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In vitro fermentation characteristics of selected oligosaccharides by swine fecal microflora[1]

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ABSTRACT: The objective of this study was to quantify the fermentation characteristics of oligosaccharides present in feed ingredients or isolated for dietary supplementation. Substrates studied included short-chain fructooligosaccharides, medium-chain fructooligosaccharides, long-chain fructooligosaccharides, raffinose, stachyose, soy solubles, granular and liquid forms of transgalactooligosaccharides, glucooligosaccharides, mannanoligosaccharides, and xylooligosaccharides. Three healthy pigs that had never received antibiotics served as sources of fecal inoculum. Each substrate was fermented in vitro; samples were taken at 0, 2, 4, 8, and 12 h, and pH change and short-chain fatty acid (SCFA) and gas production determined. Gas production at 12 h did not differ ($P > 0.05$) among all fructooligosaccharides, transgalactooligosaccharides, soy solubles, and xylooligosaccharides. Raffinose, stachyose, and raffinose + stachyose fermentation resulted in the greatest ($P < 0.05$) gas production at 12 h of all substrates tested. The rate of gas production was greatest ($P < 0.05$) for stachyose and least ($P < 0.05$) for glucooligosaccharides and mannanoligosaccharides. Substrate did not affect ($P > 0.05$) time to attain maximal rate of gas production. The pH at 12 h for all fructooligosaccharides and xylooligosaccharides did not differ ($P > 0.05$). The pH values at 12 h for raffinose, stachyose, and raffinose + stachyose were highest ($P < 0.05$) compared with all other substrates. Total SCFA production at 12 h was similar for all fructooligosaccharides and transgalactooligosaccharides, glucooligosaccharides, and soy solubles. Total SCFA production was greatest ($P < 0.05$) for xylooligosaccharides, stachyose, and raffinose + stachyose, and least ($P < 0.05$) for mannanoligosaccharides and raffinose. Stachyose fermentation resulted in the greatest ($P < 0.05$) rate and earliest time to attain maximal rate of SCFA production. All oligosaccharides studied were readily fermentable but varied in amount and type of SCFA produced. Fermentation of the pure forms of oligosaccharides contained in soy solubles resulted in greater gas production and higher pH compared with soy solubles. The oligosaccharides in the soy solubles matrix seemed to behave differently than their pure counterparts. The high rates of fermentation of most oligosaccharides tested indicate that they may serve as fermentable carbohydrate sources in the terminal small intestine or large intestine of swine.

Key Words: Fermentation, In Vitro, Oligosaccharides, Pigs

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Introduction

Much of the negative perception concerning oligosaccharides, or more specifically, soy oligosaccharides, stems from assumed depression in nutrient digestibilities and the increase in gas production resulting from fermentation of these substrates (Hata et al., 1991). Intestinal gases (H_2, CO_2, and CH_4) originate from colonic fermentation of the nondigestible oligosaccharides, raffinose and stachyose (Delzenne and Roberfroid, 1994). Yet oligosaccharide fermentation yields products that are beneficial to the host, namely the short-chain fatty acids (**SCFA**) acetate, propionate, and butyrate (Buddington, 2001). Short-chain fatty acids have been implicated in a number of important physiological events. These include utilization of butyrate by rat colonic epithelial cells as the preferred energy substrate (Roediger, 1982) and prevention of colon cancer in humans (Young and Gibson, 1991). Additionally, growing swine can obtain as much as 12% of their ME requirement from SCFA (Imoto and Namioka, 1978; Kass et al., 1980).

Lactobacilli and bifidobacteria ferment carbohydrates through a pathway mediated by the glycolytic

[1]The soy solubles used in this experiment were graciously donated by J. C. Russett of Central Soya, Fort Wayne, IN.

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Table 1. Analyzed composition (as-fed basis) of soy solubles

Item	%
DM	92.50
OM	90.20
N	2.37
Amino acids	
IDAA[a]	5.31
DAA[b]	6.62
TAA[c]	11.93
Sucrose	24.45
Lactose	0.00
Galactooligosaccharides	
Raffinose	3.96
Stachyose	15.94
Verbascose	0.69
Total galactooligosaccharides	20.59

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

enzymes they produce in which the main end products are SCFA (Zani et al., 1974). These bacteria, in addition to other bacterial species, are capable of hydrolyzing glycosidic linkages that mammalian enzymes are incapable of hydrolyzing. Yazawa et al. (1978) reported that all species of bifidobacteria of human origin, except *B. bifidum*, utilize raffinose and stachyose. These galactooligosaccharides have a higher specificity for utilization by bifidobacteria than other intestinal strains, such as *E. coli* (Kawamura et al., 1968). Therefore, these bacteria, which inhabit the large intestine of swine, should efficiently utilize galactooligosaccharides. The objective of this study was to evaluate the fermentation characteristics resulting from the fermentation of galactooligosaccharides in comparison with other oligosaccharides used in animal nutrition.

