

Supplemental Fructooligosaccharides and Mannanooligosaccharides Influence Immune Function, Ileal and Total Tract Nutrient Digestibilities, Microbial Populations and Concentrations of Protein Catabolites in the Large Bowel of Dogs^{1, 2}

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ABSTRACT The goal of this study was to examine whether supplemental fructooligosaccharides (FOS) and (or) mannanooligosaccharides (MOS) influenced indices of gut health of dogs. Adult female dogs ($n = 4$) surgically fitted with ileal cannulas were fed a dry, extruded, kibble diet twice daily. At each feeding, the following treatments were administered: 1) Control (no FOS or MOS); 2) 1 g FOS; 3) 1 g MOS; or 4) 1 g FOS + 1 g MOS. Fecal, ileal and blood samples were collected during the last 4 d of each 14-d period to measure protein catabolite concentrations, microbial populations, immune characteristics and nutrient digestibilities. Treatment means were compared using preplanned orthogonal contrasts. Dogs supplemented with MOS had lower ($P = 0.05$) fecal total aerobes and tended to have greater ($P = 0.13$) *Lactobacillus* populations. Ileal immunoglobulin (Ig) A concentrations were greater ($P = 0.05$) in dogs supplemented with FOS + MOS vs. control. Lymphocytes (% of total white blood cells) were greater ($P < 0.05$) in dogs supplemented with MOS. Serum IgA concentrations also tended ($P = 0.13$) to be greater in dogs supplemented with MOS. Dogs supplemented with FOS and FOS + MOS had lower ($P < 0.05$) fecal total indole and phenol concentrations. Dogs supplemented with MOS tended to have lower ileal DM ($P = 0.149$) and OM ($P = 0.146$) digestibilities vs. control. Results of this study suggest that dietary supplementation of FOS and MOS may have beneficial effects on colonic health and immune status of dogs. *J. Nutr.* 132: 980-989, 2002.

KEY WORDS: • dogs • oligosaccharides • intestinal microbiota • colon health

Diet has an effect on the bacterial population of the colon. Both source and level of dietary protein influence the occurrence of pathogens in canine feces (1). Many dog foods contain high concentrations of protein, which can lead to an increased colonic presence of undigested amino acids (AA)⁴ and fecal putrefactive compounds (2). Increasing the protein flow to the colon provides more fermentative substrates for pathogenic species such as *Clostridium* spp., which are known

for their ability to degrade AA and produce fecal odor (3). Deamination, decarboxylation and deamination-decarboxylation reactions produce several putrefactive compounds including ammonia, amines, branched-chain fatty acids (BCFA), indoles, phenols and sulfur-containing compounds (4,5). Many of these protein catabolites not only result in fecal odor, but also may contribute to colon carcinogenesis (6,7) and exacerbate intestinal diseases (8).

Gibson and Roberfroid (9) introduced the concept of "prebiotics," which alter the microbial populations of the gut, and consequently, improve the health of the host. By definition, a prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and (or) activity of one or a limited number of bacteria in the colon, and thus improves host health (9). The most common prebiotics studied are fructooligosaccharides (FOS). The general term "FOS" may include all nondigestible oligosaccharides composed of fructose and glucose units. Specifically, FOS refers to short chains of fructose units bound by β -(2-1) linkages attached to a terminal glucose unit. Supplementation of FOS has been shown to enhance gut health in many ways. For example, FOS supplementation has been shown to increase numbers of beneficial bacteria such as bifidobacteria

¹ Presented in part at the Alltech, Inc. 3rd Annual Pet Nutrition Seminar held in conjunction with the 17th Annual Feed Industry Symposium, April 8-11, 2001, Lexington, KY [Swanson, K. S. (2001) Effects of mannanooligosaccharides (MOS) and fructooligosaccharides (FOS) on immune function and fecal odor components in the canine.] and the Waltham International Symposium, August 6-7, 2001, Vancouver, Canada [Swanson, K. S., Grieshop, C. M., Flickinger, E. A., Merchen, N. R. & Fahey, G. C., Jr. (2001) Effects of supplemental fructooligosaccharides and mannanooligosaccharides on colonic microbial populations, immune function and fecal odor components in the canine. The Waltham International Symposium Abstracts, p. 59 (abs.).]

² Funded in part by Alltech, Inc., Nicholasville, KY 40356.

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⁴ Abbreviations used: AA, amino acids; BCFA, branched-chain fatty acids; CBC, complete blood count; cfu, colony forming units; CP, crude protein; DM, dry matter; FOS, fructooligosaccharides; GC, gas chromatography; Ig, immunoglobulin; MOS, mannanooligosaccharides; OM, organic matter; SCFA, short-chain fatty acids; WBC, white blood cells.

(10) and has been used to prevent and treat constipation (11). The effects of FOS on fecal protein catabolite concentrations, however, have been virtually ignored.

The role of mannanoligosaccharides (MOS) in pathogen resistance and modulation of the immune system is not completely understood. Attachment of pathogens to epithelial cells of the gut is an essential step in the infection process. Lectins, carbohydrate-binding proteins, are found on the exterior of cells and are associated with fimbrial adhesins of bacteria. Lectins bind to the epithelial cells of the gut by attaching to oligosaccharide components of glycoconjugate receptors. Type-1 fimbrial adhesins, which are common on numerous species of *Escherichia coli* and *Salmonella*, are specific for mannan residues (12,13). Therefore, mannans aid in the resistance of pathogenic colonization by acting as receptor analogs for Type-1 fimbriae and decrease the number of available binding sites (14).

Mannans also have been reported to modulate the immune system. Supplementation of mannans has been reported to increase immunoglobulin (Ig) A concentration in cecal contents of rats (15), increase bile IgA and systemic IgG in turkeys (16), and increase neutrophil activity in dogs (17) and fish (18). Secretory IgA is important in mucosal immunity because it inhibits the attachment and penetration of bacteria in the lumen, increases mucus secretion (19) and prevents inflammatory reactions that would cause damage to the epithelial tissues (20).

