whereas an inulin supplementation tended to increase total tract CP digestibility (Barry et al., 2009).

Cats require greater concentrations of dietary protein in part to meet their unique AA requirements. This leads to an active population of clostridial species throughout the gastrointestinal tract that are the major species using AA as fermentative substrates (Barker, 1981). Consequently, several putrefactive compounds, including ammonia, biogenic amines, branched-chain fatty acids (BCFA), indole, and phenol, may be pro-

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Table 1. Ingredient composition of diets containing select carbohydrates and fed to adult cats (as-fed basis)

Ingredient, %	Treatment			
	Control	scFOS ¹	GOS ²	scFOS + GOS
Poultry by-product, low ash	36.48	36.48	36.48	36.48
Brewer's rice	27.76	27.76	27.76	27.76
Poultry fat	12.00	12.00	12.00	12.00
Yellow corn, ground	10.00	10.00	10.00	10.00
Dried egg	5.00	5.00	5.00	5.00
Solka-Floc ³	4.00	3.50	3.50	3.00
Test carbohydrate ⁴	0.00	0.50	0.50	1.00
Liquid digest	2.00	2.00	2.00	2.00
Sodium bisulfate	1.00	1.00	1.00	1.00
Potassium chloride	0.65	0.65	0.65	0.65
Salt	0.65	0.65	0.65	0.65
Mineral premix ⁵	0.18	0.18	0.18	0.18
Vitamin premix ⁶	0.18	0.18	0.18	0.18
Taurine supplement ⁷	0.10	0.10	0.10	0.10

¹scFOS: short-chain fructooligosaccharides.

²GOS: galactooligosaccharides.

³Solka-Floc: International Fiber Corporation, North Tonawanda, NY.

⁴Test carbohydrate: scFOS, NutraFlora P-95 short-chain fructooligosaccharides, and GOS, Purimune galactooligosaccharides (GTC Nutrition, Golden, CO).

⁵Provided per kilogram of diet: vitamin A, 18 kIU; vitamin D₃, 2.7 kIU; vitamin E, 0.144 kIU; vitamin K, 2.16 mg; thiamin, 30.6 mg; riboflavin, 30.6 mg; pantothenic acid, 50.4 mg; nicotinic acid, 124.2 mg; pyridoxine, 30.6 mg; biotin, 0.108 mg; folic acid, 1.08 mg; and vitamin B₁₂, 115 µg.

⁶Provided per kilogram of diet: manganese (as MnCO₃), 18.0 mg; iron (as C₆H₈O₇.xFe), 135.0 mg; copper [as Cu₂(OH)₂CO₃], 18.0 mg; zinc (as ZnCO₃), 180.0 mg; iodine (as KIO₃), 1.8 mg; selenium (as Na₂SeO₃), 396.0 µg; and cobalt (as CoSO₄), 3.8 µg.

⁷Provided per kilogram of diet: taurine, 2.1 g.

duced. Large quantities of putrefactive compounds may play an important role in causing disease of the large bowel, including colorectal cancer (Johnson, 1977). The objective of this study was to determine the effects of low level prebiotic inclusion [0.5% scFOS, 0.5% galactooligosaccharides (**GOS**), and 0.5% scFOS + 0.5% GOS] on nutrient digestibility, fermentative metabolite concentrations, and large bowel microbial ecology of healthy adult cats.

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.

Animals and Diets

Eight domestic shorthair castrated male cats 2.8 yr of age (4.94 ± 0.30 kg of BW) at the start of the study were utilized. All cats were individually housed in stainless-steel cages in a temperature-controlled room with a 16 h light:8 h dark cycle. Food was provided to maintain BW from the beginning of the study. Food offered and refusals were weighed daily to assess food intake. Water was provided ad libitum throughout the study. Body weight and BCS (Laflamme, 1997) were determined weekly.