Materials and Methods

Substrates and Donors

The substrates used in this study were as follows: 1) fructans: short-chain fructooligosaccharides (GTC, Golden, CO), medium-chain fructooligosaccharides (oligofructose; Orafti, Tienen, Belgium), long-chain fructooligosaccharides (inulin; Orafti); 2) galactooligosaccharides: pure raffinose (Sigma Chemical, St. Louis, MO), pure stachyose (Sigma Chemical), pure raffinose + stachyose combination, soy solubles (Table 1; Central Soya, Gibson City, IL), granular transgalactooligosaccharides (Borculo Domo Ingredients, Borculo, The Netherlands), liquid transgalactooligosaccharides (Borculo Domo Ingredients); and 3) glucooligosaccharides (Bioecolia; Solabia, Pantin Cedex, France), mannanoligosaccharides (Alltech, Nicholasville, KY), and xylooligosaccharides (Xyloligo 95; Suntory, Tokyo, Japan). Three healthy pigs (approximately 25 kg) served as sources of feces from which the inoculum was prepared. The donors consumed a standard corn–soybean meal-based diet appropriate for their stage of growth for 2 wk prior to fecal collection. The pigs originated from an antibiotic-free herd and were housed in a temperature-controlled room with no exposure to other pigs or antibiotics for the duration of this study. The pigs selected from the antibiotic-free herd had no exposure to in-feed antibiotics for the duration of their life cycle; in addition, these three pigs never received injected antibiotics at any time prior to or during the study. The University of Illinois Institutional Animal Care and Use Committee approved all experimental procedures prior to experiment initiation (protocol No. 00199).

Experimental Design

One hundred fifteen milligrams of each substrate was fermented in vitro for 0, 2, 4, 8, and 12 h with the fecal microflora obtained from each of the three pigs, the exception being that 5.34 mg of pure raffinose and 17.86 mg of pure stachyose were used to simulate the concentrations of these oligosaccharides in the soy solubles ingredient tested. The substrate, raffinose + stachyose, was the combination of 5.34 mg of raffinose and 17.86 mg of stachyose. The experiment was designed as a randomized complete block with the three fecal donors serving as blocks. Treatments were allotted in a 12 × 5 factorial arrangement with 12 substrates and five incubation times. Each block × treatment combination was assayed using triplicate fermentation tubes. Freshly voided feces from each of the three pigs were used to inoculate all substrate + time combinations in triplicate. Triplicate tubes containing no substrate were fermented with each inoculum source and time point 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 fermentations is presented in Table 2. All components except for the vitamin 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.

Feces from the three donors were collected in plastic bags, which were sealed after expressing excess air, and maintained at 37°C until inoculum was prepared (within 5 min). Each fecal sample was diluted 1:10 (wt/vol) in anaerobic dilution solution (Bryant and Burkey, 1953) by blending for 15 s in a Waring blender under

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Table 2. Composition of medium used for in vitro fermentation

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	296.1
	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; $CaCl_2·H_2O$, 0.16; $MgCl_2·6H_2O$, 0.12; $MnCl_2·4H_2O$, 0.06; $CoCl_2·6H_2O$, 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; $FeSO_4·7H_2O$, 200; $ZnSO_4·7H_2O$, 10; $MnCl_2·4H_2O$, 3; H_3PO_4, 30; $CoCl_2·6H_2O$, 20; $CuCl_2·2H_2O$, 1; $NiCl_2·6H_2O$, 2; $Na_2MoO_4·2H_2O$, 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.

a stream of CO_2. Blended, diluted feces were 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 feces. Tubes were incubated at 37°C with periodic mixing for the respective fermentation times. At the appropriate time, 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 prior to 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 0, 2, 4, 8, and 12 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 *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 gas–liquid chromatography. 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. Lactate concentration was determined using a method adapted from Barker and Summerson (1941) and a Dionex Technical Bulletin (Dionex, Sunnyvale, CA).

The composition of soy solubles was analyzed by the University of Illinois and the Quality Assurance Laboratory of Central Soya; results are presented in Table 1. The methods for these analyses are as described by Smiricky et al. (2002).

Calculations and Statistical Analyses

Data were analyzed as a randomized complete block design with fecal donor serving as block. Treatments, which were factorially arranged, included substrate and length of fermentation. Therefore, donor, substrate, time, and substrate × time were used in the statistical model. All ANOVA were performed according to the GLM procedures of SAS (SAS Inst., Inc., Cary, NC). 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 least significant difference (Milliken and Johnson, 1984).

