Selected Indigestible Oligosaccharides Affect Large Bowel Mass, Cecal and Fecal Short-Chain Fatty Acids, pH and Microflora in Rats

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ABSTRACT Certain indigestible oligosaccharides may benefit gastrointestinal tract health via fermentation and proliferation of desirable bacterial species. The purpose of this study was to elucidate effects of selected oligosaccharides on cecal and fecal short-chain fatty acid (SCFA) concentration, pH, total large bowel wet weight and wall weight, and gut microbiota levels in rats. Fifty male Sprague-Dawley rats were randomly assigned to one of five treatments: 1) control diet; 2) control diet + 5% microcrystalline cellulose (5% CC); 3) control diet + 5% CC + 6% fructooligosaccharides; 4) control diet + 5% CC + 6% oligofructose; or 5) control diet + 5% CC + 6% xylooligosaccharides. The control diet consisted of (dry matter basis) 20% protein, 65% carbohydrate, 10.5% fat, vitamin and mineral mixes. The duration of the study was 14 d. The oligofructose- and fructooligosaccharide-containing diets resulted in higher cecal butyrate concentrations compared with the control, cellulose and xylooligosaccharide diets. Generally, total cecal SCFA pools were higher while pH was lower from ingesting oligosaccharide-containing diets compared with control or cellulose diets. Cecal total weight and wall weight were higher from oligosaccharide consumption, whereas colonic total wet weight was higher for rats consuming xylooligosaccharides compared with other treatments; colon wall weight was unaffected by treatments. Cecal bifidobacteria and total anaerobes were higher whereas total aerobes were lower in rats fed oligosaccharide diets compared with those fed the control diet. Cecal lactobacilli levels were unaffected by treatment. Dietary incorporation of fermentable, indigestible oligosaccharides, by providing SCFA, lowering pH, and increasing bifidobacteria, may be beneficial in improving gastrointestinal health. J. Nutr. 127: 130–136, 1997.

KEY WORDS: oligosaccharides • short-chain fatty acids • bifidobacteria • rats

Increased interest in fermentable fiber has occurred in recent years due to the beneficial effects on the human gastrointestinal tract resulting from their consumption. Research has focused on the colon and the products of colonic fermentation, namely, the short-chain fatty acids (SCFA)\(^3\) acetate, butyrate and propionate. Colonocytes are purported to be sustained by SCFA derived from bacterial fermentation, with butyrate oxidation providing more than 75% of the oxygen consumed by human colonic tissue (Roediger 1980a). Limited evidence (Roediger 1980a) indicates that butyrate is the preferred energy substrate of rat colonic mucosa. Impaired utilization of SCFA has been implicated in ulcerative colitis (UC), suggesting an energy-deficient state (Roediger 1980b). Mucosal cells demonstrated an absence of butyrate oxidation, reflecting a metabolic defect in the mucosa of UC patients. Moreover, Harig et al. (1989) inferred that diversion colitis represented an inflammatory state resulting from a nutritional deficiency that may be effectively treated with enemas containing SCFA, the missing nutrients.

It may be advantageous to provide indigestible oligosaccharides as an indirect source of SCFA to the large bowel. Potential substrates include fructooligosaccharides (FOS; also called neosugar, commercial name NutraFlora®; Golden Technologies Company, Westminster, CO), oligofructose (also called fructan-based oligosaccharides; commercial name Rafi lose®, Rafinerie, Brussels, Belgium), and xylooligosaccharides (XOS; commercial name Xylooligo, Suntory, Tokyo, Japan). Fructooligosaccharides, oligofructose and XOS have been shown to be indigestible by human enzymes in the small intestine, but are extensively fermented in the large bowel (Hidaka et al. 1986, Mitsuoka et al. 1987, Okazaki et al. 1990) to SCFA which can be absorbed and metabolized by the host. These oligosaccharides have been implicated in increasing the densities of bifidobacteria (Hidaka et al. 1991b, Okazaki et al. 1990) and lactobacilli (Williams et al. 1994) in the gastrointestinal tract, both of which are considered beneficial intestinal bacteria.

