A Novel Galactooligosaccharide Mixture Increases the Bifidobacterial Population Numbers in a Continuous In Vitro Fermentation System and in the Proximal Colonic Contents of Pigs In Vivo[1]

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ABSTRACT Prebiotics are nondigestible food ingredients that encourage proliferation of selected groups of the colonic microflora, thereby altering the composition toward a more beneficial community. In the present study, the prebiotic potential of a novel galactooligosaccharide (GOS) mixture, produced by the activity of galactosyltransferases from *Bifidobacterium bifidum* 41171 on lactose, was assessed in vitro and in a parallel continuous randomized pig trial. In situ fluorescent hybridization with 16S rRNA-targeted probes was used to investigate changes in total bacteria, bifidobacteria, lactobacilli, bacteroides, and *Clostridium histolyticum* group in response to supplementing the novel GOS mixture. In a 3-stage continuous culture system, the bifidobacterial numbers for the first 2 vessels, which represented the proximal and traverse colon, increased ($P < 0.05$) after the addition of the oligosaccharide mixture. In addition, the oligosaccharide mixture strongly inhibited the attachment of enterohepatic *Escherichia coli* ($P < 0.01$) and *Salmonella enterica* serotype Typhimurium ($P < 0.01$) to HT29 cells. Addition of the novel mixture at 4% (wt:wt) to a commercial diet increased the density of bifidobacteria ($P < 0.001$) and the acetate concentration ($P < 0.001$), and decreased the pH ($P < 0.001$) compared with the control diet and the control diet supplemented with inulin, suggesting a great prebiotic potential for the novel oligosaccharide mixture. J. Nutr. 135: 1726–1731, 2005.

KEY WORDS: • *intestinal microflora* • *prebiotic* • *galactooligosaccharides*

Probiotics, which are live microbial dietary additives thought to confer health advantages, have a long history of use in humans and animals (1). Although numerous publications exist showing that probiotics are active in the gut after ingestion (2,3), inconsistency in scientific reports related to health benefits in human studies are attributed mainly to survivability problems of the organisms after ingestion (4,5). To overcome this survivability issue, the concept of prebiotics was introduced. These are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health (6).

Although any dietary material that enters the large intestine, including resistant starch, dietary fibers, proteins, and lipids, can be considered as a candidate prebiotic, in practice, prebiotics are currently confined to oligosaccharides (7), which can induce a specific colonic fermentation by benign or potentially health-promoting indigenous bacteria, e.g., lactic acid–producing flora (5).

Among the various oligosaccharides tested for potential prebiotic application, galactooligosaccharides (GOS)[3] have attracted interest, mainly because these are the compounds in human milk that have been associated with the improved colonic health of breast-fed infants (8).

Diets enriched with GOS increase populations of *Bifidobacterium* and *Lactobacillus* species (9) and their fermentation products in the colon. The latter are mainly SCFAs, which improve the energy supply to the colonic epithelium, and facilitate calcium and magnesium absorption (9). Moreover, glycoconjugates containing GOS have been implicated in interactions between epithelial and bacterial cells (10), implying that these compounds also have the capacity to inhibit the binding of pathogens to cell surfaces by acting as competitive receptors.

The aim of this work was to evaluate the potential of a novel GOS mixture to modulate the colonic microbiota in a beneficial way and possibly function as "decoy" oligosaccharide binding receptors for 3 gastrointestinal pathogens in vitro. In addition, the prebiotic potential of the mixture was studied in weaned pigs by monitoring microbial changes in the composition of the fecal and colonic microbiota, colonic pH, and colonic SCFA concentration.

MATERIALS AND METHODS

The GOS mixture used in this study was produced from the activity of galactosyltransferases from *Bifidobacterium bifidum* NCIMB 41171 on lactose (11). The final product was in dry powder form consisting of (wt:wt) 45% lactose, 9.9% disaccharides [Gal (β 1–3)- Glc; Gal (β 1–3)- Gal; Gal (β 1–6)- Gal; Gal (α 1–6)- Gal], 23.1%

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[3] Abbreviations used: EPEC, enteropathogenic *E. coli*; FISH, fluorescent in situ hybridization; Gal, galactose; Glc, glucose; GOS, galactooligosaccharide; VTEC, verocytotoxic *E. coli*.