The data for gas and SCFA production were fitted to a logistic model equation to determine the rate of production and the time to attain maximal rate of production for each substrate. This function is frequently used to model biological, and especially microbial, growth (Koops and Grossman, 1993). It is a sigmoidal curve that can describe accelerating and, after passing through an inflection point, decelerating phases of growth (Koops and Grossman, 1991). The time at which maximal rate of gas or SCFA production occurred was calculated according to the following equation:

$$Y = A/\{1 + e^{[-[t - C)/B]}\}$$

where Y = gas or SCFA production, A = asymptote, or maximal gas or SCFA production, t = incubation time in hours, C = time in hours at which the rate of gas or SCFA production is maximum (the inflection point),

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Table 3. Gas production, rate of gas production, and time to attain maximal rate of gas production for oligosaccharides fermented with swine fecal microflora

Item[a]	Gas production, mL/g of OM in sample				Rate of gas production, mL·g OM in sample^{-1}·h^{-1}	Time to attain maximal rate of gas production, h
	2 h	4 h	8 h	12 h		
Short-chain fructooligoaccharides	36.4	85.3	209.9	230.1	28.9	5.0
Medium-chain fructooligosaccharides	34.4	72.1	184.8	243.0	27.3	6.1
Long-chain fructooligosaccharides	28.9	66.4	170.8	231.5	27.2	6.0
Raffinose[b]	635.5	961.2	1,225.0	1,227.9	—	—
Stachyose	224.8	337.4	472.3	510.3	45.5	3.0
Raffinose + stachyose	189.0	332.4	458.4	465.6	60.2	2.9
Soy solubles	52.2	128.9	175.4	191.4	39.0	3.2
Granular transgalactooligosaccharides	32.4	71.2	177.3	210.5	27.7	5.6
Liquid transgalactooligosaccharides	38.8	78.5	212.9	249.4	34.2	5.7
Glucooligosaccharides	31.2	50.0	111.4	186.2	19.0	8.2
Mannanoligosaccharides	27.9	48.5	91.7	155.3	14.9	8.3
Xylooligosaccharides	18.1	39.8	158.7	215.3	33.1	6.5
SEM[c]			18.5		3.5	1.9
LSD[d]			54.6		10.3	5.6

[a]The interaction of substrate × time was significant ($P < 0.05$) for gas production.
[b]Raffinose fermentation did not follow a logistic model; therefore, rate of gas production and time to attain maximal rate of gas production could not be determined.
[c]Standard error of the mean.
[d]Least significant difference between any two mean values in the same column ($P < 0.05$).

and B = a measure of the duration of gas or SCFA production expressed in milliliters and millimoles per gram of OM, respectively.

Variables (A, B, and C) were estimated for each donor and substrate combination using nonlinear regression (NLREG; Sherrod, 2002). The model explained 95% or more of the variation in gas and SCFA production in all cases.

Additionally, maximal rates of gas and SCFA production were estimated using the derivative of the logistic function according to the following equation:

$$\frac{A \times e^{(C + t)/B}}{B \times [e^{(C/B)} + e^{(t/B)}]^2} = dY/dt$$

where A = asymptote, or maximal gas or SCFA production; t = time in hours; C = time in hours at which maximal rate of gas or SCFA production occurs; and B = measure of the duration of gas or SCFA production expressed as milliliters and millimoles per gram of OM, respectively.

Results

Gas Production

Gas production data, rate of gas production, and time to attain maximal rate of gas production are reported in Table 3. The fructans produced similar amounts of gas at similar rates when compared to each other at each time point. Additionally, the fructans reached maximal rate of gas production at similar times (average 5.7 h).

Gas production by the galactooligosaccharide substrates was more variable. Gas production increased over time for granular transgalactooligosaccharides and liquid transgalactooligosaccharides, but no differences ($P > 0.05$) between these substrates occurred at any individual time point. Soy solubles produced similar ($P > 0.05$) amounts of gas at all time points when compared with granular transgalactooligosaccharides and liquid transgalactooligosaccharides, except at 4 h. Granular transgalactooligosaccharides and liquid transgalactooligosaccharides had similar ($P > 0.05$) rates of gas production (28 and 34 mL·g OM^{-1}·h^{-1}), as well as times ($P > 0.05$) to attain maximal rate of gas production (5.6 and 5.7 h). Stachyose and raffinose + stachyose produced more ($P < 0.05$) gas at all time points when compared with all other substrates except raffinose. Raffinose fermentation produced the most ($P < 0.05$) gas of any substrate tested, yet raffinose fermentation did not follow a logistical model for gas production; therefore, rate of production of gas and time to attain maximal rate of gas production could not be calculated. The time to attain maximal rate of gas production was similar for raffinose + stachyose and soy solubles (2.9 and 3.2 h, respectively). But the rate of gas production was higher (60.2 mL·g OM^{-1}·h^{-1}) for raffinose + stachyose than for soy solubles (39 mL·g OM^{-1}·h^{-1}).

