A dose-response experiment evaluating the effects of oligofructose and inulin on nutrient digestibility, stool quality, and fecal protein catabolites in healthy adult dogs


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ABSTRACT: In this experiment, three concentrations (0.3, 0.6, and 0.9% of diet, as-fed basis) of two fructans, oligofructose (OF) and inulin, were tested against a 0% supplemental fructan control. Seven ileal-cannulated adult female dogs were fed a meat-based, kibbled diet and assigned to treatments in a 7 × 7 Latin square design. Dietary supplementation of fructans had no effect on nutrient intakes or ileal digestibilities. Total-tract digestibilities of DM, OM, and CP decreased (P < 0.05) as a result of dietary OF and inulin supplementation. Dogs fed the control diet had a DM total-tract digestibility of 83.0%. The percentages of fecal DM for dogs fed the control and 0.3, 0.6, and 0.9% OF were 36.6, 33.3, 32.8, and 31.7%, respectively. When compared with the control, OF (P < 0.01) and inulin (P < 0.01) supplementation increased fecal ammonia concentrations. Higher fecal short-chain fatty acid (SCFA; P < 0.10) and isovalerate concentrations (P < 0.01) were noted for dogs fed both fructans. Total fecal SCFA for dogs fed the control diet and 0.3, 0.6, and 0.9% OF were 406.4, 529.9, 538.3, and 568.8 μmol/g of feces (DM basis), respectively. Dogs fed 0.3, 0.6, and 0.9% inulin had total fecal SCFA of 472.2, 468.8, and 471.5 μmol/g of feces (DM basis), respectively. Linear increases were observed in putrescine (P < 0.11), cadaverine (P < 0.07), spermidine (P < 0.12), and total amines (P < 0.05) in feces of dogs fed OF. Lower fecal phenol (P < 0.08) and total phenol (P < 0.04) concentrations occurred in dogs fed inulin, along with a linear decrease (P < 0.08) in total phenols with OF supplementation. Total fecal phenols for dogs fed the control, 0.3, 0.6, and 0.9% inulin were 3.03, 1.86, 1.97, and 2.23 μmol/g of feces (DM basis), respectively. Low-level dietary inclusion of inulin and OF positively affected indices known to be associated with gut health of the dog without seriously compromising nutrient digestibility or stool quality. Overall, the 0.9% OF treatment resulted in the best responses, including no adverse effect on nutrient intakes, ileal digestibilities, or stool quality, as well as increased fecal SCFA and decreased fecal phenols. The biological responses due to inulin were more variable.

Key Words: Dogs, Fructans, Inulin, Nutrient Digestibility, Oligofructose, Protein Catabolites


Introduction

The colonic microflora of dogs represents a rich ecosystem, composed of a wide range of metabolically active microorganisms that play an important role in influencing the health of the host. The colonic microflora are capable of fermenting complex carbohydrates and proteins to produce multiple products, including short-chain fatty acids (SCFA) and many protein catabolites. The types and amounts of fermentative products produced depend on the colonic balance of bacterial species and on the substrate available for the microorganisms to utilize (Gibson and Roberfroid, 1995).

Fructooligosaccharides (FOS)—including inulin, oligofructose, and short-chain FOS—are examples of dietary constituents that beneficially alter microbial populations in the gut and help prevent the invasion of pathogenic bacteria. These fructans have many functional and nutritional properties that may have application for companion animal nutrition. In addition to their bifidogenic properties, fructans have been shown to decrease fecal odor components, reduce blood cholesterol, prevent or inhibit the occurrence of some types of cancer, enhance vitamin synthesis, increase mineral absorption, and stimulate the immune system (Jenkins et al., 1999). However, potential adverse side-effects, such as diarrhea and flatulence, also may occur in animals consuming high levels of FOS or at moderate levels of ingestion in unadapted animals.

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Little is known concerning the optimal concentration of fructans to supplement to the canine diet in order to maximize their beneficial effects and minimize their adverse side-effects. The objective of this study was to investigate the effects of selected oligofructose and inulin concentrations on nutrient intake, nutrient digestibilities, stool quality, and fecal protein catabolites in healthy adult dogs fed a meat-based kibbled diet.

Materials and Methods

Animals

Seven purpose-bred adult female dogs (Butler Farms USA, Inc, Clyde, NY) with hound bloodlines were used. Dogs were surgically prepared with T-cannulas proximal to the ileocecal junction according to Walker et al. (1994). Dogs were housed individually in 1.2- × 3.1-m clean floor pens in a temperature-controlled room (21°C) at the animal facility of the Edward R. Madigan Laboratory, on the University of Illinois campus. A 16-h light:8-h dark cycle was used. All dogs were allowed free access to water. The surgical and animal care procedures were approved by the Institutional Animal Care and Use Committee, University of Illinois at Urbana-Champaign.