The purpose of this study was to evaluate the in vivo effects of selected oligosaccharides on cecal and fecal SCFA concentration, pH, total large bowel wet weight and wall weight, and concentrations of intestinal microbiota.
TABLE 1

Ingredient composition of dietary treatments fed to rats

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>Cellulose</th>
<th>Oligofructose</th>
<th>FOS2</th>
<th>XOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>19.7</td>
<td>19.7</td>
<td>19.7</td>
<td>19.7</td>
<td>19.7</td>
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<tr>
<td>L-Cystine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>64.75</td>
<td>59.75</td>
<td>53.75</td>
<td>53.75</td>
<td>53.75</td>
</tr>
<tr>
<td>Fat</td>
<td>10.5</td>
<td>10.5</td>
<td>10.5</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Oligofructose</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Fructooligosaccharide</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Xylooligosaccharide</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Choline</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamins3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Minerals4</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Gross energy, MJ/kg diet</td>
<td>18.43</td>
<td>18.44</td>
<td>18.68</td>
<td>18.80</td>
<td>18.76</td>
</tr>
</tbody>
</table>

1 Protein was casein hydrolysate (MW < 500). Carbohydrate supplied as dextrose. Fat supplied as medium-chain triglycerides (from fractionated coconut oil with 8.0, 0.08%; 8.0, 58.50%; 10.0, 41.02%; 12.0, 0.40%) and soybean oil (75/25). Cellulose was microcrystalline cellulose (Avicel). Choline supplied as choline bitartrate.

2 FOS, fructooligosaccharides; XOS, xylooligosaccharides.

3 Vitamins = Vitamin mix, AIN-93-VX (94047). Vitamin contribution to diet (mg/kg): thiamin, 5; riboflavin, 6; pyridoxine, 6; nicotinic acid, 30; pantothenate, 15; folic acid, 0.75; biotin, 0.2; cyanocobalamin, 0.025; all-rac-a-tocopherol acetate (500 IU/g), 150; and cholecalciferol (400,000 IU/g), 2.5.

4 Minerals = Mineral mix, AIN-93G-MX (940046). Mineral contribution to diet (mg/kg): Ca, 5000; P, 1561; K, 3600; Na, 1019; Cl, 1571; S, 300; I, 0.2; Fe, 35; Mg, 507; Zn, 30; Cu, 6; Mn, 10; Mo, 0.15; Se, 0.15; Cr, 1; Si, 5; F; 1; Ni, 0.5; B, 0.5; Li, 0.1; and V, 0.1.

MATERIALS AND METHODS

Animal selection. Fifty male Sprague-Dawley rats (average weight, 411 ± 13 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN). Prior to the experiment, the rats were fed a standard nonpurified diet. Animals were individually housed in stainless steel wire-bottom cages in an environmentally controlled room (25°C) with a 12-h light:dark cycle. Rats were given free access to water. The animal use protocol was reviewed and approved by the Laboratory Animal Care Advisory Committee of the University of Illinois.

Experimental design. Rats were randomly assigned to one of five dietary treatments (10 rats/treatment). Due to space limitations, rats were divided into two blocks of 25 rats per block representing 5 rats per treatment per block. Rats were given free access to powdered semi-elemental diets. Oligosaccharides were added to partially replace the dextrose component of the control diet. Treatments were as follows: 1) control diet; 2) control diet + 5% microcrystalline cellulose (5% CC); 3) control diet + 5% CC + 6% FOS (NutraFlora®, Golden Technologies Company); 4) control diet + 5% CC + 6% xylooligosaccharide (Raf®tose®, Raffinerie®); and 5) control diet + 5% CC + 6% XOS (XYLOlogioS 5, Suntory) (Table 1). Fructooligosaccharides are produced on a commercial basis by fermenting granulated sucrose in water with an enzyme isolated from a pure strain of Aspergillus niger. The organism produces the β-fructofuranosidase (fructosyltransferase) enzyme which links additional fructose units onto the fructose end of sucrose molecules to produce 1-kestose (GF2), nystose (GF3), and 1-β-fructofuranosylfructose (GF4) (Hidaka et al. 1991a). Oligofructose is produced via enzymatic hydrolysis of inulin. The hydrolysis results in a wide array of oligosaccharides such as GF2, GF3 and GF4 as well as oligosaccharides containing only fructose (e.g., F2, F3, F4). Xylooligosaccharides are obtained from birch wood xylan hydrolyzed by a Trichoderma-derived xylanase enzyme. The primary components of XOS are xylose, xylotriose and xylotetraose. Diets were formulated to meet or exceed the nutrient requirements of rats (NRC 1995). Diets differed in energy content. The calculated metabolizable energy (ME) content of the diets was 18.09, 17.25, 16.62, 16.62 and 16.62 MJ/kg for treatments 1–5, respectively. The ME values were calculated using 16.74 kJ/g for protein and carbohydrate, 37.66 kJ/g for fat and 6.28 kJ/g (Roberfroid et al. 1993) for oligosaccharides. The duration of the study was 14 d. Food intake was determined daily while body weight was determined every 3 d.

