ABSTRACT: Blends of fermentable oligosaccharides in combination with nonfermentable fiber, cellulose, were evaluated for their ability to serve as dietary fibers in dog foods. Using a 6 × 6 Latin square design, 6 diets were evaluated that contained either no supplemental fiber, beet pulp, cellulose, or blends of cellulose, fructooligosaccharides, and yeast cell wall added at 2.5% of the diet. Six ileal-cannulated dogs were fed 175 g of their assigned diet twice daily. Chromic oxide served as a digestibility marker. Nutrient digestibility, fecal microbial populations, fermentative end products, and immunological indices were measured. Total tract DM and OM digestibilities were lowest (P < 0.05) for the cellulose treatment. Crude protein digestibility was lower (P < 0.05) for the treatments containing carbohydrate blends. The cellulose treatment had the lowest (P < 0.05) concentration of bacteria, and all diets containing fermentable fiber had greater (P < 0.05) fecal bifidobacteria concentrations compared with the diets without supplemental fermentable fiber. Lactobacilli concentrations tended to be greater (P < 0.08) in treatments containing fermentable fiber compared with the cellulose treatment. Bifidobacteria and lactobacilli concentrations were similar for the beet pulp treatment compared with the fermentable oligosaccharide blends. Total fecal short-chain fatty acid concentration was greater for the beet pulp treatment (P < 0.05) compared with the control and cellulose treatments. The treatments containing fermentable fiber had greater (P < 0.05) fecal butyrate concentrations compared with cellulose and control treatments. Immune indices were not affected by treatment. Our results suggest that dog foods containing blends of fermentable and nonfermentable carbohydrates produce similar physiological results as dog food containing beet pulp as a fiber source. Therefore, blends of these carbohydrates could be useful substitutes for beet pulp in dog foods.

Key words: beet pulp, dietary fiber, dog, intestinal microbiota, oligosaccharide

INTRODUCTION

Commercial pet foods contain significant amounts of carbohydrates. These carbohydrates are either digestible (mostly starch) or nondigestible (generally classified as dietary fiber). Dietary fiber is physiologically important, because it affects gastric emptying (Russell and Bass, 1985), digesta transit time (Burrows et al., 1982; Fahey et al., 1990a), fecal bulk (Fahey et al., 1992; Sunvold et al., 1995b), and short-chain fatty acid (SCFA) production in the intestine (Muir et al., 1996; Silvio et al., 2000), depending on fiber type and source.

Beet pulp is often used in pet foods because of its fermentation characteristics (Sunvold et al., 1995a) and its desirable effect on stool consistency (Fahey et al., 1992; Sunvold et al., 1995b). Beet pulp typically contains about 60 to 80% total dietary fiber (TDF; Fahey et al., 1990b; Sunvold et al., 1995a,b), of which about 80% is insoluble (Sunvold et al., 1995c); however, beet pulp tends to vary in quality.

Fermentable carbohydrates such as fructooligosaccharides (FOS) and mannanoligosaccharides (MOS) from yeast cell wall (YCW) have been evaluated for use in dog foods. Both are fermented by intestinal microbiota of dogs but MOS at a more moderate rate than FOS (Vickers et al., 2001). Additionally, FOS and MOS may increase lactobacilli and bifidobacteria concentrations (Swanson et al., 2002c) and decrease production of putrefactive compounds (phenols and indoles; Swanson et al., 2002b). The purity and consistency of FOS and MOS products may make them useful as fiber sources in pet foods.

In this study, nondigestible, but fermentable, oligosaccharides were evaluated as potential replacements for more traditional dietary fiber sources. The blends tested contained both fermentable and nonfermentable fibers to create a balance of insoluble and soluble fiber.
components. Additionally, the effects of these fermentable carbohydrates on intestinal health, intestinal microbiota, and immune status of the animal were investigated.

**MATERIALS AND METHODS**

**Animals and Diets**

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

Six purpose-bred adult female dogs (Marshall Bioreources, North Rose, NY) with hound bloodlines, an average initial BW of approximately 23 kg, and an average age of 4.5 yr were surgically prepared with an ileal T-shaped cannula according to Walker et al. (1994). After the surgery, dogs were closely monitored daily for clinical abnormalities and given a 2-wk recovery period before the beginning 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 brewers rice, poultry by-product meal, and poultry fat constituting the main ingredients of the dry, extruded kibble diets (Table 1). The diet formulation was milled at Lortscher Agri Service Inc. (Bern, KS) and extruded at Kansas State University’s Bioprocessing and Industrial Value-Added Program facility (Manhattan) under the direction of Pet Food and Ingredient Technology Inc. (Topeka, KS). All fiber treatments were incorporated into the diets before extrusion. A total of 6 diets were prepared with the following fiber sources incorporated:

1) control diet – no supplemental fermentable carbohydrate (formulated to analyze as approximately 1.5% TDF);
2) as (1) + 2.5% cellulose (a highly refractory, poorly fermentable carbohydrate);
3) as (1) + 2.5% beet pulp (a moderately fermentable fiber source);
4) as (1) + 1.0% cellulose + 1.5% short-chain FOS (Nutraflora P-95, GTC Nutrition, Golden, CO; CF);
5) as (1) + 1.0% cellulose + 1.2% short-chain FOS + 0.3% YCW (Safmannan, LeSaffre Yeast Corp., Milwaukee, WI; CFY1); and
6) as (1) + 1.0% cellulose + 0.9% short-chain FOS + 0.6% YCW (CFY2).

Dogs were offered 175 g of their assigned diet twice daily (0800 and 2000). Chromic oxide was used as a digestion marker. On d 6 through 14 of each period, dogs were dosed with 0.5 g of Cr₂O₃ at each feeding via a gelatin capsule, for a total of 1.0 g of marker/d. Fresh water was available at all times.