The other substrates tested, glucooligosaccharides, mannanoligosaccharides, and xylooligosaccharides, produced similar ($P > 0.05$) amounts of gas when compared with each other at 2 and 4 h. However, at 8 and 12 h of fermentation, mannanoligosaccharides produced less ($P < 0.05$) gas when compared to xylooligosaccharides. Furthermore, time to attain maximal rate of gas production for these substrates did not differ ($P > 0.05$). Rate of gas production was similar ($P > 0.05$) between glucooligosaccharides and mannanoligosac-

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Table 4. The pH after various times of in vitro fermentation of oligosaccharides with swine fecal microflora

	Incubation time, h			
Item[a]	2	4	8	12
Short-chain fructooligosaccharides	6.3	6.1	5.1	5.1
Medium-chain fructooligosaccharides	6.3	6.1	5.3	5.1
Long-chain fructooligosaccharides	6.3	6.1	5.3	5.1
Raffinose	6.4	6.5	6.4	6.4
Stachyose	6.4	6.4	6.3	6.4
Raffinose + stachyose	6.4	6.3	6.3	6.3
Soy solubles	6.2	5.9	5.8	5.8
Granular transgalactooligosaccharides	6.3	6.0	5.3	5.3
Liquid transgalactooligosaccharides	6.3	6.2	5.6	5.6
Glucooligosaccharides	6.4	6.3	5.9	5.5
Mannanoligosaccharides	6.4	6.3	6.2	6.1
Xylooligosaccharides	6.5	6.3	5.5	5.0
SEM[b]	0.1			
LSD[c]	0.3			

[a]The interaction of substrate × time was significant ($P < 0.05$) for pH value.
[b]Standard error of the mean.
[c]Least significant difference between any two mean values in the same column ($P < 0.05$).

charides, but was higher ($P < 0.05$) for xylooligosaccharides.

Gas production was highest ($P < 0.05$), on average, for the pure soy oligosaccharides (i.e., raffinose, stachyose, and raffinose + stachyose) at all time points. Gas production by the other galactooligosaccharides was not different ($P > 0.05$) from the fructans or glucooligosaccharides, mannanoligosaccharides, or xylooligosaccharides at 2 and 4 h. However at 8 and 12 h, gas production by mannanoligosaccharides was lower ($P < 0.05$) than that for the galactooligosaccharides and the fructans. Rate of gas production was similar ($P > 0.05$) for the fructans, soy solubles, both transgalactooligosaccharide forms, and xylooligosaccharides. Glucooligosaccharides and mannanoligosaccharides had the lowest ($P < 0.05$), and raffinose + stachyose the highest ($P < 0.05$), rate of gas production. Time to attain maximal rate of gas production did not differ ($P > 0.05$) among substrates.

Changes in pH

Generally, pH decreased as time of fermentation increased (Table 4). All substrates had similar pH values (6.5) at 0 h. No differences ($P > 0.05$) in pH were noted among fructans at any time point evaluated.

The pH values for the galactooligosaccharide substrates were not different ($P > 0.05$) from each other at 2 h (average = 6.3). However at 4 h, fermentation of soy solubles resulted in the lowest ($P < 0.05$), and raffinose, stachyose, and raffinose + stachyose fermentations the highest ($P < 0.05$), pH values. At 8 and 12 h, fermentation of granular transgalactooligosaccharides resulted in the lowest ($P < 0.05$), and raffinose, stachyose, and raffinose + stachyose the highest ($P < 0.05$), pH values.

Fermentations of glucooligosaccharides, mannanoligosaccharides, and xylooligosaccharides resulted in similar ($P > 0.05$) pH values at both 2 and 4 h. However, at 8 and 12 h, xylooligosaccharide fermentation resulted in the lowest ($P < 0.05$) pH, followed by glucooligosaccharides, with mannanoligosaccharides having the highest ($P < 0.05$) pH.

Overall, pH values for the fructans, granular transgalactooligosaccharides, and xylooligosaccharides at 12 h were similar ($P < 0.05$). The pH values for fermentations of glucooligosaccharides, soy solubles, and liquid transgalactooligosaccharides at 12 h were similar ($P > 0.05$) but lower ($P < 0.05$) than pH values for fermentations of mannanoligosaccharides and the pure galactooligosaccharides.

SCFA Production

Short-chain fatty acid production data are reported in Table 5. Whereas lactate concentration was measured for all substrates tested, there was no measurable lactate present as a result of oligosaccharide fermentation. The fructans resulted in similar ($P > 0.05$) concentrations of acetate, propionate, butyrate, and total SCFA when compared to each other at each time point.

Production of SCFA by galactooligosaccharides yielded more variable results. Acetate production at 2, 4, and 8 h was lowest ($P < 0.05$) for soy solubles, granular transgalactooligosaccharides, and liquid transgalactooligosaccharides, and highest ($P < 0.05$) for raffinose. At 12 h, acetate production was highest ($P < 0.05$) for raffinose, stachyose, and raffinose + stachyose. Fermentation of soy solubles, granular transgalactooligosaccharides, and liquid transgalactooligosaccharides resulted in the least ($P < 0.05$) amount of propionate at 2 and 4 h. Raffinose fermentation at 2 and 4 h resulted in the highest ($P < 0.05$) propionate production, yet at 8 and 12 h, fermentation of raffinose resulted in no propionate production. Soy solubles, granular transgalactooligosaccharides, and liquid transgalactooligo-

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Table 5. Acetate, propionate, butyrate, and total short-chain fatty acid (SCFA) production after various times of in vitro fermentation of oligosaccharides with swine fecal microflora