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trisaccharides [Gal (β 1–6)-Gal (β 1–4)- Glc; Gal (β 1–3)- Gal (β 1–4)- Glc], 11.55% tetrasaccharides [Gal (β 1–6)- Gal (β 1–6)- Gal (β 1–4)- Glc], and 10.45% pentasaccharides [Gal (β 1–6)- Gal (β 1–6)- Gal (β 1–6)- Gal (β 1–4)- Glc]. Oligosaccharide fractions for the adhesion assay were purified by gel filtration on a Biogel P2 (Pharmacia) column eluted at 3 mL/min with water. Inulin was supplied by Orafti; all other chemicals and media preparations used in these investigations were from Sigma and Oxoid.

In vitro gut model. Physicochemical conditions in the colon were replicated in a 3-stage continuous fermenter, representing the proximal, transverse, and distal colon (12), inoculated with 10% (wt:v) fecal homogenate from healthy human volunteers in a growth medium without and with 1% (wt:v) of the synthesized GOS mixture. The growth medium contained the following ingredients: starch, 8 g/L; mucin (porcine gastric), 4 g/L; casein, 3 g/L; peptone water, 5 g/L; tryptone water, 5 g/L; bile #3, 0.4 g/L; yeast, 4.5 g/L; $FeSO_4$, 0.005 g/L; NaCl, 4.5 g/L; KCl, 4.5 g/L; KH_2PO_4, 0.5 g/L; $MgSO_4 \cdot 7H_2O$, 1.25 g/L; $CaCl_2 \cdot 6H_2O$, 0.15 g/L; $NaHCO_3$, 1.5 g/L; Tween, 80 1 mL; Hemin, 0.05 g/L; and cysteine · HCl, 0.8 g/L. The system was operated at a retention time of ~36 h. After inoculation, the gut model was left overnight to equilibrate before the medium pump was switched on, and the system was run for a cycle of at least 10 d before medium containing GOS (6 g/d) was introduced for a further cycle of 10 d. Samples (5 mL) were removed at the beginning and end of each cycle.

Bacterial enumerations by fluorescence in situ hybridization (FISH). Differences in bacterial populations were assessed by FISH using oligonucleotide probes labeled with the fluorescent dye Cy3 (Eurogentec UK) and designed to target specific diagnostic regions of bacterial 16S rRNA, as previously described (13). The molecular probes for specific bacterial genera used were Bac 303 for *Bacteroides* spp. (14), Bif 164 for *Bifidobacterium* spp. (15), Chis 150 for bacteria belonging to the *Clostridium histolyticum* group (16), and Lab 158 for *Lactobacillus/Enterococcus* spp. (17). Total bacterial counts were obtained by staining with the nucleic acid stain 4,6-diamidino-2-phenylindole.

Oligosaccharide sensitivity assay. The HT29 cell line was obtained from European Collection of Cell Cultures for Applied Microbiology and Research. Cell stocks were cultured at 37°C in humidified 5% CO_2 in air in DMEM supplemented with 5% (v:v) fetal bovine serum, 0.1 mol/L penicillin, 0.1 mol/L streptomycin, nonessential amino acids (×100), and 0.2 mol/L α-glutamine. Cells were refed every 48 h and passaged before confluence was reached. Serum standard medium (1% v:v) supplemented with different concentrations of oligosaccharides (0.01, 0.1, 1, 10, 100 mmol/L) was used for the oligosaccharide sensitivity assay as described by Olano-Martin et al. (18) to determine any toxic effects of the oligosaccharide mixture on the HT29 cells. The percentage of cell survival was calculated as: % survival = (mean absorbance of treated cells/mean absorbance of control) × 100.

