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Fructan supplementation of senior cats affects stool metabolite concentrations and fecal microbiota concentrations, but not nitrogen partitioning in excreta

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ABSTRACT: Fructan supplementation of a commercially available canned cat food was evaluated using senior (≥ 9 yr) cats to assess nitrogen (N) partitioning in excreta and stool metabolite and microbiota concentrations. Oligofructose (OF) or SynergyC (OF+IN) were added to the diet individually at 1% (dry weight basis). Cats were acclimated to the control diet for 7 d and then were randomly assigned to 1 of 3 treatment groups for 21 d ($n = 6$). Feces and urine were collected on d 22 through 28. No differences were observed in food intake; fecal output, DM percentage, score, pH, or short- or branched-chain fatty acids, fecal and urinary ammonia output, urinary felinine concentrations, or N retention. Supplemental OF+IN tended to decrease N digestibility ($P = 0.102$) and *Bifidobacteria* spp. ($P = 0.073$) and decrease fecal indole ($P < 0.05$), tyramine ($P < 0.05$), and *Escherichia coli* ($P < 0.05$) concentrations. Both fructan-supplemented treatments decreased ($P < 0.05$) fecal histamine concentrations. The tendency to a lower apparent N digestibility was likely due to increased colonic microbial protein synthesis of fructan-supplemented cats. Fructan supplementation may benefit senior cats as it modulates stool odor-forming compounds and decreases some protein catabolites and pathogenic gut microbiota concentrations without affecting N retention.

Key words: cat, fermentation endproducts, inulin, nutrient digestibility, oligofructose, protein catabolites

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INTRODUCTION

Prebiotic research in the cat is sparse when compared to that reported in other nonruminant species. As the cat is a true carnivore, it has been somewhat overlooked as a candidate for prebiotic supplementation. However, the cat is a prime target for prebiotic research given that several disease-related, odiferous protein catabolites are known to be derived from fermentation of amino acids, and since the cat has a high protein requirement, it is likely that these compounds are generated in high concentrations. As such, senior cats would be an ideal population in which to study these effects as these animals may experience reduced crude protein digestibility (Perez-Camargo, 2004), which may contribute to further N loss to the large intestine and may impact the bacterial population that resides there.

The odors associated with indoor waste elimination concern most cat owners. Short-chain fatty acids (**SCFA**) and branched-chain fatty acids (**BCFA**) are associated with fermentation of carbohydrates and protein in the large intestine, respectively (Macfarlane and Cummings, 1991), and can cause unpleasant odors. Of the two, SCFA benefit intestinal health by providing energy to the colonocytes (Macfarlane and Cummings, 1991). Other sources of fecal odor include phenols, indoles, and biogenic amines, all of which result from amino acid fermentation in the large intestine (Macfarlane and Cummings, 1991). These compounds are not well studied in the cat; however, they appear to be affected by fructan supplementation of dogs (Propst et al., 2003; Swanson et al., 2002a,b). The objective of this study was to determine the effects of supplementing senior cats with two prebiotic fructans on N partitioning, nutrient digestibility, fecal odor components, and fecal microbiota concentrations.

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MATERIALS AND METHODS

Eighteen senior cats (≥ 9 yr old) were randomized into 3 treatment groups, equalized for age (average initial age = 11.4 ± 1.6 yr), gender (3 castrated male and 3 intact females per group), and body weight (BW = 5.11 ± 1.46 kg). Cats were housed individually in stainless steel metabolism crates (0.61 m × 0.61 m × 0.61 m) with plastic flooring. Cats were provided a modified litter box to assist in the collection of feces and urine. Cats were housed in an environmentally controlled room with free access to water. All animal care procedures were approved by the Institutional Animal Care and Use Committee, University of Illinois at Urbana-Champaign.