Although some information is available on the effects of feeding FOS and MOS in selected species, there is a paucity of information in companion animals. In this experiment, we investigated whether supplemental FOS and (or) MOS influenced nutrient digestibilities, immune function, and microbial populations and protein catabolite concentrations in the large bowel of dogs.

MATERIALS AND METHODS

Animals and diets. Purpose-bred adult female dogs ($n = 4$; Butler Farms USA, Clyde, NY) with hound bloodlines, an average initial body weight of 22.5 kg (range, 21.1–23.9 kg) and average age of 3.3 y were surgically prepared with ileal cannulas according to Walker et al. (21). The surgical and animal care procedures were approved by the University of Illinois Campus Laboratory Animal Care Advisory Committee before initiation of the experiment. After surgery, dogs were closely monitored and given a 2-wk recovery period before 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 facility of the Edward R. Madigan Laboratory on the University of Illinois campus. The main ingredients of the dry, extruded, kibble diet (Table 1) were fructooligosaccharide free, and included poultry by-product meal, brewer's rice and poultry fat. The formulation resulted in a diet containing high concentrations of protein (36.8%), fat (20.9%) and ash (13.0%) and low total dietary fiber (4.8%). The diet was prepared by Wenger Manufacturing (Sabetha, KS). Dogs were offered 200 g diet twice daily (0800 and 2000 h).

At each feeding, the following treatments were administered via gelatin capsules: 1) Control (no supplemental FOS or MOS); 2) 1 g FOS; 3) 1 g MOS; or 4) 1 g FOS + 1 g MOS. The FOS supplement (Fortifeed) was obtained from GTC Nutrition (Golden, CO). The MOS supplement (Bio-MOS) was obtained from Alltech (Nicholasville, KY). Chromic oxide was used as a digestion marker. On d 6 through 14 of each period, dogs were dosed with 0.5 g Cr_2O_3 at each feeding via gelatin capsule for a total of 1.0 g marker/d. Fresh water was available at all times.

Sample collection. A 4×4 Latin-square design with 14-d periods was used. A 10-d adaptation phase preceded a 4-d collection of feces and ileal effluent. Ileal effluent was collected 3 times/d, with an interval of 4 h between collections. Individual ileal collections

TABLE 1

Ingredient and chemical composition of the diet fed to ileal cannulated dogs¹

Ingredient	g/kg
Poultry by-product meal	445.0
Brewers rice	321.0
Poultry fat	157.0
Beet pulp	40.0
Dehydrated egg	22.4
Sodium chloride	6.5
Potassium chloride	4.3
Choline chloride	1.3
Vitamin premix	1.2
Mineral premix	1.2
Analyzed composition	
Dry matter, %	92.2
	% of DM
Organic matter ²	87.0
Ash	13.0
Crude protein	36.8
Fat	20.9
Total dietary fiber	4.8
Gross energy, kJ/g	22.3

¹ Provided per kg of diet: vitamin A, 4.31 mg; vitamin D, 25.58 μg ; vitamin E, 72.04 mg; vitamin K, 0.63 mg; thiamin, 8.38 mg; riboflavin, 12.90 mg; pantothenic acid, 19.86 mg; niacin, 103.29 mg; pyridoxine, 7.06 mg; choline, 2,377.69 mg; biotin, 128.79 μg ; folic acid, 1,271.53 μg ; vitamin B-12, 172.13 μg ; manganese, 20.45 mg; iron, 300.80 mg; copper, 19.50 mg; cobalt, 2.53 mg; zinc, 183.43 mg; iodine, 7.89 mg; selenium, 0.23 mg.

² Organic matter = dry matter (DM) – ash.

were 1 h in length. Sampling times on the remaining 3 d were rotated 1 h from the previous day's collection time. For example, sampling times on d 1 took place at 0800, 1200 and 1600 h; on d 2, samples were collected at 0900, 1300 and 1700 h. Ileal samples were collected by attaching a sterile sampling bag (Fisher Scientific, Pittsburgh, PA) to the cannula barrel and around the hose clamp with a rubber band. Before attachment of the bag, the interior of the cannula was scraped clean with a spatula and digesta discarded. During collection of ileal effluent, dogs were encouraged to move freely. To deter the dogs from pulling the collection bag from the cannula, Bite-Not collars (Bite-Not Products, San Francisco, CA) were used during collection times. After ileal effluent collection, the cannula plug was put in place and the cannula site was cleaned with a dilute Betadine solution.

Total feces excreted during the collection phase of each period were removed from the floor of the pen, weighed, composited, and frozen at -20°C . On d 14 of each period, a fresh fecal sample was collected within 15 min of defecation for the measurement of fermentation end products [ammonia, biogenic amines, BCFA, indoles, lactate, phenols, short-chain fatty acids (SCFA)], IgA, bacterial enumeration and pH. During the 4-d collection phase, all fecal samples were scored according to the following system: 1 = hard, dry pellets; small, hard mass; 2 = hard, formed, dry stool; remains firm and soft; 3 = soft, formed, and moist stool; 4 = soft, unformed stool; assumes shape of container; 5 = watery; liquid that can be poured.

On d 14, a blood sample (10 mL) was collected via jugular puncture into nonheparinized evacuated tubes for use in determination of serum Ig concentration. Another 10 mL of blood was collected in an evacuated tube containing EDTA for complete blood count [CBC; RBC, hemoglobin, hematocrit, platelet, total white blood cell (WBC), neutrophil, eosinophil, basophil, lymphocyte and monocyte] determination.

Sample handling. Ileal samples were frozen at -20°C in their individual bags. At the end of the experiment, all ileal effluent samples were combined 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). Feces 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).