Extruded kibble diets were formulated using oligosaccharide-free ingredients, including poultry by-

product meal, brewers rice, and poultry fat (Table 1). The diets were extruded at Kansas State University's Bioprocessing and Industrial Value-Added Program Facility (Manhattan, KS) under the direction of a consulting firm (Pet Food and Ingredient Technology Inc., Topeka, KS). Short-chain fructooligosaccharides (NutraFlora P-95 short-chain fructooligosaccharides, GTC Nutrition, Golden, CO) and GOS (Purimune galactooligosaccharides, GTC Nutrition) were included in the diet before extrusion in exchange for cellulose. Four diets were prepared: 1) control [35% CP, 20% fat, 4% solka floc (International Fiber Corporation, North Tonawanda, NY) as the fiber source]; 2) control + 0.5% scFOS + 3.5% solka floc; 3) control + 0.5% GOS + 3.5% solka floc; and 4) control + 0.5% scFOS + 0.5% GOS + 3% solka floc.

Experimental Design

A replicated 4×4 Latin square with 21-d periods was used. Cats were adapted to the diet for 14 d, followed by a 7-d total fecal collection phase. Total feces were collected, scored, weighed, and frozen at -20°C until further analysis. During the 7-d collection phase, 1 fresh fecal sample was collected within 15 min of defecation for measurement of pH, DM, protein catabolites, and microbiota enumeration. All fecal samples were scored for consistency based on the following 5-point scale: 1 = hard, dry pellets, small hard mass; 2 = hard, formed stool that remains firm; 3 = soft, formed, and

moist stool that retains its shape; 4 = soft, unformed stool that assumes the shape of the container; and 5 = watery, liquid stool that can be poured.

Sample Handling

Fecal samples were dried at 55°C in a forced-air oven. Diets and dried feces were ground through a 2-mm screen in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ). On d 15 of each period, 1 fresh fecal sample was collected during the 7-d collection phase within 15 min of defecation and an aliquot was immediately transferred into sterile cryogenic vials (Nalgene, Rochester, NY), snap-frozen in liquid nitrogen, and frozen at -80°C until DNA extraction. Additional aliquots for phenol, indole, and biogenic amine analyses were frozen at -20°C immediately after collection, and aliquots for ammonia, short-chain fatty acid (SCFA), and BCFA determinations were placed in 5 mL of 2 N HCl and also stored at -20°C. Remaining fecal samples were frozen at -20°C.

Chemical Analyses

Diets and feces were analyzed for DM, OM, and ash (AOAC, 2000). Crude protein was determined according to AOAC (2000; Leco Nitrogen/Protein Determinator, model FP-2000, Leco Corporation, St. Joseph, MI). Fat concentration was determined by acid hydrolysis (AACC, 1983) followed by ether extraction (Budde, 1952). Total dietary fiber was determined according to Prosky et al. (1985, 1992). Gross energy was analyzed by use of an oxygen bomb calorimeter (model 1261, Parr Instrument, Moline, IL). Short-chain fatty acids and BCFA concentrations were determined by gas chromatography (Erwin et al., 1961; Hewlett-Packard 5890A series II gas chromatograph, Palo Alto, CA) and a glass column (180 cm × 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 with a flow rate of 75 mL/min. Oven, detector, and injector temperatures were 125, 175, and 180°C, respectively. Fecal ammonia concentrations were determined using spectrophotometry according to methods of Chaney and Marbach (1962). Phenol and indole concentrations were measured using gas chromatography, and biogenic amine concentrations were measured by HPLC according to the methods described by Flickinger et al. (2003).

Microbial Analyses

Fecal microbial populations were analyzed using the method described by Middelbos et al. (2007b). Briefly, total DNA was extracted from fresh fecal samples that had been stored at -80°C using the bead beater method (Yu and Morrison, 2004) followed by a DNA stool mini kit (QIAamp, Qiagen, Valencia, CA) according to manufacturer's instructions. The quantity and quality