Diets

Three dietary treatments were utilized in this study: a control treatment that consisted of a commercially available canned cat diet (Whiskas chicken and tuna dinner; Mars Petcare, Franklin, TN) with no supplemental fructan (**Control**), a treatment that consisted of the control diet with 1% oligofructose with an average DP = 4 and a DP range from 2 to 7 (Orafti-P95; BENEO-Group, Tienen, Belgium) added on a dry weight basis (**OF**) and a treatment that consisted of the control diet with 1% of an experimental chicory fructan composed of chains with a DP of 2 to 10 (60%) and of DP > 10 (28%; SynergyC; BENEO-Group, Tienen, Belgium) added on a dry weight basis (oligofructose + inulin, **OF+IN**). SynergyC was designed to distribute prebiotics throughout the colon to selectively stimulate intestinal fermentation in both the proximal (DP = 2 to 10 fraction) and more distal parts (DP > 10 fraction) of the intestine. The ingredients of the commercial diet as they appear on the label are listed in Table 1. This diet was chosen as it contained low concentrations of intrinsic fiber and would allow for quantitative delivery of fructans. The fructan treatments were incorporated into the diet by batch blending (approximately 3 min) in a Hobart industrial mixer (model A-200 DT; Hobart Corp., Troy, OH). To maintain diet consistency, the control diet was blended in the same fashion as the supplemented diets.

Experimental Design

A completely randomized design was used. All cats were fed the control diet for a baseline period of 7 d. Due to the high variation in starting weights of senior cats, they were fed to meet their individual metabolic energy requirements (**MER**; based on the equation of Radicke, 1995; NRC, 2006). Cats then were assigned randomly to 1 of the 3 dietary treatments for the remainder of the study. Cats were fed once daily to meet 100% of their MER on d 8 to19, followed by 90% of their MER on d 20 to 28 to allow for assessment of N balance and fecal metabolite concentrations as affected by treatment. Food refusals were weighed back daily. Body weight was recorded on d 1, then weekly until the end of the study.

Table 1. Ingredients as listed in order on the commercial canned cat food used in the experiment

Ingredients
Meat byproducts
Sufficient water for processing
Poultry by-products
Chicken
Fish (source of taurine)
Tuna
Guar gum
Sodium tripolyphosphate
Potassium chloride
Titanium dioxide
DL-methionine
Carrageenan
Choline chloride
Vitamin E and D_3 supplements
Zinc sulfate
Thiamine mononitrate (Vitamin B_1)
Ferrous sulfate
Sodium nitrate (for colour retention)
Manganese sulfate
Yellow #6
Folic acid
Pyridoxine hydrochloride (Vitamin B_6)
Yellow #5
Menadione sodium bisulfite complex (source of Vitamin K activity)

Urine and Fecal Collection

Litter boxes were constructed by drilling approximately 400 holes 2 mm in diameter in the bottom of one litter box, sufficient to allow urine to freely flow through the bottom of the litter pan while supporting the weight of the cat using the box. To this pan, 6 tapped spacers 2.5 cm in length (4 size #6, 2 size #8, and 2 size #10) were attached under the rim of the litter pan with corresponding sizes of stainless steel screws. This modified litter box was placed inside an unmodified litter pan to collect total urine excreted. Approximately 1.36 kg of 6-mm glass beads (Fischer Scientific, Inc., Pittsburgh, PA) were added to the modified litter box to allow for separation of feces from urine and to allow the cat to perform its natural behavior of covering its feces. Cats utilized the experimental litter box throughout the entire experiment to not change the environment of the cat once the experiment had begun.

Urine was collected on d 22 through 28 for analysis of urinary N and ammonia concentrations. Urine was acidified by placing 10 mL of 2N HCl in the unmodified litter

pan and was collected twice daily. All urine was pooled for each cat by collection period, sealed in clean 1-L Nalgene bottles, and stored at −4°C for subsequent analyses.