Diets

Three concentrations (equivalent to 0.3, 0.6, and 0.9% of the diet, as-fed basis) of two fructans, oligofructose (Raftilose P95; ORAFTI, Tienen, Belgium), and inulin (Rafilne HPF; ORAFTI), were tested against a control (no supplemental fructan). Oligofructose was purer than inulin (75% pure); therefore, doses of inulin were increased by a factor of 1.25 in order to compare similar concentrations of the active ingredients of both fructans. Dogs were administered the fructans by gelatin capsule twice daily with the following doses: 1) control (no oligofructose or inulin); 2) 1.5 g/d oligofructose; 3) 3 g/d oligofructose; 4) 4.5 g/d oligofructose; 5) 1.9 g/d inulin; 6) 3.8 g/d inulin; or 7) 5.6 g/d inulin to achieve dietary concentrations of 0.3, 0.6, or 0.9% active ingredients based on the amount of diet offered per day. All animals consumed a poultry by-product meal-based kibbled diet (Table 1) prepared under commercial conditions (Extruder model X-85, Wenger Manufacturing, Sabetha, KS) and formulated to meet or exceed AAFCO (2001) recommendations. The basal diet contained, on average, 92.9% DM, 93.3% OM, 32.7% CP, and 23.5% fat. The diet contained highly digestible, fructooligosaccharide-free ingredients, with poultry by-product meal, breuer’s rice, and poultry fat constituting the main ingredients of the diet. Beet pulp was included in the diet and provided a level of moderately fermentable fiber in the diet in order to prevent constipation.

Experimental Design

Dogs were randomly assigned to diets in a 7 × 7 Latin square design with 14-d periods. Total duration of the experiment was 98 d. During each period, d 1 through 10 constituted the diet adaptation phase and d 11 through 14 constituted the collection phase, during which time fecal and ileal samples were collected. Dogs were offered 250 g of the kibbled diet and one-half of their allotted fructan dosage at 0800 and 2000 daily. Chromic oxide was used as a digestion marker. On d 6 through 14 of each period, dogs were dosed with 0.5 g of chromic oxide in a gelatin capsule at 0800 and 2000 for a total of 1 g of marker per day.

Sampling Procedures

During the collection phase, ileal effluent was collected for 4 d, three times per day, with an interval of 4 h between collections. Individual ileal collections were delayed 1 h from the previous day’s collection times. For example, on d 1, sampling took place for 1 h at 0800, 1200, and 1600; on d 2, samples were collected for 1 h at 0900, 1300, and 1700; and so forth. Ileal effluent was collected by attaching a Whirl-Pak bag (Pioneer Container Corp., Cedarburg, WI) to the cannula barrel using a rubber band. Before attachment of the bag, the interior of the cannula was scraped clean with a spatula and any digesta discarded. Dogs wore Bite-Not collars (Bite Not Products, Inc., San Francisco, CA) during the collection to prevent them from removing the bags. During collection of ileal effluent, dogs were encouraged to move around freely. After each collection, ileal effluent samples were frozen at −20°C. At the end of the period, ileal samples were composited prior to lyophilization for further analysis.

Total feces were collected from the pen floor during the 4-d collection phase, scored, and weighed at the time of collection. Scoring was determined as follows: 1 = hard, dry pellets; small, hard mass; 2 = hard, formed,

| Ingredient composition of the basal diet (as-fed basis) fed to ileal cannulated dogs |
|---------------------------------|---|
| Ingredient | % |
| Poultry by-product meal | 44.51 |
| Brewer’s rice | 32.10 |
| Poultry fat | 15.70 |
| Beet pulp | 4.00 |
| Dehydrated egg | 2.24 |
| Sodium chloride | 0.65 |
| Potassium chloride | 0.43 |
| Choline chloride | 0.13 |
| Vitamin premix | 0.12 |
| Mineral premix | 0.12 |

aProvided per kilogram of diet: choline, 2,284.2 mg.

bProvided per kilogram of diet: vitamin A, 11.0 KIU; vitamin D₃, 0.9 KIU; vitamin E, 57.5 IU; vitamin K, 0.6 mg; thiamin, 7.6 mg; riboflavin, 11.9 mg; pantothenic acid, 18.5 mg; niacin, 93.2 mg; pyridoxine, 6.6 mg; biotin, 12.4 mg; folic acid, 1,142.1 μg; vitamin B₁₂, 164.9 μg.

bProvided per kilogram of diet: vitamin A, 11.0 KIU; vitamin D₃, 0.9 KIU; vitamin E, 57.5 IU; vitamin K, 0.6 mg; thiamin, 7.6 mg; riboflavin, 11.9 mg; pantothenic acid, 18.5 mg; niacin, 93.2 mg; pyridoxine, 6.6 mg; biotin, 12.4 mg; folic acid, 1,142.1 μg; vitamin B₁₂, 164.9 μg.

bProvided per kilogram of diet: manganese (as MnSO₄), 17.4 mg; iron (as FeSO₄), 284.3 mg; copper (as CuSO₄), 17.2 mg; cobalt (as CoSO₄), 2.2 mg; zinc (as ZnSO₄), 166.3 mg; iodine (as KI), 7.5 mg; selenium (as Na₂SeO₃), 0.2 mg.
dry stool; remains firm and soft; 3 = soft, formed moist; softer stool that retains shape; 4 = soft, unformed; stool assumes shape of container; and 5 = watery; liquid that can be poured. Feces were frozen at –20°C and then composited by dog at the end of each period.