of DNA were determined using a spectrophotometer (NanoDrop ND-1000, NanoDrop Technologies, Wilmington, DE). Quantitative PCR was performed for determination of *Bifidobacterium* spp., *Lactobacillus* spp., *Escherichia coli*, and *Clostridium perfringens*. Specific primers, previously used in our laboratory, were used for *Bifidobacterium* spp. (Matsuki et al., 2002), *Lactobacillus* spp. (Collier et al., 2003), *E. coli* (Malinen et al., 2003), and *C. perfringens* (Wang et al., 1994). Briefly, a 10- μ L final volume contained 5 μ L of mixture (2 × SYBR Green PCR Master Mix, Applied Biosystems, Foster City, CA), 15 pmol of the forward and reverse primers for the bacterium of interest, and 10 ng of extracted fecal DNA. Standard curves were obtained by harvesting pure cultures of the bacterium of interest in the log growth phase in triplicate, followed by serial dilution. Bacterial DNA was extracted from each dilution using a DNA stool mini-kit (QIAamp, Qiagen) and amplified with the fecal DNA to create triplicate standard curves (ABI PRISM 7900HT Sequence Detection System, Applied Biosystems, Foster City, CA). Colony-forming units in each dilution were determined by plating on specific agars; lactobacilli MRS (Difco, Becton, Dickinson and Co., Franklin Lakes, NJ) for lactobacilli, reinforced clostridial medium (*Bifidobacterium* spp., *C. perfringens*), and Luria Bertani medium (*E. coli*). The calculated log cfu per milliliter of each serial dilution was plotted against the cycle threshold to create a linear equation to calculate cfu per gram of dry feces.

Complete Blood Count

A complete blood count (CBC) was determined for each cat on d 21 of each period. Approximately 2 mL of blood was taken from the femoral vein and placed into 2.5-mL EDTA tubes for CBC analysis at the University of Illinois College of Veterinary Medicine Veterinary Diagnostic Lab.

Calculations

Apparent total tract nutrient digestibilities were calculated as nutrient intake (g/d) minus fecal nutrient output (g/d); this value then was divided by nutrient intake (g/d) and multiplied by 100. Metabolizable energy (kcal/g) was calculated on a DM basis using the following equation (AAFCO, 2009): $[(3.33 \times \% \text{ CP}) + (8.93 \times \% \text{ acid hydrolyzed fat}) + (3.33\% \text{ carbohydrate})]/100$, where carbohydrate is equal to $100 - (\% \text{ ash}) - (\% \text{ CP}) - (\% \text{ acid hydrolyzed fat}) - (\% \text{ total dietary fiber})$.

Statistical Analysis

The continuous variable data were analyzed by the MIXED procedure (SAS Inst. Inc., Cary, NC). For the statistical model, the random effects were animal and period, whereas the fixed effect was treatment. Least squares means were separated using LSD with a Tukey

Table 2. Chemical composition of extruded cat diets containing select carbohydrates

Item, % DM basis unless noted	Treatment			
	Control	scFOS ¹	GOS ²	scFOS + GOS
DM, %	95.2	95.0	94.8	95.0
OM	92.8	92.5	92.4	92.7
CP	34.6	35.1	35.3	34.0
Acid hydrolyzed fat	21.2	20.2	20.2	21.1
Total dietary fiber (TDF), uncorrected ³	8.2	7.6	7.8	7.3
TDF, corrected ⁴	8.2	8.1	8.3	8.3
GE, kcal/g	5.4	5.4	5.5	5.4
ME, ⁵ kcal/g (calculated)	4.0	4.0	3.9	4.0

¹scFOS: short-chain fructooligosaccharides.

²GOS: galactooligosaccharides.

³These values were determined using the TDF assay that cannot quantify scFOS and GOS.

⁴These values were determined by adding the amount of scFOS and GOS present in each diet to the TDF (uncorrected) value.

⁵ME was calculated using the following equation: $[(3.33 \times \% \text{ CP}) + (8.93 \times \% \text{ acid hydrolyzed fat}) + (3.33 \times \% \text{ carbohydrate})]/100$, where carbohydrate (% N-free extract) is equal to $100 - (\% \text{ ash}) - (\% \text{ CP}) - (\% \text{ acid hydrolyzed fat}) - (\% \text{ total dietary fiber})$ when all values are expressed on a DM basis (AAFCO, 2009).

adjustment. A single degree of freedom contrast was conducted to test the effect of supplementation treatments (average of 3 prebiotic supplemented diets) compared with the control treatment. Differences among treatment level least squares means with a probability of $P \leq 0.05$ were accepted as statistically significant, whereas mean differences with P -values ranging from 0.06 to 0.10 were considered trends.