Fresh fecal samples were collected within 15 min of defecation and an aliquot was immediately transferred to a preweighed Carey-Blair transport media container (Meridian Diagnostics, Cincinnati, OH) for subsequent bacterial enumeration (total anaerobes, total aerobes, *Bifidobacterium*, *Lactobacillus*, *C. perfringens* and *E. coli*). Additional aliquots were used for pH measurement and determination of protein catabolites and fecal IgA. One aliquot (used to measure SCFA, BCFA, ammonia and lactate) was acidified and stored at -20°C until analysis. Additional aliquots were used for the determination of biogenic amines, indoles, phenols and IgA concentrations.

Chemical analyses. Diets, feces, and ileal samples were analyzed for dry matter (DM), organic matter (OM), and ash using AOAC (22) methods. Crude protein (CP) was calculated from Kjeldahl N values (23). Total lipid content was determined by acid hydrolysis followed by ether extraction according to AACC (23) and Budde (24). Total dietary fiber concentration was determined according to Prosky et al. (25,26). Ammonia concentrations were measured according to the method of Chaney and Marbach (27). Chromium concentration was analyzed according to Williams et al. (28) using an atomic absorption spectrophotometer (Model 2380, Perkin-Elmer, Norwalk, CT). SCFA and BCFA concentrations were determined via gas chromatography (GC) according to Erwin et al. (29). Briefly, concentrations of acetate, propionate, butyrate, valerate, isovalerate and isobutyrate were determined in the supernate of acidified fecal aliquots using a Hewlett-Packard 5890A Series II gas chromatograph (Palo Alto, CA) and a glass column (180 cm \times 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco, 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. Lactate concentrations were measured by the spectrophotometric method described by Barker and Summerson (30). Phenol and indole concentrations were determined via GC according to Flickinger et al. (31). Biogenic amine concentrations were determined via HPLC according to Flickinger et al. (31).

Microbial populations were determined by serial dilution (10^{-1} to 10^{-7}) of fecal samples in anaerobic diluent before inoculation onto petri dishes of sterile agar as described by Bryant and Burkey (32). Total anaerobe and total aerobe agars were prepared according to Bryant and Robinson (33) and Mackie et al. (34). The selective media for bifidobacteria (BIM-25) were prepared using reinforced clostridial agar (BBL Microbiology Systems, Cockeysville, MD) according to Muñoa and Pares (35). *Lactobacilli* were grown on Rogosa SL agar (Difco Laboratories, Detroit, MI). *E. coli* were grown on EMB agar (Difco Laboratories, Detroit, MI). Agars used to grow *C. perfringens* were prepared according to the FDA Bacteriological Analytical Manual (36). Samples for total anaerobes, *Bifidobacterium*, *Lactobacillus* and *C. perfringens* were inoculated, diluted and incubated anaerobically (73% N_2 :20% CO_2 :7% H_2) at 37°C . Total aerobes and *E. coli* were incubated aerobically at 37°C . Plates were counted between 24 and 48 h after inoculation. Colony forming units (cfu) were defined as distinct colonies measuring at least 1 mm in diameter.

Immunological analyses. Ileal and fecal IgA concentrations were determined according to Nara et al. (37). Briefly, fresh ileal and fecal samples were sealed in sterile sampling bags with excess air removed and stored at -20°C . Samples were lyophilized and crushed with a mortar and pestle. Samples (2 g) were placed in a glass Erlenmeyer flask along with 20 mL PBS solution, pH 7.2. Samples were mixed for 30 min at room temperature and then centrifuged at $20,000 \times g$ for 30 min at 4°C . The supernatant was collected and ileal and fecal IgA concentrations were determined using a radial immunodiffusion kit (ICN Biomedicals, Aurora, OH).

After blood was collected in nonheparinized evacuated tubes, samples were centrifuged at $2060 \times g$ for 20 min at 4°C and the serum collected. Serum IgA, IgG and IgM concentrations were determined

using radial immunodiffusion kits (ICN Biomedicals). The blood collected in evacuated tubes containing EDTA was used for CBC 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 Cr/g 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 the ileal nutrient flow (output, g/d), divided by nutrient intake (g/d). The same calculations were performed with fecal samples to determine total tract nutrient digestibilities.

Statistical analyses. Data were analyzed by the General Linear Models procedure of SAS (SAS Institute, Cary, NC). The experimental design was a 4×4 Latin-square design. Four sequences of diets (one sequence per dog) were used (ABDC, BCAD, CDBA, and DACB), in which A was the control, B was the FOS treatment, C was the MOS treatment and D was the FOS + MOS treatment. The statistical model included the effect of animal, period and treatment. Treatment least-squares means were compared using preplanned orthogonal contrasts. Contrasts include MOS-supplemented vs. control, FOS-supplemented vs. control, and FOS + MOS-supplemented vs. control. A probability of $P < 0.05$ was accepted as significant although mean differences with $P < 0.15$ were accepted as trends and results are discussed accordingly.

RESULTS

Food intake and fecal characteristics. Supplementation of FOS and MOS did not affect appetite or fecal characteristics because food intake (g/d, as-is), fecal output (g/d, as-is), fecal DM percentage and fecal scores did not differ among treatments (Table 2). A trend ($P = 0.088$) for increased fecal pH was observed when dogs were supplemented with MOS vs. control. No other differences in fecal pH were apparent.

Nutrient digestibilities. No differences ($P > 0.05$) in ileal or total tract digestibilities of DM, OM or CP were detected, but trends were apparent (Table 3). The supplementation of FOS did not influence ileal nutrient digestibilities. However, during supplementation with MOS, dogs tended to have lower ileal DM ($P = 0.149$) and OM ($P = 0.146$) digestibilities vs. control. Ileal nutrient digestibilities by dogs fed MOS were lower than for control dogs for DM (55.0 vs 67.7) and OM (63.6 vs 74.1). Supplementation of FOS and (or) MOS did not affect total tract macronutrient digestibilities.