Adhesion assay. HT29 cells were grown in 12-well tissue culture plates to >90% confluence using the standard medium. For the final cell feeding before performing the assay, antibiotic-free medium was used. PBS (0.5 mL), containing each test oligosaccharide (10 mol/L), was added in 3 wells (PBS without any oligosaccharide was included as a control) and 0.5 mL of a 1/1000 dilution of the pathogen culture [enteropathogenic *Escherichia coli* (EPEC) O26 NCTC 08620, verocytotoxic *E. coli* (VTEC) O157:H7 VT$^-$ PHLS NCO12900, or *Salmonella typhimurium* NCFIMB 10248] was added to all wells. The plates were placed on a rocker mixer and incubated aerobically at 37°C for 2 h. The culture was aspirated and after 3 washes with sterile PBS (1 mL/well), 70 μL trypsin/EDTA solution was added to each well, mixed, and allowed to stand for 5 min at 37°C. PBS (1 mL) was added to each well and mixed by pipette to ensure that all of the cells were removed from the bottom of the well and that clumps were broken up. Cell suspension (1 mL) was plated out on plate count agar and incubated at 37°C for 24 h. After incubation, the colonies were enumerated and the inhibition of adhesion was calculated as the ratio of bacteria (cfu/L) present in the sample compared with the control (PBS).

Animals, diets and feeding study. Weaned male pigs (n = 40; mean live weight of 14.7 ± 2.6 kg; 28 d old) were purchased from JSR Genetics. On arrival, they were housed in 4 groups of 10 for 7 d to allow them time to settle after transport, and to acclimate to the unit and diet. During the acclimation period, pigs were fed the NEG diet[4] (Deltawean 15 NGP pellets, ABN), a complete, antibiotic-free diet for growing pigs. After this period, the pigs were transferred to individual pens randomly grouped within the same unit. Pigs were bedded on sawdust throughout the study; straw was also provided as an environmental enrichment as were "toys" to help alleviate boredom. The study was conducted in accordance with the guidelines of the Ethics & Research Committee at the University of Reading.

After acclimation, pigs were fed 1 of the following 4 diets: NEG alone, NEG supplemented with the novel GOS mixture (1.6% by weight), the novel GOS mixture (4% by weight), or inulin (1.6% by weight). The novel GOS (55% wt:wt synthesized oligosaccharides) and inulin additives were weighed separately for each pig and stored in sealed mini-grip bags; they were added to the control feed at the time of the morning feeding. This ensured that each individual pig received the prescribed dose and eliminated potential variability due to inadequate mixing.

Sample collection. The pigs were fed the dietary treatments for a mean of 34 d (33–35 d), after which they were killed by an injection of a lethal dose of sodium barbiturate (15 mL) into an ear vein. Each pig then underwent laparotomy and ties were placed on the large bowel, ~2 cm downstream of the ileocecal junction and ~2 cm upstream of the rectum. With minimum disturbance, the entire large intestine was removed as quickly as possible to a tray containing ice and transported to an adjacent laboratory. The proximal and distal sections of the large intestine were then freed from the mesentery and clamps placed to isolate the semiliquid contents of the proximal and distal colon. Samples of proximal and distal colonic contents were obtained for bacterial enumeration, SCFA, and pH measurement. Fecal samples from each pig were obtained at the beginning and at the end of the feeding period.

Analysis of SCFA and lactic acid. Production of lactic, acetic, propionic, and butyric acids in the colonic contents was quantified. Samples were centrifuged at 1500 × g for 15 min and the resultant supernatant used for analysis. A Model 1050 UV HPLC (Hewlett Packard) with an integrated oven compartment (50°C) and data system was used. Sample injection (20 μL) was performed using an autosampler. The column was a prepacked Aminex HPX-87-H strong cation-exchange resin column (150 × 7.8 mm i.d.), fitted with an ion exclusion microguard refill cartridge (Bio-Rad). The eluent was 0.005 mol/L sulfuric acid.