RESULTS

Chemical Composition of Diets

The chemical composition of the diets is presented in Table 2. Analyzed DM, OM, and GE concentrations, and calculated ME values, were similar among dietary treatments. Analyzed CP and acid hydrolyzed fat concentrations were close to the desired 30 and 20% values, respectively (as-is basis). The total dietary fiber (TDF) assay cannot quantify scFOS and GOS because these oligosaccharides do not precipitate in 78% ethanol. Therefore, the TDF values for the scFOS-, GOS-, and scFOS + GOS-supplemented diets were less than for the control diet. Corrected TDF values (uncorrected TDF values + amount of oligosaccharide added to each treatment) were similar among diets (Table 2).

Food Intake and Apparent Total Tract Nutrient Digestibility

Food (as-is basis) and DMI, fecal output and DM concentration, and apparent total tract nutrient digestibilities are presented in Table 3. Food intake, DMI, fecal output, and fecal DM concentration were not different among treatments; however, fecal output tended ($P = 0.08$) to be greater for cats fed the scFOS + GOS treatment compared with the GOS treatment. Dry matter, OM, acid hydrolyzed fat, and GE digestibilities were similar among treatments. Crude protein digest-

ibility was decreased ($P < 0.05$) when cats consumed the scFOS + GOS treatment compared with those offered the other treatments.

Fecal Characteristics and Fermentation Metabolites

Fecal pH, score, and concentrations of ammonia, 4-methylphenol, and indole are presented in Table 4. Fecal score was not different among treatments. Fecal pH was decreased ($P < 0.05$) when cats were fed the scFOS + GOS treatment compared with the control and GOS treatments. Fecal pH was decreased ($P = 0.03$) when scFOS + GOS ($P = 0.02$) were supplemented compared with the control. Fecal concentrations of ammonia, 4-methylphenol, and indole were not different among treatments.

Fecal SCFA and BCFA concentrations are presented in Table 5. Fecal concentrations of acetate were greater when cats consumed the scFOS + GOS treatment compared with GOS ($P < 0.05$) and tended to be greater ($P = 0.09$) for the scFOS + GOS treatment compared with the control. Fecal concentrations of butyrate were tended to be greater ($P = 0.09$) for cats consuming the scFOS + GOS treatment compared with the control treatment. Fecal concentrations of total SCFA tended ($P = 0.06$) to be greater when cats consumed the scFOS + GOS treatment compared with control and GOS treatments. Fecal concentrations of valerate tended to be greater ($P = 0.07$) for cats fed the scFOS + GOS treatment compared with the control. Fecal concentrations of total BCFA tended ($P = 0.06$) to be greater when cats were fed the scFOS + GOS treatment compared with the control. Fecal propionate, isobutyrate, and isovalerate concentrations were not different among treatments.

Fecal biogenic amine concentrations are presented in Table 6. Most fecal biogenic amine concentrations were not different among treatments; however, fecal tryptam-

Table 3. Food intake, DMI, fecal output, fecal DM concentration, and nutrient digestibilities for cats supplemented with short-chain fructooligosaccharides (scFOS), galactooligosaccharides (GOS), or both

Item	Treatment					P-value	
	Control	scFOS	GOS	scFOS + GOS	SEM	Main effect	Control vs. supplementation
Food intake, g/d (as-is basis)	58.7	58.3	57.6	59.3	0.6	0.16	0.63
DMI, g/d	55.9	55.4	54.7	56.3	0.6	0.11	0.49
Fecal output, g (as-is basis)	173.1 ^{cd}	168.0 ^{cd}	158.9 ^d	183.1 ^c	7.9	0.08	0.67
Fecal DM, %	43.8	44.4	43.6	41.1	1.4	0.41	0.66
Apparent total tract digestibility, %							
DM	81.4	81.7	82.7	81.2	0.6	0.25	0.43
OM	84.4	84.8	85.6	84.4	0.5	0.30	0.36
CP	84.2 ^a	84.0 ^a	84.7 ^a	81.9 ^b	0.5	0.01	0.28
Acid hydrolyzed fat	95.7	95.5	95.9	95.5	0.2	0.32	0.78
GE	85.9	86.2	87.1	85.8	0.4	0.14	0.34

^{a,b}Values lacking a common superscript letter within each row are different ($P \leq 0.05$).