	Acetate				Propionate				Butyrate				Total SCFA			
Item[a]	2 h	4 h	8 h	12 h	2 h	4 h	8 h	12 h	2 h	4 h	8 h	12 h	2 h	4 h	8 h	12 h
	mmol/g of OM in sample															
Short-chain fructooligosaccharides	0.2	0.8	2.2	2.2	0.2	0.6	2.3	2.3	0.1	0.2	0.8	1.2	0.5	1.6	5.3	5.7
Medium-chain fructooligosaccharides	0.2	0.7	1.9	2.3	0.2	0.6	2.0	2.6	0.1	0.2	0.9	1.2	0.5	1.5	4.8	6.1
Long-chain fructooligosaccharides	0.2	0.7	1.7	2.3	0.2	0.7	2.0	2.7	0.1	0.2	0.8	1.0	0.5	1.6	4.5	6.0
Raffinose	3.2	4.7	4.0	3.6	2.6	3.0	–0.3	–0.8	0.8	1.2	0.7	0.7	6.6	8.9	4.4	3.5
Stachyose	2.1	3.2	3.4	3.1	1.4	2.4	2.6	2.5	0.4	1.0	1.1	1.1	3.9	6.6	7.1	6.7
Raffinose + stachyose	1.8	3.0	3.4	3.3	1.3	2.6	2.9	2.2	0.4	1.0	1.2	1.2	3.5	6.6	7.5	6.7
Soy solubles	0.5	1.6	2.2	2.4	0.4	1.3	1.7	1.9	0.1	0.3	0.8	0.7	1.0	3.2	4.7	5.0
Granular transgalactooligosaccharides	0.4	1.0	2.2	2.4	0.3	0.9	1.8	2.1	0.1	0.3	0.9	1.0	0.8	2.2	4.9	5.4
Liquid transgalactooligosaccharides	0.2	0.7	2.0	2.0	0.3	0.7	2.5	2.5	0.1	0.1	0.8	0.9	0.6	1.5	5.3	5.4
Glucooligosaccharides	0.3	0.7	1.9	2.7	0.2	0.6	1.4	2.2	0.0	0.2	0.5	0.5	0.5	1.5	3.8	5.4
Mannanoligosaccharides	0.2	0.6	0.9	1.6	0.2	0.5	0.7	1.4	0.0	0.1	0.3	0.4	0.4	1.2	1.9	3.4
Xylooligosaccharides	0.2	0.7	2.5	3.7	0.2	0.7	1.9	2.7	0.0	0.1	0.4	0.5	0.4	1.5	4.8	6.9
SEM[b]	0.2				0.2				0.2				0.3			
LSD[c]	0.6				0.6				0.6				0.9			

[a]The interaction of substrate × time was significant ($P < 0.05$) for SCFA production.
[b]Standard error of the mean.
[c]Least significant difference between any two mean values in the same column ($P < 0.05$).

saccharides produced the least ($P < 0.05$) butyrate at 2 and 4 h. Fermentation of raffinose resulted in the highest ($P < 0.05$) production of butyrate at 2 and 4 h. Butyrate production at 8 and 12 h did not differ ($P > 0.05$) among galactooligosaccharide substrates. Total SCFA production was similar and lowest ($P < 0.05$) at 2 h for soy solubles, granular transgalactooligosaccharides, and liquid transgalactooligosaccharides. At 2 and 4 h, fermentation of raffinose resulted in the most ($P < 0.05$) total SCFA production. However, by 12 h, fermentation of raffinose resulted in the lowest ($P < 0.05$) total SCFA production. Fermentation of stachyose and raffinose + stachyose resulted in moderate total SCFA production at 2 h when compared with all other galactooligosaccharide substrates. However, at 8 and 12 h, fermentation of stachyose and raffinose + stachyose resulted in the highest ($P < 0.05$) total SCFA production of all the galactooligosaccharide substrates tested.

Fermentation of glucooligosaccharides, mannanoligosaccharides, and xylooligosaccharides resulted in similar ($P > 0.05$) acetate, propionate, and butyrate concentrations when compared to each other at 2 and 4 h. However, at 8 and 12 h, mannanoligosaccharide fermentation produced the least ($P < 0.05$) acetate and propionate, and xylooligosaccharides produced the most ($P < 0.05$) acetate. Propionate production was similar ($P > 0.05$) between xylooligosaccharides and glucooligosaccharides at 8 and 12 h. Butyrate production was similar ($P > 0.05$) for each substrate at all time points. Total SCFA production was similar ($P > 0.05$) for glucooligosaccharides, mannanoligosaccharides, and xylooligosaccharides at 2 and 4 h. However, at 8 and 12 h, mannanoligosaccharide fermentation resulted in the lowest ($P < 0.05$), and xylooligosaccharide fermentation the highest ($P < 0.05$), total SCFA production.