On d 13 or 14 of each period, the dogs were checked every 15 min in order to obtain a freshly voided fecal sample for protein catabolite component (ammonia, amines, branched-chain fatty acids, phenols, and indoles) analysis. One aliquot of feces (5 g) was acidified with 2 N HCl for ammonia, short-chain fatty acid (SCFA), and branched-chain fatty acid (BCFA) analysis. For amines, 2 g of sample was weighed in duplicate into 50-mL centrifuge tubes. For phenols and indoles, 2 g of sample was weighed in duplicate into 16-mL centrifuge tubes. All samples were immediately stored at –20°C until analyses could be performed in order to minimize any loss of volatile components.

Any feed refusals during the collection phase were collected at 0800 and 2000 daily and weighed. At the end of each period, feed refusals were composited and frozen at –20°C in order to calculate feed intake for that period.

Before analyses, ileal effluent was freeze-dried in a Tri-Philizer MP microprocessor-controlled lyophilizer (PTS Systems, Inc., Stone Ridge, NY). Feed refusals and feces were dried at 55°C. All samples were ground through a 2-mm screen in a Wiley mill (Model 3375-E10, Thomas-Wiley, Swedesboro, NJ) in preparation for chemical analyses.

**Chemical Analyses**

Diet, ileal, fecal, and feed refusal samples were analyzed for DM and ash concentrations according to AOAC (1985). Feces and ileal samples were analyzed for chromium (Cr) content according to Williams et al. (1962) using an atomic absorption spectrophotometer (model 2380, Perkin-Elmer, Norwalk, CT). Crude protein was determined from Leco total nitrogen values according to AOAC (1995). Fat content was determined by acid hydrolysis (AACC, 1983) followed by ether extraction according to Budde (1952).

Indoles and phenols were extracted by mixing 2 g of feces with 5 mL of methanol containing 500 ppm 5-methylandole (internal standard). The feces-methanol mixture was covered with parafilm, mixed well, and incubated for 1 h at 4°C with frequent mixing. Tubes then were centrifuged at 29,000 × g for 15 min at 4°C and the supernatant collected. The remaining pellet was mixed again with 5 mL of methanol and extracted as detailed above. The two supernatant fractions were combined for gas chromatographic (GC) analysis. Individual concentrations of indole, phenol, and p-cresol were determined using a Hewlett-Packard (Palo Alto, CA) 5890A Series II gas chromatograph and a Nukol fused silica capillary column (60 m × 0.32 mm i.d.). Helium was used as the carrier gas with a flow rate of 100 mL/min. Oven temperature was 200°C and detector and injector temperatures were both 220°C. Fecal samples for SCFA and BCFA were acidified and diluted 1:5 with 25% meta-phosphoric acid. After 30 min, samples were centrifuged at 20,000 × g for 20 min. The supernatant was transferred into microfuge tubes and frozen at –20°C. Following freezing, the supernatant was thawed, centrifuged at 13,000 × g for 10 min, and analyzed for SCFA and BCFA concentrations via gas chromatography according to Erwin et al. (1961). Briefly, concentrations of acetate, propionate, butyrate, valerate, isovalerate, and isobutyrate were determined in the supernatant fluid of acidified fecal aliquots using a Hewlett-Packard 5890A Series II gas chromatograph 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 gas with a flow rate of 75 mL/min. Oven temperature, detector temperature, and injector temperature were 125, 175, and 180°C, respectively. Biogenic amines (phenylethylamine, putrescine, cadaverine, and spermidine) were derivatized with dansyl chloride and quantified using high performance liquid chromatography (HPLC) as described by Flickinger et al. (2003). Concentrations of ammonia in feces were determined colorimetrically by spectrophotometry using the method of Chaney and Marbach (1962).

**Calculations**

Dry matter flow (g/d) of ileal effluent and fecal DM output were calculated by dividing Cr intake (mg/d) by ileal or fecal Cr concentration (mg Cr/g sample). Nutrient flows were calculated by multiplying the DM flow by the concentration of the nutrient in the ileal or fecal DM. Ileal and total-tract nutrient digestibilities were calculated by subtracting the nutrient flow (g/d) from the nutrient intake (g/d) and then dividing this value by nutrient intake (g/d).

