Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characteristics of growing pigs

M. R. Smiricky-Tjardes, C. M. Grieshop, E. A. Flickinger, L. L. Bauer and G. C. Fahey, Jr.

J ANIM SCI 2003, 81:2535-2545.

Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characteristics of growing pigs[1]

M. R. Smiricky-Tjardes, C. M. Grieshop, E. A. Flickinger, L. L. Bauer, and G. C. Fahey, Jr.[2]

Department of Animal Sciences, University of Illinois, Urbana 61801

ABSTRACT: The objective of this study was to evaluate dietary galactooligosaccharide (Gal OS) addition on swine nutrient digestibility, ileal and fecal bacterial populations, and ileal short-chain fatty acid (SCFA) production, and to determine their impact on ileal fermentative characteristics in vitro. Twelve T-cannulated pigs (BW = 25 kg) were fed a diet free of Gal OS for 21 d. On d 22, ileal digesta samples were collected for an in vitro fermentation experiment (Exp. 1). Substrates included: raffinose/stachyose combination (R + S), soy solubles (SS), and transgalactooligosaccharides (TOS). Also included were the non-OS components of SS and TOS. Nine pigs (three donors per treatment) served as ileal effluent donors. Each substrate was fermented in vitro for 6 h, and pH and SCFA and gas production were determined. Pigs then were allotted to three treatments: a Gal OS-free control diet and the control diet with either 3.5% added Gal OS from SS or TOS. Diets, feces, and digesta samples collected weekly for 6 wk on d 6 (feces) and 7 (digesta) were analyzed for DM, OM, CP, and chromic oxide concentrations. Feces and ileal digesta were analyzed for bifidobacteria and lactobacilli populations. Ileal digesta samples were analyzed for SCFA. On d 64, a second in vitro fermentation experiment (Exp. 2) was conducted using ileal effluent from three pigs per treatment and the same substrates used in Exp. 1. In vivo results showed that ileal and total tract DM and OM digestion were decreased ($P < 0.05$) by addition of both SS and TOS to the diet. Ileal and total-tract N digestibilities were decreased ($P < 0.05$) by dietary addition of SS. Fecal bifidobacteria and lactobacilli were increased ($P < 0.05$) by addition of SS and TOS to the diet. Ileal propionate and butyrate concentrations were greater ($P < 0.05$) for pigs fed diets containing both sources of Gal OS. In vitro results showed that fermentation data were not affected by donor animal adaptation to treatment. For both in vitro experiments, gas and SCFA production were higher ($P < 0.05$) for R + S than for SS or TOS. Fermentation of R + S resulted in a higher pH ($P < 0.05$) than did SS or TOS. Fermentation of non-OS components of SS and TOS resulted in more ($P < 0.05$) gas and SCFA production, and pH values that did not differ ($P > 0.05$) compared to SS and TOS. The Gal OS used in this study were prebiotics, increasing beneficial bacteria in vivo and SCFA concentrations both in vivo and in vitro.

Key Words: Bacteria, Digestibility, Fermentation, Oligosaccharides, Pigs

 J. Anim. Sci. 2003. 81:2535–2545

Introduction

The presence of gut bacteria can influence the nutrition and health of pigs positively or negatively. Interaction and/or competition of beneficial bacteria with potentially pathogenic bacteria can decrease or prevent colonization of the gastrointestinal tract with these species.

Changes in the diet of the pig can alter fermentative activity of bacteria in the gastrointestinal tract. Introduction of fermentable substrates into the diet could increase proliferation of beneficial bacteria (Mosenthin and Zimmermann, 2000). Due to the ability of some indigestible OS to promote favorable microflora, the term "prebiotic" was introduced to describe these compounds (Gibson and Roberfroid, 1995). Growth of these bacteria could limit invasion of pathogenic bacteria into the intestine. Additionally, these beneficial bacteria function by controlling the pH of the intestine through release of lactic and acetic acids (Modler et al., 1990). Release of these short-chain fatty acids (**SCFA**) also restricts growth of many pathogenic bacteria (Rasic, 1983).

Galactooligosaccharides (**Gal OS**) are present in significant quantities in the swine diet matrix. Soy oligosac-

[1]The soy-soluble product used in this experiment was graciously donated by J. C. Russett of Central Soya, Fort Wayne, IN.

[2]Correspondence: 132 Animal Sciences Laboratory, 1207 W. Gregory Dr. (phone 217-333-2361; fax 217-244-3169; E-mail: gcfahey@uiuc.edu).

Received June 25, 2002.
Accepted June 10, 2003.

charides (**soy OS**) are partially fermented by the action of colonic microflora (Smiricky-Tjardes et al., 2003). These Gal OS may function as selective growth factors for beneficial bacteria. Therefore, Gal OS could potentially be useful as prebiotics to promote growth of beneficial bacteria in the pig intestine. If prebiotics improve intestinal health, the necessity of subtherapeutic antibiotic supplementation may be diminished. Therefore, the objective of this study was to compare the effect of dietary inclusion of Gal OS on ileal and total-tract nutrient and OS digestibilities, ileal and fecal bifidobacteria and lactobacilli concentrations, ileal SCFA concentrations, and fermentation characteristics of selected Gal OS sources using ileal effluent as inoculum.

Materials and Methods

In Vivo Experiment

Animal and Diets. Twelve crossbred pigs (BW = 30 ± 2 kg; PIC 326 sire line × C22 dams; PIC, Franklin, KY) were used in this experiment. The pigs had been surgically fitted with a simple-T cannula approximately 12 cm anterior to the ileo-cecal junction, according to procedures adapted from Sauer et al. (1983). Adaptations included the cannula design and anesthetics. Nylon cannulas, with a smooth outer ring, plug, and screw cap were used. The cannula barrel diameter was widened to allow for collection of greater volumes of digesta. The flange was widened and smoothed to allow increased stability when the cannula was exteriorized between the last two ribs. Before use of halothane anesthesia, pigs were sedated with 1.5 mL of an i.m. mixture of tiletamine HCl, zolazepan HCl (100 mg/mL telazol), ketamine HCl (50 mg/mL), and xylazine HCl (50 mg/mL; Fort Dodge Animal Health, Fort Dodge, IA). The University of Illinois Institutional Animal Care and Use Committee approved all experimental procedures before experiment initiation (protocol No. 01243). Pigs were housed in individual metabolism crates in a temperature-controlled room. Antibiotics and ceftiofur sodium (Excenel; Pharmacia Animal Health, Kalamazoo, MI; 3 mg/kg BW) were administered once per day i.m. in the neck directly after surgery and for 3 d following surgery.

