Low-level fructan supplementation of dogs enhances nutrient digestion and modifies stool metabolite concentrations, but does not alter fecal microbiota populations

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ABSTRACT: Five ileal-cannulated adult dogs were utilized in a 5 × 5 Latin square design to determine the effects of fructan type and concentration on nutrient digestibility, stool metabolite concentrations, and fecal microbiota. Five diets were evaluated that contained cellulose alone or with inulin or short-chain fructooligosaccharides (scFOS) each at 0.2 or 0.4% of the diet. Dogs were fed 175 g of their assigned diet twice daily. Chromic oxide served as a digestibility marker. Nutrient digestibility; ileal and fecal pH and ammonia concentrations; ileal IgA concentrations; and fecal short- and branched-chain fatty acid concentrations, microbiota, and concentrations of phenol, indole, and biogenic amines were measured. No differences were observed in ileal pH or ammonia or fecal concentrations of indole or valerate. Ileal DM, OM, and CP digestibility coefficients; total tract DM and OM digestibility coefficients; and fecal concentrations of phenylethylamine increased linearly (P < 0.05), and fecal concentrations of phenol decreased linearly (P < 0.05) with inulin supplementation. Total tract DM and OM digestibility coefficients increased linearly (P < 0.05), and fecal phenol concentration decreased linearly (P < 0.05) with scFOS supplementation. Total tract DM and OM digestibility coefficients as well as fecal butyrate and isobutyrate concentrations increased quadratically (P < 0.05) with scFOS supplementation. Although a greater level of inclusion is needed to modify gut microbiota populations, low-level inclusion of inulin or scFOS is effective in modifying key nutritional outcomes in the dog.

Key words: dog, fructan, microbiota, nutrient digestibility, phenols, protein catabolite

INTRODUCTION

Fructans have been investigated for their intestinal health benefits at moderate to large concentrations in the canine diet. Several beneficial effects have been observed in these investigations, including increased beneficial microbial populations with a commensurate decrease in potentially pathogenic populations (Zentek et al., 2003) and decreased fecal putrefactants such as phenols, indoles, ammonia, and some biogenic amines (Swanson et al., 2002a,b; Propst et al., 2003). By altering intestinal microflora in favor of a more beneficial population, increased short-chain fatty acids (SCFA) are produced that increase intestinal energy supply and provide a decreasingly favorable environment for growth of potential pathogens (Macfarlane and Cummings, 1991). Fructan supplementation also has demonstrated the capacity to decreased aberrant crypt foci growth, a precursor to colon cancer (Reddy et al., 1997; Rowland et al., 1998). By decreasing potential pathogens, putrefactants also are reduced, thereby alleviating some risk of intestinal diseases associated with increased putrefactants in the intestine.

In canines, no detrimental effects on fecal quality have been observed with inulin supplementation up to a concentration of 7% (Diez et al., 1998). Fecal scores decreased with 6% fructooligosaccharide (FOS) supplementation, although not outside of a normal range (Twomey et al., 2003). These concentrations may be near the upper limit in regard to gastrointestinal tolerance and, potentially, physiological benefits to the
dog because these studies reported decreased fecal DM percentages. However, there is no functional lower-limit to fructan supplementation published to date, and no direct comparison of inulin and short-chain FOS (scFOS) at low dietary concentrations has been published. Therefore, the objective of this research was to determine the effects of 0.2 and 0.4% inulin and scFOS on ileal and total tract nutrient digestibility, ileal IgA concentration, stool protein catabolite concentrations, and microbiota in feces of healthy, adult dogs.

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

Five purpose-bred adult female dogs (Marshall Bioreources, North Rose, NY) with hound bloodlines, an average initial BW of approximately 21.5 ± 2.1 kg, and an average initial age of 5.5 yr were surgically fitted with an ileal T-shaped cannula according to Walker et al. (1994) at least 4.5 mo before the start of the experiment. Dogs were housed individually in kennels (2.4 × 1.2 m) in a temperature-controlled room with a 16-h light:8-h dark cycle at the animal care facility of the Edward R. Madigan Laboratory on the University of Illinois campus.