Microbial populations. Total anaerobes and total aerobes averaged ~ 11.0 and 8.2 cfu \log_{10}/g fecal DM across treatments, respectively (Table 4). *Bifidobacterium* and *C. perfringens* were present in similar concentrations to one another, ranging from ~ 9.5 to 10.0 cfu \log_{10}/g feces DM. *Lactobacillus* and *E. coli* were present in lower concentrations, usually ranging from 8.0 to 9.0 cfu \log_{10}/g feces DM. Total aerobe concentrations were decreased ($P = 0.054$) by ~ 1 log unit in dogs fed MOS vs. control. Dogs supplemented with MOS also tended ($P = 0.126$) to have higher concentrations of fecal *Lactobacillus*. Dogs supplemented with FOS + MOS had lower ($P = 0.088$) total anaerobe concentrations compared with the control treatment. *Bifidobacterium*, *E. coli* and *C. perfringens* concentrations were not different among treatments.

Immune characteristics. Ileal and fecal IgA concentrations (mg IgA/g CP and mg IgA/g DM) are presented in Table 5. Although all treatments resulted in numeric increases in ileal IgA concentrations vs. control, only dogs supplemented with FOS + MOS had levels that were significantly greater ($P < 0.05$). Dogs fed FOS + MOS had increased IgA concentrations on a protein basis ($P = 0.052$) and on a DM basis ($P = 0.062$) vs. control. Fecal IgA concentrations, which were

TABLE 2

Nutrient intake, fecal output and fecal characteristics of dogs supplemented with fructooligosaccharides (FOS) and/or mannanoligosaccharides (MOS)¹

Item	Treatment				SEM ³	Contrasts ²		
	Control	FOS	MOS	FOS + MOS		MOS vs. C	FOS vs. C	F + M vs. C
Intake, g/d (as is)	374	388	377	376	6.2	0.771	0.174	0.813
Fecal output, g/d (as is)	167	166	165	167	10.8	0.897	0.925	0.987
Fecal DM, %	38.0	39.0	38.2	38.2	0.86	0.846	0.410	0.838
Fecal score	2.9	2.8	2.9	2.9	0.11	0.762	0.570	0.762
Fecal pH	6.76	6.67	7.27	6.93	0.18	0.088	0.739	0.535

¹ Values are means, $n = 4$.

² Preplanned contrasts with P -value for each comparison: MOS vs. C = MOS-supplemented vs. control; FOS vs. C = FOS-supplemented vs. control; F + M vs C = FOS- + MOS-supplemented vs. control.

³ Pooled SEM.

only ~15% of that in ileal samples, were not different among treatments.

Total WBC, neutrophil and lymphocyte numbers were not different among treatments (Table 6). When lymphocyte data were expressed as a percentage of total WBC, dogs supplemented with MOS had increased ($P \leq 0.05$) serum lymphocytes vs. control dogs. No differences were observed in serum IgG or IgM concentrations among treatments. There was a trend for increased serum IgA concentrations in dogs supplemented with MOS ($P = 0.135$) vs. control. Dogs on all treatments had RBC, hemoglobin, hematocrit, platelet, eosinophil, basophil and monocyte concentrations that fell within normal ranges for dogs (38).

Fecal protein catabolite concentrations. Due to the relatively low concentrations in which these compounds are found in feces and the high variability among samples, no differences ($P > 0.05$) among treatments were observed in any of the individual or total biogenic amines (Table 7). There were trends toward increased tryptamine ($P = 0.114$) and tyramine ($P = 0.147$) concentrations in feces as a result of FOS supplementation.

Of the four indoles measured in this experiment, only indole itself was quantified in all fecal samples (Table 8). In fact, the other three indoles (2-methyl indole, 3-methyl indole and 2,3-dimethyl indole) were not present in high enough concentrations to be measured in any of the fecal samples. A dramatic decrease in fecal indole concentration was observed

in dogs supplemented with FOS ($P = 0.074$) and FOS + MOS ($P = 0.082$). In fact, indole concentrations for dogs fed FOS and FOS + MOS were decreased by almost 50%, dropping from 2.44 $\mu\text{mol/g}$ with the control treatment to 1.23 and 1.27 $\mu\text{mol/g}$ in dogs supplemented with FOS and FOS + MOS, respectively. Phenols measured in this experiment included phenol, *p*-cresol and 4-ethylphenol. Although each phenol measured was not present in all of the samples, most samples contained at least one phenol. Because of this, only total phenol ($\mu\text{mol/g}$ feces DM) data were analyzed statistically. Due to the high variability in phenol concentrations among samples, no differences ($P > 0.05$) were observed among treatments. However, similar to the indole data, a numeric decrease in total phenols of >50% was observed in the treatments with FOS and FOS + MOS vs. control. When indoles and phenols were summed, resulting in a total indole and phenol concentration, a difference occurred with FOS ($P = 0.028$) and FOS + MOS ($P = 0.031$) supplementation vs. control. Again, a decrease of ~50% was observed.

Fecal SCFA, BCFA, lactate and ammonia concentrations. No differences in fecal acetate, propionate, butyrate or total SCFA concentrations were observed among treatments (Table 9). All treatments resulted in SCFA molar ratios that fall within the normal range for dogs, with acetate, propionate and butyrate representing ~63, 26, and 11% of total SCFA, respectively. No differences in fecal lactate, valerate, isovaler-

TABLE 3

Nutrient digestibilities by dogs supplemented with fructooligosaccharides (FOS) and/or mannanoligosaccharides (MOS)¹

Item	Treatment				SEM ³	Contrasts ²		
	Control	FOS	MOS	FOS + MOS		MOS vs. C	FOS vs. C	F + M vs. C
Ileal digestibility, %								
Dry matter	67.7	65.4	55.0	61.4	5.39	0.149	0.776	0.444
Organic matter	74.1	72.1	63.6	68.8	4.44	0.146	0.761	0.429
Crude protein	66.2	64.7	53.7	60.8	5.75	0.173	0.857	0.526
Total tract digestibility, %								
Dry matter	73.3	73.0	72.7	75.3	0.94	0.652	0.776	0.191
Organic matter	82.0	81.6	81.7	83.2	0.64	0.727	0.677	0.236
Crude protein	75.9	75.2	75.7	77.7	0.82	0.840	0.551	0.164

¹ Values are means, $n = 4$.