Total feces excreted were collected on the aforementioned days of urine collection for determination of apparent nutrient digestibility. On d 26 through 28, fresh voided feces were collected into sterile sampling bags (Whirl-Pac; Pioneer Container Corp., Cedarburg, WI) within 15 min of defecation and processed immediately to minimize any loss of volatile components. Fecal samples were weighed before measuring pH, using an AP10 pH meter from Denver Instrument (Fischer Scientific, Inc., Pittsburgh, PA) with a FUTURA refillable combination AgCl electrode with a rugged (semi-flat) bulb (Beckman Instruments, Inc., Fullerton, CA). Fecal consistency was scored using a scale of 1 to 5, with 1 being a hard, dry pellet; 2 being hard, formed, dry stool that remains firm and soft; 3 being soft, formed, moist stool that retains its shape; 4 being soft, unformed, pudding-like stool that assumes the shape of a container; and 5 being watery, liquid stool that can be poured. Fecal aliquots were sealed in clean centrifuge tubes and stored at -20°C for subsequent analyses of amines, phenols, and indoles. A fecal aliquot was acidified with 5 mL of 2N HCl, then sealed in a clean 60-mL Nalgene bottle (Nalgene Nunc Int'l Corp., Rochester, NY) for subsequent analyses of SCFA, BCFA, and ammonia concentrations. Also, a fecal sample was sealed in a sterile cryovial, snap frozen in liquid N, and stored at −80°C for subsequent fecal microbial analysis. Remaining feces excreted during the collection period were collected from the litter boxes, weighed, composited, and frozen at -20°C for subsequent analyses. Fecal samples were composited by cat and period, then dried at 55°C in a forced-air oven. After drying, fecal samples were ground through a 2-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ). Excess hair was removed from ground fecal samples using a series of sieves until as many hair fragments as possible were removed.

Chemical Analyses

Diet and fecal samples were analyzed for dry matter (**DM**), organic matter (**OM**; AOAC, 2006), Leco N (AOAC, 2006), acid hydrolyzed fat (**AHF**; Budde, 1952; AACC, 1983), and gross energy (**GE**; Parr Instrument Co., Moline, IL; Parr Instrument Manuals). Diet samples also were analyzed for total dietary fiber (**TDF**) content (Prosky et al., 1992) and free monosaccharides and oligosaccharides (Smiricky et al., 2002). Urine samples were analyzed for felinine concentrations at the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratory using a felinine standard (L-felinine; US Biologicals, Swampscott, MA) in a physiological amino acid method (Deyl et al., 1986; Fekkes, 1996; Le Boucher et al., 1997). All procedures were performed in duplicate. To maintain quality control during chemical analyses, the error between duplicate samples was determined, and if it exceeded 5%, the assay was repeated. Fresh feces were analyzed for DM, OM (AOAC, 2006), Leco N (AOAC, 2006), pH, SCFA and BCFA (Erwin et al., 1961), ammonia (Chaney and Marbach, 1962), phenols, indoles, and biogenic amines (Flickinger et al., 2003). Urine was analyzed for Leco N and ammonia concentrations using the same methods as described above.

Microbial Analyses

Microbial populations were analyzed using methods described by Middelbos et al. (2007b) with minor adaptations. Briefly, fecal DNA was extracted from freshly collected samples that had been stored at −80°C until analysis, using the repeated bead beater method described by Yu and Morrison (2004) followed by a QIAamp DNA stool mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitative PCR was performed for *Bifidobacterium* spp., *Lactobacillus* spp., *E. coli*, and *C. perfringens*. Specific primers were used for bifidobacteria (Matsuki et al., 2002), lactobacilli (Collier et al., 2003), *E. coli* (Malinen et al., 2003), and *C. perfringens* (Wang et al., 1994). Amplification was performed according to DePlancke et al. (2002). Briefly, a 10-μL final volume contained 5 μL of 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 QIAamp DNA stool mini-kit and amplified with the fecal DNA to create triplicate standard curves using an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). Colony forming units (**cfu**) in each dilution were determined by plating on specific agars: lactobacilli MRS (Difco) for lactobacilli, reinforced clostridial medium (bifidobacteria, *C. perfringens*), and Luria Bertani medium (*E. coli*). The calculated log cfu/mL of each serial dilution was plotted against the cycle threshold to create a linear equation to calculate cfu/g dry feces.

Statistical Analyses

Data were analyzed using a completely randomized design. Data for fecal DM, fecal OM, pH, ammonia, phenols, indoles, biogenic amines, SCFA, and BCFA were analyzed using Mixed model procedures of SAS (SAS Inst. Inc., Cary, NC). Outlier data were removed

Table 2. Nutrient analyses of commercial canned cat food used in the experiment.