Statistical analyses. All data were expressed as means ± SD. For the in vitro gut model experiment, means before and after the treatment were analyzed by paired Student's t test. For the adhesion assay and pig feeding experiment, the data were examined for equality by Levene's test; if there was a difference ($P < 0.05$), the data were log-transformed. One-way ANOVA was performed to determine the effect of the test oligosaccharides on the adhesion of the pathogenic bacteria to HT29 cells. Significant differences from the control (PBS) were determined by Dunnett's test. The effects of diet on *1*) proximal colonic microbiota, *2*) distal colonic microbiota, *3*) fecal microbiota, *4*) proximal colonic SCFA concentration, and *5*) distal colonic SCFA concentration were determined by 1-way ANOVA. Significant differences between diet groups were determined by Tukey's Honestly Significant Difference test for comparison of means within a factor. All statistical analyses were performed using the SPSS package program version 11.5.0 and differences were considered significant at $P < 0.05$.

RESULTS

In vitro gut model system

The in vitro gut model is a system for screening the effect of dietary intervention on the colonic microbiota composi-

[4] A commercial diet containing (g/kg): oil 33, protein 192, lysine 13.2, fiber 28, ash 48, moisture 138, vitamin premix 1.2 (vitamin A, 9500 IU; vitamin E, 100 IU; cholecalciferol, 1850 IU; sodium selenite, 0.0003; cupric sulfate, 0.17; antioxidant premix, 6 (BHA, 1; BHT, 1; ethoxyquin, 4).

tion. GOS supplementation did not affect population numbers of total bacteria, lactobacilli, bacteroides, or clostridia (**Table 1**). Numbers of bifidobacteria increased by 0.9 $\log_{10}$ cells in the first vessel ($P < 0.01$) (representing the proximal colon), and similarly in the second vessel, the transverse colon (0.7 $\log_{10}$ cells; $P < 0.01$) (Table 1). Numbers of lactobacilli (0.2 $\log_{10}$ cells) tended to be greater ($P = 0.16$) in the second vessel after addition of the novel GOS.

Oligosaccharide sensitivity assay

Addition of the GOS mixture, up to 10 mmol/L, to the growth medium had no toxic effect on HT29 cells after 24 and 48 h of incubation. Cell viability was reduced in the presence of 100 mmol/L of GOS after 24 h ($P < 0.001$) and 48 h ($P < 0.001$) of incubation

Adhesion assay

The GOS addition, as a mixture and as individual fractions, did not affect the adhesion of *E. coli* 0157:H7 (VT^-) ($P = 0.194$). A very strong inhibition (almost 90%) of attachment of EPEC ($P < 0.001$) and *Salmonella enterica* serotype Typhimurium ($P < 0.001$) to HT29 cells occurred in the presence of the novel GOS mixture. We attributed this inhibition to the disaccharide fraction of the mixture, which had identical anti-adhesive properties ($P = 0.001$ and $P < 0.001$, respectively). Apart from the disaccharide fraction, only the fraction containing tetra- and pentasaccharides inhibited attachment (60% against *S. enterica* serotype Typhimurium $P < 0.05$).

Clinical measurements

Dietary intake and pig growth. All pigs consumed the entire volume of feed provided each day. The final body weight was 42.5 ± 3 kg and did not differ among experimental groups at any time of the study. One pig was euthanized on welfare grounds after ~3 wk of the study.

Bacterial population changes in colonic contents and feces. Major bacterial genera in the proximal and distal colonic contents (**Table 2**), and feces of pigs were measured (**Table 3**). Total numbers of bacteria for the same colonic regions did not differ among treatments (proximal colon $P = 0.876$; distal colon $P = 0.994$; feces $P = 0.989$), but did differ among sample type ($P < 0.05$).

The *Clostridium histolyticum* group populations did not differ among the treatments in the proximal ($P = 0.94$) or distal ($P = 0.266$) colonic content or feces ($P = 0.072$). Bifidobacterial numbers were unaffected by the addition of 1.6% (wt:wt) GOS ($P = 0.556$) or inulin ($P = 0.228$) compared with the control diet but were greater than in all other groups in the pigs fed 4% (wt:wt) GOS ($P < 0.001$). The number of bacteroides was greater in pigs fed inulin than in pigs fed the control diet ($P < 0.01$) or 4% GOS ($P < 0.05$). The lactobacilli population did not differ among the NEG-, 1.6% GOS-, and inulin-fed groups ($P = 0.152$). Pigs fed 4% GOS had more lactobacilli than pigs fed the NEG diet ($P < 0.01$), but did not differ from pigs fed 1.6% GOS ($P = 0.234$) or inulin ($P = 0.315$) (Table 2). In the distal colonic content samples, the groups did not differ in bacteroides ($P = 0.161$) and clostridia ($P = 0.266$) population numbers.