**Sample Collection**

A 6 × 6 Latin square design with 14-d periods was used. A 10-d adaptation phase preceded a 4-d collection

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Table 1. Composition of diets containing select dietary fiber sources and fed to adult dogs (as-fed basis)

| Ingredient, % | Control | Cellulose | Beet pulp | CF¹ | CFY¹² | CFY²² |
|---------------|---------|-----------|-----------|-----|-------|-------|
| Brewers rice  | 45.22   | 42.72     | 42.72     | 42.72| 42.72 | 42.72 |
| Poultry by-product meal | 37.00   | 37.00     | 37.00     | 37.00| 37.00 | 37.00 |
| Poultry fat   | 14.00   | 14.00     | 14.00     | 14.00| 14.00 | 14.00 |
| Dried egg     | 2.40    | 2.40      | 2.40      | 2.40 | 2.40  | 2.40  |
| Salt          | 0.45    | 0.45      | 0.45      | 0.45 | 0.45  | 0.45  |
| Potassium chloride | 0.56   | 0.56      | 0.56      | 0.56 | 0.56  | 0.56  |
| Choline chloride⁴ | 0.13   | 0.13      | 0.13      | 0.13 | 0.13  | 0.13  |
| Vitamin mix⁵  | 0.12    | 0.12      | 0.12      | 0.12 | 0.12  | 0.12  |
| Mineral mix⁶  | 0.12    | 0.12      | 0.12      | 0.12 | 0.12  | 0.12  |
| Cellulose     | —       | 2.50      | —         | 1.00 | 1.00  | 1.00  |
| Beet pulp     | —       | —         | 2.50      | —   | —     | —     |
| Fructooligosaccharides⁷ | —   | —         | —         | 1.50 | 1.20  | 0.90  |
| Yeast cell wall⁸ | —     | —         | —         | —   | 0.30  | 0.60  |

¹CF = 1% cellulose + 1.5% fructooligosaccharides.
²CFY1 = 1% cellulose + 1.2% fructooligosaccharides + 0.3% yeast cell wall.
³CFY2 = 1% cellulose + 0.9% fructooligosaccharides + 0.6% yeast cell wall.
⁴Provided the following per kilogram of diet: choline, 2,284.2 mg.
⁵Provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D₃, 900 IU; 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, 8.6 mg; biotin, 12.4 mg; folic acid, 1,142.1 μg; and vitamin B₁₂, 164.9 μg.
⁶Provided the following per kilogram of diet: manganese (MnSO₄), 17.4 mg; iron (FeSO₄), 284.3 mg; copper (CuSO₄), 17.2 mg; cobalt (CoSO₄), 2.2 mg; zinc (ZnSO₄), 166.3 mg; iodine (KI), 7.5 mg; and selenium (Na₂SeO₃), 0.2 mg.
⁷Nutraflora P-95, GTC Nutrition, Golden, CO.
⁸Safmannan, LeSaffre Yeast Corp., Milwaukee, WI.
of feces and ileal effluent. Ileal effluent was collected 3 times/d, at 4-h intervals. Each collection was 1 h in length. Sampling times were rotated 1 h from the previous day’s collection time. For example, sampling times on the first collection day were 0800, 1200, and 1600; on the second day, samples were collected at 0900, 1300, and 1700, etc. Ileal samples were collected by attaching a sterile sampling bag (Whirlpack, 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.

Although nutrient digestibility was calculated based on digestion marker recovery, total feces excreted during the collection phase of each period was removed from the floor of the pen, weighed, and composited to obtain the most representative sample. Fecal samples were frozen at −20°C until analysis. On d 14 of each period, a fresh fecal sample was collected within 15 min of defection for the measurement of pH and bacterial enumeration. All fecal samples during the 4-d collection period were frozen at −20°C until analysis. On d 14 of each collection day, a blood sample (5 mL) was collected via jugular puncture into nonheparinized evacuated tubes to obtain serum immunoglobulin A, G, and M concentrations. Another 5-mL blood sample was collected in evacuated tubes with EDTA for a complete blood count (total white blood cells, neutrophil, eosinophil, lymphocyte, and monocyte).

Sample Handling

Ileal 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 and 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 and diets were dried at 55°C in a forced-air oven. After drying, diets, fecal samples, and ileal samples were ground through a 2-mm screen in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ).

On d 14 of each period, fresh fecal samples were collected within 15 min of defection, and an aliquot was immediately transferred to a preweighed Cary-Blair transport media container (Meridian Diagnostics Inc., Cincinnati, OH) for subsequent bacterial enumeration (total anaerobes, total aerobes, bifidobacteria, lactobacilli, Clostridium perfringens, and Escherichia coli). Aliquots of fresh feces were transferred to sterile cryogenic vials (Nalgene, Rochester, NY) and frozen at −80°C until DNA extraction for microbial analysis (bifidobacteria, lactobacilli, C. perfringens, and E. coli). 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 10 mL of 2 N hydrochloric acid for SCFA, branched-chain fatty acids (BCFA), and ammonia analyses. Additional aliquots were used for pH measurement and fresh fecal DM determination.

Chemical Analyses

Diets, feces, and ileal samples were analyzed for DM, OM, and ash using AOAC (2000) methods. Crude protein was calculated from Leco total N values (AOAC, 2000). Amino acid concentrations in the diets were analyzed at the University of Missouri Experiment Station Chemical Laboratories using a Beckman 6300 AA analyzer (Beckman-Coulter Inc., Fullerton, CA) according to AOAC (2000) methods. Total lipid content (acid hydrolyzed fat, AHF) was determined by acid hydrolysis followed by ether extraction according to AACC (1983) and Budde (1952). Dietary fiber concentrations [TDF, soluble dietary fiber (SDF), and insoluble dietary fiber (IDF)] were determined according to Prosky et al. (1984, 1992). Gross energy was measured using an oxygen bomb calorimeter (model 1261, Parr Instruments, Moline, IL). Chromium concentrations in digesta and fecal samples were analyzed according to Williams et al. (1962) using atomic absorption spectrophotometry (model 2380, Perkin-Elmer, Norwalk, CT). Short-chain fatty acid and BCFA 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. Phenol and indole concentrations were determined using gas chromatography according to the methods of Flickinger et al. (2003). Biogenic amine concentrations were measured by HPLC according to the methods of Flickinger et al. (2003).

Microbial Analyses

Microbial populations were determined by serial dilution and plating and by DNA extraction from fecal samples, followed by quantitative PCR (qPCR) and denaturing gradient gel electrophoresis techniques as described in detail elsewhere (Middelbos et al., 2007). The purpose of using 2 distinct methods for microbial
analysis was to compare the 2 techniques under the same conditions (the same fecal samples).