Item	Amount
Dry Matter, %	22.6
	—% DM basis—
Organic matter	88.8
Crude protein (N × 6.25)	41.3
Acid hydrolyzed fat	40.7
Total dietary fiber	3.78
Insoluble	1.74
Soluble	2.04
	— kcal/g—
Gross energy	6.4
Metabolizable energy, calculated[a]	5.0

[a]Metabolizable energy was calculated using the following equation:

$$\left[\begin{array}{l}(14.65\times\%\,crude\ protein)+\\(35.58\times\%\,acid\ hydrolyzed\ fat)+\\(14.65\times\%\,carbohydrate)\end{array}\right]/100$$

where % carbohydrate is equal to
[100– (% ash)– (% crude protein)–
(% acid hydrolyzed fat)– (% total dietary fiber)]
when all values are expressed on a DM basis (AAFCO, 2010).

Table 3. Carbohydrate composition of commercial cat food alone and with supplemental fructans

	Treatment[1]		
Item	Control	OF	OF+IN
Free sugars		mg/g DM	
Sucrose	0.0	0.0	4.1
Fructose	0.2	0.9	1.3
Total	0.2	0.9	5.4
Free oligosaccharides		mg/g DM	
Kestose	0.0	0.7	0.0
Nystose	0.0	1.3	0.1
Fructofuranosylnystose	0.2	3.5	6.2
Inulin	0.0	8.4	13.0
Total	0.2	13.9	19.2

[1]Control = no supplemental fructan; OF = 1% oligofructose; OF+IN = 1% chicory pulp, oligofructose + inulin.

from analysis after analyzing data through the Univariate procedure of SAS to produce a normal probability plot based on residual data and visual inspection of the raw data. Outlier data were defined as data points 3 or more standard deviations from the mean value. This accounted for 1 cat on each of the 3 treatments, and these cats were completely removed from the data set. Stool consistency data were analyzed as categorical data by the GLIMMIX procedure of SAS. The model contained the fixed effect of diet and the random effect of cat. Treatment differences for all statistical analyses were assigned using the least significant difference calculated from standard errors using the Mixed models procedure. Significant differences were analyzed at $P < 0.05$, and tendencies were mentioned if $P \leq 0.10$.

RESULTS

The basal diet contained 5 sources of protein, including meat byproducts, poultry byproducts, chicken, fish, and tuna. These ingredients also provided dietary fat. No grains or grain byproducts were present in the diet. The diets contained 2 sources of fermentable substrate, namely, guar gum and carrageenan, and were supplemented with appropriate concentrations of vitamins and minerals.

Nutrient composition for the basal diet is presented in Table 2. All diets contained over 40% crude protein and fat when expressed on a DM basis, as well as approximately 11% ash. Gross energy content was high (6.4 kcal/g). Diets OF and OF+IN contained supplemental fructan at 1% (DM basis). Fiber analyses indicated that the basal diet contained 3.78% TDF on a DM basis. As fructans are not quantified by the TDF method, the amount of fiber provided in the treatment diets was 4.78% (1.74% insoluble dietary fiber; 3.04% soluble dietary fiber).

Free monosaccharide and oligosaccharide concentrations of the diet are presented in Table 3. The control diet contained a small amount of free fructose and minimal oligosaccharides, while diets OF and OF+IN contained slightly more free monosaccharides. The OF diet contained 2.3% inulin-type fructans and OF+IN contained 2.9%. Due to the composition of the chicory fructans, OF contains more short-chain oligosaccharides than OF+IN, as is reflected in the analyses.

No statistically significant differences were noted among treatments in DM intake (mean = 30.0 g/d), OM intake (mean = 26.5 g/d of DM), metabolizable energy intake (mean = 151.0 kcal/d of DM), fecal output on a wet or dry basis (mean = 19.3 and 7.5 g/d, respectively), fecal pH (mean = 6.94), fecal score (mean = 2.5 out of 5), DM digestibility (mean = 75.5%), or OM digestibility (mean = 82.7%; data not shown).