Pigs fed 4% GOS had greater numbers of bifidobacteria than pigs fed NEG ($P < 0.05$) or 1.6% GOS ($P < 0.05$), but did not differ from pigs fed inulin ($P = 0.533$). The numbers of lactobacilli did not differ among the 1.6% GOS-, 4% GOS-, and inulin-fed groups ($P = 0.752$), all of which had more lactobacilli than the NEG group ($P < 0.01$) (Table 2).

Fecal clostridia population numbers did not differ among the groups ($P = 0.072$). Pigs fed inulin had higher numbers of fecal bacteroides than those fed NEG ($P < 0.05$), but did not differ from those fed 1.6% GOS ($P = 0.207$) or 4% GOS groups ($P = 0.245$). Fecal lactobacilli did not differ among the 1.6% GOS-, 4% GOS-, and inulin-fed groups, all of which had higher numbers than pigs fed the NEG diet ($P < 0.05$). Fecal bifidobacteria populations in pigs fed inulin were higher than in pigs fed NEG ($P < 0.001$) or 1.6% GOS ($P < 0.05$), but did not differ from those in pigs fed 4% GOS ($P = 0.735$) (Table 3).

Lactic acid and SCFA concentration in colonic contents

Lactic, acetic, propionic, and butyric acid concentrations in the proximal and distal colonic contents of pigs were determined (**Table 4**). In the proximal colonic contents, lactic acid concentration did not differ between pigs fed the NEG diet and those fed the inulin supplement ($P = 0.814$). Pigs fed 4% GOS had greater lactic acid concentration than pigs fed NEG ($P < 0.05$) and inulin ($P = 0.058$). Addition of 1.6% GOS resulted in the highest lactic acid concentration (6.62 mmol/L) among the treatment groups ($P < 0.001$). No lactic acid was detected in the distal colonic samples.

In the proximal colonic samples, the acetic acid concen-

TABLE 1

Bacterial populations as determined by FISH in an in vitro gut model using the novel GOS as a substrate at 7 g/d[1,2]

	Vessel 1			Vessel 2			Vessel 3		
Time, *d*	1	11	21[3]	1	11	21[3]	1	11	21[3]
	$\log_{10}$ cells/L of culture								
Total bacteria	12.4 ± 0.3	12.7 ± 0.3	12.6 ± 0.2	12.4 ± 0.2	12.5 ± 0.3	12.6 ± 0.3	12.6 ± 0.2	12.5 ± 0.3	12.5 ± 0.3
Bifidobacterium spp.	11.1 ± 0.2	11.0 ± 0.2	11.9 ± 0.3*	11.0 ± 0.2	11.0 ± 0.3	11.7 ± 0.3*	11.2 ± 0.3	11.2 ± 0.3	11.4 ± 0.2
Lactobacillus spp.	10.4 ± 0.2	10.6 ± 0.2	10.6 ± 0.2	10.3 ± 0.2	10.3 ± 0.3	10.5 ± 0.3	10.4 ± 0.2	10.3 ± 0.3	10.3 ± 0.3
Bacteroides spp.	11.0 ± 0.4	11.2 ± 0.3	11.2 ± 0.3	11.0 ± 0.4	11.1 ± 0.3	11.1 ± 0.4	10.8 ± 0.4	10.8 ± 0.2	10.8 ± 0.3
Clostridium histolyticum group	9.9 ± 0.2	9.0 ± 0.4	9.8 ± 0.2	9.8 ± 0.2	9.8 ± 0.2	9.7 ± 0.3	10.0 ± 0.3	10.0 ± 0.2	9.9 ± 0.3

[1] Values are means ± SD, $n = 5$ (data were log-transformed). * Different from d 11 (steady state before treatment), $P < 0.05$.
[2] The GOS supplement contains 55% wt:wt synthesis product.
[3] Main effect of GOS after 10 d of supplementation.