Pigs were removed from in-feed antibiotics and fed an oligosaccharide-free control diet (Table 1) for 21 d before project initiation. Fourteen days after the last antibiotic injection and 35 d after withdrawal from in-feed antibiotics, digesta were collected to serve as the source of inoculum for in vitro fermentation (Exp. 1) and pigs were assigned to dietary treatment. Three isonitrogenous diets were used in this experiment (Table 1). Diets were formulated to meet or exceed nutrient requirements of 25- to 50-kg pigs (NRC, 1998) and to contain 17% CP (as-fed basis). The diets included: 1) a Gal OS-free casein-cornstarch control (**Gal OS-free**); 2) casein-cornstarch diet + 17% SS (**SS**); and 3) casein-cornstarch diet + 6% TOS (**TOS**). The SS and TOS served as sources of supplemental Gal OS. The composition of SS (Central Soya, Gibson City, IL) and TOS (Borculo Domo Ingredients, Borculo, The Netherlands) is presented in Table 2. Soy solubles contained 3.96% raffinose, 15.94% stachyose, 24.45% sucrose, and 11.93% total AA. Transgalactooligosaccharides contained 58.3% galactooligosaccharides, 20% lactose, and 18% glucose. Chromic oxide was included (0.5%, as-fed) in all experimental diets and served as an inert marker for digestibility calculations.

Pigs were fed twice daily (0800 and 2000 h, equal portions at each meal). Initial feeding amount was determined on the basis of $0.09 \times BW^{0.75}$ and the amount was increased 150 g in each subsequent experimental period. Water was provided for ad libitum consumption from a low-pressure drinking nipple.

Each experimental period lasted 7 d and included a 5-d adaptation period and a 2-d collection period. Fecal collections occurred on d 6, and ileal digesta collections occurred on d 7. Digesta were collected continuously from 0800 to 2000 into polyethylene tubing (5 cm × 25 cm; Rand Materials Handling Equipment Co., Inc., Pawtucket, RI) that was emptied every hour into plastic containers and stored at −10°C until the end of the collection. After collection, digesta were thawed, pooled by pig, and a subsample was freeze-dried. Feces and digesta were collected for all microbiological analyses within 15 min of excretion. Individual aliquots were immediately transferred to preweighed Cary-Blair transport media containers (Meridian Diagnostics, Cincinnati, OH) for subsequent bacterial enumeration. Pigs remained on their respective dietary treatment for 6 wk to evaluate whether dietary adaptation to inclusion of Gal OS occurred.

Chemical Analyses. Diets and freeze-dried digesta were ground in a coffee mill (Mr. Coffee, Bedford Heights, OH). Diets, digesta, and feces were analyzed for DM, OM, and N (method No. 999.03) using AOAC (1995) methodology. Chromium was quantified in the diets, digesta, and feces by the method of Fenton and Fenton (1995). Raffinose and stachyose concentrations of diets, digesta, and feces were quantified by HPLC according to Smiricky et al. (2002). Transgalactooligosaccharide concentrations (method no. 2001.01) of diets, digesta, and feces were quantified by HPLC using AOAC (2001) methodology.

Microbiological Analyses. Microbial populations were determined by serial dilution (10^{-1} to 10^{-7}) in anaerobic diluent before inoculation onto petri dishes of sterile agar as described by Bryant and Burkey (1953). Bifidobacteria and lactobacilli present in the fresh ileal and fecal samples were enumerated. The selective medium for bifidobacteria (BIM-25) was prepared using reinforced clostridial agar (BBL Microbiology Systems, Cockeyville, MD) according to the method described by Muñoa and Pares (1988). Lactobacilli were cultured on Rogosa SL agar (Difco Laboratories, Detroit, MI). Inoculating drops of three appropriate dilutions onto their respective plates maximized counting precision of the microbiota. After adsorption of the droplets, the plates were inverted and incubated anaerobically (95% CO_2/5% H_2) at 39°C for 48

Table 1. Ingredient and chemical composition (%, as-fed basis) of experimental diets fed to pigs[a]

Ingredient	Dietary treatment		
	Gal OS-free	SS	TOS
Cornstarch	47.55	33.20	41.55
Casein	20.00	17.00	20.00
Sucrose	20.00	20.00	20.00
Corn oil	2.00	2.00	2.00
Dicalcium phosphate	3.00	3.00	3.00
Calcium carbonate	0.45	0.45	0.45
Sodium chloride	0.35	0.35	0.35
Cellulose[b]	5.00	5.00	5.00
Vitamin premix[c]	0.30	0.30	0.30
Trace mineral premix[d]	0.30	0.30	0.30
Potassium carbonate, 55%	0.45	0.45	0.45
Magnesium oxide, 58%	0.05	0.05	0.05
Choline chloride[e]	0.05	0.05	0.05
Chromic oxide	0.50	0.50	0.50
Soy solubles[f]	—	17.35	—
Transgalactooligosaccharides[g]	—	—	6.00
Analyzed composition, %			
Dry matter	92.20	93.65	92.69
Nitrogen	3.02	2.97	2.92
Total galactooligosaccharides	0.00	4.78	3.50

[a]Dietary treatments are as follows: Gal OS-free = galactooligosaccharide-free diet; SS = soy solubles-containing diet; and TOS = transgalactooligosaccharide-containing diet.
[b]Solka Floc, International Fiber Corp., North Tonawanda, NY.
[c]Provided per kilogram diet: 2,000 IU of vitamin A; 300 IU of vitamin D_3; 20 IU of vitamin E; 1.0 mg of vitamin K (menadione); 4 mg of thiamine; 15 mg of niacin; 4 mg of riboflavin; 12 mg of pantothenic acid; 15 μg of vitamin B_{12}; 2 mg of pyridoxine; 0.1 mg of d-biotin; 0.5 mg of folic acid; and 0.06 g of choline.
[d]Provided per kilogram diet: 90 mg of Fe (iron sulfate); 5 mg of Mn (manganese oxide); 8 mg of Cu (copper sulfate); 0.20 mg of I (potassium iodate); 0.21 mg of Se (sodium selenite); and 90 mg of Zn (zinc sulfate).
[e]Provided per kilogram diet: 270 mg of choline.
[f]Provided per kilogram diet: 6.9 g of raffinose, 27.7 g of stachyose, and 1.2 g of verbascose.
[g]Provided per kilogram diet: 35 g of transgalactooligosaccharides.

h. Colony counts were made after 24 to 48 h of incubation to determine colony-forming units per gram sample.