When comparing all oligosaccharides tested at the 12 h fermentation time, mannanoligosaccharide fermentation produced the least amounts ($P < 0.05$) of acetate and propionate when compared to all other substrates. Fermentation of the fructans, soy solubles, granular transgalactooligosaccharides, liquid transgalactooligosaccharides, and glucooligosaccharides resulted in similar ($P > 0.05$) acetate and propionate productions. Fermentation of pure galactooligosaccharides and xylooligosaccharides resulted in the highest ($P < 0.05$) acetate production. Glucooligosaccharide, mannanoligosaccharide, and xylooligosaccharide fermentation resulted in the lowest ($P < 0.05$) butyrate concentration, whereas fermentation of all other substrates resulted in similar ($P > 0.05$) butyrate concentrations. Total SCFA production was similar ($P > 0.05$) for the fructans, soy solubles, both transgalactooligosaccharides forms, and glucooligosaccharides. Fermentation of raffinose and mannanoligosaccharides resulted in the lowest ($P < 0.05$) total SCFA production. Stachyose, raffinose + stachyose, and xylooligosaccharide fermentation resulted in the most ($P < 0.05$) total SCFA production at 12 h.

Rate of SCFA production and time to attain maximal rate of SCFA production data are presented in Table 6. Rates of acetate, propionate, and butyrate production and time to attain maximal rate of production were

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Table 6. Rate of production and time to attain maximal rate of production of acetate, propionate, and butyrate as a result of in vitro fermentation of oligosaccharide substrates with swine fecal microflora

	Rate of production, mmol·g OM in sample^{-1}·h^{-1}			Time to attain maximal rate of production, h		
Item	Acetate	Propionate	Butyrate	Acetate	Propionate	Butyrate
Short-chain fructooligosaccharides	0.5	0.5	0.2	4.9	7.1	5.4
Medium-chain fructooligosaccharides	0.3	0.4	0.2	5.7	6.0	6.5
Long-chain fructooligosaccharides	0.3	0.4	0.2	6.0	6.2	6.2
Raffinose[a]	—	—	—	—	—	—
Stachyose	1.2	0.8	0.3	1.6	1.8	2.4
Raffinose + stachyose	0.7	1.1	0.3	1.9	2.1	2.7
Soy solubles	0.6	0.6	0.2	3.4	3.5	4.2
Granular transgalactooligosaccharides	0.4	0.2	0.2	4.5	6.6	5.0
Liquid transgalactooligosaccharides	0.5	0.6	0.2	4.8	5.0	6.5
Glucooligosaccharides	0.3	0.2	0.1	7.8	7.5	5.5
Mannanoligosaccharides	0.2	0.1	0.1	8.2	8.3	7.1
Xylooligosaccharides	0.5	0.4	0.1	7.3	6.5	6.7
SEM[b]		0.1			0.7	
LSD[c]		0.3			2.1	

[a]Raffinose fermentation did not follow a logistic model; therefore, rate of short-chain fatty acid production and time to attain maximal rate of short-chain fatty acid production could not be determined.
[b]Standard error of the mean.
[c]Least significant difference between any two mean values in the same column ($P < 0.05$).

similar ($P > 0.05$) among the fructans (average 0.4, 0.4, and 0.2 mmol·g OM in sample^{-1} ·h^{-1} and 5.5, 6.4, and 6.0 h, respectively).

Raffinose fermentation did not follow a logistical model for SCFA production; therefore, rate of production of SCFA and the time to attain maximal rate of SCFA production could not be calculated. Fermentation of granular transgalactooligosaccharides resulted in the lowest ($P < 0.05$) rates of acetate and propionate production of the galactooligosaccharide substrates. Stachyose fermentation resulted in the highest ($P < 0.05$) rate of acetate production and raffinose + stachyose the highest ($P < 0.05$) rate of propionate production. Rate of butyrate production was similar ($P > 0.05$) among all galactooligosaccharide substrates.

Fermentation of mannanoligosaccharides resulted in the lowest ($P < 0.05$), and xylooligosaccharides the highest ($P < 0.05$), rates of acetate and propionate production. Rate of butyrate production was similar ($P > 0.05$) for glucooligosaccharides, mannanoligosaccharides, and xylooligosaccharides.

Overall, rates of acetate production were similar and lowest ($P < 0.05$) for glucooligosaccharides, mannanoligosaccharides, medium-chain fructooligosaccharides, and long-chain fructooligosaccharides. Fermentation of the galactooligosaccharides (except granular transgalactooligosaccharides) resulted in the highest ($P < 0.05$) rates of acetate and propionate production when compared to other substrates. The rates of propionate production were similar and lowest ($P < 0.05$) for glucooligosaccharides and mannanoligosaccharides. Fermentation of the fructans and xylooligosaccharides resulted in moderate and similar ($P > 0.05$) rates of propionate production. Rates of butyrate production did not differ ($P > 0.05$) among substrates.

Discussion

Because all oligosaccharides are inherently different in properties, such as chain length, sugar composition, and bond types, different fermentation characteristics may occur. It was the aim of this study to determine differences in fermentation characteristics among oligosaccharides sources present in feed ingredients or isolated and available for dietary supplementation purposes.