Collection of samples. On d 1 through 13, rats were given free access to dietary treatments. On the eve of d 13, food was removed for 8–10 h. Following food deprivation, rats were given free access to their respective diet for 2 h and intake recorded. The starving and refeeding were done to assure procurement of an appropriate sample from the cecum and colon for analysis. Rats were killed at 3 h postprandial by placement in a CO2 chamber. A ventral midline incision was made and the cecum and colon excised. Immediately after removal, cecum and colon with contents were weighed to determine total weight. Cecal and colonic contents were collected, pH measured using a Fisher Scientific Accumet® 1001 pH meter (Fisher Scientific, Pittsburgh, PA) with a MI-410 microcombination pH electrode probe attached (Microelectrodes, Londonderry, NH), and a 0.4-g aliquot was immediately processed for SCAFA analysis. Cecal contents were measured in the material (pellets) taken from the lower colon to the anus. The remaining cecal and fecal contents were immediately placed in preweighed Carey-Blair transport media to maintain an anaerobic environment (Meridian Diagnostics, Cincinnati, OH) and stored under liquid nitrogen for later microbiota enumeration. Following removal of the appropriate samples, the tissues were cleaned with water, blotted dry and weighed to determine cecal and colonic total wall weight. Stool consistency was documented daily by the investigators as formed (pelleted) or unformed.

Chemical analyses. Cecal and fecal samples were acidified with 250 g/L metaphosphoric acid using a mixture of 0.4 g sample:0.8 mL acid:2.8 mL distilled H2O and centrifuged at 25,900 × g for 20 min. The supernatant was decanted and frozen at −20°C in microfuge tubes. Following freezing, the supernatant was thawed, centrifuged in microfuge tubes at 13,000 × g for 10 min, and analyzed for lactate concentration colorimetrically (Barker and Summerson 1941) and for SCFA concentration (acetate, propionate and butyrate) via gas chromatography (Erwin et al. 1961). Briefly, concentrations of acetate, propionate and butyrate were determined in the supernatant using a Hewlett-Packard (Wilmington, DE) 5890A Series II gas chromatograph and a glass column (180 cm × 4 mm i.d.) packed with 100 g/L SP-1200/10 g/L H2PO4 on 80/100 mesh Chromosorb WAW (Supelco, Bellefonte, PA). Nitrogen was used as the carrier gas with a flow rate of 75 mL/min. Oven temperature was 125°C, detector temperature was 175°C and injector temperature was 180°C.

Cecal and fecal samples stored in liquid nitrogen for microbiota enumeration were thawed at room temperature and serially diluted
with dilution solution (Bryant and Burkey 1953) under a CO₂
environment. Bifidobacterium species (ssp.) were enumerated from the
diluted samples and inoculated onto petri dishes containing a selective
and differential medium, Bifidobacterium iodoacetate medium 25
(BIM-25), in an anaerobic environment. The medium composition
(g/L) is as follows: reinforced clostridial agar (BBL Microbiology Sys-
tems, Cockeysville, MD), 51; nalidixic acid, 0.02; polymyxin B sulfate,
0.0085; kanamycin sulfate, 0.05; iodoacetate acid, 0.025; and 2,3,5-
triphényltétrazolium chloride, 0.025 (Muñoz and Pares 1988). The
basal agar was autoclaved and allowed to cool to 55–60°C. Filtered
sterilized antibiotics, iodoacetate, and 2,3,5-triphényltétrazolium
chloride then were added. Lactobacillus spp. were enumerated from the
diluted samples and inoculated onto petri dishes containing Ro-
gosa SL agar (Difco Laboratories, Detroit, MD). The agar was prepared
according to manufacturer’s instructions. Total anaerobic and aerobic
counts were enumerated using diluted samples according to Bryant
and Robinson (1961) and Mackie et al. (1978). The composition of the
medium used for total culturable counts is presented in Table 2.
For total anaerobic counts, all components were mixed in a round-
bottomed flask before melting in a microwave. After melting, the
medium was saturated with CO₂, and the NaHCO₃ solution was
added. The medium then was autoclaved for sterilization, and allowed
to cool to 55–60°C. Immediately before pouring the plates under
anaerobic conditions, the glucose and Na₂S-cysteine solution were
added while the medium was maintained under anaerobic conditions.
For aerobic counts, the total cuttable counts medium was prepared as
for the anaerobic counts excluding Resazurin solution, NaHCO₃,
and Na₂S-cysteine. In addition, the medium was not saturated with
CO₂ and plates were poured in aerobic conditions. Counting precision
of the microbiota was maximized by inoculating drops of three appro-
priate dilutions onto their respective plates. A repeating dispenser
was used to dispense six 10-µL droplets onto the agar plates. After
adsorption of the droplets, the plates were inverted and incubated at
38°C inside a cabinet in an anaerobic chamber. Colony counts were
made after the respective days of incubation, and colony forming
units (CFU) per gram of wet sample were calculated.