TABLE 2

Bacterial populations in proximal and distal colon contents of weaned pigs fed a commercial diet alone (NEG) or supplemented with 1.6 or 4% (wt:wt) GOS or 1.6% (wt:wt) inulin after 4 wk of treatment[1,2]

	Proximal colon					Distal colon				
	NEG	1.6% GOS	4% GOS	Inulin	*P*	NEG	1.6% GOS	4% GOS	Inulin	*P*
	log$_{10}$ *cells/g wet wt*									
Total bacteria	8.61 ± 0.24	8.68 ± 0.22	8.62 ± 0.21	8.67 ± 0.25	0.876	8.83 ± 0.28	8.81 ± 0.21	8.80 ± 0.22	8.80 ± 0.29	0.994
Bifidobacterium spp.	7.13 ± 0.25^{b}	7.30 ± 0.26^{b}	7.87 ± 0.26^{a}	7.37 ± 0.32^{b}	<0.001	7.03 ± 0.25^{b}	7.05 ± 0.26^{b}	7.41 ± 0.27^{a}	7.58 ± 0.32^{a}	<0.001
Lactobacillus spp.	6.94 ± 0.23^{b}	7.17 ± 0.26^{ab}	7.38 ± 0.24^{a}	7.19 ± 0.24^{ab}	0.007	6.60 ± 0.34^{b}	6.96 ± 0.21^{ab}	7.16 ± 0.35^{a}	7.05 ± 0.30^{a}	0.002
Bacteroides spp.	7.57 ± 0.21^{b}	7.76 ± 0.21^{ba}	7.65 ± 0.33^{b}	7.97 ± 0.24^{a}	0.003	7.77 ± 0.27	7.88 ± 0.24	7.82 ± 0.26	8.04 ± 0.32	0.161
Clostridium histolyticum group	8.12 ± 0.23	8.08 ± 0.25	8.13 ± 0.28	8.07 ± 0.27	0.940	8.05 ± 0.41	8.15 ± 0.15	8.29 ± 0.19	8.18 ± 0.23	0.266

[1] Values are means ± SD, $n = 10$ (data were log-transformed). For each colonic region, means in a row without a common superscript differ, $P < 0.05$.
[2] GOS supplement contains 55% (wt:wt) synthesis product.

tration was significantly higher for pigs fed 4% GOS than for any other treatment ($P < 0.01$). In the distal colonic contents, the acetic acid concentration was greater in pigs fed inulin than in pigs fed NEG ($P < 0.01$), 1.6% GOS ($P < 0.01$), or 4% GOS ($P < 0.05$).

Propionic acid concentration did not differ among the treatment groups in the distal colonic contents ($P = 0.311$). In the proximal colonic contents, the propionic acid concentration was lower in pigs fed 1.6% GOS than in pigs fed NEG ($P < 0.01$), 4% GOS ($P = 0.129$), or inulin ($P = 0.582$). Concentrations of butyric acid concentration (10–11 mmol/L) did not change in response to the dietary treatments ($P = 0.944$) in the proximal colonic content. In the distal colonic contents, pigs fed inulin had a higher butyric acid concentration than pigs fed NEG ($P = 0.068$), 1.6% GOS ($P < 0.05$) or 4% GOS ($P = 0.234$).

pH of colonic contents

Pigs fed 4% GOS had lower proximal colonic pH than pigs fed NEG ($P < 0.05$), 1.6% GOS ($P < 0.05$), or inulin ($P < 0.001$). Pigs fed inulin had higher pH than pigs fed 1.6% GOS ($P < 0.05$). The distal colonic pH did not differ among the treatment groups ($P = 0.995$) (**Table 5**).