Nitrogen digestibility tended to be lower ($P = 0.102$) for cats fed the OF+IN treatment (Table 4). No statistical differences were noted among treatments in N intake; total N excreted; N retained; fecal N or ammonia excreted; or urinary N, felinine, or ammonia excreted during the treatment period.

Indole concentrations decreased ($P < 0.05$) in cats consuming the OF+IN treatment (Table 5). Fecal concentrations of histamine decreased ($P < 0.05$) in fructan-supplemented cats, and concentration of tyramine decreased ($P < 0.05$) in the OF+IN treatment in contrast with the control. Fecal concentrations of acetate,

Table 4. Nitrogen balance and ammonia and feline concentrations for cats fed supplemental fructans

Item	Treatment[1]			SEM[2]	*P* value
	Control	OF	OF+IN		
Nitrogen intake, g/d DM basis	2.2	1.8	2.0	0.2	0.556
Fecal N excreted, g/d DM basis	0.3	0.4	0.4	0.1	0.951
Urinary N excreted, g/d/DM basis	1.4	1.1	0.9	0.2	0.263
Total N excreted, g/d DM basis	1.7	1.4	1.2	0.3	0.514
Nitrogen digestibility, %	85.4[d]	80.2[cd]	77.0[c]	2.5	0.102
N retained					
g/d	0.5	0.4	0.5	0.1	0.898
% of N intake	27.7	28.0	30.0	6.6	0.961
Fecal ammonia excreted, mg/g feces	5.7	4.3	4.1	1.1	0.574
Urinary ammonia excreted, mg/d	57.9	54.6	50.4	12.3	0.678
Urinary felinine excreted, mg/d	14.2	16.6	12.4	4.6	0.789

[c,d]Superscripts in the same row denote a trend ($P \leq 0.10$) among treatments.

[1]Control = no supplemental fructan; OF = 1% oligofructose; OF+ IN = 1% chicory pulp, oligofructose + inulin.

[2]Standard error of the mean.

Table 5. Fecal concentrations of phenols, indoles, and biogenic amines produced by cats fed supplemental fructans.

Item	Treatment[1]			SEM[2]	*P* value
	Control	OF	OF+IN		
	——— umol/g fecal DM———				
Short- and branched chain fatty acids					
Acetate	126.6	89.7	101.3	35.6	0.759
Propionate	26.1	29.7	40.4	8.3	0.462
Butyrate	55.4	52.4	62.0	9.1	0.771
Isobutyrate	13.0	9.0	10.3	1.6	0.244
Isovalerate	23.8	19.6	17.6	4.4	0.545
Valerate	17.6	21.4	14.2	5.2	0.631
Phenols/Indoles					
Phenol	0.4	0.8	0.9	0.4	0.621
4-methyl phenol	2.5	3.1	1.8	0.5	0.183
Indole	6.2[b]	5.9[b]	3.4[b]	0.5	0.004
3-methyl indole	0.4	0.2	0.4	0.2	0.856
Biogenic amines					
Cadaverine	3.6	3.6	3.5	1.3	1.003
Histamine	2.8[b]	0.6[a]	0.1[a]	0.6	0.021
Phenylethylamine	1.5	1.0	0.1	0.8	0.479
Putrescene	7.3	3.7	3.0	1.6	0.192
Spermidine	6.7	2.2	0.1	2.1	0.158
Tryptamine	2.3	1.2	0.8	0.7	0.331
Tyramine	4.0[b]	1.7[ab]	0.1[a]	0.8	0.044

[a,b]Superscripts in the same row denote a difference ($P < 0.05$) among treatments.

[1]Control = no supplemental fructan; OF = 1% oligofructose; OF+ IN = 1% chicory pulp, oligofructose + inulin.

[2]Standard error of the mean.

propionate, butyrate, isobutyrate, isovalerate, valerate, fecal phenol, 4-methyl phenol, 3-methyl indole, tryptamine, phenylethylamine, putrescine, and spermidine were not different among treatments.