^{c,d}Values lacking a common superscript letter within each row are different ($P \leq 0.10$).

ine concentrations were greater ($P = 0.02$) when cats consumed the scFOS + GOS treatment compared with the GOS and scFOS treatments alone.

Fecal Microbiota

Fecal concentrations of select microbiota are presented in Table 7. Fecal *Bifidobacterium* spp. concentrations were greatest ($P < 0.05$) in the scFOS + GOS treatment. Fecal concentrations of *Bifidobacterium* spp. were greater ($P < 0.05$) when cats were fed the scFOS, GOS, or scFOS + OS treatments compared with the control. Fecal concentrations of *Lactobacillus* spp., *E. coli*, and *Clostridium perfringens* were not different among treatments.

CBC

White blood cell counts are presented in Table 8. There were no differences in neutrophils or total white blood cells concentrations among treatments, whereas blood lymphocyte concentrations (expressed as a per-

centage) tended ($P = 0.10$) to be less for cats fed the scFOS treatment compared with those fed the control.

DISCUSSION

A control diet was formulated using ingredients known to be free of endogenous oligosaccharides that were of high nutritive value. Solka floc (cellulose) was used as the control fiber because it is essentially inert and not subject to microbial fermentation. Test oligosaccharides (scFOS and GOS) were added to the diet at 0.5% at the expense of the cellulose. A mixture of the 2 oligosaccharides each at 0.5%, resulting in an experimental treatment containing 1% oligosaccharide, also was tested. This was done to test potential synergies between the 2 oligosaccharides at the same concentrations as were fed individually, and to evaluate whether a greater dietary concentration might exert a stronger response on the outcome variables measured.

Dietary composition was similar across diets except for TDF. As expected, the differences in TDF were ob-

Table 4. Fecal pH, score, and concentrations of ammonia, 4-methylphenol, and indole for cats supplemented with short-chain fructooligosaccharides (scFOS), galactooligosaccharides (GOS), or both

Item	Treatment					P-value	
	Control	scFOS	GOS	scFOS + GOS	SEM	Main effect	Control vs. supplementation
Fecal Score ¹	2.7	2.7	2.7	2.8	0.1	0.81	0.75
pH	6.7 ^a	6.4 ^{ab}	6.6 ^a	6.0 ^b	0.2	0.03	0.05
Ammonia, $\mu\text{mol/g}$ of DM	110.0	125.7	100.6	129.3	11.9	0.16	0.46
4-Methylphenol, $\mu\text{mol/g}$ of DM	2.2	2.3	2.4	2.4	0.4	0.93	0.56
Indole, $\mu\text{mol/g}$ of DM	1.9	1.7	1.8	1.9	0.2	0.85	0.59

^{a,b}Values lacking a common superscript letter within each row are different ($P \leq 0.05$).

¹All fecal samples were scored for consistency based on the following scale: 1 = hard, dry pellets, small hard mass; 2 = hard, formed stool that remains firm; 3 = soft, formed, and moist stool that retains its shape; 4 = soft, unformed stool that assumes the shape of the container; and 5 = watery, liquid stool that can be poured.