**Immunological Analyses**

Ileal immunoglobulin A concentrations were measured according to the methods of Nara et al. (1983). Freshly collected ileal fluid was frozen at −20°C in sterile collection bags. The frozen samples were lyophilized and crushed using a mortar and pestle. A 2-g aliquot of each lyophilized and crushed sample was suspended in 20 mL of PBS solution (pH 7.2) and mixed for 30 min at room temperature. Samples then were centrifuged at 20,000 × g for 30 min at 4°C. The supernatant was collected and ileal immunoglobulin A concentrations determined using radial immunodiffusion kits (MP Biomedicals, Aurora, OH).

After blood was collected in nonheparinized evacuated tubes, samples were centrifuged at 2,000 × g for 20 min at 4°C, and the serum was collected. Serum immunoglobulin A, immunoglobulin G, and immunoglobulin M concentrations were determined using radial immunodiffusion kits. The blood collected in evacuated tubes containing EDTA was used for complete blood count determination, which was performed on a Cell-Dyn 3500 hematology analyzer (Abbott Laboratories, Abbott Park, IL).

**Calculations**

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 effluent). Ileal nutrient flows were calculated by multiplying DM flow by the concentration of the nutrient in the ileal DM. Ileal nutrient digestibilities were calculated as nutrient intake (g/d) minus ileal nutrient flow (output, g/d), and this value was then divided by nutrient intake (g/d). Similar calculations were performed on fecal samples to determine total tract nutrient digestibilities.

**Statistical Analysis**

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 experimental design was a 6 × 6 Latin square. The statistical model included the random effects of animal and period and the fixed effect of treatment. All treatment least squares means were compared with each other, and the Tukey adjustment was used to control for experimentwise error. Differences among 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. Dry matter, OM, and GE concentrations were consistent among diets, but the CP and AHF concentrations varied slightly. Total dietary fiber, IDF, and SDF varied consistently with the added fiber components.

Nutrient intakes and apparent ileal and total tract nutrient digestibilities are presented in Table 3. Feed refusals were minimal, and nutrient intakes were similar among treatments. Crude protein intake varied slightly with the CP concentrations in the diet. The intakes for TDF, IDF, and SDF also varied according to their respective concentrations in the treatment diets.

Total tract DM digestibility was lower \( (P < 0.05) \) for the cellulose treatment compared with the control treatment (83.1 vs. 86.2%). Trends were noted for DM digestibility between the CF and control treatment \( (P = 0.06; 84.1 \text{ vs. } 86.2\%) \) and between the CFY2 and cellulose treatments \( (P = 0.10; 83.2 \text{ vs. } 85.1\%) \). Organic matter digestibility was decreased \( (P < 0.05) \) for the cellulose, CF, and CFY1 treatments compared with control (88.7, 89.8, and 90.0 vs. 91.7%, respectively). Additionally, the cellulose treatment had decreased \( (P < 0.05) \) OM digestibility compared with the beet pulp and CFY2 treatments and tended \( (P = 0.09) \) to have decreased OM digestibility compared with the CFY1 treatment (88.7 vs. 90.0%). Crude protein digestibility was decreased \( (P < 0.05) \) for the treatments supplemented with fermentable oligosaccharides (−84.7%) compared with the control, beet pulp, and cellulose treatments (−86.8%). Fat digestibility, in general, was high (96 to 97%) but was decreased \( (P < 0.05) \) for the CFY1 treatment compared with the control and cellulose treatments. Gross energy digestibility was decreased \( (P < 0.05) \) for the cellulose, CF, and CFY1 treatments compared with control (89.8, 90.6, and 90.5 vs. 91.9%, respectively).

Total dietary fiber digestibility for the beet pulp treatment (39.1%) was greater \( (P < 0.05) \) than for the cellulose (11.5%), CF (15.3%), and CFY1 (14.2%) treatments. Total dietary fiber digestibility values for the control (27.3%) and CFY2 (25.2%) treatments were not different from any of the other treatments.

Complete blood counts and serum and ileal immunoglobulin concentrations are presented in Table 4. No differences were detected among treatments in white blood cell counts or immunoglobulin concentrations.

Fecal microbial concentrations are presented in Table 5. According to serial dilution and plating methods, bifidobacteria concentrations were greatest for the CF treatment, and the only difference detected was between the CF and cellulose treatments \( (P < 0.05) \). Trends were noted for decreased bifidobacteria concentrations for the control treatment compared with CF \( (P = 0.06) \) and CFY2 compared with the cellulose treatment \( (P = 0.09) \). *Clostridium perfringens*, *E. coli*, and lactobacilli concentrations were not different among treatments, although trends \( (P = 0.07) \) were detected for decreased lactobacilli concentrations in the control and cellulose treatments compared with the CF treatment. Total aerobic bacteria concentrations were greater \( (P < 0.05) \) for the CF treatment compared with the cellulose treatment and tended \( (P = 0.06) \) to be...
greater for the CF treatment compared with the control treatment (10.0 vs. 9.0 \( \log_{10} \text{cfu/g} \)). Total anaerobic bacterial concentrations were greater \( (P < 0.05) \) for the CF and CFY2 treatments compared with the cellulose treatment. Additionally, there was a trend \( (P = 0.09) \) for greater total anaerobic bacterial concentrations for the CF treatment compared with the control.

Fecal bacterial enumeration by qPCR did not indicate significant differences in \( C. \) perfringens or \( E. \) coli populations among treatments. Bifidobacteria concentrations, however, were greater \( (P < 0.05) \) in the treatments supplemented with fermentable substrate (beet pulp, CF, CFY1, and CFY2). The CF and CFY2 treatments resulted in greater \( (P < 0.05) \) lactobacilli concentrations compared with the cellulose treatment. Additionally, trends for increased lactobacilli concentrations were noted with the beet pulp \( (P = 0.07) \) and CFY1 \( (P = 0.08) \) treatments compared with the cellulose treatment.

Analysis of total fecal bacterial DNA is presented in Table 6. The mean Dice’s scores given in the table represent comparisons between 2 treatments and, thus, cannot be separated in a classical statistical manner. The pooled SEM indicates overall variation present in the matrix of comparisons.

