In Vitro Fermentation of Swine Ileal Digesta Containing Oat Bran Dietary Fiber by Rat Cecal Inocula Adapted to the Test Fiber Increases Propionate Production But Fermentation of Wheat Bran Ileal Digesta Does Not Produce More Butyrate

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ABSTRACT This experiment evaluated three hypotheses: i) production of propionate is increased during fermentation of substrate containing oat bran (OB); ii) production of butyrate is increased during fermentation of substrate containing wheat bran (WB) and iii) results of in vitro fermentations using physiological substrates and inocula agree with in vivo data. Ileal digesta collected from swine fed OB and WB were the substrates. Digesta was fermented for 0–96 h in an anaerobic in vitro system using inocula prepared from ceca of rats fed the same fiber sources. Carbohydrate and short-chain fatty acid (SCFA) contents in the fermentations were measured by gas chromatography. Fermentation of WB digesta did not produce more n-butyrate (P > 0.05) and was significantly slower (P < 0.05) than fermentation of OB digesta. OB digesta fermentation produced a significantly greater (P < 0.05) molar proportion of SCFA as propionate. Bacterial mass increased more and was maintained longer during fermentation of OB digesta than the WB digesta. Our results indicate that dilution of undigested WB fiber and not n-butyrate production is one mechanism by which WB may protect colonic mucosa; propionate production is increased during fermentation of β-glucan in OB; and in vitro system using physiological sources of inoculum and substrate containing WB and OB yields results that agree with in vivo findings in humans and rats. J. Nutr. 130: 585–593, 2000.

KEY WORDS: dietary fiber • in vitro fermentation • WB • OB • swine • rats

Increased amounts of short-chain fatty acids (SCFA) and unfermented material in the colonic lumen are two processes by which dietary fiber is suggested to function in human health and disease prevention. Butyrate, one of the SCFA generated during fermentation of dietary fiber, has been shown to have trophic properties (Sakata 1987). In addition, it has been shown to be an antitumor agent using several cultured cell lines (Bugaut and Bentejac 1993) and a rat model (McIntyre et al. 1993). Molecular bases for this antitumor property are being defined (Smith et al. 1998, Velazquez et al. 1997). Wheat bran (WB) has been proposed as protective in colon cancer because a higher proportion of butyrate is generated by dietary fiber, has been proposed as an inhibitor of hepatic synthesis of cholesterol (Anderson et al. 1990). Various clinical and experimental studies have demonstrated that OB, but not WB, reduces serum cholesterol levels (Anderson et al. 1990). Propionate, another SCFA generated during fermentation of dietary fiber, has been proposed as an inhibitor of hepatic synthesis of cholesterol (Anderson et al. 1990).
1990, Shinnick and Marlett 1993). However, the many experiments, using a variety of animal and in vitro models and protocols, that have been conducted to evaluate the possible hypocholesterolemic action of propionate have yielded conflicting and inconsistent results (Bugaut and Bentejac 1993).

One objective of this research was to compare and contrast the rate and extent of fermentations of OB and WB and the accompanying SCFA production to distinguish their hypothesized antineoplastic and hypocholesterolemic functions. Fermentation in the large intestine of monogastric species is a dynamic process that involves over 400 species of microbes (Savage 1983) and material much more complex than the test fibers usually employed in in vitro fermentation studies (Cumminings 1981). Further, a single measurement of fermentation, typical of most in vivo studies, might not detect elevations in specific SCFA in an ongoing process. Therefore, we also used this experiment to evaluate the ability of an in vitro system using physiological substrates and microflora to predict what is known about fiber digestibility in humans. The physiological system used ileal digesta as the substrate, andecal microflora previously exposed to the substrate as the source of inoculum, because prior exposure of the inoculum to the substrate to be fermented, as well as the source of inoculum, has been shown to influence fermentation (Monsma and Marlett 1995, 1996).

MATERIALS AND METHODS

Experimental design. The ileal digesta that was to be fermented was collected from swine fed diets containing either WB or OB and in which cannulae had been surgically implanted in the terminal ileum. Cecal contents of rats that had been fed WB or OB were the inocula source used in an in vitro anaerobic incubation system. Nonlinear regression was used to characterize and quantitate the carbohydrate remaining and the SCFA present at time points during 96 h of fermentation to determine the rates and extent of change in the production of SCFA and the fermentation of carbohydrate. The experimental design was a 2 x 6 blocked factorial of two substrates the production of SCFA and the fermentation of carbohydrate. The 96 h of fermentation to determine the rates and extent of change in the inocula source used in an in vitro anaerobic incubation system. (Monsma and Marlett 1995, 1996).