Table 5. Fecal short-chain fatty acid (SCFA) and branched-chain fatty acid (BCFA) concentrations for cats supplemented with short-chain fructooligosaccharides (scFOS), galactooligosaccharides (GOS), or both

Item, $\mu\text{mol/g}$ of DM	Treatment					P-value	
	Control	scFOS	GOS	scFOS + GOS	SEM	Main effect	Control vs. supplementation
Acetate	166.5 ^{ab,c}	173.8 ^{ab,cd}	159.8 ^{b,cd}	219.4 ^{a,d}	15.3	0.05	0.32
Propionate	51.7	53.1	53.1	68.7	5.9	0.17	0.34
Butyrate	28.5 ^c	31.4 ^{cd}	35.4 ^{cd}	42.4 ^d	3.8	0.09	0.02
Total SCFA	246.7 ^d	258.4 ^{cd}	248.3 ^d	330.6 ^c	23.2	0.06	0.24
Isobutyrate	5.6	5.9	5.6	7.1	0.7	0.15	0.32
Isovalerate	9.0	9.4	9.3	10.9	1.2	0.41	0.39
Valerate	14.4 ^c	15.1 ^{cd}	18.2 ^{cd}	20.5 ^d	1.7	0.07	0.09
Total BCFA	31.3 ^d	32.8 ^{cd}	32.4 ^{cd}	40.9 ^c	3.6	0.06	0.19

^{a,b}Values lacking a common superscript letter within each row are different ($P \leq 0.05$).

^{c,d}Values lacking a common superscript letter within each row tended to be different ($P \leq 0.10$).

served because the scFOS and GOS (alone and in combination) did not precipitate in 78% ethanol, so they were unable to be quantified accurately using the TDF method (Prosky et al., 1985). However, when TDF values were corrected for the supplemental test prebiotics, values were similar among treatments.

Dry matter, OM, acid hydrolyzed fat, and GE apparent total tract digestibility values were not different among treatments. However, CP digestibility decreased with 1% scFOS + GOS supplementation, which was likely due to the production of greater bacterial biomass in the large bowel. This occurred only at 1% supplementation, indicating an effect of greater oligosaccharide concentration and not of individual oligosaccharides supplemented at lesser dietary concentrations. This hypothesis was supported by the increased fecal output data noted for the combination treatment.

Hesta et al. (2001) reported that cats fed inulin- (3 and 6%) or 3% oligofructose-supplemented diets had less total tract CP digestibility values. However, after correcting the value for fecal bacterial N content, CP digestibility was not different among treatments. Barry et al. (2009) reported that total tract CP digestibility tended to be linearly increased for dogs supplemented with 0.2 or 0.4% inulin; however, values were not affected by 0.2 or 0.4% scFOS supplementation.

As expected, fecal DM content and score were not affected by dietary treatments. Barry et al. (2010) found that fecal DM was not affected in cats fed a 4% oligofructose-supplemented diet. In dogs, Barry et al. (2009) reported that low inclusion (0.2 and 0.4%) of scFOS or inulin did not affect fecal quality. In the present experiment, fecal output tended to be greater in cats fed the scFOS + GOS-supplemented diet compared with the GOS diet. Hesta et al. (2005) noted that cats supplemented with 3.11% oligofructose tended to have a greater fecal moisture and output compared with the control.

Fecal pH was decreased when cats were fed the scFOS + GOS treatment compared with the control and GOS treatments. Decreased fecal pH likely resulted from lactic acid and SCFA production from carbohydrate fermentation. Hesta et al. (2001) reported that cats fed a 6 or 9% oligofructose-supplemented diet had decreased fecal pH compared with the control and 3% oligofructose treatments.

Fecal ammonia concentrations were not different among treatments. Similar results were observed by Flickinger et al. (2003) when dogs were supplemented with scFOS at 0.5, 1.5, or 3% dietary concentrations, and by Barry et al. (2009) when dogs were supplemented with inulin or scFOS (0.2 and 0.4%).

Table 6. Fecal biogenic amine concentrations for cats supplemented with short-chain fructooligosaccharides (scFOS), galactooligosaccharides (GOS), or both

Item, $\mu\text{mol/g}$ of DM	Treatment					P-value	
	Control	scFOS	GOS	scFOS + GOS	SEM	Main effect	Control vs. supplementation
Cadaverine	242.5	231.4	290.0	351.9	81.0	0.56	0.55
Putrescine	45.4	36.4	48.4	60.4	12.0	0.55	0.84
Histamine	11.2	10.7	13.3	15.7	3.4	0.42	0.49
Tryptamine	27.3 ^{ab}	24.1 ^a	19.6 ^a	57.6 ^b	8.6	0.02	0.53
Tyramine	49.2	32.9	50.4	28.0	7.8	0.13	0.21
Spermidine	16.1	19.3	18.9	19.4	2.8	0.83	0.37
Spermine	3.6	4.2	3.3	3.5	1.6	0.94	0.99

^{a,b}Values lacking a common superscript letter within each row are different ($P \leq 0.05$).