**Statistical analyses.** Data were analyzed by ANOVA for a ran-
domized complete block design according to the General Linear Mod-
els procedure of SAS (SAS 1992). Model sums of squares were sepa-
rated initially into block, treatment and block × treatment effects.
No block × treatment interactions were significant at the P < 0.05
level; therefore, the final model consisted of block and treatment.

**TABLE 2**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration in medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar, bacto</td>
<td>12 g</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Starch, rice</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.8 g</td>
</tr>
<tr>
<td>Casitone, bacto</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Na-DL-lactate solution¹</td>
<td>20 mL</td>
</tr>
<tr>
<td>Solution A²</td>
<td>75 mL</td>
</tr>
<tr>
<td>Solution B³</td>
<td>75 mL</td>
</tr>
<tr>
<td>Clarified rumen fluid</td>
<td>400 mL</td>
</tr>
<tr>
<td>Resazurin solution⁴</td>
<td>1 mL</td>
</tr>
<tr>
<td>Distilled H₂O</td>
<td>370 mL</td>
</tr>
<tr>
<td>After melting: 91 g/L NaHCO₃ (wt/v)</td>
<td>36 mL</td>
</tr>
<tr>
<td>After sterilization: 250 g/L Glucose (wt/v), Na₂S/Cysteine solution⁵</td>
<td>20 mL</td>
</tr>
</tbody>
</table>

¹ Composition (mL/L): Na lactate (600 g/L), 167.
² Composition (g/L): K₂PO₄, 6.0; NaN₃C₆H₅O₇·2H₂O, 2.0.
³ Composition (g/L): NaCl, 12.0; (NH₄)₂SO₄, 12.0; KH₂PO₄, 6.0; CaC₂O₄, 1.2; MgSO₄·7H₂O, 2.46; Na₂C₆H₅O₇·2H₂O, 20.0.
⁴ Resazurin, 1 g/L in distilled H₂O.
⁵ Composition (g/L): Cysteine HCl·2H₂O, 12.5 g; 0.4 N NaOH, 500 mL; Na₂S·9H₂O, 12.5 g.

When significant (P < 0.05) differences were detected, individual
means were compared by the least significant difference method
(Carmer and Swanson 1973). Correlation data were analyzed ac-
cording to the Pearson Correlation (CORR) procedure of SAS (SAS

**RESULTS**

**Effects of diet on intake and body weight.** Intake ranged from
~15–18 g/d with an average of 16.8 g/d and fluctuated daily. On d 2, 3, 6 and 8, significant treatment differences (P < 0.05) were noted; these may have been caused by normal intake fluctuations that have little biological importance. Ini-
itial body weights ranged from 410 to 412 g with final body
weights ranging from 416 to 420 g. Body weights of rats on
all treatments initially declined after feeding the experimental
diets because of a change in diet (nonpurified diet vs. powdered
diet). However, after 4 d, body weights began to increase over
time for all treatment groups. No significant differences among
groups were noted in body weight even though ME content
of the diets differed.

**Short-chain fatty acid concentrations and molar propor-
tions.** The addition of oligofructose resulted in higher (P < 0.05) fecal acetate concentrations compared with the other
treatments, whereas propionate, butyrate and lactate were un-
affected by treatment (Table 3). Although the ME intake
was lower for rats fed oligosaccharide diets, the addition of
oligofructose or FOS resulted in higher (P < 0.05) cecal buty-
rate concentrations compared with XOS, control, and cellu-
lose treatments, whereas lactate concentration was higher (P
< 0.05) for rats fed XOS. Cecal concentrations of acetate and
propionate were unaffected by treatment.

The cecal SCFA pool, expressed as µmol/cecum, was al-
tered due to oligosaccharide treatments. The cecal pools of
acetate and butyrate were higher (P < 0.05) in rats consuming
oligosaccharide compared with the control and cellulose diets
that provided higher ME, whereas lactate was higher (P
< 0.05) in rats consuming XOS compared with the other
 treatments. Cecal propionate also was higher (P < 0.05) in rats
consuming oligosaccharides compared with those consuming
cellulose. Total cecal SCFA were dramatically higher (P
< 0.05) as a result of oligosaccharide consumption, which sup-
plied lower ME.