² See Table 2 for contrasts.

³ Pooled SEM.

TABLE 4

Fecal microbial populations for dogs supplemented with fructooligosaccharides (FOS) and/or mannanoligosaccharides (MOS)¹

Item	Treatment				SEM ³	Contrasts ²		
	Control	FOS	MOS	FOS + MOS		MOS vs. C	FOS vs. C	F + M vs. C
<i>cfu log₁₀/g fecal DM⁴</i>								
Total anaerobes	11.09	10.98	11.06	10.93	0.06	0.741	0.194	0.088
Total aerobes	8.67	8.35	7.68	8.19	0.24	0.054	0.400	0.224
Bifidobacterium	9.72	9.76	9.68	9.56	0.13	0.860	0.818	0.431
Lactobacillus	8.48	8.79	9.16	8.82	0.27	0.126	0.457	0.406
E. coli	8.32	8.04	8.25	7.16	0.60	0.932	0.746	0.217
C. perfringens	9.88	9.84	10.00	9.96	0.12	0.484	0.816	0.626

¹ Values are means, $n = 4$.

² See Table 2 for contrasts.

³ Pooled SEM; due to a missing data point, a weighted SEM was calculated for total aerobes.

⁴ cfu, colony-forming unit; DM, dry matter.

ate, isobutyrate, total BCFA or ammonia were observed among treatments (Table 9).

DISCUSSION

This experiment was performed to examine whether supplemental MOS and (or) FOS influenced immune function, nutrient digestibilities at the ileum and in the total gastrointestinal tract, and microbial populations and protein catabolite concentrations in the large bowel of dogs. Overall, the supplementation of MOS generally had beneficial effects on microbial populations and systemic immune characteristics, whereas FOS supplementation decreased the concentrations of selected protein catabolites formed in the large bowel. The combination of FOS + MOS tended to enhance local and systemic immune characteristics and decreased the concentrations of putrefactive compounds found in feces. Data suggested that MOS supplementation may decrease ileal DM and OM digestibility.

As expected, supplementation of FOS and (or) MOS did not influence food intake, fecal output, fecal DM percentage or fecal scores. Mannanoligosaccharide supplementation tended to increase ($P = 0.088$) fecal pH compared with dogs fed control diets. Fructooligosaccharide supplementation did not influence fecal pH.

In a rat study, a decrease in cecal pH was observed in FOS-supplemented rats vs. rats consuming a basal diet after 2

(5.6 vs. 7.5), 8 (6.8 vs. 7.5) and 27 wk (6.6 vs. 7.5) of supplementation (39). These authors did not measure fecal pH. Campbell et al. (40) reported significant decreases in cecal pH in rats fed diets containing short-chain FOS (i.e., Nutraflora) or oligofructose, but no differences in fecal pH were observed among treatment groups. The difference between cecal and fecal pH may be explained by the much lower SCFA concentrations measured in feces, which is likely due to the high absorption rate of SCFA in the colon (41). The absence of differences in fecal pH in the current experiment also is likely due to the rapid absorption of SCFA from the colon.

Ileal nutrient digestibilities were lower than expected. The lower DM digestibility may be explained in part by the high ash content (13%) of the diet. This high ash level was most likely due to the high inclusion rate of an animal by-product (44.5% poultry by-product meal), which varies considerably in amount of bone (i.e., ash) included in the final product. Murray et al. (42) reported a wide variation in the concentrations of OM, CP, AA and fat in animal by-products due to the origin of raw materials used and (or) rendering conditions used to prepare the material.

Supplementation with FOS did not appear to influence ileal nutrient digestibilities. However, MOS supplementation tended to decrease ileal DM ($P = 0.149$) and OM ($P = 0.146$) digestibilities, which appears to be due mainly to a decrease in CP digestibility. Total tract digestibilities appeared to be un-

TABLE 5

Ileal and fecal immunoglobulin (Ig) A concentrations for dogs supplemented with fructooligosaccharides (FOS) and/or mannanoligosaccharides (MOS)¹

Item	Treatment				SEM ³	Contrasts ²		
	Control	FOS	MOS	FOS + MOS		MOS vs. C	FOS vs. C	F + M vs. C
Ileal IgA, ⁴ mg/g DM	3.40	3.91	4.03	4.90	0.46	0.376	0.468	0.062
Ileal IgA, mg/g CP	8.22	9.74	9.77	12.22	1.17	0.383	0.394	0.052
Fecal IgA, mg/g DM	0.64	0.55	0.56	0.63	0.52	0.334	0.290	0.927
Fecal IgA, mg/g CP	1.64	1.46	1.51	1.74	0.14	0.530	0.385	0.638

¹ Values are means, $n = 4$.

² See Table 2 for contrasts.

³ Pooled SEM.

⁴ CP, crude protein; DM, dry matter.