Calculations and Statistical Analyses. Apparent ileal digestibility coefficients were calculated according to the following formula:

$$\text{AID } (\%) = 100 - [(Cr_F/Cr_D) \times (N_D/N_F) \times 100]$$

where AID is the apparent ileal digestibility of DM, OM, N, or Gal OS; Cr_F is the concentration of chromium in the feed; Cr_D is the concentration of chromium in the digesta; N_D is the concentration of nutrient in digesta; and N_F is the concentration of nutrient in the feed. A colony forming unit was defined as a distinct colony measuring at least 1 mm in diameter. Colony forming units per gram of sample (DM basis) were calculated as:

$$\text{cfu/g} = \frac{(\text{mean cfu}) \times (\text{dilution}) \times (\text{diluent dilution})}{(\text{g of sample, DM basis}) \times (\text{mL in droplet})}$$

The data were analyzed using the GLM procedures of SAS (SAS Inst., Inc., Cary, NC). Analysis of variance was performed according to a repeated measures design (Steele and Torrie, 1980). The model included the effects of period, pig, and diet. The least squares means for apparent digestibility and bacterial populations for the experimental diets were compared using Fisher's LSD procedure (Milliken and Johnson, 1984). For all statistical analyses, an alpha level of 0.05 was used to determine statistical significance.

In Vitro Experiments

Substrates and Donors. The substrates used in these studies were a pure raffinose/stachyose combination (**R + S;** Sigma Chemical, St. Louis, MO), soy solubles (**SS;** Table 2; Central Soya, Gibson City, IL), and granular transgalactooligosaccharides (**TOS;** Table 2; Borculo Domo Ingredients, Borculo, The Netherlands). Tubes containing 5.34 mg of pure raffinose and 17.86 mg of pure stachyose were used to simulate the concentrations of these OS in the SS ingredient tested. The substrate, R + S, was the combination of 5.34 mg of R and 17.86 mg of S. Additionally, tubes containing the constituent monosaccharides, disaccharides, and AA present in SS and TOS were prepared to determine the fermentative characteristics of these components. These were termed "non-OS SS components" and "non-OS TOS components". The "non-OS components" are defined as components other than oligosaccharides. It was hypothesized that these components would be completely digested before the terminal ileum by hydrolytic means, and thus

Table 2. Analyzed composition (%, as-fed basis) of galactooligosaccharide-containing compounds added to the diets of pigs

Item	Soy solubles	Transgalactooligosaccharides
	%	
DM	93.86	94.23
OM	90.20	100.00
N	2.37	0.00
Amino acids		
Arg	0.89	—
His	0.35	—
Ile	0.52	—
Leu	0.83	—
Lys	0.60	—
Met	0.17	—
Phe	0.72	—
Thr	0.43	—
Trp	0.24	—
Val	0.56	—
IDAA[a]	5.31	—
Ala	0.53	—
Asp	1.52	—
Cys	0.25	—
Glu	2.31	—
Gly	0.49	—
Pro	0.57	—
Ser	0.45	—
Tyr	0.50	—
DAA[b]	6.62	—
TAA[c]	11.93	0.00
Monosaccharides		
Glucose	0.41	18.61
Galactose	0.00	1.06
Fructose	0.65	0.00
Disaccharides		
Sucrose	24.45	0.00
Lactose	0.00	19.74
Oligosaccharides		
Raffinose	3.96	0.00
Stachyose	15.94	0.00
Verbascose	0.69	0.00
Total galactooligosaccharides	20.59	58.30

[a]Sum of indispensable amino acids.
[b]Sum of dispensable amino acids.
[c]Sum of total amino acids.

would not be a factor in the fermentation process taking place at the terminal ileum. However, it was our intent to quantify response criteria associated with the fermentation when these components were present as substrates.

Fourteen days after the last antibiotic injection, digesta were collected to serve as the source of inoculum for in vitro fermentation Exp. 1, and pigs were assigned to dietary treatment as described in the in vivo portion. The pigs had no exposure to other pigs or antibiotics for the duration of the study. In vitro fermentation Exp. 2 was conducted using three donors per dietary treatment. These pigs were the same nine used for in vitro fermentation Exp. 1 before their consumption of dietary Gal OS.

Experimental Design. One hundred and fifteen milligrams of each substrate was fermented in vitro for 6 h with ileal microflora obtained from each of the nine pigs, the exception being that 5.34 mg of pure raffinose and 17.86 mg of pure stachyose (R + S substrate) were used to simulate the concentrations of these Gal OS in the SS ingredient tested. The experiment was designed as a randomized complete block with donor serving as block. Treatments were allotted in a five × four factorial arrangement with five substrates and four incubation times. Each block × treatment combination was assayed using duplicate fermentation tubes. Freshly voided ileal effluent from each of the pigs was used to inoculate all substrate × time combinations in duplicate. Duplicate tubes containing no substrate were fermented with each inoculum source to enable appropriate corrections for gas production and SCFA production not arising from the substrates.

Fermentation Procedures. The composition of the semi-defined medium used for the in vitro fermentation experiments, as used by Campbell and Fahey (1997), is presented in Table 3. All components except for the vitamin

Table 3. Composition of medium used for in vitro fermentation Experiments 1 and 2 using pig ileal inoculum

Component	Concentration in medium
	mL/L
Solution A[a]	330.0
Solution B[b]	330.0
Trace mineral solution[c]	10.0
Water-soluble vitamin solution[d]	20.0
Folate:biotin solution[e]	5.0
Riboflavin solution[f]	5.0
Hemin solution[g]	2.5
Short-chain fatty acid mix[h]	0.4
Resazurin[i]	1.0
Distilled H_2O	296.0
	g/L
Na_2CO_3	4.0
Cysteine HCl·H_2O	0.5
Trypticase	0.5
Yeast extract	0.5

[a]Composition (g/L): NaCl, 5.4; KH_2PO_4, 2.7; $CaCl_2 \cdot H_2O$, 0.16; $MgCl_2 \cdot 6H_2O$, 0.12; $MnCl_2 \cdot 4H_2O$, 0.06; $CoCl_2 \cdot 6H_2O$, 0.06; $(NH_4)_2SO_4$, 5.4.

[b]Composition (g/L): K_2HPO_4, 2.7.

[c]Composition (mg/L): ethylenediaminetetraacetic acid (disodium salt), 500; $FeSO_4 \cdot 7H_2O$, 200; $ZnSO_4 \cdot 7H_2O$, 10; $MnCl_2 \cdot 4H_2O$, 3; H_3PO_4, 30; $CoCl_2 \cdot 6H_2O$, 20; $CuCl_2 \cdot 2H_2O$, 1; $NiCl_2 \cdot 6H_2O$, 2; $Na_2MoO_4 \cdot 2H_2O$, 3.

[d]Composition (mg/L): thiamin·HCl, 100; d-pantothenic acid, 100; niacin, 100; pyridoxine, 100; p-aminobenzoic acid, 5; vitamin B_{12}, 0.25.

[e]Composition (mg/L): folic acid, 10; d-biotin, 2; NH_4HCO_3, 100.

[f]Composition: riboflavin, 10 mg/mL in 5 mmol/L of HEPES.

[g]Composition: hemin, 500 mg/mL in 10 mmol/L of NaOH.

[h]Composition: 250 mL/L each of *n*-valerate, isovalerate, isobutyrate, and DL-α-methylbutyrate.