**Total large bowel weight, wall weight and pH.** Gut pH,
total weight and wall weights of the colon and cecum are pre-
sent in Table 4. Total weight of the colon and cecum was higher (P < 0.05) in rats consuming the XOS-containing
diet compared with the other treatments. In addition, rats fed
FOS or oligofructose had a higher (P < 0.05) total cecal
weight compared with rats fed the control and cellulose diets.
Colonic wall weight was unaffected by treatment. Cecal wall
weight was higher (P < 0.05) as a result of oligosaccharide
consumption. Fecal pH was lower (P < 0.05) in rats consum-
ing XOS compared with the other treatments. Cecal pH
was dramatically lower (P < 0.05) in rats consuming XOS
compared with all other treatments, and was lower (P < 0.05) in
rats receiving the oligofructose and FOS treatments compared
with those receiving the higher ME control and cellulose treat-
ments. Stool consistency was formed (pelleted) throughout
the study with no visible differences noted among groups.

**Bacterial concentrations.** Fecal bifidobacteria concentra-
tions were greatest (P < 0.05) as a result of ingestion of XOS
and were higher (P < 0.05) for rats fed FOS and cellulose
compared with the higher ME control (Table 5). Lactoba-
icillus spp. were lower (P < 0.05) in rats fed XOS or oligofructose
compared with those fed cellulose. Total aerobes were lower
(P < 0.05) due to ingestion of oligosaccharides compared with
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TABLE 3

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Cellulose</th>
<th>Oligofructose</th>
<th>FOS</th>
<th>XOS</th>
<th>SEM</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal SCFA, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Acetate</td>
<td>7.83b</td>
<td>5.32b</td>
<td>12.90a</td>
<td>7.22b</td>
<td>7.79b</td>
<td>1.53</td>
<td>0.0121</td>
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<tr>
<td>Propionate</td>
<td>2.00</td>
<td>1.56</td>
<td>3.11</td>
<td>1.88</td>
<td>2.01</td>
<td>0.49</td>
<td>NS</td>
</tr>
<tr>
<td>Butyrate</td>
<td>1.74</td>
<td>1.22</td>
<td>3.05</td>
<td>2.12</td>
<td>1.29</td>
<td>0.48</td>
<td>NS</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.17</td>
<td>0.22</td>
<td>0.20</td>
<td>0.15</td>
<td>0.33</td>
<td>0.07</td>
<td>NS</td>
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<tr>
<td>Cecal SCFA, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>29.52</td>
<td>26.16</td>
<td>35.71</td>
<td>44.64</td>
<td>35.34</td>
<td>4.35</td>
<td>NS</td>
</tr>
<tr>
<td>Propionate</td>
<td>5.95</td>
<td>5.09</td>
<td>4.69</td>
<td>5.44</td>
<td>4.90</td>
<td>0.69</td>
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</tr>
<tr>
<td>Butyrate</td>
<td>5.64b</td>
<td>5.19b</td>
<td>9.74a</td>
<td>10.53a</td>
<td>5.95b</td>
<td>1.13</td>
<td>0.0022</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.27b</td>
<td>0.19b</td>
<td>0.30b</td>
<td>0.54b</td>
<td>1.30a</td>
<td>0.26</td>
<td>0.0235</td>
</tr>
<tr>
<td>Total SCFA4</td>
<td>69.61b</td>
<td>58.85b</td>
<td>165.33a</td>
<td>187.76a</td>
<td>201.88a</td>
<td>24.76</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

1 Values are means of 10 rats.
2 FOS, fructooligosaccharides; XOS, xylooligosaccharides.
3 NS = Not significant (P > 0.05). abc Means in the same row not sharing superscript letters differ (P ≤ 0.05).
4 Total SCFA = acetate + propionate + butyrate + lactate.