Oligosaccharide-free ingredients were used in diet formulation, with poultry by-product meal, brewers rice, and poultry fat constituting the main ingredients of the dry, extruded kibble diets (Table 1). The diet was milled at Lortscher Agri Service Inc. (Bern, KS) and extruded at Kansas State University’s Bioprocessing and Industrial Value-Added Program Facility (Manhattan, KS) under the direction of Pet Food and Ingredient Technology Inc. (Topeka, KS). A Wenger X20/E325 extruder was used with a Wenger 4800 Series Dryer/Cooler (Wenger, Kansas City, MO). Extrusion temperature started at 95°C when the diet left the preconditioning step. The kibble entered the dryer at 104°C and passed through the dryer for 12 min at this temperature (6 min each on 2 belts). They were allowed to pass through the remainder of the dryer (5 min) without applied heat so that the extruded diet would remain slightly warm when the poultry fat was added. Fructan treatments were incorporated into the diets before extrusion. Five diets were prepared, and their compositions are presented in Table 1. Dogs were offered 175 g of their assigned diet twice daily (0800 and 2000 h) to maintain BW and ideal BCS (4 to 5 on a 9-point scale). Chromic oxide was included in the diet at 0.2% of the basal mix and was used as a digestion marker. Fresh water was available at all times.

Sample Collection

A 5 × 5 Latin square design with 18-d periods was used. A 14-d adaptation phase preceded a 4-d collection of feces and ileal effluent. Ileal effluent was collected 3 times/d at 4-h intervals. Each collection was 1 h in length, and sampling times were rotated 1 h from the
collection from the previous day. For example, sampling times on the first collection day were 0800, 1200, and 1600 h; on the second day, samples were collected at 0900, 1300, and 1700 h, etc. Ileal samples were collected by attaching a sterile sampling bag (Whirlpac, Fisher Scientific, Pittsburgh, PA) to the cannula barrel with a rubber band. Before attachment of the bag, the cannula plug was removed, the interior of the cannula scraped clean, and old digesta discarded. During collection of ileal effluent, the dogs were encouraged to move around freely. To prevent the dogs from pulling the collection bag from the cannula, Bite-Not collars (Bite-Not Products, San Francisco, CA) were used during collections as needed. After ileal effluent collection, the cannula plug was put in place, and the cannula site was cleaned with a dilute betadine solution.

Total feces excreted during the collection phase of each period were removed from the floor of the pen on d 15 through 18, weighed, and composited to obtain a representative sample. On d 15 of each period, a fresh fecal sample was collected within 15 min of defecation for the measurement of pH, protein catabolites, and microbial enumeration. Day 15 was chosen for fresh fecal collection to minimize the potential for inaccuracy within the fecal sample due to concurrent ileal digesta sampling. All fecal samples during the 4-d collection phase were scored for consistency according to the following system: 1 = hard, dry pellets in a small, hard mass; 2 = hard, formed stool that remains firm and soft; 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**

An aliquot of fresh feces was immediately transferred into sterile cryogenic vials (Nalgene, Rochester, NY) and frozen at −80°C until DNA extraction for microbial analysis. Aliquots for analysis of phenol, indole, and biogenic amine concentrations were frozen at −20°C immediately after collection. One aliquot was collected and put in 5 mL of 2 N hydrochloric acid for SCFA, branched-chain fatty acid (BCFA), and ammonia analyses. Additional aliquots were used for pH measurement and fresh fecal DM determination. Remaining fecal samples were frozen at −20°C for further analyses.

On d 17 of each period, fresh ileal effluent samples were collected within 15 min of removing the collection bags. Procedures mentioned above for fecal sampling were followed using ileal effluent for ammonia and pH analyses. Remaining ileal effluent samples were frozen at −20°C in their individual bags. At the end of the experiment, all ileal effluent samples were composited for each dog for each period, then refrozen at −20°C. Before analysis, ileal effluent was lyophilized in a Dura-Dry MP microprocessor-controlled freeze-drier (FTS Systems, Stone Ridge, NY). Composited fecal samples were dried at 55°C in a forced-air oven. After drying, fecal and ileal samples were ground through a 2-mm screen in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ).