**Statistics**

Data were analyzed by ANOVA as a 7 × 7 Latin square by the General Linear Models procedure of SAS (SAS Inst., Inc., Cary, NC). The statistical model included the effects of animal, period, and treatment. Treatment least squares means were compared using planned contrasts. Contrasts included the following: 1) control diet vs. all oligofructose concentrations, 2) control diet vs. all inulin concentrations, 3) linear effects of oligofructose concentrations, 4) linear effects of inulin concentrations, 5) quadratic effects of oligofructose concentrations, and 6) quadratic effects of inulin concentrations. To minimize variation, the inulin and oligofructose treatments were analyzed separately, with both data sets including the control diet. Therefore, the standard error of the mean is presented for each oligosaccharide. Although P < 0.05 was the accepted level of statistical significance, trends between P > 0.06 and P < 0.15 also are presented and discussed.
Power tests were conducted using a two-tailed test with $\alpha = 0.05$ and $\beta = 0.20$. The following standard deviations and desired detected differences were used for power calculations of total-tract DM digestibility, fecal ammonia concentrations, and fecal amine concentrations, respectively: 2.5 and 5 percentage units; 9.4 and 25 μmol/g; 0.4 and 0.8 μmol/g. These calculations indicated that between 5 and 20 observations should have allowed the detection of relevant differences among means at a level of $P < 0.05$. Because the ileally cannulated dog model is expensive and difficult to maintain long-term, a limited number of animals was available. A Latin square design was employed in order to gain maximal statistical power despite the small number of animals. Furthermore, reporting responses in the range of 0.06 < $P$ < 0.15 allows the authors to discuss results that may be biologically important.

Results and Discussion

Nutrient Intakes

Intakes of DM, OM, CP, and fat by dogs did not differ among treatments (Table 2). Dogs readily ingested the diet when supplemented with either fructan compared to the control.

Apparent Ileal Digestibility

Ileal digestibilities of DM, OM, CP, and fat did not differ among treatments. A high SEM may have prevented the detection of significant differences among treatments in ileal nutrient digestibility. Similar work done in our lab by Flickinger et al. (2003) and Swanson (2002) had lower ileal digestibility SEM (DM, 4.37; OM, 3.31; CP, 4.12; fat, 0.66; and DM, 5.39; OM, 4.44; and CP, 5.75, respectively) than the present experiment. Also, in this study, the ileal SEM are higher than total-tract SEM, where differences were detected. Crude protein varied most in apparent ileal digestibility (51.3 to 68.7%). As expected, ileal digestion of fat was high (approximately 95%) with little variation among treatments (oligosfructose SEM = 1.14 and inulin SEM = 0.88). Houdijk et al. (1999) found no differences in apparent ileal digestibilities of OM, CP, or fat by swine fed 0.75 or 1.50% oligofructose (Raftilose P95, ORAFTI). Similarly, Strickling et al. (2000), using dogs, determined that 0.5% dietary oligofructose (Raftifeed P75, ORAFTI) did not significantly affect apparent ileal DM or CP digestibilities. Similar ileal nutrient digestibilities across treatments indicate no impairment in hydrolytic digestion processes as a result of fructan supplementation. This is significant because ileal digestion values represent a more accurate portrayal of protein/amino acid digestion than do apparent total-tract digestion values.

Apparent Total-Tract Digestibility

When dogs were supplemented with oligofructose, total-tract digestibilities of DM ($P < 0.05$), OM ($P < 0.03$), and CP ($P < 0.02$) were lower than for dogs fed the control diet (Table 2). Total-tract digestibilities of DM ($P < 0.03$) and OM ($P < 0.03$) exhibited a quadratic decrease and a trend for a quadratic decrease for CP ($P < 0.10$) and fat ($P < 0.07$) due to oligofructose supplementation. Total-tract digestibilities of CP exhibited a linear decrease ($P < 0.05$) with oligofructose inclusion. Dogs supplemented with inulin had lower DM ($P < 0.04$), OM ($P < 0.03$), and CP ($P < 0.03$) and a trend for lower fat ($P < 0.15$) total-tract digestibilities compared to the control. Total-tract digestibilities of DM ($P <

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Table 2. Nutrient intakes and digestibilities by ileal-cannulated dogs fed diets supplemented with selected concentrations of oligofructose (OF) and inulin (I)a

| Item         | Control (C) | 0.3 | 0.6 | 0.9 | SEM | 0.3 | 0.6 | 0.9 | SEM | C vs. OF | C vs. I | OF | I | OF |
|--------------|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-------|----|---|----|
| Intake, g/d  |             |     |     |     |     |     |     |     |     |     |       |       |    |   |    |
| Dry matter   | 354         | 344 | 353 | 364 | 16.7| 313 | 339 | 359 | 22.9| 0.98| 0.53| 0.60| 0.69| 0.52| 0.20|
| Organic matter| 330        | 320 | 329 | 340 | 15.5| 292 | 316 | 335 | 21.4| 0.98| 0.53| 0.60| 0.69| 0.52| 0.20|
| Crude protein| 116         | 112 | 115 | 119 | 9.0 | 102 | 111 | 118 | 6.9 | 0.98| 0.53| 0.60| 0.69| 0.52| 0.20|
| Fat          | 83          | 81  | 83  | 86  | 6.5 | 74  | 80  | 85  | 5.0 | 0.98| 0.53| 0.60| 0.69| 0.52| 0.20|