The oligosaccharides tested in this experiment were selected based on their potential use as ingredients in various nutritional formulas that promote gastrointestinal health via SCFA production and alterations of gut microbiota. Concerning use of cecal SCFA concentrations compared with cecal SCFA pool data, values for the cecal pool appear to be a more accurate reflection of cecal fermentation when comparing oligosaccharides of various fermentabilities. This response was noted previously by Berggren et al. (1993). Other researchers also have noted that different levels of inulin fed to rats correlated better with cecal pool than with cecal concentration of SCFA (Levrat et al. 1991, Rémy et al. 1992). The present study demonstrated the ability of 6% oligosaccharide-containing diets to elevate the cecal total SCFA pool relative to

TABLE 4

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Cellulose</th>
<th>Oligofructose</th>
<th>FOS</th>
<th>XOS</th>
<th>SEM</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>2.02b</td>
<td>1.69b</td>
<td>2.01b</td>
<td>1.99b</td>
<td>2.54a</td>
<td>0.17</td>
<td>0.0251</td>
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<tr>
<td>Cecum</td>
<td>3.03c</td>
<td>2.91c</td>
<td>5.79b</td>
<td>5.14b</td>
<td>7.16a</td>
<td>0.37</td>
<td>0.0001</td>
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<td>Wall weight, g</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
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<td>1.09</td>
<td>1.14</td>
<td>1.11</td>
<td>1.12</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Cecum</td>
<td>0.74c</td>
<td>0.73c</td>
<td>1.13ab</td>
<td>1.07b</td>
<td>1.29a</td>
<td>0.06</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fecal pH</td>
<td>6.83a</td>
<td>6.84a</td>
<td>6.69a</td>
<td>6.71a</td>
<td>6.42b</td>
<td>0.06</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cecal pH</td>
<td>6.67a</td>
<td>6.70a</td>
<td>6.17b</td>
<td>6.19b</td>
<td>5.91c</td>
<td>0.07</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

1 Values are means of 10 rats.
2 FOS, fructooligosaccharides; XOS, xylooligosaccharides.
3 NS = Not significant (P > 0.05). abc Means in the same row not sharing superscript letters differ (P ≤ 0.05).
TABLE 5
Least squares means of microbiota concentrations in fecal and cecal contents of rats fed various diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Cellulose</th>
<th>Oligofructose</th>
<th>FOS2</th>
<th>XOS</th>
<th>SEM</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal microbiota, log_{10} CFU/g wet stool</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>9.1c</td>
<td>9.7b</td>
<td>9.4bc</td>
<td>9.5b</td>
<td>10.4a</td>
<td>0.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>7.2bc</td>
<td>7.9a</td>
<td>7.2bc</td>
<td>7.4ab</td>
<td>6.7c</td>
<td>0.2</td>
<td>0.0112</td>
</tr>
<tr>
<td>Total aerobes</td>
<td>8.2a</td>
<td>8.4a</td>
<td>7.4b</td>
<td>7.2b</td>
<td>7.3b</td>
<td>0.2</td>
<td>0.0005</td>
</tr>
<tr>
<td>Total anaerobes</td>
<td>8.8d</td>
<td>9.4c</td>
<td>9.9b</td>
<td>9.7bc</td>
<td>10.7a</td>
<td>0.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cecal microbiota, log_{10} CFU/g wet contents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>8.4c</td>
<td>8.7bc</td>
<td>8.9b</td>
<td>9.1b</td>
<td>9.8a</td>
<td>0.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>6.4</td>
<td>6.6</td>
<td>6.4</td>
<td>6.5</td>
<td>6.0</td>
<td>0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Total aerobes</td>
<td>7.4a</td>
<td>7.4a</td>
<td>6.6b</td>
<td>6.6b</td>
<td>6.5b</td>
<td>0.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total anaerobes</td>
<td>8.6c</td>
<td>8.8bc</td>
<td>9.2b</td>
<td>9.2b</td>
<td>10.2a</td>
<td>0.1</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

1 Values are means of 10 rats.
2 FOS, fructooligosaccharides; XOS, xylooligosaccharides; CFU, colony-forming units.
3 NS = Not significant (P > 0.05). abcd Means in the same row not sharing superscript letters differ (P ≤ 0.05).