**Chemical Analyses**

Diet, ileal, and fecal samples were analyzed for DM, OM, and ash using AOAC (2000) methods. Crude protein was calculated from Leco total N values (AOAC, 2000). Total lipid content (acid hydrolyzed fat, AHF) of the diet was determined according to AACC (1983) and Buddle (1952). Gross energy of the diet was measured using an oxygen bomb calorimeter (model 1261, Parr Instruments, Moline, IL). Chromium concentrations in diet, digesta, and fecal samples were analyzed according to Williams et al. (1962) using atomic absorption spectrophotometry (model 2380, Perkin-Elmer, Norwalk, CT). Short- and branched-chain fatty acid concentrations were determined by gas chromatography according to Erwin et al. (1961) using a 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. Ammonia concentrations were determined using spectrophotometry according to the methods of Chaney and Marbach (1962). Phenol and indole concentrations were determined using gas chromatography according to the methods of Flickinger et al. (2003a). Biogenic amines concentrations were measured by HPLC according to methods described by Flickinger et al. (2003a).

**Microbial Analyses**

Fecal microbial populations were analyzed using methods described by Middelbos et al. (2007) 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 (Nano-Drop Technologies, Wilmington, DE). Quantitative PCR was performed using specific primers for *Bifidobacteria* spp. (Matsuki et al., 2002), *Lactobacillus* spp. (Collier et al., 2003), *Escherichia coli* (Malinen et al., 2003), and *Clostridium 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 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 colony-forming units per milliliter of each serial dilution was plotted against the cycle threshold to create a linear equation to calculate colony-forming units per gram of dry feces.

**Immunological Analyses**

Ileal IgA concentrations were measured according to the methods of Nara et al. (1983). Freshly collected ileal effluent was frozen at −20°C in sterile collection bags. The frozen samples were lyophilized and ground in a Wiley mill. A 2-g aliquot of each lyophilized and ground sample was suspended in 20 mL of PBS solution (pH 7.2) and mixed for 30 min at room temperature. Samples were then centrifuged at 20,000 × g for 30 min at 4°C. The supernatant was collected and ileal IgA concentrations per milliliter of each serial dilution was plotted against the cycle threshold to create a linear equation to calculate colony-forming units per gram of dry feces.

**Calculations**

Dietary intake was calculated using recorded values from d 15 through d 18. Metabolizable energy was calculated using the following equation:

\[
\text{ME} = \frac{14.64 \times \% \text{ CP} + 35.56 \times \% \text{ acid hydrolyzed fat} + 14.64 \times \% \text{ carbohydrate}}{100},
\]

where carbohydrate is equal to 100 – (% ash) – (% CP) – (% acid hydrolyzed fat) – (% total dietary fiber) when all values are on a DM basis (AAFCO, 2009). Dry matter (g/d) recovered as ileal effluent was calculated by dividing the Cr intake (mg/d) by ileal Cr concentrations (mg of Cr/g of ileal DM). Ileal nutrient flows were calculated by multiplying DM flow by the concentration of the nutrient in the ileal DM. Ileal nutrient digestibility coefficients were calculated as nutrient intake (g/d) minus ileal nutrient flow (output, g/d), and this value then was divided by nutrient intake (g/d). Similar calculations were performed on fecal samples to determine total tract nutrient digestibility coefficients.

**Statistical Analyses**

Data for continuous variables were analyzed by the MIXED procedure, and data for discontinuous variables were analyzed by the GLIMMIX procedure (SAS Inst., Cary, NC). The statistical model included the random effects of animal and period and the fixed effect of treatment. Least squares means within fructan groupings (inulin and scFOS) were compared with the control treatment to form treatment linear and quadratic contrasts. Outlier data were removed from analysis after analyzing data through the UNIVARIATE procedure 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 SD from the mean of the raw data. Differences among treatment level least squares means with a probability of \(P < 0.05\) were accepted as statistically significant, although mean differences with \(P\)-values ranging from 0.06 to 0.10 were accepted as trends.

**RESULTS**

The chemical composition of the diets is presented in Table 2. Analyzed DM, OM, CP, AHF concentrations, and GE and calculated ME values were similar among treatments.

One dog from the 0.2% inulin group was removed from the study before the first collection period for reasons unrelated to treatment. This dog was replaced by another dog in all other periods, and as such, only 4
Data points were included for the 0.2% inulin treatment for all outcome variables.

