Galactoglucomannan oligosaccharide supplementation affects nutrient digestibility, fermentation end-product production, and large bowel microbiota of the dog[1]

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ABSTRACT: A galactoglucomannan oligosaccharide (GGMO) obtained from fiberboard production was evaluated as a dietary supplement for dogs. The GGMO substrate contained increased concentrations of oligosaccharides containing mannose, xylose, and glucose, with the mannose component accounting for 35% of DM. Adult dogs assigned to a 6 × 6 Latin square design were fed 6 diets, each containing a different concentration of supplemental GGMO (0, 0.5, 1, 2, 4, and 8%) that replaced dietary cellulose. Total tract DM and OM apparent digestibilities increased ($P < 0.001$) linearly, whereas total tract CP apparent digestibility decreased ($P < 0.001$) linearly as dietary GGMO substrate concentration increased. Fecal concentrations of acetate, propionate, and total short-chain fatty acids increased ($P \leq 0.001$) linearly, whereas butyrate concentration decreased ($P \leq 0.001$) linearly with increasing dietary concentrations of GGMO. Fecal pH decreased ($P \leq 0.001$) linearly as dietary GGMO substrate concentration increased, whereas fecal score increased quadratically ($P \leq 0.001$). Fecal phenol ($P \leq 0.05$) and indole ($P \leq 0.01$) concentrations decreased linearly with GGMO supplementation. Fecal biogenic amine concentrations were not different among treatments except for phenylethylamine, which decreased ($P < 0.001$) linearly as dietary GGMO substrate concentration increased. Fecal microbial concentrations of *Escherichia coli, Lactobacillus* spp., and *Clostridium perfringens* were not different among treatments. A quadratic increase ($P \leq 0.01$) was noted for *Bifidobacterium* spp. as dietary GGMO substrate concentration increased. The data suggest positive nutritional properties of supplemental GGMO when incorporated in a good-quality dog food.

Key words: digestibility, dog, fermentation end-product, galactoglucomannan oligosaccharide, microbiota

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INTRODUCTION

A novel galactoglucomannan oligosaccharide (**GGMO**) substrate (Previda, Temple-Inland, Diboll, TX) is derived from the fiberboard manufacturing process. During production, wood chips are steamed using increased temperature and pressure. When the pressure is released quickly, soluble wood sugars, and oligosaccharides are separated from the insoluble wood pulp and dissolve into the surrounding water. The resulting sugar solution is condensed through evaporation, resulting in a thick, molasses-like substance.

The GGMO substrate is composed of numerous types of oligosaccharides, including mannanoligosaccharides, xylooligosaccharides, and glucooligosaccharides. In addition, GGMO contain select polyphenolic compounds. The GGMO substrate has been shown to be resistant to hydrolytic digestion, but highly fermentable in vitro using canine fecal inoculum (G. C. Fahey Jr., unpublished data); however, in vivo data are lacking.

Because the GGMO substrate contains an increased concentration of select oligosaccharides and is easily fermented, it has the potential to elicit a prebiotic effect; however, the prebiotic potential has yet to be evaluated in an animal model. To be classified as a prebiotic, the substrate must "allow specific changes, both in composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (Roberfroid, 2007). The objective of this study was to evaluate nutritional effects and prebiotic potential of a spray-dried GGMO substrate when added to canine diets and tested in a dose-response experiment.

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MATERIALS AND METHODS

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

Galactoglucomannan Oligosaccharide Substrate

Production of the GGMO substrate involves wood chips, water, and pressure, but does not use strong acids or bases unlike other fiberboard production processes. This results in an ingredient potentially safe for consumption by animals. During hydrolysis, hemicelluloses are depolymerized through hydronium ions from water and other compounds such as uronic, acetic, and phenolic acids (Garrote et al., 1999). The release of pressure on the "wood chip digester" results in destruction primarily of cellulose, hemicelluloses, and lignin that releases soluble sugars into the surrounding water, along with polyphenolic compounds from lignin. The resulting water solution contains increased concentrations of sugars (3 to 4%), a concentration unsafe for disposal into wastewater streams. Thus, the sugar solution is removed from the wood chips and further condensed into a syrup with a final sugar concentration of 30 to 54% (Michalka, 2007). Sugars are mostly in the form of oligosaccharides compared with free sugars.

The GGMO syrup substrate was spray-dried (PCS P-0.1, Pulse Combustion Systems, Payson, AZ) with a contact temperature of 360°C and exit temperature of 102°C. The starting substrate was diluted to 50.25% solids before drying. Spray-drying allowed the substrate to be mixed in a diet matrix that was extruded and a kibble formed.