TABLE 6

Blood immune characteristics of dogs supplemented with fructooligosaccharides (FOS) and/or mannanoligosaccharides (MOS)¹

Item	Treatment				SEM ³	Contrasts ²		
	Control	FOS	MOS	FOS + MOS		MOS vs. C	FOS vs. C	F + M vs. C
Total WBC, ⁴ 10 ³ /μL	12.09	11.91	11.05	11.16	1.04	0.508	0.909	0.553
Neutrophil, ⁵ %	70.13	66.38	66.03	70.00	2.63	0.312	0.352	0.974
Neutrophil, 10 ³ /μL	8.54	7.87	7.30	8.00	0.85	0.342	0.600	0.670
Lymphocyte, ⁵ %	15.55	16.80	20.40	17.75	1.39	0.049	0.549	0.307
Lymphocyte, 10 ³ /μL	1.82	2.01	2.22	1.84	0.21	0.229	0.547	0.956
Serum IgA, g/L	1.93	2.13	2.33	2.30	1.64	0.135	0.421	0.157
Serum IgG, g/L	125.13	110.60	117.83	117.43	75.59	0.520	0.223	0.498
Serum IgM, g/L	7.98	8.63	8.10	8.70	5.25	0.872	0.415	0.367

¹ Values are means, $n = 4$. Ig, immunoglobulin.

² See Table 2 for contrasts.

³ Pooled SEM.

⁴ WBC, white blood cell.

⁵ Percentage of total white blood cells.

changed with MOS supplementation. In a similar experiment, MOS supplementation decreased total tract DM, OM, CP and N-free extract digestibilities (43). It is possible that mannans in the intestinal lumen may bind and agglutinate proteins, making them less digestible in the small intestine due to physical hindrance. This area requires further study to determine whether agglutination, or other unknown mechanisms, may cause the decreased ileal digestibility observed in the current experiment and the decreased total tract CP digestibility observed in the experiment of Zentek et al. (43).

Contrary to other studies, supplementation of FOS in the present study did not result in any differences in fecal microbial populations. Fructooligosaccharide supplementation has been shown to increase bifidobacteria populations in mice (44) and humans (10). It is possible that the dose of 2 g FOS/d was not high enough to change these populations in dog feces. Fecal bacterial or SCFA concentrations do not always accurately predict the fermentation taking place in the proximal colon (45). Because short-chain FOS are extensively fer-

mented by colonic bacteria (46–48), it is likely that the dose of 1 g of FOS given to the dogs twice daily was quickly fermented, beneficially affecting the microbial populations in the proximal colon without changing populations in lower regions of the large bowel or in feces.

Mannanoligosaccharide supplementation had a positive influence on microbial populations by tending to increase *Lactobacillus* ($P = 0.126$) numbers and decrease total aerobe ($P = 0.054$) concentrations. By producing lactate and bacteriocins (49), lactate-producing bacteria reduce colonic pH and decrease pathogen populations. In addition, *Lactobacillus* strains have been reported to inhibit enteropathogenic *E. coli* binding to intestinal cells (50) and decrease enzyme (β -glucuronidase, azoreductase and nitroreductase) levels responsible for the production of carcinogenic compounds (51). In the current experiment, it did not appear that *Lactobacillus* inhibited the growth of *E. coli* because concentrations of this organism were not different among treatments.

Bifidobacterium and *C. perfringens* concentrations were not

TABLE 7

Fecal biogenic amine concentrations in dogs supplemented with fructooligosaccharides (FOS) and/or mannanoligosaccharides (MOS)¹

Item	Treatment				SEM ³	Contrasts ²		
	Control	FOS	MOS	FOS + MOS		MOS vs. C	FOS vs. C	F + M vs. C
$\mu\text{mol/g fecal DM}$								
Agmatine	4.14	5.39	4.96	4.00	0.84	0.516	0.333	0.912
Cadaverine	1.16	1.95	1.64	1.80	0.63	0.607	0.410	0.496
Phenylethylamine	0.82	0.73	1.01	0.88	0.13	0.339	0.640	0.774
Putrescine	2.03	3.12	3.01	3.54	0.66	0.335	0.291	0.158
Spermidine	1.89	2.06	2.03	2.19	0.27	0.723	0.668	0.461
Spermine	0.18	0.22	0.17	0.18	0.03	0.914	0.352	0.914
Tryptamine	1.53	2.11	1.72	1.77	0.22	0.573	0.114	0.470
Tyramine	0.89	1.25	1.20	0.64	0.15	0.203	0.147	0.273
Total amines ⁴	12.63	16.82	15.74	14.99	2.36	0.387	0.255	0.505

¹ Values are means, $n = 4$.

² See Table 2 for contrasts.

³ Pooled SEM.

⁴ Total amines = agmatine + cadaverine + phenylethylamine + putrescine + spermidine + spermine + tryptamine + tyramine. Histamine was measured, but detected only in trace amounts; therefore, it was not included in calculating the concentration of total amines.

TABLE 8

Fecal indole and phenol concentrations in dogs supplemented with fructooligosaccharides (FOS) and/or mannanoligosaccharides (MOS)¹

Item	Treatment				SEM ³	Contrasts ²			
	Control	FOS	MOS	FOS + MOS		MOS vs. C	FOS vs. C	F + M vs. C	
	<i>μmol/g fecal DM</i>								
Indole	2.44	1.23	2.14	1.27	0.40	0.612	0.074	0.082	
Total phenols ⁴	0.58	0.27	0.49	0.27	0.23	0.795	0.377	0.377	
Total phenols and indoles ⁵	3.03	1.50	2.64	1.54	0.37	0.490	0.028	0.031	

¹ Values are means, $n = 4$.

² See Table 2 for contrasts.

³ Pooled SEM.

⁴ Total phenols = phenol + *p*-cresol + 4-ethyl phenol.

⁵ Total phenols and indoles = phenol + *p*-cresol + 4-ethyl phenol + indole.

different among treatments. In the current experiment, fairly high colonic *C. perfringens* concentrations were observed with all treatments, which may have been due to the high protein content (36.8%) of the diet. Although MOS supplementation has been reported to decrease fecal *C. perfringens* concentrations in dogs (52), no changes in concentrations of this organism were observed in this experiment.