Nutrient intakes were consistent (316.3 to 326.5 g of DM/d) across all treatment groups except for the 0.2% scFOS treatment (291.6 g of DM/d; Table 3). Two of 5 dogs consumed less of this treatment than the other treatments. As a result, a quadratic response ($P = 0.018$ to $0.029$) was observed in DM, OM, and CP intake for the scFOS treatments.

Ileal digestibility of DM, OM, and CP increased ($P = 0.001$ to $0.006$) linearly for dogs consuming the inulin and scFOS treatments. Total tract DM and OM digestibility coefficients increased ($P \leq 0.002$) linearly for dogs consuming the inulin treatments. Total tract CP digestibility tended to increase ($P \leq 0.08$) linearly (88.7 to 89.6%) as well. Dogs consuming the scFOS treatments demonstrated a quadratic increase ($P \leq 0.03$) in total tract DM and OM digestibility coefficients. There were no differences in total tract CP digestibility by dogs consuming the scFOS treatments.

Fecal concentrations of acetate, propionate, and total SCFA decreased ($P = 0.020$ to $0.047$) in quadratic fashion with increasing inulin addition to the diet (Table 4). Fecal isobutyrate and total BCFA decreased ($P = 0.093$ and $0.109$) in a quadratic fashion for inulin-supplemented dogs. A quadratic decrease ($P = 0.081$) in fecal acetate concentration was observed with increasing scFOS addition to the diet. However, a quadratic increase ($P = 0.004$) in fecal butyrate concentration was observed in the same treatment group (scFOS). Also observed with increasing scFOS addition to the diet was a quadratic increase in fecal isobutyrate ($P = 0.010$), isovalerate ($P = 0.072$), and total BCFA ($P = 0.027$) concentrations. No differences were observed in fecal valerate concentrations.

No differences were observed in ileal pH, ileal ammonia concentrations, ileal IgA concentrations, or fecal score (Table 5). Fecal pH tended to increase ($P \leq 0.06$) linearly (6.55 to 6.95) for dogs consuming the inulin treatments, but no differences were observed for dogs consuming the scFOS treatments. Fecal ammonia concentrations tended to increase ($P \leq 0.06$) in quadratic fashion in dogs consuming the scFOS treatments. Fecal concentrations of phenol decreased ($P \leq 0.05$) in a linear fashion for dogs consuming the inulin (39.48 to 17.57 µmol/g DM of feces) and scFOS (39.48 to 21.72 µmol/g DM of feces) treatments. Fecal concentrations of biogenic amines were generally not affected by treatment (data not shown). A linear increase ($P < 0.02$) in fecal phenylethylamine concentration was observed for dogs from 0.01 µmol/g DM of feces in dogs consuming the control diet to 0.08 µmol/g DM of feces in dogs consuming the 0.4% inulin treatment. Agmatine, histamine, and spermine were not found in quantifiable concentrations in any of the samples collected. No differences were observed in fecal tryptamine, putrescine, cadaverine, tyramine, or spermidine concentrations. No significant differences or trends were noted among treatments in fecal microbiota concentrations (Table 6).

**DISCUSSION**

In this experiment, low concentrations of scFOS and inulin were evaluated for their impact on select outcomes related to nutritional and intestinal effects in the dog. Both compounds have been shown to positively affect diet digestibility and intestinal characteristics of dogs, generally at a dietary inclusion of 1% or greater, but few low-level inclusion studies or fructan comparison studies have been conducted in the dog. Short-chain FOS is microbially derived, and its components include kestose (GF2), nystose (GF3), and fructofuranosylnystose (GF4). In contrast, inulin is extracted from chicory.
root and consists of fructose chains with a degree of polymerization of around 60 units (Flickinger et al., 2003b).

Diet composition greatly impacts results observed in any study involving fermentable fibers. In the present study, an oligosaccharide-free basal diet consisting primarily of poultry by-product meal and brewers rice was chosen. Although corn was added to the diet, it was added at a low rate of inclusion so as to allow for good kibble manufacture, but not at large enough concentrations to be considered a main ingredient. In addition, corn contains no fructans and very little soluble fiber. Protein quality and quantity may affect the composition of the gastrointestinal microbiota, as well as the end products of fermentation, should it remain undigested. Low-ash poultry by-product meal was chosen as the protein source for this study as it is a high quality protein source, readily digested, and commonly used in the petfood industry. In addition, a protein concentration of 30% was chosen to allow the test diets to reflect high-quality diets manufactured by commercial petfood companies. Cellulose was added to all treatment diets as a source of insoluble, nonfermentable fiber.