Substrate Chemical Analyses

The GGMO substrate was analyzed for DM, OM, and ash using AOAC (2006) methods. Crude protein was calculated from Leco total N values (AOAC, 2006). Total lipid content (acid-hydrolyzed fat) of the substrate was determined according to the methods of the AACC (1983) and Budde (1952). Gross energy was measured using an oxygen bomb calorimeter (model 1261, Parr Instruments, Moline, IL). Free monosaccharide and oligosaccharide concentrations were determined according to Smiricky et al. (2002). Hydrolyzed monosaccharides (i.e., sugars covalently bound to each other) were determined according to Hoebler et al. (1989) and Bourquin et al. (1990). Polyphenolic compound concentrations were determined according to Jung et al. (1983) and Titgemeyer et al. (1991).

Animals and Diets

Six female dogs with hound bloodlines (3.4 ± 0.0 yr; 22 ± 2.1 kg) were utilized. Dogs were housed in individual kennels (2.4 × 1.2 m) in a temperature-controlled room with a 16 h light:8 h dark cycle. Six diets were formulated to contain approximately 30% CP and 20% fat (as-is basis). Each diet contained a specified concentration of the GGMO substrate (0, 0.5, 1, 2, 4, or 8%), which replaced cellulose (Solka-Floc; International Fiber Corporation, North Tonawanda, NY) in the diet. Low ash poultry by-product meal, poultry fat, brewer's rice, ground corn, and vitamin and mineral premixes made up the remainder of the dry, extruded, kibble diet (Table 1). Diets were formulated to meet or exceed the NRC (2006) requirements for adult dogs at maintenance. Diets were extruded at the Kansas State University Bioprocessing and Industrial Value-Added Program facility (Manhattan, KS) under the supervision of a private consultant (Pet Food and Ingredient Technology Inc., Topeka, KS). Dogs were offered 160 g of the diet twice daily (0800 and 1700 h) to meet the required energy needs based on estimated ME of the diet. Chromic oxide (0.2%) was added to the diet as a digestibility marker. Fresh water was offered to the dogs ad libitum.

Sample Collection

A 6 × 6 Latin square design experiment with 14-d periods was conducted. The first 10 d were an adaptation period, followed by 4 d of total fecal collection. Although total tract nutrient digestibility values were based on the concentration of chromic oxide recovered in feces, total feces excreted during the collection phase of each period were taken from the pen floor, weighed, and frozen at −20°C until further analyses. All fecal samples during the collection period were subjected to a consistency score according to the following scale: 1 = hard, dry pellets, and small hard mass; 2 = hard, formed, dry stool, and remains firm and soft; 3 = soft, formed, and moist stool, and retains shape; 4 = soft, unformed stool, and assumes shape of container; and 5 = watery, liquid that can be poured.

Sample Handling

Fecal samples were dried at 55°C in a forced-air oven and ground in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) through a 2-mm screen. On d 11 of each period, fresh fecal samples were collected within 15 min of defecation. An aliquot of fresh feces was immediately transferred to sterile cryogenic vials (Nalgene, Rochester, NY) and snap-frozen in liquid nitrogen. Once frozen, vials were stored at −80°C until DNA extraction for microbial analysis. Aliquots for analysis of phenols, indoles, and biogenic amines were frozen at −20°C immediately after collection. One aliquot was collected and placed in 5 mL of 2 *N* hydrochloric acid for ammonia and short-chain fatty acid (**SCFA**) analysis. Additional aliquots were used for pH measurement and fresh fecal DM determination.

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Table 1. Ingredient and chemical composition of diets supplemented with the galactoglucomannan oligosaccharide (GGMO) substrate and fed to dogs

	Diet, % GGMO substrate					
Item	0.0	0.5	1.0	2.0	4.0	8.0
Ingredient, %						
Poultry by-product meal	39.00	39.00	39.00	39.00	39.00	39.00
Brewer's rice	27.35	27.35	27.35	27.35	27.35	27.35
Poultry fat	14.00	14.00	14.00	14.00	14.00	14.00
Ground corn	10.00	10.00	10.00	10.00	10.00	10.00
Solka-Floc[1]	8.00	7.50	7.00	6.00	4.00	0.00
GGMO (spray-dried)[2]	0.00	0.50	1.00	2.00	4.00	8.00
Salt	0.65	0.65	0.65	0.65	0.65	0.65
Potassium chloride	0.50	0.50	0.50	0.50	0.50	0.50
Chromic oxide	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[3]	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix[4]	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
DM content and chemical composition (DM basis)						
DM, %	94.3	94.1	94.1	94.0	93.8	93.6
OM, %	93.0	93.4	93.5	93.0	93.1	93.0
CP, %	31.6	31.1	31.9	32.0	31.1	32.5
Acid hydrolyzed fat, %	21.6	22.5	20.9	21.4	22.3	20.9
Total dietary fiber–uncorrected,[5] %	11.5	10.5	10.3	9.3	7.5	4.2
Total dietary fiber–corrected,[6] %	11.5	11.0	11.3	11.3	11.5	12.2
GE, kcal·g^{-1}	5.4	5.5	5.4	5.4	5.5	5.4

[1]Solka-Floc, International Fiber Corporation, North Tonawanda, NY.