DISCUSSION

The prebiotic potential of commercial GOS was demonstrated previously in various animal and human studies (9). However, the novel GOS mixture investigated here both in vitro and in vivo was the product of the galactosyltransferase activity of *Bifidobacterium bifidum* NCIMB 41171, a common bacterium in the gastrointestinal tract of healthy humans, on lactose. The normal function of the enzyme that was used in the synthesis reaction is to hydrolyze substrates; therefore, bifidobacteria that are expressing the enzyme have a competitive advantage for growth on the synthesized oligosaccharides as previously demonstrated in vitro (11). Such a preference was seen in the gut model used here, which simulated various physicochemical conditions within anatomically distinct regions of the colon (Table 1). An increase of 0.9 $\log_{10}$ cells in the bifidobacterial population numbers was observed in the first vessel, which represented the proximal colon. The effect was reduced in the second vessel and almost lost in the third,

TABLE 3

Bacterial populations in feces from pigs fed a commercial diet (NEG) or NEG supplemented with 1.6 and 4% (wt:wt) GOS and 1.6% (wt:wt) inulin before and after 4 wk of treatment[1,2]

Time	0 wk	4 wk				
		NEG	1.6% GOS	4% GOS	Inulin	*P*
		log$_{10}$ *cells/g wet wt*				
Total bacteria	8.75 ± 0.22	8.97 ± 0.24	8.99 ± 0.23	8.97 ± 0.28	8.95 ± 0.28	0.989
Bifidobacterium spp.	6.48 ± 0.29	6.91 ± 0.25^{c}	7.07 ± 0.23^{bc}	7.34 ± 0.21^{ab}	7.45 ± 0.24^{a}	<0.001
Lactobacillus spp.	6.33 ± 0.25	6.55 ± 0.23^{b}	6.93 ± 0.16^{a}	7.17 ± 0.24^{a}	6.94 ± 0.26^{a}	0.049
Bacteroides spp.	7.27 ± 0.23	7.75 ± 0.24^{b}	7.84 ± 0.27^{ab}	7.85 ± 0.22^{ab}	8.04 ± 0.18^{a}	<0.001
Clostridium histolyticum group	7.43 ± 0.33	8.04 ± 0.24	8.14 ± 0.25	8.33 ± 0.28	8.22 ± 0.23	0.072

[1] Values are means ± SD, $n = 40$ at 0 wk; $n = 10$ at 4 wk (data were log-transformed). Means in a row without a common superscript differ, $P < 0.05$.
[2] GOS supplement contains 55% wt:wt synthesis product.

TABLE 4

SCFA concentrations in the proximal and distal colon contents of weaned pigs fed a commercial diet alone (NEG) or NEG supplemented with 1.6 or 4% (wt:wt) GOS or 1.6% (wt:wt) inulin after 4 wk of treatment[1,2]

	Proximal colon					Distal colon				
	NEG	1.6% GOS	4% GOS	Inulin	*P*	NEG	1.6% GOS	4% GOS	Inulin	*P*
	μmol/g wet matter									
Lactic acid	2.62 ± 0.7^{c}	6.62 ± 1.7^{a}	4.41 ± 0.8^{b}	3.07 ± 1.1^{bc}	<0.001	ND[3]	ND	ND	ND	—
Acetic acid	41.43 ± 6.5^{b}	44.45 ± 2.8^{b}	51.85 ± 2.7^{a}	44.58 ± 3.3^{b}	<0.001	31.61 ± 5.5^{b}	30.68 ± 2.3^{b}	33.57 ± 2.2^{b}	38.54 ± 3.7^{a}	<0.001
Propionic acid	35.68 ± 5.1^{a}	27.52 ± 2.9^{b}	32.99 ± 8.4^{ab}	30.63 ± 3.6^{ab}	0.013	15.36 ± 3.5	15.27 ± 1.9	16.75 ± 3.9	17.36 ± 2.2	0.311
Butyric acid	10.51 ± 1.4	10.57 ± 1.6	11.19 ± 3.2	10.86 ± 4.0	0.944	4.81 ± 1.2^{ab}	4.48 ± 0.7^{b}	5.13 ± 1.1^{ab}	6.11 ± 1.4^{a}	0.017

[1] Values are means ± SD, $n = 10$ (data were log-transformed). For each colonic region, means in a row without a common superscript differ, $P < 0.05$.
[2] GOS supplement contains 55% (wt:wt) synthesis product.
[3] ND, not detected.

most probably because the low molecular weight of the synthesized oligosaccharides meant that they did not persist throughout the gut model.