Each mean represents the average value for seven individually cannulated dogs.
Table 3. Stool quality measurements and fecal ammonia concentrations for ileal-cannulated dogs fed diets supplemented with selected concentrations of oligofructose (OF) and inulin (I)\(^a\)

<table>
<thead>
<tr>
<th>Item</th>
<th>% OF</th>
<th>% I</th>
<th>SEM</th>
<th>C vs. OF</th>
<th>Quadratic</th>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet fecal mass, g/d</td>
<td>162</td>
<td>144</td>
<td>15.6</td>
<td>209</td>
<td>23.0</td>
<td>0.54</td>
<td>0.01</td>
</tr>
<tr>
<td>Dry fecal mass, g/d</td>
<td>57</td>
<td>57</td>
<td>4.0</td>
<td>66</td>
<td>6.7</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Dry matter, %</td>
<td>36.6</td>
<td>35.4</td>
<td>3.4</td>
<td>35.4</td>
<td>3.4</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Fecal score(^b)</td>
<td>2.6</td>
<td>2.4</td>
<td>2.7</td>
<td>2.4</td>
<td>2.7</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>Fecal ammonia, mol/g DM</td>
<td>222.5</td>
<td>292.2</td>
<td>29.6</td>
<td>292.2</td>
<td>29.6</td>
<td>1.15</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^a\)Each mean represents the average value for seven individually cannulated dogs.

\(^b\)Scores based on the following scale: 1 = hard, dry pellets; small hard mass; 2 = hard, formed dry stool; remains firm and soft; 3 = soft, formed, moist; softer stool that retains shape; 4 = soft, unformed; stool; assumes shape of container, pudding-like; 5 = watery; liquid that can be poured.

Effects of fructan supplementation in dogs

Oligofructose tended (\(P < 0.08\)) to increase wet fecal output in a linear fashion (Table 3). Compared to the control, all levels of oligofructose tended to have greater dry fecal output (\(P < 0.08\)). Inulin supplementation did not affect wet or dry fecal output. Because of an increase in bacterial biomass, fecal mass has been shown to increase when oligosaccharides are added to human and dog diets (Gibson et al., 1995; Strickling et al., 2000). There was no significant difference in the percentage of fecal DM in dogs fed the inulin treatment. Fecal DM percentage tended to be lower (\(P < 0.11\)) for oligofructose treatments in comparison to the control. A tendency for a linear decrease (\(P < 0.09\)) in fecal DM percentage was evident when dogs were supplemented with oligofructose. This response is in agreement with data reported by Diez et al. (1998), who found decreases in DM percentage for dogs fed a beef- and rice-based diet supplemented with 5% dietary oligofructose. Strickling et al. (2000) also determined that 0.5% dietary oligofructose increased the fecal moisture content in dogs. Fecal scores did not differ statistically among treatments.
Overall, these data indicate that the concentrations of fructans tested did not adversely affect stool quality. As dog owners purchase food primarily on the basis of diet acceptability and appearance of the stool, these data are important in evaluating the potential of fructan inclusion in diets.

When compared with the control, oligofructose (P < 0.01) and inulin (P < 0.01) supplementation resulted in increased fecal ammonia concentrations. A linear trend was noted for inulin (P < 0.12), although there was relatively less incremental increase in ammonia concentrations above 0.3% inulin supplementation. The highest fecal ammonia concentration for oligofructose occurred with the 0.3% treatment group, whereas the lowest concentration occurred for the control group, resulting in a quadratic effect (P < 0.04). This is in contrast to results of Flickinger et al. (2003), who determined that similar oligofructose supplementation (1.9 g/d) tended (P < 0.10) to decrease fecal ammonia concentration in dogs consuming a corn-based diet. Differences in response to fructan supplementation between that study and ours perhaps are due to the type of diet fed (plant- vs. animal protein-based). Ammonia is produced in the colon by bacterial hydrolysis of urea as well as by bacterial deamination of amino acids, peptides, and proteins (Vince et al., 1976). The higher concentrations of fecal ammonia excreted by dogs fed oligofructose and inulin may indicate a shift in N excretion pattern from urine to feces.

**Fecal Short-Chain Fatty Acid Concentrations**

Concentrations of fecal acetate, propionate, butyrate, and total SCFA were higher than control values when dogs were supplemented with oligofructose (P < 0.01) and inulin (P < 0.10; Table 4). Concentrations of acetate and total SCFA increased linearly (P < 0.01) with increasing concentrations of oligofructose, with the greatest increase noted for the 0.3% oligofructose treatment. Propionate (P < 0.04) exhibited a quadratic response and butyrate exhibited a quadratic trend (P < 0.09) with oligofructose supplementation, with the majority of the increase in SCFA concentration occurring between the control and 0.3% oligofructose treatments. Inulin resulted in a linear increase in fecal propionate (P < 0.04) and butyrate (P < 0.01) and a linear trend in total fecal SCFA (P < 0.10) concentrations. A quadratic trend (P < 0.09) for propionate was evident with inulin inclusion, with the highest concentration (111.2 μmol/g of feces, DM basis) occurring for the 0.6% treatment and the lowest concentration (109.7 μmol/g of feces, DM basis) for the 0.9% treatment. Again, the largest difference in fecal SCFA concentrations was observed between the control and 0.3% inulin treatments. These results indicate that oligofructose and inulin are highly fermentable substrates that will increase fecal SCFA concentrations at relatively low levels of inclusion in diets.