[2]Galactoglucomannan oligosaccharide (Previda), Temple-Inland, Diboll, TX.

[3]Provided per kilogram of diet: vitamin A, 17,600 IU; vitamin D_3, 1,760 IU; vitamin E, 180 IU; vitamin K, 0.88 mg; thiamine, 4.40 mg; riboflavin, 5.72 mg; pantothenic acid, 22.00 mg; niacin, 39.60 mg; pyridoxine, 3.52 mg; biotin, 0.13 mg; folic acid, 0.44 mg; and vitamin B_{12}, 0.11 mg.

[4]Provided per kilogram of diet: manganese (as $MnSO_4$), 66.00 mg; iron (as $FeSO_4$), 120 mg; copper (as $CuSO_4$), 18 mg; cobalt (as $CoSO_4$), 1.20 mg; zinc (as $ZnSO_4$), 240 mg; iodine (as KI), 1.8 mg; and selenium (as Na_2SeO_3), 0.24 mg.

[5]These values were determined using the total dietary fiber assay (Prosky et al., 1984), which cannot quantify GGMO.

[6]These values were determined by adding the amount of GGMO substrate present in each diet to the total dietary fiber (uncorrected) value.

Chemical Analyses

Diet and fecal samples were analyzed for DM, OM, and ash using AOAC (2006) methods. Crude protein and total lipid contents and GE were determined as described before. Total dietary fiber (**TDF**) was analyzed according to Prosky et al. (1984). Chromium concentrations of diet and fecal samples were analyzed according to Williams et al. (1962) using atomic absorption spectrophotometry (model 2380, Perkin-Elmer, Norwalk, CT). Fecal SCFA and branched-chain fatty acid (**BCFA**) concentrations were determined by gas chromatography according to Erwin et al. (1961) using a gas chromatograph (Hewlett-Packard 5890A series II, Palo Alto, CA) and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier with a flow rate of 75 mL·min^{-1}. Oven, detector, and injector temperatures were 125, 175, and 180°C, respectively. Fecal ammonia concentrations were determined according to the method of Chaney and Marbach (1962). Fecal phenol and indole concentrations were determined using gas chromatography according to the methods described by Flickinger et al. (2003). Biogenic amines concentrations were quantified using HPLC according to methods described by Flickinger et al. (2003).

Microbial Analyses

Fecal microbial populations were analyzed using methods described by Middelbos et al. (2007a) with minor adaptations. Briefly, fecal DNA was extracted from freshly collected samples that had been stored at −80°C until analysis, using the repeated bead beater method described by Yu and Morrison (2004) with a DNA extraction kit (QIAamp DNA Stool Mini Kit, Qiagen, Valencia, CA) according to the manufacturer's instructions. Extracted DNA was quantified using a spectrophotometer (NanoDrop ND-1000, Nano-Drop Technologies, Wilmington, DE). Quantitative PCR was performed using specific primers for *Bifidobacterium* spp. (Matsuki et al., 2002), *Lactobacillus* spp. (Collier et al., 2003), *Escherichia coli* (Malinen et al., 2003), and *Clostridium perfringens* (Wang et al., 1994). Amplification was performed according to DePlancke et al. (2002). Briefly, a 10-μL final volume contained 5 μL of 2 × SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 15 pmol of the forward and reverse primers for the bacterium of interest, and 10 ng of extracted fecal DNA. Standard curves were obtained by harvesting pure cultures of the bacterium of interest in the log growth phase in triplicate, followed by serial dilution. Bacterial DNA was extracted from each dilution using a DNA extraction kit (Qiagen)

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and amplified with the fecal DNA to create triplicate standard curves (ABI PRISM 7900HT Sequence Detection System, Applied Biosystems, Foster City, CA). Colony-forming units in each dilution were determined by plating on specific agars; lactobacilli MRS (Difco, BD, Franklin Lakes, NJ) for lactobacilli, reinforced clostridial medium (bifidobacteria, *C. perfringens*), and Luria Bertani medium (*E. coli*). The calculated log cfu per milliliter of each serial dilution was plotted against the cycle threshold to create a linear equation to calculate cfu per gram of dry feces.

Calculations

Dry matter recovery was calculated by dividing Cr intake ($mg \cdot d^{-1}$) by Cr concentrations in feces (mg Cr·g feces^{-1}). Fecal nutrient flows were calculated by multiplying DM flow by nutrient concentrations in the fecal DM. Total tract nutrient digestibilities were calculated as nutrient intake ($g \cdot d^{-1}$) minus fecal nutrient flow (output, $g \cdot d^{-1}$); this value was then divided by nutrient intake ($g \cdot d^{-1}$).