There is a clear pattern in the similarity of fecal DNA among treatments. The control treatment and cellulose treatment had a high degree of similarity \( (87\%) \), as did the treatments containing fermentable fiber sources (beet pulp, CF, CFY1, and CFY2; 85 to 88\%). The control treatment and the treatments containing fermentable fiber had slightly lower similarity \( (84 to 85\%) \), whereas the cellulose treatment resulted in the least similar fecal DNA compared with the treatments containing fermentable fibers \( (80 to 83\%) \).

Fecal pH and score, daily wet fecal output, and concentrations of fecal ammonia, SCFA, BCFA, phenol, and indole are presented in Table 7. Fecal pH was unaffected by the dietary fiber treatments, as was fecal score. Wet fecal output was not significantly different among treatments, but the beet pulp treatment tended
Table 3. Food intake and apparent ileal and total tract digestibility by dogs fed diets containing select dietary fiber sources

| Treatment | Control | Cellulose | Beet pulp | CFY12 | CFY23 | SEM |
|-----------|---------|-----------|-----------|-------|-------|-----|
| Intake, g/d DM | 296 | 278 | 302 | 282 | 263 | 285 | 18.0 |
| OM | 272 | 256 | 277 | 259 | 242 | 263 | 16.5 |
| CP | 99 | 92 | 100 | 90 | 80 | 84 | 5.7 |
| Acid hydrolyzed fat | 63 | 60 | 62 | 63 | 52 | 58 | 3.8 |
| GE, kcal/d | 1,584 | 1,499 | 1,568 | 1,511 | 1,374 | 1,497 | 95.3 |
| Total dietary fiber | 7.5 | 14.1 | 12.2 | 9.0 | 9.2 | 9.9 | 0.65 |
| Insoluble fiber | 5.0 | 11.7 | 7.8 | 6.5 | 5.9 | 6.8 | 0.49 |
| Soluble fiber | 2.5 | 2.4 | 4.2 | 2.6 | 3.2 | 3.1 | 0.19 |
| Ileal digestibility, % DM | 78.0 | 57.8 | 67.5 | 73.1 | 77.2 | 72.6 | 6.0 |
| OM | 83.9 | 76.4 | 75.7 | 76.7 | 74.8 | 79.5 | 4.6 |
| CP | 74.0 | 55.4 | 65.0 | 70.7 | 74.2 | 68.4 | 6.5 |
| Acid hydrolyzed fat | 95.9 | 93.1 | 93.6 | 96.1 | 96.0 | 95.5 | 1.0 |
| GE | 86.1 | 71.6 | 77.9 | 82.5 | 85.0 | 81.8 | 4.1 |
| Total dietary fiber | −26.2 | −36.3 | −11.5 | −12.2 | −2.6 | −40.7 | 26.2 |
| Total tract digestibility, % DM | 86.2 | 83.2 | 85.4 | 84.1 | 84.2 | 85.1 | 0.9 |
| OM | 91.7 | 88.7 | 91.0 | 89.8 | 90.0 | 90.5 | 0.6 |
| CP | 86.9 | 86.7 | 87.0 | 84.9 | 84.7 | 84.8 | 1.0 |
| Acid hydrolyzed fat | 97.2 | 97.1 | 96.6 | 96.7 | 95.9 | 96.5 | 0.3 |
| GE | 91.9 | 89.8 | 91.3 | 90.6 | 90.5 | 90.9 | 0.6 |
| Total dietary fiber | 27.3 | 11.5 | 39.1 | 15.3 | 14.2 | 25.2 | 6.2 |

Means in the same row not sharing common superscript letters are different \((P < 0.05)\).

\(1\) CF = 1% cellulose + 1.5% fructooligosaccharides.

\(2\) CFY1 = 1% cellulose + 1.2% fructooligosaccharides + 0.3% yeast cell wall.

\(3\) CFY2 = 1% cellulose + 0.9% fructooligosaccharides + 0.6% yeast cell wall.

\((P = 0.09)\) to result in greater wet fecal output compared with the CFY1 treatment.

Fecal ammonia concentration was not affected significantly by dietary treatment. Per gram of fecal DM, the beet pulp treatment resulted in the greatest fecal concentration of acetate, which was different \((P < 0.05)\) from values for the cellulose and control treatments.

Table 4. Complete blood count and serum and ileal immunoglobulin concentrations in adult dogs fed diets containing select dietary fiber sources

| Treatment | Control | Cellulose | Beet pulp | CFY12 | CFY23 | SEM |
|-----------|---------|-----------|-----------|-------|-------|-----|
| Blood cell count, thousands/\(\mu\)L Total white cells | 11.3 | 11.7 | 11.8 | 9.9 | 10.1 | 10.9 | 1.0 |
| Neutrophils | 7.3 | 7.7 | 8.4 | 6.9 | 6.4 | 7.1 | 0.8 |
| Lymphocytes | 2.5 | 2.0 | 1.9 | 1.9 | 2.3 | 2.4 | 0.3 |
| Monocytes | 1.0 | 1.1 | 0.9 | 0.6 | 0.7 | 0.7 | 0.2 |
| Eosinophils | 0.5 | 0.8 | 0.6 | 0.6 | 0.7 | 0.6 | 0.1 |
| Serum immunoglobulins, mg/dL Immunoglobulin A | 38.6 | 39.6 | 43.8 | 40.4 | 35.3 | 39.0 | 7.3 |
| Immunoglobulin G | 1,209 | 1,306 | 1,218 | 1,264 | 1,332 | 1,244 | 145.0 |
| Immunoglobulin M | 165.8 | 143.6 | 148.7 | 148.8 | 146.8 | 151.1 | 15.3 |
| Ileal immunoglobulins, mg/g of DM Immunoglobulin A | 4.3 | 4.5 | 3.4 | 4.1 | 4.7 | 4.4 | 1.3 |

\(1\) CF = 1% cellulose + 1.5% fructooligosaccharides.

\(2\) CFY1 = 1% cellulose + 1.2% fructooligosaccharides + 0.3% yeast cell wall.

\(3\) CFY2 = 1% cellulose + 0.9% fructooligosaccharides + 0.6% yeast cell wall.