Table 7. Fecal microbial populations for cats supplemented with short-chain fructooligosaccharides (scFOS), galactooligosaccharides (GOS), or both

Item, cfu log ₁₀ /g of fecal DM	Treatment				SEM	P-value	
	Control	scFOS	GOS	scFOS + GOS		Main effect	Control vs. supplementation
<i>Bifidobacterium</i> spp.	9.4 ^c	9.9 ^b	10.1 ^{ab}	10.3 ^a	0.20	0.001	0.0004
<i>Lactobacillus</i> spp.	10.7	10.7	10.8	10.9	0.14	0.27	0.19
<i>Escherichia coli</i>	10.3	10.4	10.3	10.4	0.14	0.84	0.49
<i>Clostridium perfringens</i>	9.6	9.6	9.7	9.7	0.19	0.73	0.51

^{a-c}Values lacking a common superscript letter within each row are different ($P \leq 0.05$).

Phenolic and indolic compounds are produced from aromatic AA (phenylalanine, tyrosine, and tryptophan) fermentation (Blachier et al., 2007). In the present study, only 4-methylphenol and indole were present in all fecal samples. The dietary treatments did not affect fecal concentration of phenol or indole. Flickinger et al. (2003) reported that phenol and indole were not affected by 1, 2, or 3 g/d of scFOS supplementation in dogs. In contrast, Terada et al. (1993) noted that fecal ethylphenol and indole concentrations were decreased in cats supplemented a 175 mg of lactosucrose/d. Potentially pathogenic bacteria, including *Clostridium perfringens* and *E. coli*, are responsible for the production of putrefactive compounds. In the present study, fecal populations of *C. perfringens* and *E. coli* were not affected by oligosaccharide inclusion in diets. This might explain, in part, why fecal concentrations of phenol and indole were unaffected by dietary oligosaccharide inclusion.

Fecal acetate concentrations were greater when cats were fed the scFOS + GOS treatment. Propst et al. (2003) reported that fecal acetate concentrations were increased when dogs consumed an oligofructose-supplemented diet (0.3, 0.6, and 0.9%, DM basis). Fecal lactate and acetate production have been shown to decrease pH, leading to development of an unfavorable environment for pathogenic bacteria (i.e., *C. perfringens* and *E. coli*; Macfarlane and Cummings, 1991). Fecal butyrate concentrations tended to be greater when cats consumed the scFOS + GOS treatment compared with the control. Similarly, 4% oligofructose supplementation increased fecal butyrate concentrations in

adult cats (Barry et al., 2010). Additionally, Hesta et al. (2001) noted that fecal total SCFA concentrations were greater in cats fed a 6% inulin-supplemented diet compared with control cats. Butyrate serves as an energy source for colonocytes, and greater butyrate concentrations are thought to have an important role in gut health and colonocyte proliferation (Blottière et al., 2003). In the current experiment, scFOS or GOS alone did not affect fecal acetate, propionate, butyrate, or total SCFA concentrations. This lack of difference noted after treatment with GOS or scFOS alone seems to be a function of the dietary concentration provided. However, fecal butyrate concentrations tended to be greater when cats were fed the scFOS or GOS treatment compared with the control. It seems from the data of the present study that if butyrate production is to be enhanced, a concentration between 0.5 and 1% is necessary to elicit this response.