[i]Composition: resazurin, 1 g/L in distilled H_2O.

solutions were mixed before autoclave sterilization of the medium. Filter-sterilized vitamin solutions were added just before dispensing the medium, which was maintained under anaerobic conditions at all times after preparation. Aliquots (10 mL) of medium were aseptically transferred into Balch tubes, capped with butyl rubber stoppers, and sealed with aluminum caps. All tubes were stored at 4°C for approximately 12 h to enable hydration of the substrates before initiating fermentations. Tubes were placed in a 37°C water bath approximately 30 min before inoculation.

Ileal effluent from the donors was collected in plastic bags, which were sealed after expressing excess air, and maintained at 37°C until the inoculum was prepared (within 5 min). Each ileal sample was diluted 1:10 (wt/vol) in an anaerobic dilution solution (Bryant and Burkey, 1953) by blending it for 15 s in a Waring blender under a stream of CO_2. Blended, diluted ileal effluent was filtered through four layers of cheesecloth and sealed in 125-mL serum bottles under CO_2.

Appropriate sample and blank tubes were aseptically inoculated with 1.5 mL of diluted ileal effluent. Tubes were incubated at 37°C with periodic mixing. At 6 h, tubes were removed from the 37°C incubator and processed immediately for analyses. First, gas production was determined by fluid displacement (water with 5% HCl and resazurin) at equal pressure using a manometer (Campbell and Fahey, 1997). Corrections were made for temperature, pressure, and headspace contained in the Balch tube before initiation of fermentation. Gas production (mL) was calculated as gas production from the substrate minus gas production from the blank divided by original sample weight expressed on an OM basis. The pH of tube contents was measured with a standard pH meter (Denver Instrument Co., Arvada, CO) at 6 h. Finally, a 2-mL subsample was taken from each tube for SCFA analyses.

Chemical Analyses. Samples to be analyzed for SCFA were mixed with 0.5 mL of 250 g/L of *m*-phosphoric acid, precipitated at room temperature for 30 min, and then centrifuged at 25,900 × *g* for 20 min. The supernatant was decanted and frozen at –20°C in microfuge tubes. After freezing, the supernatant was thawed and centrifuged in microfuge tubes at 13,000 × *g* for 10 min. Concentrations of SCFA were determined via GLC. Briefly, concentrations of acetate, propionate, and butyrate were determined in the supernatant of the tubes using a Hewlett-Packard 5890A Series II gas chromatograph (Palo Alto, CA) and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100 mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier gas with a flow rate of 75 mL/min. Oven temperature, detector temperature, and injector temperature were 125, 175, and 180°C, respectively. Short-chain fatty acid concentrations were corrected for the quantities of SCFA produced in the blank tubes.

Calculations and Statistical Analyses. Data were analyzed as a randomized complete block design, with ileal digesta donor serving as block. Treatments, which were factorially arranged, included substrate (in vitro Exp. 1 and 2) and donor dietary treatment (in vitro Exp. 2). Therefore, donor, substrate, donor dietary treatment, and substrate × donor dietary treatment were used in the statistical model. All ANOVA were performed according to the GLM procedures of SAS (SAS Inst., Inc.). Least squares means were reported along with the pooled SEM for all response criteria. When treatment differences were detected ($P < 0.05$), means were compared using the least significant difference method.

Results

In Vivo Experiment

The results are presented as means averaged over the six weekly collection times because there was no effect ($P > 0.05$) of week of collection on the response criteria measured in this study.

Apparent Digestibility. Apparent ileal and total-tract digestibility coefficients are presented in Table 4. Addition of both SS and TOS decreased ($P < 0.05$) apparent ileal and total-tract DM and OM digestibilities, but no differences were noted between Gal OS sources. Only SS decreased ($P < 0.05$) apparent ileal and total-tract N

Table 4. Influence of dietary galactooligosaccharides on apparent ileal and total-tract digestibility coefficients (%) by pigs

	Dietary treatment[a]			
Digestibility	Gal OS-free	SS	TOS	SEM
Ileal DM	82.6[b]	78.8[c]	79.0[c]	0.9
Total-tract DM	84.7[b]	83.9[bc]	82.3[c]	0.8
Ileal OM	86.0[b]	80.5[c]	81.8[c]	0.7
Total-tract OM	89.2[b]	87.4[bc]	86.3[c]	0.6
Ileal N	87.1[b]	83.6[c]	85.1[bc]	0.9
Total-tract N	91.1[b]	87.2[c]	89.3[bc]	0.8
Ileal Gal OS	—	77.0[b]	100.0[c]	6.0
Total-tract Gal OS	—	100.0	100.0	—

[a]Dietary treatments are as follows: Gal OS-free = galactooligosaccharide-free diet; SS = soy solubles-containing diet; and TOS = transgalactooligosaccharide-containing diet. Each mean represents four individually penned pigs per dietary treatment replicated over six different weeks of collection.
[b,c]Means in the same row without common superscripts differ ($P < 0.05$).

digestibilities. Apparent ileal Gal OS digestibility was higher ($P < 0.05$) for pigs consuming the TOS diet when compared with the SS diet. Apparent total-tract digestibility of Gal OS was 100% for SS and TOS diets.

Bacterial Populations. The effect of Gal OS on ileal and fecal bifidobacteria and lactobacilli populations is presented in Table 5. Addition of TOS increased ($P < 0.05$) fecal bifidobacteria concentrations and addition of SS further increased ($P < 0.05$) fecal bifidobacteria concentrations beyond that of the TOS treatment. Similar to the effect on bifidobacteria concentrations, fecal lactobacilli concentrations were increased ($P < 0.05$) by addition of TOS and further increased ($P < 0.05$) by addition of SS to the diet.

Ileal SCFA Concentrations. Short-chain fatty acid concentrations in ileal effluent are reported in Table 6. Dietary inclusion of the Gal OS did not affect ($P > 0.05$) acetate concentrations in ileal effluent. However, SS addition tended to increase ($P < 0.11$) both propionate and butyrate concentrations in ileal effluent. Total SCFA concentration did not differ ($P > 0.05$) among dietary treatments.

In Vitro Fermentation Experiments 1 and 2

Gas Production. For in vitro fermentation Exp. 1 (Table 7), non-OS SS and TOS components resulted in the most ($P < 0.05$) gas production. Gas production resulting from fermentation of SS and TOS was lower ($P < 0.05$) than for R + S, but not different ($P > 0.05$) from each other.

Gas production in in vitro fermentation Exp. 2 (Table 7) was less for all substrates except R + S in comparison with data from in vitro fermentation Exp. 1. After 6 wk of adaptation to dietary Gal OS, R + S fermentation resulted in the highest ($P < 0.05$) amount of gas production when compared with SS and TOS, whose values did not differ ($P > 0.05$). The non-OS SS and TOS components produced less ($P < 0.05$) gas than did R + S but more ($P < 0.05$) gas than SS and TOS substrates.