Fecal propionate concentrations were greatest for the beet pulp treatment and greater \((P < 0.05)\) than for the control, cellulose, and CF treatments. Both CFY1 and CFY2 treatments had greater \((P < 0.05)\) propionate concentrations than did the control and cellulose treatments. Additionally, the propionate concentration for the CF treatment tended \((P = 0.08)\) to be greater than
Table 5. Fecal microbial populations for dogs fed diets containing select dietary fiber sources

| Treatment       | Control | Cellulose | Beet pulp | CF1    | CFY12  | CFY23  | SEM  |
|-----------------|---------|-----------|-----------|--------|--------|--------|------|
| **Population (plating)** |         |           |           |        |        |        |      |
| Bifidobacteria  | 10.1ab  | 10.0b     | 10.3ab    | 10.6c  | 10.2ab | 10.5ab | 0.13 |
| *Clostridium perfringens* | 9.8     | 9.9       | 9.8       | 9.8    | 9.9    | 10.0   | 0.19 |
| *Escherichia coli* | 8.0     | 8.1       | 8.4       | 8.0    | 8.7    | 7.9    | 0.25 |
| Lactobacilli    | 9.3     | 9.4       | 9.6       | 10.4   | 9.8    | 10.1   | 0.34 |
| Total aerobes   | 8.6ab   | 8.9b      | 9.6ab     | 10.0a  | 9.8b   | 9.8ab  | 0.28 |
| Total anaerobes | 10.1ab  | 10.5ab    | 10.8ab    | 11.0a  | 10.9b  | 11.0a  | 0.11 |
| **Population (quantitative PCR)** |         |           |           |        |        |        |      |
| Bifidobacteria  | 7.7b    | 7.8b      | 8.9a      | 8.7a   | 9.1a   | 8.7a   | 0.30 |
| *Clostridium perfringens* | 10.7    | 10.2      | 11.3      | 11.2   | 11.2   | 11.0   | 0.38 |
| Lactobacilli    | 11.3ab  | 11.2b     | 12.0ab    | 12.2a  | 12.1ab | 12.1a  | 0.23 |
| *E. coli*       | 10.3    | 10.1      | 10.6      | 10.7   | 10.7   | 10.1   | 0.32 |

*abMeans in the same row not sharing common superscript letters are different (P < 0.05).

1CF = 1% cellulose + 1.5% fructooligosaccharides.

2CFY1 = 1% cellulose + 1.2% fructooligosaccharides + 0.3% yeast cell wall.

3CFY2 = 1% cellulose + 0.9% fructooligosaccharides + 0.6% yeast cell wall.

for the cellulose treatment. Butyrate concentrations were decreased (P < 0.05) for the control and cellulose treatments compared with treatments that were supplemented with some form of fermentable fiber where values were similar to each other. Isobutyrate concentrations were not different among treatments. Isovalerate concentrations were greater (P < 0.05) for the CF and CFY1 treatments than for the cellulose treatment. Valerate concentrations were greater (P < 0.05) for the CF and CFY1 treatments compared with the cellulose treatment. Trends were noted for decreased valerate concentrations for the cellulose compared with the beet pulp (P = 0.07) and CFY2 (P = 0.06) treatments.

Phenol, p-cresol, and indole concentrations were not affected by treatment. Phenol concentrations for the CFY2 treatment were below the detection limit for all samples. No 4-ethyl-phenol, 7-methyl-indole, 3-methyl-indole, 2-methyl-indole, or 2,3-dimethyl-indole was detected in any of the samples.

Fecal biogenic amine concentrations are presented in Table 8. Total biogenic amine concentrations were not affected by dietary treatment. The CFY2 treatment resulted in the greatest concentration of biogenic amines (6.53 μmol/g of DM), whereas the cellulose treatment resulted in the lowest concentration (2.55 μmol/g of DM). Of the individual amines, putrescine was greater (P < 0.05) for the CFY2 treatment compared with the cellulose treatment.

**DISCUSSION**

In this experiment, nondigestible, but fermentable, oligosaccharides were evaluated as potential replacements for more traditional dietary fiber sources. The blends of carbohydrates tested contained both fermentable and nonfermentable fibers to create a balance of insoluble and soluble fiber components.

The variation in CP intake was related directly to the CP concentrations in the diets. This is evident, because the DM and OM intakes were very similar. However, dietary CP concentrations in all diets were well above minimum requirements, and protein intake was...
Table 7. Fecal pH, score, and concentrations of ammonia, short-chain fatty acids, branched-chain fatty acids, phenols, and indoles for dogs fed diets containing select dietary fiber sources

| Treatment      | Control | Cellulose | Beet pulp | CF \(^1\) | CFY \(^1\) \(2\) | CFY \(^2\) \(3\) | SEM  |
|----------------|---------|-----------|-----------|-----------|----------------|----------------|------|
| pH             | 6.7     | 6.5       | 6.3       | 6.5       | 6.4            | 6.3            | 0.14 |
| Score\(^4\)    | 2.9     | 2.7       | 2.7       | 2.7       | 2.6            | 2.6            | 0.18 |
| Fecal output (as-is), g/d | 73.2    | 87.6      | 89.6      | 80.1      | 69.0           | 76.6           | 7.7  |
| Ammonia, \(\mu\)mol/g of DM | 164     | 132       | 191       | 177       | 169            | 175            | 15.1 |
| Short-chain fatty acids, \(\mu\)mol/g of DM | 172\(^b\) | 127\(^b\) | 276\(^a\) | 187\(^{ab}\) | 196\(^{ab}\) | 187\(^{ab}\) | 24.3 |
| Acetate        | 63\(^{bc}\) | 49\(^b\)  | 93\(^a\)  | 70\(^{bc}\) | 84\(^{ab}\)    | 85\(^{ab}\)    | 6.2  |
| Propionate     | 28\(^{b}\) | 21\(^b\)  | 42\(^{a}\) | 40\(^a\)   | 41\(^{a}\)      | 42\(^{a}\)      | 4.6  |
| Butyrate       | 12.7\(^{ab}\) | 9.6\(^{b}\) | 16.1\(^{ab}\) | 18.7\(^{a}\) | 16.9\(^{a}\)    | 16.3\(^{ab}\)  | 2.0  |
| Total          | 262\(^{b}\) | 197\(^{b}\) | 411\(^{a}\) | 297\(^{ab}\) | 321\(^{ab}\)   | 314\(^{ab}\)   | 33.2 |
| Branched-chain fatty acids, \(\mu\)mol/g of DM | 5.6     | 4.8       | 6.6       | 6.5       | 6.9            | 6.0            | 0.7  |
| Isobutyrate    | 10.4\(^{ab}\) | 8.3\(^{b}\) | 12.2\(^{ab}\) | 13.4\(^{a}\) | 13.5\(^{a}\)    | 11.8\(^{ab}\)  | 1.3  |
| Isovalerate    | 12.7\(^{ab}\) | 9.6\(^{b}\) | 16.1\(^{ab}\) | 18.7\(^{a}\) | 16.9\(^{a}\)    | 16.3\(^{ab}\)  | 2.0  |
| Phenols and indoles, \(\mu\)g/g of DM | 20.1     | 9.1       | 4.7       | 7.4       | 6.9            | Trace          | 8.0  |
| Phenol         | 79.9    | 50.5      | 30.6      | 40.6      | 73.7           | 19.7           | 17.0 |
| p-Cresol       | 224.1   | 193.3     | 254.5     | 239.9     | 173.7          | 201.2          | 28.4 |