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. Inc., Cary, NC). The statistical model included the random effects of animal and period and the fixed effect of treatment. Least squares means were separated using least squares differences with a Tukey adjustment and linear and quadratic contrasts. Outlier data were removed from analysis after analyzing data using the UNIVARIATE procedure to produce a normal probability plot based on residual data and visual inspection of the raw data. Outlier data were defined as data points 3 or more SD from the mean. Differences among treatment level least squares means with $P \leq 0.05$ were accepted as statistically significant, whereas mean differences with $P \leq 0.10$ were accepted as trends.

RESULTS

Substrate Composition

Dry matter and OM concentrations of the GGMO substrate were greater than 94%, whereas concentrations of CP and acid hydrolyzed fat were less than 1% (Table 2). Of the free monosaccharides, arabinose, xylose, and galactose were greatest in concentration, whereas fructose and sucrose were least. After hydrolysis, free monosaccharide concentrations were greatest for mannose, glucose, and xylose, whereas fucose and rhamnose were least. Oligosaccharide concentrations were greatest for raffinose, cellotriose, and maltopentaose, whereas cellopentaose and maltotriose concentrations were least. No free phenolic compounds were detected in the GGMO substrate. Of the bound phenolics, vanillin and sinapyl acid were greatest in concentration.

Table 2. Dry matter content and chemical composition of the spray-dried galactoglucomannan oligosaccharide (GGMO) substrate (DM basis)

Item	Concentration
DM, %	94.1
OM, %	95.9
CP, %	0.2
Acid hydrolyzed fat, %	0.9
GE, $kcal \cdot g^{-1}$	4.2
Free sugar, $mg \cdot g^{-1}$	
Fucose	1.25
Arabinose	50.76
Rhamnose	1.80
Galactose	11.88
Glucose	2.16
Sucrose	0.00
Xylose	14.47
Mannose	4.64
Fructose	0.97
Total	87.93
Hydrolyzed monosaccharide,[1] $mg \cdot g^{-1}$	
Fucose	3.44
Arabinose	36.79
Rhamnose	5.55
Galactose	76.33
Glucose	159.19
Xylose	134.00
Mannose	353.73
Total	769.03
Oligosaccharide, $mg \cdot g^{-1}$	
Cellobiose	1.64
Raffinose	2.28
Cellotriose	3.68
Maltotriose	0.43
Cellopentaose	0.19
Maltotetraose	0.92
Maltopentaose	2.09
Maltohexaose	1.12
Maltoheptaose	0.87
Total	13.22
Polyphenolic, $mg \cdot g^{-1}$	
m-Coumaric acid	0.08
p-Coumaric acid	0.07
Ferulic acid	0.09
p-Hydroxybenzoic acid	0.02
4′-Hydroxypropiophenone	0.01
Isovanillic acid	0.08
Sinapyl acid	0.92
Sinapyl alcohol	0.04
Sinapyl aldehyde	0.08
Vanillin	1.50
Total	2.89

[1]Hydrolyzed monosaccharide concentrations were corrected for free sugar concentrations.

Chemical Composition of Diets

Chemical composition of diets was similar. Crude protein concentrations were near the desired 30% value (as-is basis). Acid hydrolyzed fat concentrations were near the desired 20% value (as-is basis; Table 1). An

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Table 3. Nutrient intakes and digestibilities of diets supplemented with the galactoglucomannan oligosaccharide (GGMO) substrate and fed to dogs

	Diet, % GGMO substrate							*P*-value	
Item	0.0	0.5	1.0	2.0	4.0	8.0	SEM	Linear	Quadratic
Intake, g·d^{-1}									
DM	288	258	253	264	280	248	22	0.445	0.675
OM	268	241	236	246	261	231	21	0.432	0.702
CP	91	80	81	84	87	81	7	0.602	0.592
Acid hydrolyzed fat	62	58	53	57	63	52	5	0.310	0.776
Total dietary fiber–uncorrected[1]	33	27	26	25	21	10	2	<0.001	0.039
Total dietary fiber–corrected[2]	33	28	28	30	32	29	—	—	—
Apparent digestibility, %									
DM	78.0	78.7	77.9	79.5	82.0	81.8	0.5	<0.001	0.021
OM	81.3	82.0	81.4	82.7	85.0	85.2	0.4	<0.001	0.018
CP	84.2	83.7	82.7	82.7	81.5	77.0	0.5	<0.001	<0.001
Acid hydrolyzed fat	95.6	95.7	95.3	95.5	96.0	95.1	0.2	0.431	0.429

[1]Intake values were determined using the total dietary fiber assay that cannot quantify the GGMO substrate.

[2]Intake values were determined by multiplying DM concentration of the GGMO substrate by the dietary GGMO substrate concentration. This value then was multiplied by DM intake·d^{-1} and added to the total dietary fiber (uncorrected) value.

uncorrected TDF concentration value and a corrected TDF concentration value are reported because the TDF assay cannot quantify the GGMO substrate because oligosaccharides do not precipitate in 78% ethanol and, thus, are unable to be quantified. The TDF concentration values for diets were small except for the cellulose control treatment. To correct this problem, the dietary concentration of GGMO substrate was added to the TDF (uncorrected) value to account for the GGMO substrate not analyzed. After this correction was made, TDF concentrations increased and were similar among diets.