Following the presentation of an antigen in the gut, T cells stimulate B lymphocytes to differentiate into plasma cells with the ability to produce IgA (19), the predominant Ig isotype produced by plasma cells in the intestinal lamina propria (53). After leaving the Peyer's patches and passing through the systemic circulation, IgA⁺ B cells migrate back to the lamina propria where they are capable of secreting large amounts of the antibody. Secretory IgA is important in mucosal immunity because it inhibits the attachment and penetration of bacteria and toxins in the lumen, increases time for digestive enzymes to function, binds and prevents absorption of undigested proteins, increases mucus secretion (19) and prevents inflamma-

tory reactions that would cause damage to the epithelial tissues (20). The presence of normal IgA concentrations may play a role in some intestinal diseases because reduced IgA concentrations have been associated with humans with Crohn's disease (54) and dogs with small intestinal bacterial overgrowth (55).

In the current experiment, ileal IgA concentrations were greater ($P = 0.052$) in dogs supplemented with FOS + MOS. These results agree with other studies that have reported increased mucosal IgA concentrations (17) and IgA in cecal contents (15) of rats supplemented with MOS. Increased ileal IgA concentrations suggest an enhanced local immune capacity and greater protection against pathogenic invasion.

In the current experiment, no differences were observed in fecal IgA concentrations among treatments. Fecal IgA concentrations were only ~15% of that in ileal samples, which suggests microbial breakdown in the colon. Secretory IgA seems to be relatively resistant to intestinal proteolytic enzymes (56). However, some bacterial species (e.g., *Clostridium*

TABLE 9

Fecal short-chain fatty acid (SCFA) concentrations and molar ratios, branched-chain fatty acid (BCFA) concentrations and ammonia concentrations in dogs supplemented with fructooligosaccharides (FOS) and/or mannanoligosaccharides (MOS)¹

Item	Treatment								SEM ⁴	Contrasts ²		
	Control		FOS		MOS		FOS/MOS			MOS vs. C	FOS vs. C	F + M vs. C
	<i>μmol/g</i>	MR ³	<i>μmol/g</i>	MR	<i>μmol/g</i>	MR	<i>μmol/g</i>	MR				
Total SCFA ⁵	354.05		341.81		364.44		323.14		50.74	0.890	0.870	0.682
Acetate	226.65	64.02	212.37	62.13	231.51	63.52	199.08	61.61	37.32	0.930	0.796	0.620
Propionate	90.57	25.58	91.97	26.91	93.68	25.71	88.15	27.28	10.84	0.846	0.931	0.880
Butyrate	36.83	10.40	37.47	10.96	39.25	10.77	35.91	11.11	3.92	0.678	0.911	0.874
Lactate	1.51		7.52		1.50		1.59		2.96	0.998	0.202	0.986
Total BCFA ⁶	40.30		35.64		42.15		36.66		2.86	0.662	0.294	0.404
Valerate	21.08		17.41		22.48		20.26		1.84	0.610	0.207	0.762
Isobutyrate	8.09		7.47		8.38		6.92		0.65	0.765	0.525	0.249
Isovalerate	11.12		10.75		11.30		9.48		1.13	0.916	0.825	0.342
Ammonia	146.71		126.71		141.57		120.26		13.83	0.802	0.346	0.225

¹ Values are means, $n = 4$.

² See Table 2 for contrasts.

³ MR, molar ratios of acetate, propionate and butyrate.

⁴ SEM of SCFA, BCFA and ammonia concentrations.

⁵ Total SCFA = acetate + propionate + butyrate.

⁶ Total BCFA = valerate + isobutyrate + isovalerate.

spp.) have been shown to possess proteases capable of degrading IgA (57). Because a vast difference in colonic microbial populations among species and individual animals exists, a large difference in the potential to degrade IgA also exists. Therefore, the measurement of ileal IgA concentration is a better indicator of the local immune capacity in the gut than is fecal IgA concentration. Accordingly, Ferguson et al. (58) indicated that fecal IgA concentrations are misleading because they do not accurately represent gastrointestinal mucosal IgA secretion.

Immune characteristics measured in blood indicated slight changes in systemic immune capacity as a result of FOS and MOS supplementation. Total WBC and neutrophil concentrations were not different among treatments. However, MOS-supplemented dogs were likely to have an enhanced immune system, with increased IgA ($P = 0.135$) and lymphocyte ($P < 0.05$) concentration (% of WBC). Because serum IgG and IgM concentrations were not affected, a systemic immune response most likely did not occur and was not the cause of the increased levels of circulatory lymphocytes and IgA observed in these dogs. Rather, the trends for increased serum IgA and lymphocyte concentrations are likely due to the increased proliferation of B lymphocytes and secretory IgA occurring in the gut. Regardless of the cause, the increase in serum IgA and lymphocyte concentrations may result in an enhanced systemic immune capacity in dogs supplemented with MOS.

SCFA are the main energy source for colonocytes, in particular, butyrate, which is the preferred energy substrate of colonic epithelium (59) and may account for up to 70% of its total energy consumption (60). SCFA also decrease luminal pH and create an environment less favorable for pathogenic species. In the current experiment, the supplementation of FOS and MOS did not affect fecal acetate, propionate, butyrate or total SCFA concentrations. Fecal SCFA molar ratios also were unaffected because all treatments resulted in values that fall within the normal range for dogs, with acetate, propionate and butyrate representing ~63, 26 and 11% of the SCFA, respectively. A potential factor preventing the detection of differences in fecal SCFA concentration among treatments in the current study is the rapid absorption of SCFA by colonocytes (41). Although the measurement of SCFA concentrations in the proximal colon would be useful, sample collection in this part of the gastrointestinal tract was not possible in the current experiment.

Lactate is a major end-product of the lactate-producing species, *Lactobacillus* and *Bifidobacterium*. An increased lactate concentration often is beneficial because it decreases luminal pH and is a potent antimicrobial substance to several pathogenic species. In the current experiment, lactate concentrations were not different among treatments. Transient increases in fecal lactate concentration have been observed in animals supplemented with FOS. The 14-d periods used in the current experiment may have been long enough for lactate-consuming species, such as *Propionibacterium* spp., *Veillonella* spp., *Clostridium* spp. and sulfate-reducers (61,62), to adapt to an increased supply of lactate and normalize lactate levels before sample collection.