Gas production was similar among all substrates tested except for mannanoligosaccharides and the pure galactooligosaccharides. Fermentation of soy solubles resulted in lower amounts of gas production when compared with pure forms of galactooligosaccharides found in soy solubles. Additionally, the rate at which pure raffinose + stachyose produced gas greatly exceeded that of the soy solubles. These data indicate that raffinose and stachyose present in the soy solubles matrix behave much differently than they do in the pure form. Hence, the increase in gas production observed in fermentations of pure galactooligosaccharides may provide indirect evidence that genera other than bifidobacteria and lactobacilli were stimulated since they 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 cross feeding on bi-

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fidobacterial metabolites is unclear. Soy solubles contain 2.4% N and a significant amount of sucrose. Therefore, fermentation of the oligosaccharides in soy solubles may have been delayed due to the availability of more fermentable components. Additionally, they may be complexed and less available for fermentation when present in the whole plant matrix. Carbohydrates are the preferred substrates for fermentation and, thus, the sucrose in soy solubles would have been quickly transported via a phosphotransferase carrier system into the cell wall of bacteria and converted to fructose 1, 6 bisphosphate (Lengeler et al., 1999). This compound can then enter the Embden-Meyerhof pathway and be converted to 2 pyruvate + 2 ATP + 2 NADH (Lengeler et al., 1999). Pyruvate can be converted to SCFA, resulting in release of 1 mol of CO_2. The minimal amount of gas released as a result of sucrose fermentation may be the reason that less gas is produced by soy solubles fermentation when compared to fermentation of the pure galactooligosaccharide components of soy solubles. Additionally, gas production as a result of fermentation of raffinose was higher than for stachyose or raffinose + stachyose. Since raffinose fermentation did not follow a logistical model, and rate of production and time to attain maximal rate of production could not be calculated, the difference between raffinose and stachyose is perhaps due to a slower rate of stachyose fermentation. Stachyose contains an extra α 1-6 linked galactose unit. This additional bond that must be broken by bacterial α-galactosidase may have slowed fermentation and decreased the rate of fermentation and, thus, gas production when compared with raffinose fermentation. The potential reason that raffinose + stachyose fermentation did not result in gas production rates intermediate to raffinose and stachyose fermentation is the slower rate of fermentation as affected by the presence of stachyose and, thus, gas production did not rebound to the production level of pure raffinose.

Mannanoligosaccharide fermentation resulted in the lowest quantity of gas production, the lowest rate of gas production, and the longest time to attain maximal rate of gas production. This is coupled with high pH and low SCFA production. Unlike most of the oligosaccharides tested in this experiment, mannanoligosaccharide is a crude extract from yeast and contains 6% N, 41% total dietary fiber, 8% fat, and 44% total monosaccharides on a dry matter basis. Therefore, ingredients that do not ferment as rapidly, such as N and fat, could potentially inhibit fermentation. Additionally, the fiber component of mannanoligosaccharides could slow its fermentability.

The change in pH as a result of fructan fermentation was similar among all fructan forms. Flickinger et al. (2000) reported a 17% decrease in pH values when short-chain fructooligosaccharides were fermented for 11 h with canine fecal microflora. Similarly, in the present study, the pH value for short-chain fructooligosaccharides decreased 17% over a 12 h fermentation period.

At every time point, soy solubles fermentation resulted in a lower pH than did the pure galactooligosaccharide substrates. A potential explanation could be that fermentation of raffinose + stachyose was rapid and, once complete, proteolytic bacteria present in the fermentation vessel began consuming spent bacteria as substrates for growth, yielding end products that were of a higher pH. Fermentations of soy solubles and liquid transgalactooligosaccharides generally resulted in similar pH values, indicating potential similarity in fermentative characteristics.

Fermentation of xylooligosaccharides and the fructans resulted in the lowest pH values of all oligosaccharides tested. These data indicate that bacteria present in the large intestine of swine may more extensively ferment these substrates compared with the other oligosaccharides tested.

Short-chain fatty acid production among fructan sources was not different in this study. Flickinger et al. (2000) reported a fivefold increase in acetate and a ninefold increase in propionate and butyrate production after 11 h of short-chain fructooligosaccharides fermentation. Our data indicate greater increases in acetate (10-fold) and butyrate (11-fold) production. This may be due to the different sources of fecal inoculum (canine vs. swine). The SCFA production data presented by Flickinger et al. (2000) are consistent with data for other in vitro fermentations of short-chain fructooligosaccharides using canine fecal microflora (Sunvold et al., 1995; Vickers et al., 2001). In agreement with our data, Vickers et al. (2001) reported similar SCFA production as a result of fermentation of short-chain fructooligosaccharides and long-chain fructooligosaccharides (0.97 vs. 1.1 mmol of total SCFA/g of OM fermented, respectively) with canine fecal microflora.