the control and cellulose treatments with a higher ME content. Similar effects were demonstrated by Younes et al. (1995) in rats fed FOS and XOS at the 7.5% dietary level. Butyrate was higher for FOS- and oligofructose-fed rats compared with those fed XOS, with acetate being the primary SCFA followed by butyrate and propionate. While these oligosaccharides differ in their chemical composition, they probably are fermented similarly by the microbiota of rats because of the presence of similar constituents (glucose and fructose) for fermentation. However, butyrate (mmol/L) was produced in a higher concentration when rats were fed the FOS and oligofructose diets compared with the XOS, control, and cellulose diets. Furthermore, butyrate, expressed as μmol/cecum, was higher when rats were fed the lower ME oligosaccharide diets compared with the higher ME control and cellulose diets. This would be anticipated due to the higher amount of available fermentable fiber provided by the oligosaccharide diets. By producing a greater concentration of butyrate, the preferred energy source for colonocytes, a trophic effect may result within the gastrointestinal tract. The diets used in the present study were semi-elemental; thus, they were more highly digested. Therefore, the amount of undigested substrate reaching the large bowel would be lower, allowing less available substrate for fermentation that may lead to atrophy of the large bowel. However, butyrate (mmol/L) was produced in a higher concentration when rats were fed the FOS and oligofructose diets compared with the XOS, control, and cellulose diets. This would be anticipated due to the higher amount of available fermentable fiber provided by the oligosaccharide diets. By producing a greater concentration of butyrate, the preferred energy source for colonocytes, a trophic effect may result within the gastrointestinal tract. The diets used in the present study were semi-elemental; thus, they were more highly digested. Therefore, the amount of undigested substrate reaching the large bowel would be lower, allowing less available substrate for fermentation that may lead to atrophy of the large bowel. However, butyrate (mmol/L) was produced in a higher concentration when rats were fed the FOS and oligofructose diets compared with the XOS, control, and cellulose diets. This would be anticipated due to the higher amount of available fermentable fiber provided by the oligosaccharide diets. By producing a greater concentration of butyrate, the preferred energy source for colonocytes, a trophic effect may result within the gastrointestinal tract. The diets used in the present study were semi-elemental; thus, they were more highly digested. Therefore, the amount of undigested substrate reaching the large bowel would be lower, allowing less available substrate for fermentation that may lead to atrophy of the large bowel. However, butyrate (mmol/L) was produced in a higher concentration when rats were fed the FOS and oligofructose diets compared with the XOS, control, and cellulose diets. This would be anticipated due to the higher amount of available fermentable fiber provided by the oligosaccharide diets. By producing a greater concentration of butyrate, the preferred energy source for colonocytes, a trophic effect may result within the gastrointestinal tract. The diets used in the present study were semi-elemental; thus, they were more highly digested. Therefore, the amount of undigested substrate reaching the large bowel would be lower, allowing less available substrate for fermentation that may lead to atrophy of the large bowel.

TABLE 6
Correlation analysis of fecal and cecal data of rats fed various diets

<table>
<thead>
<tr>
<th></th>
<th>Bifidobacteria</th>
<th>Lactobacilli</th>
<th>Total aerobes</th>
<th>Total anaerobes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal r</td>
<td>0.8356***</td>
<td>0.0437</td>
<td>0.5882</td>
<td>−0.2186***</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>−0.2380</td>
<td>0.8021***</td>
<td>0.5800***</td>
<td>−0.3222*</td>
</tr>
<tr>
<td>Total aerobes</td>
<td>−0.5116*</td>
<td>0.3156*</td>
<td>0.7600***</td>
<td>−0.2549</td>
</tr>
<tr>
<td>Total anaerobes</td>
<td>0.5849***</td>
<td>−0.0747</td>
<td>−0.3040*</td>
<td>0.7317***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal r</td>
<td>0.0673</td>
<td>−0.0951</td>
<td>0.0365</td>
<td>−0.0090</td>
</tr>
<tr>
<td>Propionate</td>
<td>−0.0720</td>
<td>−0.0570</td>
<td>−0.0977</td>
<td>0.0023</td>
</tr>
<tr>
<td>Butyrate</td>
<td>−0.0382</td>
<td>−0.1330</td>
<td>0.0135</td>
<td>0.0267</td>
</tr>
<tr>
<td>Lactate</td>
<td>−0.1743</td>
<td>−0.0926</td>
<td>−0.1057</td>
<td>0.1459</td>
</tr>
</tbody>
</table>

Fecal and cecal pH correlation = 0.5384***

* P < 0.05; ** P < 0.001; *** P < 0.0001.
The elevation noted in cecal total weight and wall weight with the oligosaccharide diets may result from SCFA normalizing cell proliferation. Other researchers have demonstrated higher cecal weight and cecal wall weight caused by FOS and XOS compared with a diet with no fiber (Younes et al. 1995). Perhaps FOS and XOS increase crypt depth and cell density by providing butyrate as an energy source, resulting in higher intestinal wall weight compared with controls. When comparing differences in cecal and colonic total weights among treatments, only major differences in the former were noted. This is probably the reverse of what would be expected in humans. This discrepancy may result from the fact that rats are cecal fermentors and humans are colonic fermentors. Thus, in rats, the cecum would have the highest level of SCFA available for absorption/utilization. The acidic cecal pH resulting from ingestion of the oligosaccharide diets probably is caused by the greater level of total SCFA production. The more pronounced lower pH for rats consuming the XOS diet is likely related to the higher production of lactate.