In an in vitro assay performed by Vickers et al. (2001) using canine fecal inoculum, it was determined that short-chain FOS and inulin were more highly fermentable than other fiber substrates commonly used in canine diets (beet pulp, cellulose, soy fiber, and mannanoligosaccharides). Smiricky (2002) also performed an in vitro assay using several nondigestible oligosaccharides, but used swine fecal microflora as the inoculum source. Short-chain FOS (scFOS, GTC, Golden, CO), oligofructose (ORAFTI), and inulin (ORAFTI) produced similar concentrations of the individual (acetate, propionate, and butyrate) and total SCFA at each of the time points studied (2, 4, 8, and 12 h). Generally, SCFA production increased with time; after 12 h of fermentation, total SCFA production for short-chain FOS, oligofructose, and inulin was 5.7, 6.1, and 6.0 mmol/g OM, respectively. These levels of SCFA production were high in comparison to those for pure raffinose, soy solubles, granular and liquid transgalactooligosaccharides, but used swine fecal microflora as the inoculum source. Short-chain FOS (scFOS, GTC, Golden, CO), oligofructose (ORAFTI), and inulin (ORAFTI) produced similar concentrations of the individual (acetate, propionate, and butyrate) and total SCFA at each of the time points studied (2, 4, 8, and 12 h). Generally, SCFA production increased with time; after 12 h of fermentation, total SCFA production for short-chain FOS, oligofructose, and inulin was 5.7, 6.1, and 6.0 mmol/g OM, respectively. These levels of SCFA production were high in comparison to those for pure raffinose, soy solubles, granular and liquid transgalactooligosaccharides.
charides, glucooligosaccharides, and mannanoligosaccharides. Short-chain FOS, oligofructose, and inulin also had similar rates of acetate, propionate, and butyrate production and similar times to attain maximal rate of production, thus, demonstrating the high fermentability of these fructans.

Similar increases in SCFA production were observed in a study of dogs that consumed a high-quality kibbled diet supplemented with 4 g/d short-chain FOS (Swanson, 2002). An increase in fecal concentrations of total SCFA, propionate, and butyrate were observed with fructan supplementation in comparison to the control (514.8, 119.6, and 58.2 μmol/g of feces vs. 433.7, 83.6, and 40.8 μmol/g of feces, respectively). These studies support the conclusion that oligofructose and inulin are highly fermentable substrates that result in similar increases in SCFA production.

In addition to SCFA contributions to overall good health, individual SCFA exert specific health benefits. Propionate and butyrate can act independently to stimulate fluid absorption of calcium, magnesium, and other cations in the colon; this action also may be enhanced with the help of acetate (Topping, 1996). Propionate may enhance the absorptive capacity of the colon by stimulating proliferation of the colonic epithelium (Topping, 1996). Acetate and propionate were implicated in having regulatory effects on lipid and cholesterol metabolism; however, subsequent research rejects a major role of these SCFA in the control of plasma cholesterol (Demigne et al., 1986; Topping, 1996). Butyrate, while contributing only 15 to 20% of total SCFA, seems to make the greatest contribution to the integrity of the colon. It is the preferred energy source for colonocytes, contributing directly to energy production and producing a trophic effect on the colonic epithelium by increasing colonic crypt height and ecum size (Topping, 1996). Low concentrations of butyrate can potentially cause differentiation of mammalian cells as well as colon carcinoma cells (Roediger, 1980). Therefore, readily fermentable substrates, such as fructans, could be beneficial for the host animal due to the production of SCFA and their potential effects on optimizing gut health. Because approximately 95% of SCFA produced are rapidly absorbed from the colon, fecal analysis may not be the best response criterion reflecting SCFA status of the animal (McNeil et al., 1978). But short of sacrificing the animal to obtain colonic contents, fecal analysis remains the only viable option to assess this response.