Collection of digesta from swine. Ileal digesta was collected from five barrows (½ Duroc 1/4 Large White 1/4 Landrace, The Swine Research Facility, University of Wisconsin-Madison), in which an ‘open type’ T polyethylene cannula (Ankom, Fairport, NY) had been implanted at the terminal ileum during aseptic surgery. Surgery was performed, and the animals were housed at the Livestock Laboratory, University of Wisconsin-Madison. The custom-designed cannula consisted of a flange in the gut lumen of polyethylene tubing (2.28 cm i.d., 2.6 cm o.d., 6.18 cm long) that had been cut in half lengthwise and rounded at the ends. The T side-arm (2.28 cm i.d., 2.6 cm o.d., 7.80 cm long) of the cannula was grooved so that polyethylene support discs, which prevented movement of the cannula, could be anchored.

Each pig was unfed for 48 h prior to surgery; mean weight of the five pigs at time of surgery was 43 ± 1 kg. The pigs were premedicated, intubated, put on anesthesia (1–2% halothane) and placed in right lateral recumbency. The ileum was exteriorized through a dorsal-ventral incision (10 cm) in the paralumbar area, and the cannula was inserted through a 4-cm incision in the antimesenteric border into the terminal ileum 15-cm anterior to the ileo-cecal junction. The ileal incision was closed with nonabsorbable suture material using a purse-string suture pattern. A peritoneal support disc (5.58 cm diameter with an opening in the center matching the dimension of the cannula), to which a circle of Dacron® material (Hancock Fabrics, Madison, WI) of the same dimensions had been sutured, then was placed around the T side-arm portion of the cannula; the fabric

ring was anchored to the serosal surface of the intestine using non-absorbable suture material. The cannula then was brought through a circular incision in the abdominal wall, to which a circle of Dacron® material (Hancock Fabrics, Madison, WI) of the same dimensions had been sutured, then was placed around the T side-arm portion of the cannula; the fabric

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>Formulations of rat and swine diets and test meals containing wheat bran or oat bran</td>
</tr>
<tr>
<td><strong>Pigs</strong></td>
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<tr>
<td><strong>Maintenance diet</strong></td>
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<tr>
<td>Fiber source</td>
</tr>
<tr>
<td>Casein</td>
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<tr>
<td>Sucrose</td>
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<td>Cornstarch</td>
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<td>Fat</td>
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<td>Vitamin mix</td>
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<td>Mineral mix</td>
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<tr>
<td>Molasses</td>
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<tr>
<td>β-Methionine</td>
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<td>Choline chloride</td>
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<td>Total</td>
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1 Pig diets are presented on dry weight basis. Sources of the ingredients were: wheat bran (OB) donated by the Quaker Oats Co. (Barrington, IL); wheat bran (WB) donated by the Kellogg Co. (Battle Creek, MI); casein and sucrose, Teklad (Madison, WI); cornstarch, donated by the A.E. Staley Manufacturing Co. (Decatur, IL); soy oil; and molasses, PM Agricultural Products (Westwego, LA).

2 Rats are presented on dry weight basis. All ingredients, except for fiber sources, were from Harlan Teklad. Swine dietary fiber sources were used for the rat diets.

3 Fiber sources in the pig maintenance diet were 117 g/kg of soy meal and 248 g/kg of com. Baked muffins containing WB and OB were the fiber sources in the pig test meals. The WB and OB in the pig test meals were the fiber sources in the rat diets.

4 Fat in the pig diets was equal parts of soy oil and lard. Corn oil was the fat in the rat diets.

5 Vitamins and trace minerals were supplied in the swine diets by a mix previously described (Crenshaw 1986). Vitamins were supplied in the rat diets by the AIN-76 Vitamin Mixture (AIN 1980).

6 Swine diets also contained (g/kg): 1.8 limestone, 20.55 dicalcium phosphate and 2.78 sodium chloride. Minerals were supplied in the rat diets by the AIN-76 Mineral Mixture (AIN 1980).

7 Weight over 1000 g due to ash in fiber sources.
and maintenance diet for the swine were similar (Table 2). Test meals contained 0.5% of chromium sesquioxide (Fisher Scientific, Pittsburgh, PA) in place of a comparable amount of cornstarch to determine recovery of the test meals as ileal digesta.

Test meals were consumed within 15 min of being offered. Digesta was collected on ice for 16 h, at 2-h intervals, each of which was weighed and then frozen (-5°C). Complete collections were facilitated by using a collection gate (Ankom, Inc., Fairport, NY), a hollow tube made to fit securely into the T side-arm of the cannula. The 2-h collections of ileal digesta from each pig was thawed, blended, lyophilized, and after analysis, proportionally combined to prepare representative composites of output from each pig for fermentation.