SCFA Production. Short-chain fatty acid production data for in vitro fermentation Exp. 1 and 2 are presented in Tables 8 and 9, respectively.

In Exp. 1 (Table 8), TOS fermentation resulted in the lowest ($P < 0.05$) production of acetate and butyrate. Acetate production by other substrates was not different ($P > 0.05$). Production of propionate and butyrate was lowest ($P < 0.05$) for TOS and highest ($P < 0.05$) for R + S. Fermentation of R + S, non-OS SS components, and non-OS TOS components resulted in the highest ($P < 0.05$) SCFA production, whereas fermentation of TOS resulted in the lowest ($P < 0.05$) value.

In vitro fermentation Exp. 2 (Table 9) resulted in more total SCFA production by the substrates, with the exception of the non-OS components of SS, than for in vitro

Table 5. Influence of dietary galactooligosaccharides on ileal and fecal bacterial populations ($\log_{10}$ cfu/g of DM) of pigs

	Dietary treatment[a]			
Item	Gal OS-free	SS	TOS	SEM
Ileal bifidobacteria	11.0	11.3	11.1	0.1
Fecal bifidobacteria	9.8[b]	11.9[c]	11.1[d]	0.2
Ileal lactobacilli	10.7	10.9	10.7	0.1
Fecal lactobacilli	10.8[b]	11.6[c]	11.2[d]	0.1

[a]Dietary treatments are as follows: Gal OS-free = galactooligosaccharide-free diet; SS = soy solubles-containing diet; and TOS = transgalactooligosaccharide-containing diet. Each mean represents four individually penned pigs per dietary treatment replicated over six different weeks of collection.
[b,c,d]Means in the same row without common superscripts differ ($P < 0.05$).

Table 6. Influence of dietary galactooligosaccharides on ileal short chain fatty acid (SCFA) concentrations (μmol/g of DM) in pigs

	Dietary treatment[a]			
Item	Gal OS-free	SS	TOS	SEM
Acetate	56.0	71.3	62.1	8.3
Propionate	17.6[b]	26.8[c]	19.3[b]	3.6
Butyrate	8.2[b]	24.2[c]	11.1[b]	5.4
Total SCFA	81.8	122.3	92.5	16.7

[a]Dietary treatments are as follows: Gal OS-free = galactooligosaccharide-free diet; SS = soy solubles-containing diet; and TOS = transgalactooligosaccharide-containing diet. Each mean represents four individually penned pigs per dietary treatment replicated over six different weeks of collection.
[b,c]Means in the same row without common superscripts differ ($P < 0.11$).

fermentation Exp. 1. Fermentation of R + S resulted in the highest ($P < 0.05$) acetate, propionate, butyrate, and total SCFA production. Acetate, propionate, and butyrate production values were not different ($P > 0.05$) for SS and TOS. Fermentation of the non-OS components of TOS resulted in higher ($P < 0.05$) acetate, propionate, and butyrate production than the substrates themselves.

Changes in pH. The effect of substrate fermentation on pH for in vitro fermentation Exp. 1 and 2 is presented in Table 10.

In Exp. 1, fermentation of R + S resulted in a higher ($P < 0.05$) pH when compared with SS. Fermentation of the non-OS components of SS and TOS, and TOS itself, resulted in intermediate pH values at 6 h.

In Exp. 2, and similar to results of Exp. 1, fermentation of R + S resulted in a higher ($P < 0.05$) pH when compared with that of SS. Fermentation of the non-OS components of SS and TOS, and TOS, resulted in intermediate pH values in comparison to other substrates.

Discussion

In Vivo Experiment

Supplementation of the diet with both forms of Gal OS resulted in statistically significant depressions in ileal and total-tract DM and OM digestibilities. The TOS source was 100% OM and the SS source was 97% OM on a DM basis. Therefore, the depression in digestibility must be attributed to addition of indigestible organic ingredients to the diet, thus diluting the amount of nutrients available for hydrolytic digestion. A significant decrease in ileal and total-tract N digestibility also was noted for SS. There are two possible reasons for this. First, SS contained 2.4% N, and the experimental diets were balanced to be isonitrogenous. However, the N in SS may be present in a matrix that is less readily available to the pig before its potential fermentation in the terminal ileum. Second, 14% of bacterial cell mass is N and, during fermentation, amino acids can be assimilated into cell mass (Lengeler et al., 1999). This increase in cell mass and, in turn, increased N assimilation, could be a reason for the greater N excretion in feces and the greater depression in N digestion by pigs fed the SS-containing vs. the control diet. Alles et al. (1999) reported a significant increase in N concentration (5.6 vs. 5.3%, DM basis) of human feces when subjects consumed 15 g/d TOS compared to subjects consuming a diet containing no TOS.

Table 7. Gas production at 6 h of in vitro fermentation of Gal OS substrates with swine ileal microflora

	Gas production, mL produced at 6 h/g of OM	
Item[a]	In vitro Experiment 1	In vitro Experiment 2
R + S	80.6	103.1
Non-OS SS components	102.3	66.4
SS	41.5	16.9
Non-OS TOS components	115.1	70.0
TOS	49.8	31.9
SEM	7.1	
LSD[b]	20.9	

[a]Substrate identification: R + S = raffinose/stachyose combination; Non-OS SS components = nonoligosaccharide soy soluble components; SS = soy solubles; Non-OS TOS components = nonoligosaccharide transgalactooligosaccharide components; TOS = transgalactooligosaccharides.
[b]Least significant difference between any two mean values in the same column ($P < 0.05$).

For all nutrients investigated, there was as much as a 5.5-percentage unit decrease in digestibility. Veldman et al. (1993) reported 25% decreases in apparent ileal OM and N digestibilities when pigs were fed a SPC–cornstarch-based diet with and without added soy OS (2.8 or 0.7% soy OS) from velasse. The authors speculated that the decrease in digestibility was the result of an increase in gut osmolarity and dilution of digestive enzyme activities and substrate concentrations. Previous studies in our lab indicated that concentrations of soy OS up to 3.7% of diet DM did not affect apparent ileal N digestibility (Smiricky et al., 2002). Also, a study conducted by Zhang et al. (2001) reported no effect of up to 2.0% soy OS on total-tract nutrient digestion by pigs weighing approximately 13 kg. However, we added only 1.3% Gal OS from SS in our previous study, whereas diets in the current study contained 3.5% Gal OS in the form of SS. Additionally, the protein source (SBM vs. casein) in the two respective diets was different. These factors could impact nutrient digestion.

Kikuchi et al. (1996) reported no significant effect of 5% TOS on apparent total-tract DM digestion by rats.