Food Intake and Apparent Nutrient Digestibility

Nutrient intakes were similar ($P = 0.45$) across treatments with dogs consuming between a mean of 248 and 288 g of DM·d^{-1} (Table 3). Uncorrected TDF intake values decreased ($P < 0.001$) linearly with increased supplementation of the GGMO substrate. Total dietary fiber intake was corrected by multiplying the DM concentration of the GGMO substrate by the dietary GGMO substrate concentration. This value then was multiplied by the DM intake·d^{-1} value and added to the TDF (uncorrected) concentration value. The correction increased TDF intake (g·d^{-1}) for each treatment, and after correction, TDF intakes were similar among treatments.

Fecal DM output decreased ($P = 0.006$) linearly as the GGMO substrate concentration increased from 0 to 8% (63 to 45 g·d^{-1}, DM basis, respectively; data not shown). Dry matter and OM digestibilities were greater ($P < 0.001$) for the 4 and 8% supplemental GGMO treatments, whereas values for remaining treatments were less but similar to each other. Crude protein digestibility decreased ($P < 0.001$) quadratically as dietary GGMO substrate concentration increased. Fat digestibility was unaffected by treatment ($P = 0.43$). A corrected TDF digestibility could not be computed because there was no method to determine GGMO substrate digestibility alone.

Fermentation Metabolites

Fecal concentrations of acetate, propionate, and total SCFA increased ($P < 0.001$) linearly as supplemental GGMO concentration increased (Table 4), whereas butyrate concentration decreased ($P < 0.001$) linearly. Fecal isobutyrate, isovalerate, and total BCFA concentrations were not different among treatments (average 5.44, 8.25, and 14.8 μmol·g^{-1}, respectively). A linear increase ($P < 0.01$) in valerate was noted as the dietary GGMO substrate concentration increased.

Fecal pH decreased ($P < 0.001$) linearly as dietary GGMO substrate concentration increased, whereas fecal score increased ($P < 0.001$) quadratically (Table 5). Fecal ammonia concentrations were similar among treatments (average 2.11 mg·g^{-1}). Fecal phenol ($P < 0.05$) and indole ($P < 0.01$) concentrations decreased linearly as dietary GGMO concentration increased. Fecal biogenic amine concentrations were not different among treatments except for phenylethylamine and tryptamine, which decreased ($P < 0.001$ and $P = 0.09$, respectively) linearly as dietary GGMO substrate concentration increased. Total biogenic amine concentrations were not different ($P = 0.23$) among treatments. Across treatments, agmatine and histamine were not detected in feces.

Fecal Microbiota

Fecal microbial concentrations of *E. coli, Lactobacillus* spp., and *C. perfringens* were not different among treatments ($P = 0.91$, 0.78, and 0.82, respectively; Table 6). A quadratic increase ($P < 0.01$) was noted for *Bifidobacterium* spp. as supplemental GGMO concentration increased.

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Table 4. Concentrations (μmol·g^{-1}, DM basis) of fecal short-chain (SCFA) and branched-chain fatty acids (BCFA) for dogs fed diets containing the galactoglucomannan oligosaccharide (GGMO) substrate

	Diet, % GGMO substrate							*P*-value	
Item	0.0	0.5	1.0	2.0	4.0	8.0	SEM	Linear	Quadratic
SCFA									
Acetate	209.3	232.2	217.2	258.6	302.8	393.2	24.9	<0.001	0.003
Propionate	84.1	95.5	91.9	119.7	153.9	183.1	15.4	<0.001	0.076
Butyrate	46.0	40.6	38.6	44.3	33.6	27.2	3.4	0.001	0.201
Total SCFA	339.4	361.5	347.7	422.6	490.3	603.6	38.7	<0.001	0.014
BCFA									
Isobutyrate	5.9	5.5	5.1	4.9	5.8	5.4	0.6	0.681	0.375
Isovalerate	9.2	8.6	7.9	7.9	8.9	6.9	1.1	0.284	0.975
Valerate	1.0	1.1	0.9	1.2	1.2	1.5	0.5	0.010	0.151
Total BCFA	16.1	15.2	13.7	14.1	16.0	13.8	1.7	0.538	0.632