Oligosaccharides may also have therapeutic potential to reduce *Salmonella* spp. and *E. coli*–associated enterotoxigenic infections. The hypothesis is that these oligosaccharides act as functional mimics for bacterial receptors on intestinal cells; therefore, they competitively bind the bacteria, thereby preventing attachment to the gut epithelium. Previous studies reported that glycolipid receptors containing the galabiose disaccharide can mediate the binding of P-fibriated *E. coli* to uroepithelial cells (19) and that oligosaccharides were able to block the adherence of P-fimbriated *E. coli* to cells in vitro (20,21). Here, we used the synthesized oligosaccharides (GOS), as a mixture and as oligosaccharide fractions to study the adhesion of an EPEC, a VTEC O157:H7 (VT^{-}), and *S. enterica* serotype Typhimurium to HT29 adenocarcinoma cells in vitro. Addition of the novel GOS mixture to the assay system reduced the attachment of EPEC ($P < 0.01$) and *S. enterica* serotype Typhimurium ($P < 0.01$) but not of the VTEC ($P = 0.194$). When various fractions of the mixture were tested, only the disaccharide fraction, which also contained an α anomeric configuration, had a similar effect, although larger molecules of the tetra- and pentasaccharides showed a degree of inhibition of *S. enterica* serotype Typhimurium attachment.

The prebiotic capacity of the novel GOS was further investigated in a pig feeding trial. Pigs are considered to be a good experimental model for humans due to physiologic and metabolic similarities (22). For a food ingredient to be considered to be a prebiotic, in addition to promoting a healthier colonic microbiota, it must not be hydrolyzed or absorbed in the upper part of the gastrointestinal tract (6). The reduced pH ($P < 0.05$) in the proximal colon combined with significant increases in SCFA production ($P < 0.01$) strongly suggest that the GOS mixture was not absorbed in the small intestine, but was delivered to the colon intact. The increase in SCFA in the proximal colon was due almost entirely to acetic acid production ($P < 0.01$), the main fermentation product of bifidobacteria. This was in turn confirmed by an increase in bifidobacterial numbers, consistent with prebiotic activity. Generally, the performance of the novel mixture (high concentration) in manipulating the bacterial composition was comparable to that of inulin in the distal colon and fecal samples, but offered a significant improvement in the proximal colon.

Nondigestible oligosaccharides were suggested to benefit the health of the host by selectively stimulating the growth and activity of potentially health promoting bacteria. It has been proposed that the "second generation" prebiotics should be designed to have improved functionality by providing a more selective fermentation and/or added functionality. In this context, we showed in vitro and in vivo that this novel GOS mixture not only offers a prebiotic effect, but also selectivity toward bifidobacteria. Moreover, the GOS mixture (in vitro) has the potential to inhibit the attachment of 2 common gastrointestinal pathogens, enteropathogenic *E. coli* and *S. typhimurium*, to the colonic epithelium by acting as a decoy

TABLE 5

pH of proximal and distal colon contents of weaned pigs fed a commercial diet alone (NEG) or NEG supplemented with 1.6 or 4% (wt:wt) GOS or 1.6% (wt:wt) inulin after 4 wk of treatment[1,2]

	NEG	1.6% GOS	4% GOS	Inulin	*P*
Proximal colon	5.71 ± 0.16^{ab}	5.65 ± 0.11^{b}	5.49 ± 0.14^{c}	5.90 ± 0.27^{a}	<0.001
Distal colon	7.16 ± 0.04	7.16 ± 0.03	7.16 ± 0.04	7.12 ± 0.02	0.995

[1] Values are means ± SD, $n = 10$ (data were log-transformed). Means in a row without a common superscript differ, $P < 0.05$. ND, not detected.
[2] GOS supplement contains 55% (wt:wt) synthesis product.

for their receptor sites. However, further in vivo studies are warranted to evaluate that potential.

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