Fecal Protein Catabolites

No significant differences in isobutyrate concentrations were noted among treatments (Table 4). Valerate concentrations tended to be higher (P < 0.10) with inulin consumption in comparison to the control; also, concentrations increased linearly (P < 0.02) with inulin. Isovalerate concentrations were higher (P < 0.01) for both fructans. A linear increase (P < 0.01) also was observed in isovalerate concentrations as a result of oligofructose and inulin supplementation. A quadratic response (P < 0.02) in isovalerate concentration resulted with oligofructose inclusion, with the 0.3% treatment having the highest fecal isovalerate concentration. When dogs were fed oligofructose, total BCFA concentrations were higher for the 0.3% treatment and lowest for the 0.9% treatment, resulting in a quadratic trend (P < 0.15). These data are in contrast with Flickinger et al. (2003), who found no change in total BCFA concentration when dogs were fed a corn-based diet supplemented with 1.9 g/d oligofructose. This type of response could perhaps explain the lack of effect on BCFA concentration when dogs were supplemented with 0.5% oligofructose alone (Strickling et al., 2000). However, higher concentrations may need to be examined for possible reduction of BCFA concentrations by inulin added to animal protein-based diets because the addition of this fructan at the concentrations tested in this experiment tended to increase valerate and isovalerate concentrations. This could be due to the higher degree of polymerization of inulin, resulting in fermentation in a more distal portion of the short hindgut of the dog, with subsequent excretion of higher amounts of BCFA in feces.

Fecal concentrations of phenylethylamine exhibited a trend to be higher (P < 0.06) for dogs supplemented with inulin in comparison to the control (Table 5). Putrescine (P < 0.11), cadaverine (P < 0.07), spermidine (P < 0.12), and total amines (P < 0.05) exhibited linear increasing trends with supplemental oligofructose. This is in contrast to Flickinger et al. (2003), who found no change in fecal amine concentrations with the addition of 1.9 g/d oligofructose or increasing concentrations of dietary short-chain FOS. Differences between these studies, which tested equivalent dietary concentrations of fructans, may be due to the type of supplemental fructan (short-chain FOS vs. oligofructose and inulin) and/or to the diet matrix used.

Amines are produced by decarboxylation reactions by bacteria in the colon. Polyamines (putrescine, spermine, and spermidine) are present in all living cells and are required for the metabolic activity and growth process of tissues and organs of the body (Bardocz et al., 1993). They stimulate DNA, RNA, and protein synthesis, making them important in the maturation of the intestinal and colonic mucosa (Tabor and Tabor, 1984; Delzenne et al., 2000). On the negative side, they have been correlated with odor production and an increased incidence of colon cancer. Johnson (1977) reported that cancer patients had more bacterial species with high colonic decarboxylase production than did healthy subjects. This indicates a potential for increased amounts of amine production in the presence of high decarboxylase levels and, therefore, a possible contribution to carcinogenesis. Therefore, when cell proliferation is required, polyamines can be beneficial; however, when cell proliferation is already adequate, it may be possible for additional polyamines to elicit harmful effects, such as stimulating tumorigenesis, in the gut.
Table 5. Fecal amine concentrations for ileal-cannulated dogs fed diets supplemented with selected concentrations of oligofructose (OF) and inulin (I)\(^a\)\(^b\)

| Item               | Control (C) | 0.3 | 0.6 | 0.9 | SEM | 0.3 | 0.6 | 0.9 | SEM | C vs. OF | C vs. I | OF | I |
|--------------------|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-------|----|---|
| Phenylethylamine   | 0.33        | 1.19| 1.00| 1.34| 0.8 | 0.97| 0.74| 0.83| 0.2 | 0.35 | 0.06 | 0.38 | 0.20| 0.76| 0.24|
| Putrescine         | 4.23        | 3.78| 4.65| 6.38| 1.0 | 4.05| 4.36| 5.60| 0.9 | 0.55 | 0.66 | 0.11 | 0.27| 0.37| 0.44|
| Cadaverine         | 1.86        | 2.14| 2.32| 3.34| 0.6 | 2.00| 2.35| 2.16| 0.4 | 0.26 | 0.49 | 0.07 | 0.45| 0.56| 0.70|
| Spermidine         | 1.99        | 2.23| 2.33| 2.48| 0.2 | 2.02| 2.05| 2.08| 0.2 | 0.20 | 0.81 | 0.12 | 0.76| 0.86| 0.99|
| Total amines\(^c\)| 8.42        | 9.35|10.30|13.53|1.8 | 9.01| 9.50|10.68|1.3 | 0.22 | 0.37 | 0.05 | 0.21| 0.59| 0.82|

\(^a\)Each mean represents the average value for seven individually cannulated dogs.
\(^b\)Values expressed as \(\mu\)mol/g of feces (DM basis).
\(^c\)Total amines (phenylethylamine + putrescine + cadaverine + spermidine).

The increase in fecal amine concentrations noted in the present study may be of importance because they are implicated as cell growth regulators. It seems that oligofructose supplementation could enhance this process as a result of its effect on increasing gut concentrations of these substances. In vivo, oligofructose and inulin may be fermented more slowly and in more distal sections of the colon, which would explain the increases in amine concentrations observed in our study when oligofructose was supplemented to dogs. This does not, however, explain the lack of an amine production response with inulin supplementation. To elicit this effect, perhaps inulin is required in dietary amounts larger than those employed in the present study. Although mean values among treatments did not reach statistical significance, other researchers using higher levels of inulin supplementation have noted significant effects. Remesy et al. (1993) reported that rats fed diets containing 15% inulin had an increase in cecal crypt height and bacterial decarboxylase activity, indicating a potential increase in amine production.