DISCUSSION

The increased DM concentration of the GGMO test substrate is a result of the spray-drying process used to convert the molasses-like product into a powder form. The increased OM concentration is due to the GGMO substrate being composed mostly of carbohydrates, with free sugars and hydrolyzed monosaccharides accounting for 86% of the OM. Crude protein and acid hydrolyzed fat concentrations were very small. Free arabinose concentration was much greater (3.5 times) than the next greatest sugar concentration (xylose). However, after hydrolysis, the concentration of arabinose was much less compared with most other hydrolyzed monosaccharides. The concentration of mannose was very small in the free sugar form, but after hydrolysis, it was present in the greatest concentration and was 2.22 times greater than the next greatest sugar concentration (glucose). Mannose accounted for nearly one-half of the hydrolyzed monosaccharides present in the GGMO substrate. Low molecular weight oligosaccharides accounted for 1.4% of the OM in GGMO. Bound phenolic compounds accounted for 0.3% of the GGMO substrate and are likely derived from the lignin in the starting material. Some polyphenolic compounds may not have been accounted for due to the lack of a standard for some compounds. The GGMO substrate also may contain acetyl groups and sugar alcohols; however, analysis of these compounds was not conducted.

The increased concentrations of mannan, xylans, and glucans result from the cellulose and hemicelluloses present in the wood chips used for production of the GGMO substrate. These carbohydrates resist hydrolytic digestion in the small intestine (Flickinger et al., 2000; Asano et al., 2003), but are partially fermented in the large bowel. Several in vitro and in vivo studies have reported that they exert beneficial effects in the large bowel by increasing production of SCFA, reducing pH, and modulating microbial populations (Djouzi and Andrieux, 1997; Flickinger et al., 2000; Swanson et al., 2002; Smiricky-Tjardes et al., 2003).

Dietary composition was similar among diets except for TDF concentration. Differences in TDF concentration were expected because the GGMO substrate does

Table 5. Fecal pH and score, and concentrations (DM basis) of fecal ammonia, phenol, indole, and biogenic amines for dogs fed diets supplemented with the galactoglucomannan oligosaccharide (GGMO) substrate

	Diet, % GGMO substrate							*P*-value	
Item	0.0	0.5	1.0	2.0	4.0	8.0	SEM	Linear	Quadratic
pH	6.7	6.4	6.2	5.9	5.9	5.8	0.2	<0.001	0.243
Fecal score[1]	2.4	2.4	2.4	2.5	3.4	4.5	0.2	<0.001	<0.001
Ammonia, mg·g^{-1}	2.3	2.3	2.0	2.1	2.1	2.0	0.2	0.181	0.779
Phenol, μg·g^{-1}	112.0	101.7	58.0	90.8	70.6	43.4	26.5	0.050	0.965
Indole, μg·g^{-1}	133.5	158.4	128.4	106.6	107.5	48.5	24.6	0.009	0.204
Biogenic amine, μmol·g^{-1}									
Cadaverine	1.29	1.03	0.88	1.30	1.35	0.80	0.20	0.416	0.619
Phenylethylamine	0.07	0.07	0.03	0.00	0.00	0.00	0.01	<0.001	0.318
Putrescine	4.45	3.57	3.08	4.59	4.22	3.01	0.52	0.463	0.833
Spermidine	1.37	1.43	1.12	1.38	1.35	1.27	0.17	0.726	0.795
Spermine	0.62	0.86	0.25	0.63	0.39	0.35	0.24	0.144	0.963
Tryptamine	0.52	0.49	0.43	0.52	0.47	0.36	0.06	0.093	0.532
Tyramine	0.08	0.06	0.16	0.00	0.10	0.00	0.05	0.294	0.474
Total	8.38	7.51	5.94	8.35	8.26	5.79	0.84	0.226	0.756

[1]Based on the 5-point scale with score 1 being hard, dry pellets, and small hard mass, and score 5 being watery liquid that can be poured.

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Table 6. Fecal microbial populations of dogs consuming diets supplemented with the galactoglucomannan oligosaccharide (GGMO) substrate (cfu; $\log_{10} \cdot g^{-1}$ fecal DM)

	% GGMO substrate							*P*-value	
Item	0.0	0.5	1.0	2.0	4.0	8.0	SEM	Linear	Quadratic
Escherichia coli	10.0	9.9	10.2	9.7	10.3	10.0	0.5	0.915	0.927
Lactobacillus spp.	11.0	10.5	11.0	10.6	10.8	10.9	0.1	0.777	0.186
Bifidobacterium spp.	8.0	8.0	8.3	7.0	8.5	9.8	0.5	0.017	0.006
Clostridium perfringens	9.1	8.9	8.9	9.2	9.0	8.8	0.4	0.822	0.802

not precipitate in 78% ethanol and, thus, is unable to be analyzed properly using the TDF procedure. Nutrient intakes were high, with no significant differences noted among treatments except for TDF. However, when TDF intakes were corrected for supplemental GGMO, values were similar among diets.