Bifidobacterium spp. populations harbored by cats in this study were lower than all other microbial populations quantified, and OF+IN treatment tended to decrease ($P = 0.073$) concentrations of *Bifidobacterium* ssp. compared with the control and OF treatments (Table 6). *Escherichia coli* concentrations were decreased ($P < 0.05$) in the OF+IN treatment compared with the control and OF treatments. No differences were observed for *Lactobacillus* spp. or *C. perfringens*.

DISCUSSION

A goal of this research was to determine if supplemental dietary fructans are effective in altering concentrations of nitrogenous compounds in excreta of senior cats. A commercial canned diet was chosen as this type of diet is palatable, meets the nutritional requirements of the cat, and allows ease of fructan administration. The diet contained primarily protein and fat, along with vitamins and minerals.

While the diet chosen for this study had several beneficial properties with regard to nutrition and ease of supplementing the fructan treatments, it also may have influenced the results observed in the present study. The diet contained an intrinsic concentration of 3.78% TDF as a result of the presence of the guar gum, carrageenan, and, potentially, the collagen from the meat byproducts. Guar gum and carrageenan are considered sources of fermentable fiber. If contained in sufficient concentrations, guar gum could increase SCFA production (Henningsson et al., 2002; Stewart and Slavin, 2006) and could increase certain microbial populations within the large intestine (Tomlin et al., 1988), but carrageenan actually could decrease intestinal microbiota concentrations (Mallett et al., 1985). However, these ingredients were incorporated into the diet as the seventh and twelfth ingredients, respectively, on the label, so were present in relatively low amounts.

Analysis of the fructans indicated differences between the treatments. Some free sugar concentrations were increased by adding the fructan supplements, specifically sucrose and fructose. These monosaccharides are formed during the manufacturing of oligofructose and inulin and would not be expected to affect the overall health of the cat. Analysis of the free oligosaccharides showed the difference in chain length distribution between OF and OF+IN. The short β(2–1) fructan chains in OF are fermented faster than the long β(2–1) fructan chains in OF+IN (van der Meulen et al., 2004), and it is hypothesized that the latter would sustain a prolonged fermentation in the distal part of the colon. Furthermore,

Table 6. Fecal concentrations of microbiota produced by cats fed supplemental fructans

Item	Treatment[1]			SEM[2]	*P*-value
	Control	OF	OF+IN		
	— $\log_{10}$ cfu/g fecal DM—				
Bifidobacterium spp.	7.52[d]	7.56[d]	6.85[e]	0.21	0.073
Clostridium perfringens	12.56	12.51	12.05	0.40	0.624
Escherichia coli	11.93[b]	10.80[b]	8.42[a]	0.61	0.012
Lactobacillus spp.	10.87	10.61	9.87	0.44	0.301

[a,b]Superscripts in the same row denote a difference ($P < 0.05$) among treatments

[c,d]Superscripts in the same row denote a trend ($P \leq 0.10$) among treatments.

[1]Control = no supplemental fructan; OF = 1% oligofructose; OF+ IN = 1% chicory pulp, oligofructose + inulin.

[2]Standard error of the mean.

the method used for determining dietary inulin concentrations may account for oligosaccharides other than inulin, including hydrocolloids from guar gum and fractions of starch with DP > 7. As there was no inulin intrinsic to the canned commercial cat food used in this experiment, it was possible to determine that the concentrations of contaminating hydrocolloids was 0.9% of the diet (based on the control diet). Therefore, this value was subtracted from the inulin values presented in the data tables.

Senior cats may experience decreased crude protein digestibility (Perez-Camargo 2004). This was observed for the cats consuming the OF+IN treatment, and likely led to the observed changes in protein catabolite and microbiota concentrations. Furthermore, the protein ingredients in the diet may have influenced protein catabolite concentrations. Meat byproducts are readily digested and absorbed by cats, but collagen concentrations may have decreased protein availability for the cats consuming these diets. Undigested protein, whether due to decreased enzymatic activity or feeding of refractory protein ingredients, would escape to the large intestine and serve as a nitrogen source for the colonic microbiota.