When carbohydrate as a substrate for the microbiota of the large intestine is limiting, BCFA are produced. Short-chain fructooligosaccharides and GOS are rapidly fermented in the proximal colon, and then peptides and AA are fermented by bacteria in the transverse and distal colon to provide energy. End products of AA fermentation are BCFA, phenol, indole, and biogenic amines. Branched-chain fatty acids are generated from branched-chain AA (valine, leucine, and isoleucine) fermentation (Macfarlane et al., 1992). Similar to results of the present study, 4% oligosaccharide supplementation led to increases in fecal BCFA, and the authors stated that these increases might have resulted from

Table 8. White blood cell counts for cats supplemented with short-chain fructooligosaccharides (scFOS), galactooligosaccharides (GOS), or both

Item	Treatment				SEM	P-value	
	Control	scFOS	GOS	scFOS + GOS		Main effect	Control vs. supplementation
Total white blood cells, 10 ³ /μL	8.2	8.3	9.1	8.7	0.5	0.58	0.41
Neutrophils, %	52.0	58.5	59.0	56.4	2.7	0.21	0.05
Neutrophils, 10 ³ /μL	4.3	4.9	5.6	4.9	0.5	0.41	0.20
Lymphocytes, %	39.9 ^{ab}	33.3 ^b	33.9 ^{ab}	34.9 ^{ab}	2.8	0.10	0.02
Lymphocytes, 10 ³ /μL	3.1	2.7	2.9	3.0	0.2	0.37	0.21

^{a,b}Values lacking a common superscript letter within each row tended to be different ($P \leq 0.10$).

the rapid fermentation of the oligosaccharides in the proximal colon; then, proteolytic bacteria in the distal colon would continue fermenting AA as energy sources instead of carbohydrates (Barry et al., 2010).

Polyamines, including putrescine, spermidine, and spermine, are beneficial protein catabolites required for normal development and repair of intestinal mucosal cells (Wang and Johnson, 1990). Löser et al. (1999) noted that rats fed polyamine-deficient diets long-term had significant hypoplasia of small intestinal and colonic mucosa. Therefore, a decrease in fecal polyamine concentrations may be not desirable. Only tryptamine was affected in the present study, which was unexpected, but may be a consequence of decreased CP digestibility with the scFOS + GOS treatment.

As expected, scFOS- and GOS-supplemented diets affected fecal microbial *Bifidobacterium* spp. concentrations. One important criterion to prove oligosaccharide efficacy as a prebiotic is selective fermentation by intestinal microbiota that increases beneficial bacterial concentrations, including *Bifidobacterium* spp. and *Lactobacillus* spp. (Roberfroid, 2007). Whereas bifidobacteria populations were increased, fecal *Lactobacillus* spp., *E. coli*, and *Clostridium perfringens* were not affected by dietary treatment. Terada et al. (1993) reported that adult cats supplemented with 175 mg of lactosucrose/d had greater fecal *Bifidobacterium* spp. and *Lactobacillus* spp. and less fecal *Clostridium* spp. Supplementation with 4% oligofructose also increased fecal *Bifidobacterium* spp. in adult cats when compared with cellulose (Barry et al., 2010). Additionally, dogs fed 4 g of FOS + 2 g of MOS (Swanson et al., 2002b) or 1.5% scFOS (Middelbos et al., 2007a) had greater fecal concentrations of *Bifidobacterium* spp. and *Lactobacillus* spp. compared with unsupplemented controls. However, low inclusion of scFOS (0.2 and 0.4%) or inulin (0.2 and 0.4%) did not change canine gut microbial populations (Barry et al., 2009).

White blood cell, neutrophil, and lymphocyte concentrations were generally unaffected by dietary treatment. All blood values remained within the normal physiological range for cats. Previous studies with scFOS, yeast cell wall, or inulin reported inconsistent effects on immunological indices among studies, depending on type and concentration of prebiotic (Swanson et al., 2002a,b; Middelbos et al., 2007a; Barry et al., 2009). Greater concentrations of oligosaccharide supplementation might be necessary to alter immunological indices (Middelbos et al., 2007a; Barry et al., 2009).

Data from this experiment showed potentially positive outcomes of scFOS + GOS on health-related characteristics of cats when fed alone or in combination. The effects for the scFOS + GOS treatment likely resulted from the greater concentration provided rather than from any synergy that might exist between them. These oligosaccharides may serve as valuable nutritional interventions to improve digestive health of cats, but it is apparent that concentrations >0.5% should be used to elicit positive responses.

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