Table 8. Acetate, propionate, butyrate, and total short-chain fatty acid (SCFA) production at 6 h of in vitro fermentation of Gal OS substrates with swine ileal microflora unexposed to Gal OS (in vitro fermentation Exp. 1)[a]

Item	Acetate, mmol/g of OM	Propionate, mmol/g of OM	Butyrate, mmol/g of OM	Total SCFA, mmol/g of OM
R + S	1.0	0.5	0.5	2.0
Non-OS SS components	1.0	0.3	0.3	1.6
SS	0.7	0.1	0.2	1.0
Non-OS TOS components	0.9	0.3	0.3	1.5
TOS	0.4	0.1	0.1	0.6
SEM	0.2	0.1	0.03	0.2
LSD[b]	0.6	0.3	0.09	0.6

[a]Substrate identification: R + S = raffinose/stachyose combination; Non-OS SS components = nonoligosaccharide soy soluble components; SS = soy solubles; Non-OS TOS components = nonoligosaccharide transgalactooligosaccharide components; TOS = transgalactooligosaccharides.
[b]Least significant difference between any two mean values in the same column ($P < 0.05$).

Gabert et al. (1995) reported no significant decrease in apparent ileal DM, N, or AA digestibilities by pigs consuming a diet containing 0.5% TOS. Additionally, Houdijk et al. (1999) reported no differences in apparent ileal and total-tract DM (average, 74 and 86%, respectively), OM (avg., 77 and 89%, respectively), or N (average., 64 and 83%, respectively) digestibilities when pigs consumed a diet containing 1.5% TOS. Again, these concentrations of TOS are much lower than those used in the current study. Therefore, the depression in digestion observed in our study may be related to the high amount of indigestible OS in the diet and the subsequent increase in synthesis of bacterial cell mass.

Supplementation of the diet with either SS or TOS resulted in significant increases in fecal bifidobacteria and lactobacilli concentrations. Both soy OS and TOS are reported to be bifidogenic (Modler et al., 1990). Ileal Gal OS digestibility was 100% for pigs consuming the TOS diet and 77% for pigs consuming the SS diet. Therefore, more Gal OS were available in the proximal large intestine for fermentation in pigs consuming the SS diet. This may be why greater increases in beneficial bacteria in the large intestine of pigs consuming the SS diet were observed compared with pigs consuming the TOS diet. Total-tract Gal OS digestibility was 100% for pigs fed both Gal OS-containing diets, indicating that the microflora of the large intestine were capable of completely fermenting SS. Other researchers have obtained similar results. When human subjects consumed 15 g/d of raffinose, bifidobacteria populations increased by 0.5 $\log_{10}$ cfu/g of feces, and lactobacilli populations increased by 1.3 $\log_{10}$ cfu/g of feces (Benno et al., 1987). Hayakawa et al. (1990) reported a 0.4 $\log_{10}$ cfu/g of feces increase in bifidobacteria concentrations and a 1.2 $\log_{10}$ cfu/g of feces increase in lactobacilli concentrations when humans consumed a diet containing 7.1 g of stachyose and 2.0 g of raffinose/d.

Previous research indicates that 1.5 to 5% additions of TOS to diets increase colonic bifidobacteria and lactobacilli concentrations. Rowland and Tanaka (1993) reported increases in bifidobacteria (0.6 $\log_{10}$ cfu/g of cecal contents) and lactobacilli (0.4 $\log_{10}$ cfu/g of cecal contents)

Table 9. Acetate, propionate, butyrate, and total short-chain fatty acid (SCFA) production at 6 h of in vitro fermentation of Gal OS substrates with swine ileal microflora after a 6-wk adaptation to Gal OS-containing diets (in vitro fermentation Exp. 2)

Item[a]	Acetate, mmol/g of OM	Propionate, mmol/g of OM	Butyrate, mmol/g of OM	Total SCFA, mmol/g of OM
R + S	1.8	1.2	0.4	3.4
Non-OS SS components	1.0	0.4	0.2	1.5
SS	0.7	0.3	0.1	1.1
Non-OS TOS components	1.1	0.8	0.2	2.1
TOS	0.4	0.3	0.1	0.8
SEM	0.2	0.1	0.03	0.2
LSD[b]	0.6	0.3	0.09	0.6

[a]Substrate identification: R + S = raffinose/stachyose combination; Non-OS SS components = nonoligosaccharide soy soluble components; SS = soy solubles; Non-OS TOS components = non-oligosaccharide transgalactooligosaccharide components; TOS = transgalactooligosaccharides.
[b]Least significant difference between any two mean values in the same column ($P < 0.05$).

Table 10. pH values at 6 h of in vitro fermentation of Gal OS substrates with swine ileal microflora

	pH value	
Item[a]	In vitro Experiment 1	In vitro Experiment 2
R + S	6.3	6.2
Non-OS SS components	6.1	6.0
SS	6.0	5.7
Non-OS TOS components	6.1	6.1
TOS	6.1	5.9
SEM	0.1	
LSD[b]	0.3	

[a]Substrate identification: R + S = raffinose/stachyose combination; Non-OS SS components = nonoligosaccharide soy solubles components; SS = soy solubles; Non-OS TOS components = nonoligosaccharide transgalactooligosaccharide components; TOS = transgalactooligosaccharides.

[b]Least significant difference between any two mean values in the same column ($P < 0.05$).

when rats were fed a diet containing 5% TOS. Ito et al. (1993) reported a significant increase in bifidobacteria (0.3 $\log_{10}$ cfu/g of feces) and lactobacilli (0.7 $\log_{10}$ cfu/g of feces) concentrations when humans consumed 15 g of TOS/d. However, Gabert et al. (1995) reported no difference in lactobacilli concentrations when pigs were fed 0.5% TOS.

Bifidobacteria may comprise up to 25% of the gut flora in healthy human adults (Modler et al., 1990) and thus have a role in decreasing intestinal pH as a result of production of the SCFA. Lower pH could potentially restrict growth of pathogenic or putrefactive bacteria (Modler et al., 1990). Therefore, SS and TOS may serve as useful feed ingredients for the promotion of bifidobacteria growth in the growing pig.

Propionate and butyrate concentrations in the ileum of pigs were increased ($P < 0.11$) by the TOS treatment. Inclusion of SS in the diet increased concentrations of propionate and butyrate beyond the increase noted for TOS, perhaps due to the fermentation of their non-OS components. Minimal data have been reported on the effects of oligosaccharides on ileal SCFA concentrations. Houdijk (1998) reported no effect of TOS addition on ileal SCFA concentration. Acetate comprised 82.5% and propionate 12.5% of the total SCFA in the ileal digesta contents of pigs consuming 4% TOS. In the current study, acetate comprised 68% and propionate 22% of the SCFA present in ileal digesta of pigs consuming a 6% TOS-containing diet. These values indicate that fermentation of TOS starts before the large intestine, often considered the only site of fermentation of oligosaccharides. Furthermore, Houdijk (1998) indicated that OS fermentation might actually start as early as the stomach. He reported lower pH values of the gastric contents when 1.5% TOS was fed to growing pigs.