\(^{a,b}\)Means in the same row not sharing common superscript letters are different \((P < 0.05)\).

1. CF = 1% cellulose + 1.5% fructooligosaccharides.
2. CFY \(^1\) = 1% cellulose + 1.2% fructooligosaccharides + 0.3% yeast cell wall.
3. CFY \(^2\) = 1% cellulose + 0.9% fructooligosaccharides + 0.6% yeast cell wall.
4. Score based on the following scale: 1 = hard, dry pellets; small, hard mass; 2 = hard-formed, dry stool; remains firm and soft; 3 = soft, formed, and moist stool; retains shape; 4 = soft, unformed stool; assumes shape of container; and 5 = watery; liquid that can be poured.
5. 4-Ethyl-phenol, 7-methyl-indole, 3-methyl-indole, 2-methyl-indole, and 2,3-dimethyl-indole were not detected in any sample.

at least double the recommended CP intake on a metabolic BW basis (NRC, 2006).

Intake of TDF, IDF, and SDF also varied among treatment. This observation is not surprising, because treatment diets were formulated to contain different concentrations of dietary fiber. The control treatment had the lowest fiber intake as expected, whereas the cellulose treatment had the greatest intake, which is caused by the refractory nature of cellulose (>90% TDF; Sunvold et al., 1995a,b,c). The beet pulp treatment resulted in a greater fiber intake compared with most other treatments due to the relatively high fiber concentration in beet pulp (~60 to 80%; Fahey et al., 1990b; Sunvold et al., 1995c). The fermentable oligosaccharide

Table 8. Fecal biogenic amine concentrations in dogs fed diets containing select dietary fiber sources

| Treatment      | Control | Cellulose | Beet pulp | CF \(^1\) | CFY \(^1\) \(2\) | CFY \(^2\) \(3\) | SEM  |
|----------------|---------|-----------|-----------|-----------|----------------|----------------|------|
| Total biogenic amines\(^4,5\) | 4.01     | 2.55      | 5.11      | 4.03      | 5.14           | 6.53           | 1.55 |
| Agmatine       | 0.38    | 0.25      | 0.29      | 0.07      | 0.59           | 0.47           | 0.29 |
| Cadaverine     | 0.42    | 0.23      | 0.55      | 0.26      | 0.61           | 0.67           | 0.18 |
| Histamine      | 0.12    | 0.10      | 0.07      | 0.00      | 0.14           | 0.11           | 0.09 |
| Putrescine     | 1.02\(^{ab}\) | 0.35\(^{b}\) | 1.98\(^{ab}\) | 2.21\(^{ab}\) | 1.27\(^{ab}\)   | 3.04\(^{a}\)   | 0.73 |
| Spermidine     | 1.10    | 0.90      | 1.16      | 0.95      | 1.17           | 1.12           | 0.31 |
| Spermine       | 0.30    | 0.26      | 0.19      | 0.03      | 0.30           | 0.22           | 0.14 |
| Tryptamine     | 0.32    | 0.26      | 0.42      | 0.35      | 0.46           | 0.40           | 0.13 |
| Tyramine       | 0.36    | 0.21      | 0.45      | 0.16      | 0.52           | 0.48           | 0.18 |

\(^{a,b}\)Means in the same row not sharing common superscript letters are different \((P < 0.05)\).

1. CF = 1% cellulose + 1.5% fructooligosaccharides.
2. CFY \(^1\) = 1% cellulose + 1.2% fructooligosaccharides + 0.3% yeast cell wall.
3. CFY \(^2\) = 1% cellulose + 0.9% fructooligosaccharides + 0.6% yeast cell wall.
4. Phenylethylamine was analyzed but present only in trace amounts (~0.01 \(\mu\)mol/g of DM).
5. Total biogenic amines equal the sum of the individual amines listed in Table 8 plus phenylethylamine.
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treatments resulted in lower fiber intakes, not because fermentable carbohydrate was present in lower concentrations, but because FOS in particular do not analyze as TDF due to their low degree of polymerization and the resulting inability to precipitate in 80% ethanol.