Initially, the values associated with N retention in the present study appeared to be high for what is considered normal for the adult or senior cat. Previous research has observed N retention values for adult cats that are near zero or negative (Russell et al., 2000; Funaba et al., 2005), and one could expect N retention values in senior cats to be similar to or lower than those of adult cats. However, it is not unusual to observe N retention values higher than zero for adult cats at maintenance. Funaba et al. (1998) observed that 5 male cats retained approximately 1.25 g N/d when consuming a dry diet with 34.6% crude protein. In another study, N retention was 0.6 g/d when adult cats were fed a diet containing 20% protein (Russell et al., 2000). Given the protein concentrations of the diet in the current study, the N retention values reported were within the range previously reported in the literature.

Concentrations of indoles were increased in the present study compared with a study conducted in younger cats (Vester et al., 2009; Barry et al., 2010). Younger cats may produce lower concentrations of protein catabolites due to more efficient digestive function. Furthermore, the diets used in these studies were very different: the present study used a canned commercial diet with higher protein and fat concentrations, whereas Vester et al. (2009) used beef- and horse-based diets that were high in raw protein and fat and Barry et al. (2010) used dry, kibble diets with moderate protein and fat concentrations. Indole concentrations were increased compared with those measured in younger cats fed beef- and horse-based diets with higher protein concentrations (Vester et al., 2009). Compared with the study performed in younger cats, tyramine concentrations were decreased and histamine concentrations were increased in the present study. This could be due to differences in diet composition, in addition to differences in diet digestibility, as biogenic amines are generated from amino acid fermentation by the colonic microbiota. Indole, histamine, and tyramine are considered putrefactive in the intestine and can be carcinogenic (Cummings and Macfarlane, 1991). Further potential detrimental health effects of histamine and tyramine have been related to diet-induced migraines and allergies (Millichap and Yee, 2003). In addition, indole has been shown to have a toxic effect against the growth of lactic acid bacteria after 24 h of incubation (Nowak and Libudzisz, 2006) and to play a role in renal failure, with patients suffering from this condition being commonly encouraged to take prebiotic or probiotic supplementation (Dou et al., 2004; Evenepoel et al., 2009). Putrescine, spermidine, and spermine are viewed as markers of a healthy intestine when present in low concentrations as they are involved in the process of apoptosis and normal cell turnover (Delzenne et al., 2000), cell proliferation and maturation in neonatal rats, and have a trophic effect in the gastrointestinal tract of adult rats (Dufour et al., 1988). Spermidine decreased in both treatment groups, which could be considered detrimental to fructan supplementation of senior cats. However, histamine concentrations decreased with both fructans and indole and tyramine decreased with the OF+IN treatment, so the decrease in spermidine is offset, in part, by these occurrences. Despite the current research and information available in this field, the physiological relevance of these metabolites to the host remains largely unknown and controversial. The rapid advancement of microbiome and metabolome technologies may bring new insights to this field and a more definitive physiological role of these catabolites to health.

As bifidobacteria are generally observed in low concentrations in cats (Terada et al., 1993; Buddington and Paulsen, 1998), the concentrations observed in the present study are not unusual. However, the trend toward

decreased bifidobacteria concentrations in the feces is unusual with regard to supplemental fructans. Typically, concentrations of bifidobacteria increase with fructan supplementation in cats and dogs (Middelbos et al., 2007a; Barry et al., 2010). The cats consuming the OF+IN diet may have initially had a low population of bifidobacteria, or the bifidobacteria present in these cats may not have been able to outcompete other microbiota present in the colon. *Escherichia coli* populations were expected to decrease with fructan supplementation, as was observed in the present study and another study (Barry et al., 2010).

In conclusion, fructan supplementation of senior cats has the potential to modify odor component production. One percent supplementation of OF+IN decreased apparent N digestibility, an indication of greater microbial growth in the large bowel. Microbial populations measured in this study were altered by fructan supplementation, and protein catabolites were generally reduced, indicating the potential for improved colonic health. In practical application, a concentration of greater than 1% dietary fructan supplementation might be recommended to modulate microbial populations and increase production of beneficial endproducts of fermentation such as SCFA. This also may reduce excretion of urinary N at the expense of fecal N and, potentially, reduce ammonia and felinine concentrations, leading to less urinary odor production.

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