A quadratic decrease in food intake was observed for the scFOS group due to a decrease in food intake of the dogs fed the 0.2% scFOS diet. This observation was not anticipated and is difficult to explain as inclusion of 0.2% scFOS should not drastically change diet acceptability or appearance. We view this result as spurious; however, it could affect nutrient digestibility. If this were the case, nutrient digestibility at the terminal ileum would have increased quadratically because the dogs on the 0.2% diet consumed less food. In the data presented, digestibility did not increase quadratically.

### Table 4. Concentrations of fecal short-chain (SCFA) and branched-chain fatty acids (BCFA) for dogs fed diets containing fructans

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet</th>
<th>Contrast (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.2% Inulin</td>
</tr>
<tr>
<td>Short-chain fatty acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>284.73</td>
<td>187.74</td>
</tr>
<tr>
<td>Propionate</td>
<td>92.47</td>
<td>68.43</td>
</tr>
<tr>
<td>Butyrate</td>
<td>40.13</td>
<td>34.57</td>
</tr>
<tr>
<td>Total SCFA</td>
<td>410.54</td>
<td>289.86</td>
</tr>
<tr>
<td>Branched-chain fatty acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>11.02</td>
<td>8.91</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>13.64</td>
<td>11.75</td>
</tr>
<tr>
<td>Valerate</td>
<td>19.17</td>
<td>18.25</td>
</tr>
<tr>
<td>Total BCFA</td>
<td>43.64</td>
<td>38.67</td>
</tr>
</tbody>
</table>

1Values expressed as micromoles per gram of feces (DM basis).
2PSEM = pooled SEM.
3scFOS = short-chain fructooligosaccharides.

### Table 5. Ileal and fecal pH, concentrations of ileal and fecal ammonia, concentrations of ileal IgA, fecal score, and fecal concentrations of phenols and indoles for dogs fed diets containing fructans

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet</th>
<th>Contrast (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.2% Inulin</td>
</tr>
<tr>
<td>Ileal pH</td>
<td>7.20</td>
<td>7.29</td>
</tr>
<tr>
<td>Ammonia, mg/g of DM</td>
<td>2.41</td>
<td>2.88</td>
</tr>
<tr>
<td>IgA, mg/g of DM</td>
<td>16.64</td>
<td>18.47</td>
</tr>
<tr>
<td>Fecal pH</td>
<td>6.55</td>
<td>6.67</td>
</tr>
<tr>
<td>Score</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Ammonia, mg/g of DM</td>
<td>3.38</td>
<td>3.29</td>
</tr>
<tr>
<td>Phenol, µmol/g of DM</td>
<td>39.48</td>
<td>38.15</td>
</tr>
<tr>
<td>Indole, µmol/g of DM</td>
<td>478.68</td>
<td>407.32</td>
</tr>
</tbody>
</table>

1PSEM = pooled SEM.
2scFOS = short-chain fructooligosaccharides.
but linearly, at the terminal ileum, whereas a quadratic increase was observed in the total tract. As the quadratic effects appear in apparent total tract DM and OM digestibility, it is difficult to speculate on the exact cause of the increase as the microbiota utilize and, thus, affect the composition of the nutrients excreted in feces. One possibility is that the microbiota in the large intestine were able to increase fermentation of available substrates in the dogs consuming the 0.2% treatment. This could explain the increase in some of the fermentation end products for this treatment group as compared with the 0.0 and 0.4% treatment groups.

Ileal DM, OM, and CP digestibility coefficients increased linearly for scFOS and inulin treatments. A similar trend was observed for dogs consuming inulin and oligofructose, a hydrolytic product of inulin, when fed at 0.3 to 0.9% dietary concentrations (Propst et al., 2003). Total tract DM, OM, and CP digestibility coefficients increased linearly in dogs consuming inulin, and increased quadratically (DM and OM) in dogs consuming scFOS in agreement with data of Howard et al. (2003), who supplemented dogs with 1.5% scFOS, and Bosch et al. (2009), who supplemented dogs with 2.0% inulin in combination with 8.5% beet pulp. However, this contrasts with several other reports of decreased or unchanged nutrient digestibility with fructan supplementation (Swanson et al., 2002a,b; Flickinger et al., 2003a; Propst et al., 2003). Reduced concentrations of phenol decreased linearly with inulin and scFOS supplementation and increased quadratically (DM and OM) in dogs consuming scFOS in agreement with Flickinger et al. (2003a), Propst et al. (2003). Branched-chain fatty acids also were affected, but in a different manner, with quadratic increases noted for isobutyrate, valerate, and total BCFA with scFOS supplementation. Perhaps more endogenous protein, microbial protein, or a combination of the 2 reached the descending colon where they subsequently were fermented by proteolytic microbiota. Fecal ammonia data support this contention.