The high total tract nutrient digestibility for all diets is consistent with the dietary formulation that contained high-quality ingredients. Total tract DM digestibility was greater for the control and beet pulp treatment compared with cellulose treatment. Cellulose increases DM output and can thereby decreases DM digestibility. Muir et al. (1996) reported that increasing concentrations (2.5 to 7.5%) of Solka floc depressed total tract DM, OM, and CP digestibility while not affecting ileal nutrient digestibility. Total tract OM digestibility was greater for the control treatment than for the cellulose, CF, and CFY1 treatments. This could possibly be attributed to TDF composition, because the cellulose, CF, and CFY1 treatments numerically had the lowest TDF digestibilities. This would also account for the difference in OM digestibility between the beet pulp and CFY2 treatments compared with cellulose. Digestible energy responded similarly to OM digestibility, with the exception of the difference between the CFY2 and cellulose treatments, and may be related to TDF digestibility. Total tract CP digestibility was greater for the control, cellulose, and BP treatments compared with the CF, CFY1, and CFY2 treatments. The high CP digestibility of the control and cellulose treatments (compared with the treatments with fermentable oligosaccharides) is probably due to the lack of fermentable substrate and a resulting limited formation of bacterial biomass. Increased synthesis of bacterial biomass and the subsequently decreased CP digestibility is the result of GE derived from oligosaccharide fermentation. This effect will, in turn, slightly affect DM and OM digestibility, as was noted herein. The high CP digestibility of the beet pulp treatment is slightly surprising, because bacterial counts were high for this treatment (discussed later). Although the control and cellulose treatments had statistically greater AHF digestibilities compared with the CFY1 treatment, the small numerical difference (<1.3%) is not likely to affect the animal. The greater TDF digestibility by dogs fed the beet pulp treatment compared with the cellulose, CF, and CFY1 treatments is not surprising, because this treatment contained the greatest concentration of soluble fiber. The refractory nature of cellulose resulted in lower TDF digestibility by dogs fed the cellulose treatment and is probably responsible for the decreased TDF digestibility of the CF and CFY1 treatments. It is unlikely that the presence of FOS and MOS from YCW contribute to differences in TDF digestibility, because these oligosaccharides are too soluble to analyze as TDF. The digestibility of TDF for the beet pulp treatment is comparable to previous work from our laboratory (Fahey et al., 1990b), and although beet pulp at greater concentrations (>7.5%) may affect nutrient digestibility, the 2.5% concentration used here does not have this drawback.

Immunological indices were not affected by dietary treatment in this study, although previous research with fermentable oligosaccharides in dogs (O’Carra, 1997; Swanson et al., 2002b,c; Grieshop et al., 2004; Middelbos et al., 2007) suggested that white blood cell counts and immunoglobulin concentrations may be altered. All values reported herein, however, are within physiological ranges for adult dogs. Perhaps greater concentrations of fibrous constituents are necessary to affect immunological outcomes.

Fecal microbial populations were affected by dietary treatment. Bifidobacteria concentrations were significantly greater for the CF treatment compared with the cellulose treatment. In combination with the tendencies of greater bifidobacteria counts for the CF compared with the control treatment and the CFY2 treatment compared with the cellulose treatment, these observations suggest preference of bifidobacteria for treatments containing FOS and MOS. The effect of fermentable fiber is more striking when qPCR is used for microbial analysis. Both the control and cellulose treatments had significantly decreased bifidobacteria counts compared with the beet pulp, CF, CFY1, and CFY2 treatments. These results suggest that blends of fermentable oligosaccharides, but also beet pulp, are able to increase bifidobacteria in the canine intestine. Clostridium perfringens and E. coli concentrations were not affected by dietary treatment according to microbiota analysis by both serial dilution and plating and qPCR.

Using serial dilution and plating, lactobacilli concentrations tended to be greater for the CF treatment than for the control and cellulose treatments. As with bifidobacteria, these results are not surprising, because the available substrate for fermentation favors lactobacilli proliferation. Analysis using qPCR provides stronger support for the CF treatment effect. The CF treatment had significantly greater lactobacilli concentrations than the cellulose treatment, and trends existed for all treatments containing fermentable components to have greater lactobacilli counts than the cellulose treatment. The observed differences between the treatments without and with added fermentable components were 0.7 to 1.0 log counts (or a 5- to 10-fold increase in number of cells), which could be considered a biologically significant difference.

Total anaerobic and aerobic bacterial counts were significantly lower for the cellulose treatment compared with the CF treatment, and total anaerobic bacteria were also lower for the cellulose treatment compared with the CFY2 treatment. Additionally, the CF treatment also tended to have greater total aerobic bacterial counts compared with the control treatment. The inert fiber, cellulose, is likely the reason why this dietary treatment resulted in lower bacterial counts compared with some of the treatments containing fermentable substrates.

This is the first report that beet pulp inclusion in dog diets produced bifidobacteria and lactobacilli populations in similar concentrations as diets containing fer-
mendable oligosaccharides. The latter treatments would have a theoretical advantage in their potential to increase lactic acid-producing bacteria such as bifidobacteria and lactobacilli because of the selective fermentation of FOS and MOS by these species. However, these data suggest that beet pulp as a fiber source is just as potent in increasing beneficial gut bacteria as are prebiotic oligosaccharides. This finding may have implications for the use of beet pulp as a fiber source in control diets used in studies evaluating possible prebiotic compounds. Indeed, the 2 microbial enumeration methods used here tended to agree on the order of the treatments in terms of fecal microbial concentrations. However, with the qPCR method, the effect of treatment on bifidobacteria and lactobacilli was much more distinct compared with the plating method, despite the greater variation for bifidobacteria. Given the composition of the fiber substrates, the patterns in bifidobacteria and lactobacilli were as expected.

Differences in *C. perfringens* concentrations among treatments were larger (although not significant) using qPCR enumeration compared with plating methods. A possible reason for this is the difficulty of growing *C. perfringens* from a fecal inoculum, because the cultivation is susceptible to fungal contamination and fast overgrowth of the colonies on the plate. This makes distinguishing separate colonies difficult and may impair the detection of differences in colony-forming units.

It is interesting to note that, even though the trends between methods are similar, the absolute numbers of colony-forming units enumerated are markedly different. In the case of bifidobacteria, the plating colony-forming unit counts are greater than the qPCR colony-forming unit counts. Normally, qPCR tends to report greater colony-forming units because it also accounts for dead (but intact) bacterial cells, whereas plating is able only to analyze viable cells in the fecal sample. Nevertheless, the observation that different analytical methods yield similar differences in bacterial concentrations among treatments (i.e., the absence of a method × treatment interaction) is much more important than the actual numbers they generate. Based on the results noted here, the 2 methods yield similar results, although qPCR appears to have more discriminatory power.