Microflora metabolize nitrogenous compounds that enter the colon into putrefactive catabolites such as ammonia, biogenic amines and phenols, which are implicated as the major odor components of feces (63,64). More importantly, many of these protein catabolites may have negative influences on gut health. For example, high concentrations of ammonia are suspected to disturb the mucosa cell cycle and contribute to colon carcinogenesis (6,7,65). Phenol has been reported to promote skin cancer (66) and exacerbate ulcerative colitis (8).

Phenols are usually excreted in urine after glucuronide or sulfate conjugation, which occurs in the large intestinal mucosa or liver (67). However, little is known about phenol metabolism in the colon.

The metabolism of N in the colon by microflora may be modified by the availability of substrate, particularly by dietary carbohydrate (69,70). Fermentable carbohydrates, including FOS, may decrease the concentration of putrefactive compounds by providing gut microflora with an additional energy supply. In the colon, bacteria act as N sinks, utilizing the undigested protein and its metabolites in the presence of energy for their protein synthesis (71). Bacteria use ammonia as a major source of N, and other forms of protein or AA are deaminated to ammonia before being used metabolically (72). Carbohydrates (e.g., FOS, resistant starch, dietary fiber) serve as the energy source required to produce microbial protein. When energy (carbohydrate) supplies are limited, bacteria ferment AA to SCFA and ammonia to obtain energy (73). However, if an available energy source is provided, the luminal concentrations of nitrogenous compounds decrease and the concentrations of fecal N (bacterial mass) increase (71,74).

Decreased protein catabolite concentrations due to oligosaccharide supplementation have been reported in rats and dogs. Terada et al. (75) reported decreased fecal ammonia, phenol, indole, skatole and ethylphenol concentrations after 14 d of lactosucrose supplementation. Zentek et al. (43) reported decreased fecal ammonia excretion in dogs supplemented with MOS. In rats, several experiments have reported decreased fecal ammonia concentrations after oligosaccharide consumption (69,76,77).

In agreement with Terada et al. (75), dogs supplemented with FOS and FOS + MOS in the current experiment had decreased concentrations of fecal phenols and indoles. This implies that the supplementation of FOS influences the catabolism and (or) excretion of aromatic AA reaching the colon. Higher doses of FOS may be required to generate significant decreases in the concentrations of ammonia, isobutyrate, isovalerate, valerate and total BCFA measured in feces. No significant differences in biogenic amines were observed among treatments. Trends for increased tryptamine ($P = 0.114$) and tyramine ($P = 0.147$) concentrations observed in FOS-supplemented dogs were unexpected. The decrease in phenol and indole concentrations in combination with the increase in biogenic amine concentrations may suggest that FOS supplementation influences the metabolism of not only aromatic AA, but all AA present in the large bowel. More research is required in this area before any definitive conclusions can be made. Because most of the protein catabolites are present at low concentrations in feces, variance among samples is high. In future experiments, greater animal numbers would assist in detecting differences in protein catabolite concentrations among treatments. Because bacteria possess a number of inducible and repressible enzymes, changes in metabolic activity of intestinal flora can occur without appreciable changes in actual numbers or types of organisms in the gut (51). Therefore, the measurement of fecal enzyme activity levels also may assist in determining the metabolic changes occurring in the large intestine from oligosaccharide supplementation.

In the current experiment, positive effects of supplementing FOS and MOS were observed in healthy adult dogs. It is likely that the health benefits of feeding FOS and (or) MOS would be even more beneficial in populations of elderly dogs, young weanling puppies or dogs under stress. During weaning, a rapid shift in microbial populations occurs in the gut. Beneficial species such as bifidobacteria and lactobacilli decrease,

whereas *E. coli* and *C. perfringens* increase, creating an unfavorable colonic microbial community. Huis in 't Veld and Havenaar (78) reported dramatic decreases (~1000-fold) in lactobacilli numbers in piglets the day after weaning. At the same time, *E. coli* populations increased far above the concentration of lactobacilli. Mathew et al. (79) also reported decreased lactobacilli and increased coliforms shortly after weaning. Elderly populations also would benefit from increased populations of beneficial bacteria and intestinal IgA. Geriatric dogs have been shown to possess a poor microbial balance. Benno et al. (80) reported greater populations of *C. perfringens* and streptococci and lower populations of bacteroides, eubacteria, bifidobacteria and lactobacilli in old vs. young Beagles. Goldin and Gorbach (51) reported higher levels of β -glucuronidase, nitroreductase and azoreductase in old vs. young rats, increasing the potential for the production of compounds known to promote cancer. Similar to other species, the immune system of dogs declines with age, accompanied by decreases in mitogen stimulation, chemotaxis and phagocytosis occurring (81,82).

To conclude, FOS and MOS are prebiotics that are likely to have a positive influence on indices of gut health in dogs. Mannan oligosaccharides tend to enhance microbial populations and modulate systemic immune function. Fructooligosaccharides decrease concentrations of putrefactive compounds measured in feces, improving gut health. The combination of FOS + MOS tends to enhance local and systemic immune capacity in addition to decreasing fecal protein catabolite concentrations. Therefore, FOS and MOS may be used in dog diets to improve gut health by altering microbial populations positively, enhancing immune capacity and decreasing concentrations of putrefactive compounds. The use of these prebiotics might be most beneficial in geriatric dogs, young weanling puppies or dogs under stress, all of which may have compromised immune systems or undesirable microbial communities in the gut.

ACKNOWLEDGMENT

The authors thank Galen Rokey from Wenger Manufacturing (Sabetha, KS) for his assistance in diet preparation.

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