The higher number of cecal Bifidobacterium spp. observed in rats whose diets were supplemented with lower ME oligosaccharides is in agreement with the work of several other research groups (Gibson et al. 1995, Hidaka et al. 1986 and 1991b, Mitsuoka 1990, Mitsuoka et al. 1987, Okazaki et al. 1990). The higher number of bifidobacteria and total anaerobes with a concomitant lowering in total aerobes suggests a shift towards anaerobic bacterial species in the large intestine. The XOS diet resulted in the most pronounced elevation in bifidobacteria. Okazaki et al. (1990) indicate that XOS actively support the growth of bifidobacteria, while not being utilized by other bacteria in the large intestine. Moreover, the primary end-products of Bifidobacterium spp. during fermentation are acetate and lactate. Thus, the higher lactate noted in rats fed the XOS diet may be a result of the unique fermentation pattern of bifidobacteria. Additionally, the cellulose diet elicited effects similar to those of the FOS diet in fecal bifidobacteria, lactobacilli and total anaerobes, whereas in the cecum, the cellulose diet was intermediate between the control, FOS and oligofructose diets. The effects of the cellulose diet remain unclear; however, this may be a result of rats having a longer transit time when fed the cellulose diet, allowing for increased fermentation of cellulose to elicit a similar microflora pattern as highly fermentable FOS.

The higher Bifidobacterium spp. population may result in changes in the microbial ecology of the colon that are detrimental to other anaerobic bacterial species (Gibson and Wang 1994). Oligofructose, commercially known as Rafitlose® (Raffinerie), and inulin, commercially known as Raftiline® (Raffinerie), fed at 15 g/d to human subjects led to significant increases in bifidobacteria and decreases in potential pathogens (Gibson et al. 1995). This is an effective strategy for increasing bifidobacteria in the distal intestine, and the mechanism by which Bifidobacterium spp. are thought to be inhibitory is related to the higher production of acetic and lactic acids during fermentation of FOS, oligofructose and XOS. Increased acid production results in a lower pH which prevents enteric colonization of potentially pathogenic microorganisms and growth of putrefactive bacteria (Gibson and Roberfroid 1995). However, Gibson and Wang (1994) indicated that acidity may not be the sole mechanism of inhibition. In a batch culture system held at pH 7, Bifidobacterium spp. were able to inhibit growth of Escherichia coli and Clostridium perfringens. Gibson and Wang (1994) theorized that bifidobacteria may be able to exert an inhibitory effect not necessarily related to acid production. In addition, May et al. (1994) showed that high SCFA levels inhibit growth of, and toxin A production by, C. difficile independent of pH. Furthermore, bifidobacteria stimulate immune function, particularly against malignant cells, produce B-complex vitamins, restore the normal intestinal flora during antibiotic therapy and reduce blood ammonia levels (Gibson and Roberfroid, 1995). Regardless of the inhibitory effect on potential pathogens, the higher amount of Bifidobacterium spp. demonstrated in the cecum of rats in the present study caused by ingestion of oligosaccharides may promote beneficial effects within the gastrointestinal tract.

The concentrations of SCFA in cecal and fecal contents were not correlated, which would be expected because SCFA are rapidly and extensively (95–99%) absorbed in the large bowel. Comparing fecal and cecal data in this experiment, it appears that changes in gut microbiota may be obtained through fecal sampling; however, to obtain meaningful SCFA and pH values, fecal samples must be collected because rats are cecal fermentors.

The data indicate that FOS, oligofructose and XOS at 6% of the diet produced higher SCFA in rats. Thus, oligosaccharides may serve as an indirect energy source for the large bowel. Furthermore, by serving as an indirect energy source for the large bowel, indigestible oligosaccharides may play a critical role in maintaining mucosal cell differentiation and, therefore, integrity of the gastrointestinal mucosa. These oligosaccharides are preferentially fermented by Bifidobacterium spp. which may be useful in promoting gastrointestinal health via restoration of normal flora following antibiotic therapy, preventing colonization of potential pathogens by lowering pH, and enhancing the immune system. Thus, providing these oligosaccharides as ingredients in nutritional formulas could benefit the health of the gastrointestinal tract.

**LITERATURE CITED**