The similarity in total fecal DNA is most striking between the cellulose treatment and the treatments containing fermentable oligosaccharides, with values of 80 to 81%. This was expected, because the cellulose treatment does not allow for extensive bacterial growth as was outlined above. Among the 3 treatments containing fermentable oligosaccharides (CF, CFY1, and CFY2), similarity of fecal DNA was high (87 to 88%). The beet pulp treatment was most similar to the CF treatment (88%), but similarity with the CFY1 and CFY2 treatments was lower (85 to 86%). This latter observation could be due to fermentation characteristics associated with each treatment, which could indicate that beet pulp stimulates similar bacterial species as FOS, but a mixture of FOS and YCW does so to a lesser extent. Although no quantitative inferences can be made based on these data, it is evident that low-level fiber supplementation is able to alter total fecal bacterial DNA composition. This is likely the result of substrate-specific alteration of bacterial populations in the intestine.

Fecal pH and fecal score were not affected by dietary treatment. Increased fermentation and the resulting SCFA production are thought to lower pH and possibly increase fecal water content. However, the pH effect may be limited, because the dog colon absorbs SCFA rapidly, and absorption increases as SCFA concentrations increase (Herschel et al., 1981). The beet pulp treatment tended to have a greater wet fecal output compared with the CFY1 treatment. Compared with other fiber sources, beet pulp has been reported to increase fecal output (Fahey et al., 1992; Sunvold et al., 1995b), but the inclusion level of beet pulp used herein is up to 80% lower than in previously published work. Additionally, fecal output increases linearly with beet pulp inclusion level (Fahey et al., 1990b), and, therefore, the low inclusion level of fiber used here may not have been sufficient to increase fecal bulk significantly in excess of that for a very low fiber diet (the control treatment).

Fecal ammonia concentrations were not affected by dietary treatment, but fecal SCFA and BCFA were affected. The beet pulp treatment had significantly greater acetate, propionate, and butyrate concentrations per gram of DM compared with the control and cellulose treatments. The high concentration of SCFA in the beet pulp treatment is likely caused by the fact that beet pulp contains a blend of several fermentable substrates, all with different fermentation rates. By the time of defecation, it is possible that fermentation activity is greater for beet pulp than for the treatments containing no fermentable substrates (control and cellulose). Oligosaccharides such as MOS and, especially, FOS are highly fermentable (compared with natural fibers) and are rapidly used up once they enter the large intestine. Combined with the fast and efficient intestinal absorption of SCFA, this may affect SCFA presence in feces (at least numerically) in comparison with beet pulp. It has been estimated that only 5% of SCFA produced in the intestinal tract are excreted in feces (McNeil et al., 1978). A very interesting effect noted in this experiment is the significantly greater butyrate concentrations for all treatments containing fermentable components compared with the control and cellulose treatments. Butyrate is the main fuel source of colonocytes, and high butyrate concentrations are thought to be associated with gut health and colonocyte proliferation (Roediger, 1995). However, this butyrate production may not be solely due to altered bifidobacteria and lactobacilli concentrations, because these bacteria produce mainly lactate. Lactate, however, can be used by other species such as *Eubacterium* spp. to form.
butyrate. Unfortunately, neither lactate nor *Eubacterium* or other lactate-consuming bacterial concentrations were measured in this experiment. Nevertheless, if the increased butyrate concentrations are indigenous to the type of fermentable carbohydrate blends used here, it would be of interest to investigate how lactate produced by bifidobacteria and lactobacilli is further metabolized to butyrate by other bacterial species. Regardless of the mechanism of butyrate formation, our data suggest that fermentable fiber sources are able to produce large amounts of butyrate compared with diets containing no supplemental fiber or cellulose.

Branched-chain fatty acids are generated when energy is limiting in the large intestine. In this experiment, the CF treatment had significantly greater fecal BCFA concentrations compared with the cellulose treatment. The CF treatment also had significantly greater total bacterial counts. The rapid fermentation of FOS in the proximal colon may have resulted in a limited energy environment in the distal region, leading to an increased catabolism of AA with the end result being greater BCFA concentrations. Previous research with fermentable carbohydrates (inulin, oligofructose, and short-chain FOS) in our laboratory reported mixed effects on fecal BCFA concentrations ranging from no effect to a decrease in BCFA, depending on type and concentration of the oligosaccharide (Swanson et al., 2002a,b; Flickinger et al., 2003; Propst et al., 2003).

Fecal biogenic amine concentrations reported here are comparable to results previously reported by our laboratory in adult dogs (Swanson et al., 2002a,b; Flickinger et al., 2003; Propst et al., 2003). Additionally, the pattern of biogenic amine concentrations is similar to previously reported data with putrescine and spermidine and accounts for at least 31% (but often >50%) of total biogenic amines, suggesting significant decarboxylation of Met, Arg, and ornithine, the parent AA of putrescine and spermidine (and spermine). Although total amine concentration is not statistically different among treatments, the numerically decreased concentration for the cellulose treatment compared with the other treatments is in agreement with other indices of bacterial activity (e.g., SCFA concentrations and fecal bacterial counts).

Although polyamines (cadaverine, putrescine, spermidine, and spermine) have been linked to the incidence of colorectal cancers (Milovic and Turchanowa, 2003), they are required for normal development and repair of intestinal mucosal cells throughout the intestinal tract (Wang and Johnson, 1990; Wang et al., 1991; Löser et al., 1999). It is, therefore, undesirable to have significantly decreased polyamine concentrations in the intestinal lumen, because depletion of (intracellular) polyamines affects epithelial cell apoptosis. The exact mechanisms and effects of polyamine depletion on apoptosis, however, are not yet completely understood, and research in this area has yielded contradictory results (Seiler and Raul, 2005).

In conclusion, fermentable carbohydrates used in blends with nonfermentable fiber result in similar responses in digestive physiology as beet pulp, which is regarded as one of the superior natural fiber sources in pet foods. Especially compared with a diet supplemented with cellulose only, it is evident that fermentable fiber plays an important role in the canine intestine. Although nutrient digestibility may be slightly decreased when using fermentable fiber blends, this effect is offset by beneficial properties. For example, fermentable fiber stimulates bacterial growth and fermentation, thereby increasing production of butyrate. It appears, however, that fermentable fiber also may increase BCFA concentrations. Nevertheless, if a fiber blend containing fermentable oligosaccharides can be produced economically and at a constant quality, it may be an alternative to the use of beet pulp, which is known to vary in consistency and quality.

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