It is unusual that the greatest concentration (8%) of a material such as GGMO did not affect nutrient intake. In addition, dogs did not demonstrate any adverse effects such as emesis, signs of gastric distress, or severe diarrhea, to the greater dietary concentrations of GGMO. However, fecal scores for dogs fed the 8% GGMO treatment were unacceptably large, indicating production of loose stool. This was not the case for dogs fed the remaining treatments. The ability of the dog to safely consume a diet with such an increased concentration of fermentable substrate indicates the potential utility of the GGMO. The 4 to 8% concentrations tested far exceed practical levels of dietary inclusion, but our intention was to conduct a study where tolerance could be assessed along with key nutritional/microbiological outcomes. Results indicate that concentrations of GGMO (4 to 8%) are well-tolerated by dogs.

Digestibility coefficients were increased for all nutrients, in part due to the better quality ingredients incorporated in the diet. Dry matter and OM digestibility differences were due mainly to the presence of cellulose, a 0% fermentable insoluble dietary fiber. This lack of fermentability increases DM and OM output in feces, thus decreasing DM and OM digestibility. Muir et al. (1996) found that adding Solka-Floc (7.5%) to diets decreased total tract DM and OM digestibilities in dogs. A similar response was reported by Middelbos et al. (2007b) when select fiber substrates were tested. The diet containing cellulose resulted in decreased DM and OM digestibilities compared with those containing fermentable substrates (fructooligosaccharides, yeast cell wall, or their combination).

Crude protein digestibility decreased as the GGMO concentration increased perhaps because of an increase in microbial biomass production in the large bowel. Increased fermentation in the large bowel would stimulate growth of microbiota, which would be excreted in feces in the form of microbial protein. Several studies have reported reduced apparent CP digestibility because of inclusion of fermentable substrates such as pectin, galactooligosaccharides, mannanoligosacharides, and fructooligosaccharides (Flickinger et al., 2000; Silvio et al., 2000; Zentek et al., 2002; Middelbos et al., 2007b, respectively).

As the dietary concentration of GGMO substrate increased, SCFA concentrations in feces increased, indicative of increased fermentation in the large bowel. However, butyrate concentration decreased overall as a result of GGMO substrate addition to the diet. This decrease could be explained by the rapid fermentation of GGMO in the large bowel, probably in the proximal colon, allowing butyrate, an energy substrate for colonocytes, to be absorbed during passage through the tract rather than be excreted in feces (Topping and Clifton, 2001). Swanson et al. (2002) noted no differences in butyrate concentrations after feeding fermentable fibers (fructooligosaccharides, 1 g; mannanoligosacharides, 1 g; and fructooligosaccharides and mannanoligosacharides, 1 g each) to dogs. These authors stated that this lack of difference could be due to rapid absorption of butyrate by colonocytes. Measurement of SCFA concentrations, particularly butyrate, in the proximal colon would have been useful but impractical in the in vivo dog model. Another possible explanation relates to the mixture of oligosaccharides affecting SCFA production. Englyst et al. (1987) demonstrated in vitro that fermentation of select oligosaccharides results in different quantities of SCFA produced. The oligosaccharides found in the GGMO substrate could possibly ferment to predominantly acetate and propionate with less butyrate. This would explain the linear increase in acetate and propionate concentrations, and linear decrease in butyrate concentration, as dietary GGMO concentration increased. The linear decrease in fecal pH was due to the greater production of SCFA.

Peptides and AA entering the large bowel serve as potential fermentative substrates for the microbiota, especially when energy is limiting. If carbohydrate fermentation occurs rapidly, fermentation likely takes place in the proximal colon (Topping and Clifton, 2001). This leaves little carbohydrate to be fermented in the transverse and distal colon. Bacteria then must ferment peptides and AA for energy. End products of AA fermentation include BCFA, phenol and indole compounds, and biogenic amines. Branched-chain fatty acids result from fermentation of branched-chain AA (valine, leucine, and isoleucine; Macfarlane et al., 1992). The addition of the dietary GGMO substrate did not affect fecal ammonia or BCFA concentrations with the exception of valerate, which made up less than 8% of the total BCFA.

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Phenolic compounds result from the fermentation of aromatic AA (phenylalanine, tyrosine, and tryptophan; Hughes et al., 2000). Fecal phenol and indole concentrations decreased linearly as dietary GGMO concentration increased, indicative of a decrease in AA catabolism by colonic microbiota. Fecal samples were analyzed for 8 different phenol and indole compounds (phenol, 4-methyl phenol, 4-ethyl phenol, indole, 7-methyl indole, 3-methyl indole, 2-methyl indole, and 2,3 dimethyl indole); however, only phenol and indole were detected. The decrease in phenol and indole concentrations could result from the GGMO substrate providing sufficient fermentable energy throughout the large bowel for the microbiota, thus preventing AA from being needed as an energy source. Interestingly, concentrations of phenol in this experiment were much greater than values noted by Middelbos et al. (2007b) who fed a diet containing cellulose (1% of diet), fructooligosaccharides (0.9, 1.2, or 1.5% of diet), and yeast cell wall (0.3 or 0.6% of diet), a source of mannanoligosacharides, to dogs. But, phenol concentrations in the current study were less than those noted by Propst et al. (2003) who reported numerical increases in these metabolites after addition of oligofructose or inulin (0.3, 0.6, or 0.9% of diet) to diets fed to dogs. Swanson et al. (2002) noted a decrease in indole concentration with supplementation (1 $g \cdot dog^{-1} \cdot d^{-1}$) of fermentable substrates (fructooligosaccharides, mannanoligosacharides, or fructooligosaccharides + mannanoligosacharides) to the diet. Middelbos et al. (2007b) reported greater concentrations of indole compared with concentrations observed in the current study. Indole concentration was not affected by addition of fructooligosaccharides plus yeast cell wall to canine diets. Differences in phenol and indole concentrations among studies could be attributed to the TDF concentration of the diets. This would alter the amount of fermentable substrate entering the large bowel and thus alter phenol and indole production.