Table 6. Fecal indole and phenol concentrations for ileal-cannulated dogs fed diets supplemented with selected concentrations of oligofructose (OF) and inulin (I)\(^a\)\(^b\)

| Item    | Control (C) | 0.3 | 0.6 | 0.9 | SEM | 0.3 | 0.6 | 0.9 | SEM | C vs. OF | C vs. I | OF | I |
|---------|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-------|----|---|
| Indole  | 20.03       | 19.69| 17.18|20.19| 2.406|21.53|17.07|19.61|2.607| 0.71  | 0.83  | 0.84| 0.62| 0.55| 0.85|
| Phenol  | 2.11        | 1.62 | 1.59 | 1.71 | 0.452| 1.03 | 1.28 | 1.29 | 0.425| 0.37  | 0.08  | 0.50| 0.25| 0.56| 0.23|
| \(p\)-Cresol | 0.92     | 2.40 | 0.62 | 0.32 | 0.800| 0.84 | 0.69 | 0.94 | 0.496| 0.79  | 0.86  | 0.19| 0.96| 0.24| 0.75|
| Total phenols\(^c\) | 3.03 | 4.02 | 2.20 | 2.03 | 0.627| 1.86 | 1.97 | 2.23 | 0.395| 0.69  | 0.04  | 0.08| 0.20| 0.43| 0.10|

\(^a\)Each mean represents the average value for seven individually cannulated dogs.
\(^b\)Values expressed as \(\mu\)mol/g of feces (DM basis).
\(^c\)Total phenols (phenol + \(p\)-cresol).
fructan supplementation and in unadapted subjects, complaints of abdominal pain, bloating, and flatulence are common. In dogs, Russell (1998) determined that 1% short-chain FOS and 1.5, 3.0, and 5.0% supplemental chicory (a natural source of inulin) were capable of increasing bifidobacteria and, at times, SCFA production while decreasing Clostridium perfringens concentrations. However, poor stool quality in dogs was noted with the addition of 3 and 5% chicory, indicating a problem in using these high levels in dog feeding. Studies also exist that examine lower levels of supplemental dietary fructans, such as the current study and those discussed previously, but the physiological responses are varied. For example, Swanson (2002) used dogs supplemented with 4 g/d short-chain FOS (equivalent to 0.7% of the diet) and found that dogs had greater bifidobacteria and lower Clostridium perfringens concentrations compared to those fed sucrose. Dogs supplemented with short-chain FOS also tended to have higher fecal total SCFA and valerate concentrations, lower total phenol concentrations, but no effect on fecal ammonia or biogenic amine concentrations was observed. In another experiment by Swanson (2002), dogs were supplemented with 2 g/d of short-chain FOS (which constituted approximately 0.5% of the diet) and fewer bifidogenic responses were noted. Fecal indole and total phenol concentrations decreased; however, no differences were found in fecal SCFA, BCFA, bacteria, or total amine concentrations.

In summary, supplementation of fructans in the present study resulted in positive effects on indices known to be associated with gut health in dogs consuming a meat-based diet. No decreases in ileal nutrient digestibilities or negative effects on fecal volume or fecal score resulted from oligofructose or inulin feeding. Increases in fecal SCFA concentrations increased linearly with inclusion of both fructans, but more clearly with oligofructose ($P < 0.01$) than with inulin ($P < 0.10$). The quadratic trend observed in total BCFA concentration with the addition of oligofructose suggests that supplementation at the 0.9% level or greater may decrease concentrations of these odor-producing compounds. Dogs fed inulin had lower fecal phenol and total phenol concentrations. Also, a linear decrease in total phenols with oligofructose supplementation was observed. Total amine concentration increased for dogs supplemented with oligofructose. The overall effects on fecal concentrations of SCFA and protein catabolites varied between fructans and among fructan concentrations. In this experiment, more definitive beneficial effects and minimal adverse side-effects were noted for dogs fed the 0.9% oligofructose treatment, whereas the responses due to the concentrations of inulin tested were more variable.

Implications

The present study demonstrates clearly that oligofructose and inulin affect several aspects of diet digestibility. Total-tract dry matter and crude protein digestibilities were slightly decreased by the inclusion of the products, whereas short-chain fatty acid production was clearly increased with the products. However, differences between the various inclusion levels were less clear, suggesting the need for continued research. Fructans can be added to diets in their natural form (e.g., chicory) or as partially purified ingredients (e.g., short-chain fructooligosaccharides, oligofructose, inulin). It also is important to consider the type of diet (animal-vs. plant-based) to which the fructan is supplemented and whether the diet contains natural sources of fructooligosaccharides (e.g., wheat, wheat by-products) or other oligosaccharides (e.g., galactooligosaccharides in soy products). These factors may lead to differences among studies, making it difficult to identify an appropriate dosage of fructan to be included in diets both from physiologic and economic standpoints.

Literature Cited