In Vitro Fermentation Experiments

Gas production in vitro using ileal contents collected before dietary consumption of Gal OS by pigs was not different for SS and TOS fermentations. However, fermentation of R + S resulted in much greater concentrations of gas. This result also was noted by Smiricky-Tjardes et al. (2003) when R + S was fermented using swine fecal microflora. Equal concentrations of pure Gal OS behaved much differently in vitro than when present in the SS matrix. The increase in gas production observed with R + S may be indirect evidence of species other than bifidobacteria and lactobacilli being stimulated by the presence of these substrates, as these genera do not produce gas during homolactic fermentation (Lengeler et al., 1999). Homolactic-fermenting organisms, such as bifidobacteria and lactobacilli, produce exclusively D- or L-lactate from hexoses (Lengeler et al., 1999). Whether growth of other genera is a direct result of fermentation processes or from crossfeeding on bifidobacterial metabolites is unclear. This also may be the mechanism by which the non-OS components of SS and TOS generated the largest amounts of gas when compared to the SS and TOS substrates.

Soy solubles and TOS produced less gas in in vitro Exp. 2 when compared with in vitro Exp. 1. The decrease in gas production in vitro may be due to a decrease in the concentrations of bacterial species in the ileal effluent whose fermentation results in gas production.

The non-OS components of SS and TOS were included in the in vitro fermentation experiments to identify whether gas and SCFA production was truly a result of OS fermentation vs. fermentation of more readily available ingredients (e.g., mono- and disaccharides and AA) present in the substrates. Fermentation of the non-OS components resulted in more gas and SCFA production than the substrates themselves. Fermentation of these components likely was rapid, generating high amounts of SCFA. Proteolytic fermentation by different bacterial genera results in gas production and end products with a more basic pH. This could explain why the pH values obtained as a result of fermentation of the non-OS components were higher than those for SS and TOS and not different from the pH values obtained for R + S. In vivo, these non-OS components should not reach the terminal ileum as they should be digested and absorbed anterior to this site.

Total SCFA production was highest for R + S and the non-OS components of SS and TOS. Smiricky-Tjardes et al. (2003) reported increased SCFA production by, and more rapid fermentation of, R + S when compared with SS in an in vitro experiment using swine fecal microflora. It appeared that Gal OS in the SS matrix were fermented more slowly than when pure R and S were present in the same concentrations. In this study, SCFA production resulting from fermentation of SS was numerically higher than SCFA production from TOS. These data are not different from those of the in vivo study, indicating that ileal SCFA concentrations were higher for pigs consuming the SS diet when compared with the TOS diet.

Production of SCFA in in vitro fermentation Exp. 2 was higher than that for in vitro fermentation Exp. 1, indicating that there may have been an increase with

time in the concentrations of bacteria in the ileal effluent responsible for fermenting these substrates to SCFA. Kikuchi-Hayakawa et al. (1997) reported a 19% increase in total SCFA production after 24 h of in vitro fermentation with cecal contents from rats consuming a diet containing 5 vs. 0% TOS.

Substrates that produce relatively large amounts of SCFA may be beneficial to the host animal because SCFA play many important roles in vivo. Butyrate has been reported to be the preferential energy source of colonocytes in rats (Roediger, 1982). Hindgut fermentors utilize acetate as a fuel source for peripheral tissues (Cummings, 1991). Propionate has been suggested to spare AA that would be used in gluconeogensis in the postabsorptive state (Demigne and Remesy, 1991). Additionally, SCFA can contribute up to 28% of the total maintenance requirements of pigs (Imoto and Namioka, 1978). Therefore, substrates that are readily fermented to SCFA would bathe the intestinal lumen in these organic acids (potentially important for optimal gut health) and, ultimately, as a result of their efficient absorption, be beneficial to the host animal. Analyses of intestinal contents and feces for SCFA concentration may not be a good indicator of production since less than 5% of the bacterially derived SCFA appear in feces due to efficient colonic uptake (McNeil et al., 1978).

Fermentation of R + S resulted in the highest, and SS the lowest, pH at 6 h during in vitro fermentation Exp. 1. Fermentation of TOS resulted in an intermediate pH. These data are not different from those of Smiricky-Tjardes et al. (2003), who reported that R + S fermentation resulted in a higher pH than did SS. A potential explanation could be that fermentation of R + S resulted in proliferation of proteolytic bacteria due to their rapid fermentation, and these proteolytic bacteria began consuming spent bacteria as substrates resulting in end-products with a more basic pH.

In in vitro fermentation Exp. 2, similar to results of in vitro fermentation Exp. 1, R + S fermentation resulted in the highest pH and SS the lowest. Overall, pH values during in vitro fermentation Exp. 2 were lower than for in vitro fermentation Exp. 1. This may be a result of higher concentrations of SCFA produced during Exp. 2 vs. 1. Fermentation of TOS resulted in a lower pH than did R + S and the non-OS components of SS and TOS. Fermentation of R + S was rapid and, therefore, growth of bifidobacteria and lactobacilli may have stopped. Therefore, proteolytic bacterial growth and fermentation due to the presence of AA in the spent bacteria yielded more basic end products than did fermentations of TOS or SS. The non-OS components of SS also may have resulted in proteolytic bacterial growth and fermentation due to the presence of AA in the substrate.

In conclusion, in vivo and in vitro data suggest that both SS and TOS are fermented by bifidobacteria and lactobacilli as indicated by increases in bacterial populations and SCFA production. Increases in bacterial populations occurred across all treatments during the 6-wk experiment; thus, donor animal dietary treatment did not impact in vitro fermentation characteristics in Exp. 2. Soy solubles appear to be more effective at increasing intestinal concentrations of bifidobacteria, lactobacilli, and SCFA. Transgalactooligosaccharides increased beneficial bacteria without effecting a large depression in N digestion. In vitro, the pure Gal OS were fermented more rapidly and to a greater extent than SS. Transgalactooligosaccharide fermentation was intermediate between that of R + S and SS. These data indicate that SS may actually be more effective as a prebiotic substrate in the terminal small intestine or proximal large intestine than its pure counterparts.

Implications

Galactooligosaccharides increase concentrations of gut beneficial bacteria, bifidobacteria and lactobacilli, as well as concentrations of short-chain fatty acids, but decrease nutrient digestibilities. Both sources of galactooligosaccharides tested in this experiment are prebiotics; however, due to the low purity of soy solubles, the inclusion rate is high and may be impractical for swine diets. Nonetheless, in vitro and in vivo, soy solubles resulted in the greatest short-chain fatty acid production. Many positive roles have been established for the short-chain fatty acids in animal health, and dietary inclusion of soy solubles and transgalactooligosaccharides could potentially affect gut health of pigs in a positive manner. These improvements in intestinal health that result from dietary galactooligosaccharide inclusion might provide protection against putrefactive bacteria; thus, galactooligosaccharides might be a potential substitute for subtherapuetic levels of antibiotics.