No differences in fecal biogenic amine concentrations were noted among treatments, except for phenylethylamine. This indicates that the supplemental GGMO substrate does not affect biogenic amine production by the colonic microbiota. Biogenic amine concentrations in this study were greater than those noted by Swanson et al. (2002) and Middelbos et al. (2007b), but were comparable, if not slightly less, than concentration values noted by Propst et al. (2003) who evaluated fermentable carbohydrates (fructooligosaccharides + yeast cell wall; fructooligosaccharides, mannanoligosacharides, and fructooligosaccharides + mannanoligosacharides; oligofructose and inulin, respectively) fed to dogs. In all studies, a numerical increase in total biogenic amine concentration was noted when the dietary concentration of fermentable substrate was increased. Overall, supplemental GGMO did not alter protein fermentation in the large bowel as indicated by a lack of change in fecal ammonia, BCFA, or biogenic amine concentrations.

Biogenic amines, such as putrescine, spermine, and spermidine are beneficial metabolites due to their ability to modulate apoptosis and cellular turnover (Chen et al., 2003; Guo et al., 2005; Seiler and Raul, 2005). Increases, or lack of change in amine concentration after dietary intervention, may be viewed as beneficial to colonic health.

One requirement of a fermentable substrate to be declared a prebiotic is that it must result in an increase in beneficial bacteria (e.g., *Bifidobacterium* spp. and *Lactobacillus* spp.), a decrease in harmful bacteria (e.g., *E. coli* and *C. perfringens*) concentrations, or appropriate changes in both (Roberfroid, 2007). Fecal microbial populations were unaffected by addition of the GGMO substrate except for *Bifidobacterium* spp. whose concentration increased quadratically. A prebiotic effect often is characterized by a 1 log unit increase in concentration of a beneficial bacterium in the fermentative compartment (Roberfroid et al., 1998). An approximate 2 log unit increase was noted between the control and 8% GGMO substrate treatment, indicating the prebiotic potential of the GGMO substrate in the dog, but at an excessive dietary concentration. A 1 log unit decrease was noted when comparing the control and the 2% GGMO treatment. Numerous factors exist that may influence changes in microbial populations such as pH, transit rate, fiber substrate composition, and microbial interactions (El Oufir et al., 1996; Fons et al., 2000; Scott et al., 2008). It is likely that a combination of these factors altered the large bowel environment to one that was not favorable for the growth of *Bifidobacterium* spp., thus the decrease in concentration at this level of supplementation.

Swanson et al. (2002) and Middelbos et al. (2007a) observed no change in fecal bacterial populations when dogs were fed 0.05 to 1% yeast cell wall, a source of mannanoligosaccharides. Strickling et al. (2000) noted numeric changes, less than 0.43 log $cfu \cdot g^{-1}$ DM, in populations of *C. perfringens, Bifidobacterium* spp., and *E. coli* after dogs were fed 0.5% yeast cell wall and xylooligosaccharide. Authors noted that lactobacilli populations increased by 1.02 and 0.83 log $cfu \cdot g^{-1}$ DM after dogs were fed yeast cell wall and xylooligosaccharide, respectively; however, these changes were considered insignificant. It is possible in these studies that the dose used was insufficient to elicit an effect on the microbial populations. The microbiological data from our study do not support use of the GGMO substrate as an effective prebiotic substrate, especially at the concentrations that normally would be included in commercial diets (0.5 or 1.0%).

Increases in nutrient digestibility and fecal SCFA concentrations, in addition to decreased CP digestibility, digesta pH values, and phenol and indole concentrations, indicate an active large bowel fermentation when supplemental GGMO is fed to dogs. Data presented here provide evidence of the positive nutritional properties, but not necessarily prebiotic potential, of supple-

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mental GGMO when incorporated in a high quality dog food. Because of an increased concentration of mannan, continued research on its pathogen-binding capability and its potential as an immunomodulatory agent is necessary to determine its efficacy as a dietary supplement affecting canine health and well being.

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