Undigested protein is available for microbial fermentation in the colon and, as a result, phenols, indoles, and biogenic amines may be formed. Phenol and indole concentrations in feces indicate protein fermentation in the large intestine. Given that phenols and indoles potentially interact with other putrefactants in the intestine to form carcinogens (Macfarlane and Cummings, 1991), reducing these compounds could positively affect the intestinal health of the dog. Beneficial biogenic amines, such as putrescine, spermine, and spermidine, serve as markers of cell death and apoptosis and are indicators of cell turnover (Guo et al., 2005; Seiler and Raul, 2005; Linsalata and Russo, 2008). However, amines such as cadaverine indicate putrefaction and can be detrimental to overall intestinal health (Macfarlane and Cummings, 1991).

Concentrations of phenol decreased linearly with increasing fructan supplementation, in agreement with published literature (Propst et al., 2003). Indole concentration was not affected by fructan supplementation in agreement with Flickinger et al. (2003a), Propst et al. (2003), and Swanson et al. (2002a). Reduced con-
cations of biogenic amines were observed across all treatments, and only 1 of the 6 measured increased slightly due to treatment.

Concentrations of ileal IgA were not modified with fructan supplementation. Similar results were observed by Grieshop et al. (2003a) when supplementing oligofructose at 0.6% of the diet, as well as by Propst et al. (2003) when supplementing inulin or oligofructose at 0.3, 0.6, or 0.9% of the diet. However, when supplementing moderate to large concentrations of fructans, several authors observed decreasing fecal scores or fecal DM percentages, indicating increased stool moisture (Diez et al., 1998; Flickinger et al., 2003a; Twomey et al., 2003). Because fecal quality and score are very important to pet owners, any negative change in fecal score could be viewed as detrimental to the use of an ingredient. Low-level fructan supplementation does not appear to change fecal quality.

Changes in intestinal microbial composition are closely associated with fructan supplementation. In general, increased Bifidobacterium spp. and Lactobacillus spp. have been observed with a decrease in Clostridium spp. and other protein-fermenting microbiota in feces (Zentek et al., 2003). The microbial populations observed in feces in the present study were not affected by treatment. This indicates that 0.2 and 0.4% supplementation of scFOS and inulin fall below the concentration where microbiota are affected. Although time needed to adapt microbiota to a fiber source remains unknown, this is not deemed to be a factor in this study. Several authors have measured microbial populations after 10 d of dietary adaptation with measurable changes observed. In the present study, the adaptation period was modified to 14 d. This should have allowed ample time for intestinal microbial communities to adapt to dietary fructan sources. Perhaps inulin and scFOS are fully fermented in the proximal colon, leaving no fructans for fermentation in the distal colon and, thus, no measurable change in fecal microbiota.

In conclusion, inulin and scFOS significantly modified ileal and total tract nutrient digestibility, SCFA and BCFA concentrations in feces, and stool protein catabolites in the feces of healthy, adult dogs. As a practical application of this research, high nutrient digestibility coefficients are critical when dogs are housed indoors for extended periods of time. In addition, decreased stool protein catabolites result in a less offensive stool odor and are beneficial to intestinal health because they decrease the potential for disease in the large intestine. However, the intestinal microbiota were not affected by treatment in this experiment, leading to the conclusion that greater concentrations of supplemental fructans are necessary to affect microbiota concentrations in feces. Many commercial dog foods contain fructans at concentrations even less than those studied in this experiment. It is important to establish threshold levels at which biological responses might be expected. From these data and those in the literature, the full beneficial effects of fructans probably will not be experienced unless dietary concentrations are above 0.4% of dry food.

LITERATURE CITED


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