Stachyose and raffinose + stachyose fermentations produced more SCFA, had higher rates of SCFA production, and attained a maximal rate of SCFA production earlier when compared with other oligosaccharides. Galactooligosaccharides in the soy solubles matrix are fermented more slowly than pure raffinose and stachoyse present in the same concentrations as in soy solubles. Therefore, in the ingredient matrix, galactooligosaccharides may be more efficacious as fermentative substrates in the large intestine when compared with their pure counterparts because they are more slowly fermented, and thus, may escape degradation by terminal small intestinal microflora and be available to serve as fermentative substrates in the large intestine of swine. Additionally, at 8 and 12 h, fermentation of raffinose resulted in no propionate formation, indicating that bacteria contributing to fermentation were not generating propionate or the propionate in the fermentation vessels was metabolized.

Fermentation of mannanoligosaccharides resulted in less production of SCFA when compared with all other substrates except raffinose. Also, rate of SCFA production was lowest for mannanoligosaccharides, indicating a lower fermentative capacity. In agreement with our

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data, Vickers et al. (2001) reported substantially lower acetate (0.89 vs. 2.3 mmol/g of OM sample), propionate (0.3 vs. 0.9 mmol/g of OM), and butyrate (0.2 vs. 0.3 mmol/g of OM) values when mannanoligosaccharide was fermented for 12 h when compared to short-chain fructooligosaccharides.

Flickinger et al. (2000) reported lower amounts of SCFA production when glucooligosaccharides were fermented by canine microflora over an 11-h period in comparison to our data. They reported a fourfold increase in acetate, an eightfold increase in propionate, and a fourfold increase in butyrate production when glucooligosaccharides was fermented. Our results show an eightfold increase in acetate, a 10-fold increase in propionate, and a fivefold increase in butyrate production when glucooligosaccharides was fermented with swine fecal microflora over a 12-h period.

Short-chain fatty acids play several important roles in animal metabolism. For example, Roediger (1982) reported that butyrate was the preferential energy source of colonocytes in rats. Also, nonruminant hindgut fermentors utilize acetate largely as a fuel source for peripheral tissues (Cummings, 1991). Propionate has been suggested to spare amino acids 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 energy requirements of pigs (Imoto and Namioka, 1978). Therefore, substrates that produce high amounts of SCFA in vitro, namely the fructans, xylooligosaccharides, and the pure galactooligosaccharides, may do the same in vivo. However, dietary inclusion of pure galactooligosaccharides may result in problems, namely high amounts of gas production and fermentation rates that are too rapid. Substrates resulting in lower amounts of gas production and moderate to high quantities of SCFA, such as soy solubles and the fructans, may be better substrates for fermentation in vivo.

The differences in amounts of SCFA produced by oligosaccharides tested in our study when compared with previous studies may be a result of the source of fecal inoculum. Flickinger et al. (2000) reported data utilizing canine fecal donors, whereas our study utilized swine fecal donors. Different potential concentrations of bacteria known to ferment oligosaccharides may have been a factor in the increased SCFA production noted in our study.

In conclusion, fermentation of most of the oligosaccharides in this study resulted in a rapid decline in pH and an increase in organic acid production. This indicates that most of these oligosaccharides are rapidly fermentable. Higher amounts of gas and SCFA production were synonymous with faster rates of production and earlier times to attain maximal rate of production. Lower amounts of gas and SCFA production were indicative of slower rates of production and longer times to attain maximal rates of production. The greater rate of fermentation of the pure galactooligosaccharides indicates that these oligosaccharides may serve as fermentable substrates in the terminal small intestine or proximal large intestine. Fermentation of mannanoligosaccharides resulted in the lowest SCFA production, indicating that this substrate is fermentable but not to the same extent as the other oligosaccharides tested. Fermentation of xylooligosaccharides resulted in the highest SCFA production by 12 h when compared with the pure galactooligosaccharides and soy solubles; however, time to attain maximal rate of SCFA production, when compared with that of the pure galactooligosaccharides and soy solubles, was longer. The slower rate of fermentation of xylooligosaccharides when compared with galactooligosaccharides and soy solubles may indicate that it is a better source of fermentable substrate in the distal portion of the gastrointestinal tract, such as in the transverse and descending regions of the large intestine.

Implications

Oligosaccharide fermentation patterns obtained in vitro might be used to predict behavior in vivo. The type of oligosaccharide fermented influences the nature of the fermentative end products. Higher amounts of gas and short-chain fatty acid production were indicative of faster rates of production and earlier times to attain maximal rate of production. Lower amounts of gas and short-chain fatty acid production were indicative of slower rates of production and longer times to attain maximal rates of production. Many positive roles have been established for short-chain fatty acids in human and animal nutrition. Oligosaccharides such as fructooligosaccharides or galactooligosaccharides that are rapidly fermentable might optimize bacterial growth and metabolism in the terminal small intestine and proximal large intestine. Oligosaccharides, such as xylooligosaccharides that are slowly fermented, may be more suitable as substrates for microbes inhabiting the distal large intestine.

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