Literature Cited

Alles, M. S., R. Hartemink, S. Meyboom, J. L. Harryvan, K. M. J. Van Laere, and F. M. Nagenast. 1999. Effect of transgalactooligosaccharides on the composition of the human intestinal microflora and of putative risk markers for colon cancer. Am. J. Clin. Nutr. 69:980–991.

AOAC. 1995. Official Methods of Analysis. 15th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

AOAC. 2001. Official Methods of Analysis. 16th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Benno, Y., K. Endo, N. Shiragami, K. Sayama, and T. Mitsuoka. 1987. Effects of raffinose intake on human fecal microflora. Bifido. Micro. 6:59–63.

Bryant, M. P., and L. A. Burkey. 1953. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. J. Dairy Sci. 36:205–217.

Campbell, J. M., and G. C. Fahey, Jr. 1997. Psyllium and methylcellulose fermentation properties in relation to insoluble and soluble fiber standards. Nutr. Res. 17:619–629.

Cummings, J. H. 1991. Production and metabolism of short-chain fatty acids in humans. Pages 11–17 in Short-Chain Fatty Acids: Metabolism and Clinical Importance. Rep. 10th Ross Conf. Med. Res. A. F. Roche, ed. Ross Laboratories, Columbus, OH.

Demigne, C., and C. Remesy. 1991. Hepatic metabolism of short-chain fatty acids. Pages 17–23 in Short-Chain Fatty Acids: Metabolism and Clinical Importance. Rep. 10th Ross Conf. Med. Res. A. F. Roche, ed. Ross Laboratories, Columbus, OH.

Fenton, T. W., and M. Fenton. 1995. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631–634.

Gabert, V. M., W. C. Sauer, R. Mosenthin, M. Schimitz, and R. Ahrens. 1995. The effect of oligosaccharides and lactitiol on the ileal digestibilities of amino acids, monosaccharides and bacterial populations and metabolites in the small intestine of weanling pigs. Can. J. Anim. Sci. 75:99–107.

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr. 125:1401–1412.

Hayakawa, K., J. Mitzutani, K. Wada, T. Masai, I. Yoshihara, and T. Mitsuoka. 1990. Effects of soybean oligosaccharides on human fecal flora. Microbiol. Ecol. Health Dis. 3:293–303.

Houdijk, J. G. M. 1998. Effects of non-digestible oligosaccharides in young pigs diets. Ph.D. Diss., Agricultural Univ. of Wageningen, Wageningen, The Netherlands.

Houdijk, J. G. M., M. W. Bosch, S. Tamminga, M. W. A. Verstegen, E. B. Berenpas, and H. Knoop. 1999. Apparent ileal and total-tract nutrient digestion by pigs as affected by dietary nondigestible oligosaccharides. J. Anim. Sci. 77:148–158.

Imoto, S., and S. Namioka. 1978. VFA production in the pig large intestine. J. Anim. Sci. 47:467–478.

Kikuchi, H., C. Andrieux, M. Riottot, M. Bessaada, F. Popot, P. Beaumatin, and O. Szylit. 1996. Effect of two levels of transgalactosylated oligosaccharide intake in rats associated with human faecal microflora on bacterial glycolytic activity, end-products of fermentation and bacterial steroid transformation. J. Appl. Bacteriol. 80:439–446.

Kikuchi-Hayakawa, H., M. Kimura, and W. Masaaki. 1997. Adaptation of rate of organic acid production of hindgut bacteria to chronic intake of galactooligosaccharide in the rat. J. Nutr. Sci. Vitaminol. 43:357–368.

Lengeler, J. W., G. Drews, and H. G. Schlegel, ed. 1999. Biology of the Prokaryotes. Blackwell Science, New York.

McNeil, N. I., J. H. Cummings, and W. P. T. James. 1978. Short chain fatty acid absorption by the human large intestine. Gut 26:819–822.

Milliken, G. A., and D. E. Johnson. 1984. Analysis of Messy Data. Vol. 1. Van Nostrand Reinhold Co., New York.

Modler, H. W., R. C. McKellar, and M. Yaguchi. 1990. Bifidobacteria and bifidogenic factors. Can. Inst. Food Sci. Technol. J. 23:29–41.

Mosenthin, R., and B. Zimmermann. 2000. Probiotics and prebiotics in pig nutrition—Alternatives for antibiotics? Pages 29–50 in Selected Topics in Animal Nutrition, Biochemistry and Physiology, R. Mosenthin, ed. University of Alberta, Edmonton, Alberta, Canada.

Muñoa, F. J., and R. Pares. 1988. Selective medium for isolation and enumeration of *Bifidobacterium* spp. Appl. Environ. Microbiol. 54:1715–1718.

NRC. 1998. Nutrient Requirements of Swine. 10th ed. Natl. Acad. Press, Washington, DC.

Rasic, J. L. 1983. The role of dairy food containing bifido and acidophilus bacteria in nutrition and health. N. Eur. Dairy J. 4:80–88.

Roediger, W. E. W. 1982. Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology 83:424–432.

Rowland, I. R., and R. Tanaka. 1993. The effects of transgalactosylated oligosaccharides on gut flora metabolism in rats associated with human faecal microflora. J. Appl. Bacteriol. 74:667–674.

Sauer, W. C., H. Jorgensen, and R. Berzins. 1983. A modified nylon bag technique for determining apparent digestibilities of protein in feedstuffs for pigs. Can. J. Anim. Sci. 63:233–237.

Smiricky-Tjardes, M. R., E. A. Flickinger, C. M. Grieshop, L. L. Bauer, M. R. Murphy, and G. C. Fahey, Jr. 2003. In vitro fermentation characteristics of selected oligosaccharides by swine fecal microflora. J. Anim. Sci 81:2505–2514.

Smiricky, M. R., C. M. Grieshop, D. M. Albin, J. E. Wubben, V. M. Gabert, and G. C. Fahey, Jr. 2002. The influence of soy oligosaccharides on apparent and true ileal amino acid digestibilities and fecal consistency in growing pigs. J. Anim. Sci. 80:2433–2441.

Steele, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw-Hill Publishing Co., New York.

Veldman, A., W. A. G. Venn, D. Barug, and P. A. van Paridon. 1993. Effect of alpha-galactosides and alpha-galactosidase in feed on ileal piglet digestive physiology. J. Anim. Physiol. Anim. Nutr. 69:57–65.

Zhang, L., D. Li, S. Qiao, J. Wang, L. Bai, Z. Wang, and I. K. Han. 2001. The effect of soybean galactooligosaccharides on nutrient and energy digestibility and digesta transit time in weanling piglets. Asian-Aust. J. Anim. Sci. 14:1598–1604.