**Collection of cecal inocula from rats.** The design of the experiment to collect cecal contents from rats adapted to the test fibers has been described (Monsma and Marlett 1995). Forty retiired breeder rats were used (Harlan Sprague Dawley, Indianapolis, IN) and were individually housed in wire-bottom cages. One group (n = 20), mean initial body weight of 453 ± 5 g, were fed purified diet (AIN 1980) in which cellulose was replaced with OB fiber (Table 1). The second group (n = 20), initial mean body weight of 447 ± 2 g, were fed purified diets containing WB. Amounts of purified ingredients were adjusted to account for macronutrients contributed by the brans (Monsma and Marlett 1995). Mean daily food intakes and weight gains during the 12 d of feeding for the OB group (26 ± 3 g, 1.6 ± 0.2 g) and WB group (24 ± 1 g, 1.4 ± 0.1 g) were not significantly different. Contents of four ceca, that were harvested and pooled in an anaerobic chamber, were used to prepare the inocula solution (Monsma and Marlett 1995) for the ileal digesta from a single pig.

**In vitro fermentations.** Duplicate aliquots (1.52–1.55 g dry wt providing 2250 μmol of total carbohydrate) of ileal digesta composite from each pig were fermented in an anaerobic chamber for 3, 6, 12, 24, 48 and 96 h, using 67.5 mL of sterile buffer and 7.5 mL of inoculum solution for each flask (Monsma and Marlett 1995). At the designated time, flasks were removed from the incubation chamber, and an aliquot (2 mL) was taken for SCFA analysis. The remaining volume was frozen (-70°C) within 0.5 h to stop fermentation (Shell Freezemobile, The Vitris Company, Gardiner, NY) and lyophilized to dryness for subsequent analysis.

**Chemical analyses.** The swine maintenance diet and test meals were analyzed for dietary fiber by an enzymatic-gravimetric procedure (AOAC Method 985.29 1990), and crude protein (N X 6.25), crude fat and starch, as previously described (Monsma et al. 1992). The chromium in the test meals was measured by the procedure described by Gunbaca et al. (1974). An enzymatic-colorimetric method was used to determine the (1→3),(1→4)-β-D-glucan content of the OB test meal (Shinnick et al. 1988).

Each 2-h collection of ileal digesta was analyzed for chromium, wet and dry weight, and crude protein contents to determine that the digesta collected during each 2-h period contained constituents which were present in previous and subsequent collections. The composites of ileal digesta were analyzed for crude protein, crude fat, starch, ash, neutral and amino sugars by gas chromatography (Monsma et al. 1992), and for the OB digesta, (1→3),(1→4)-β-D-glucan.

Terminated fermentations and inooluron solutions were analyzed for neutral and amino sugars, including β-glucan for those containing OB, and Klason lignin, β-glucan, non-β-glucan glucose, arabinose, xylose and muramic acid accounted for 83% of the total carbohydrate present in the ileal digesta and therefore, are the only carbohydrates reported.

The non-β-glucan-derived glucose was calculated as the difference between total glucose and β-glucan-derived glucose. Non-β-glucan-derived glucose, xylose and arabinose were used to estimate the cellulose and arabinosylan, respectively, the apparent major polysaccharides in WB and OB dietary fiber (Marlett 1993). The amino sugar muramic acid, a component of the peptidoglycan murein found only in the cell wall skeleton of bacteria (Schlegel 1988), was measured to estimate changes in microbial mass during fermentation.

Fermentation of the polysaccharides was assessed as the disappearance of the primary monosaccharides in each polysaccharide, which was calculated as the difference between the amount of the monosaccharide measured at the fermentation start and the amount remaining at fermentation of a fermentation.

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**Statistical analyses.** The statistical model used was the General Linear Model procedure. Regression curve fittings were performed using TableCurve 2D (1994). Data were tested for homoscedasticity by Bartlett’s test (Zar 1974) and square root transformation if necessary. ANOVA was performed using GB-STAT (1994). When significant (P < 0.05) differences were observed, means were compared by the Fischer’s protected least significant difference method. Data are reported as means ± SEM.

Composition of swine ileal digesta, performance of the rats that provided the cecal inocula, and the SCFA and sugar contents of the fermentations terminated at each time point were compared by one-way ANOVA.

A nonlinear regression procedure was used to fit the disappearance of β-glucan and non-β-glucan glucose, arabinose and xylose to the first-order exponential curve, \( y = a(1 + e^{-bx}) \). The initial rates of disappearance (μmol/h) of the individual carbohydrates were calculated by multiplying the maximum carbohydrate disappearance by the fractional rate constant (Monsma and Marlett 1996). Two-way ANOVA was used to compare the main effects of carbohydrate and ileal digesta and their interaction on the initial rate and maximum disappearance of the individual carbohydrates.

Acetate, propionate, n-butyrate, i-butyrate, i-valerate and n-valerate were measured in duplicate by gas chromatography as previously described (Monsma and Marlett 1995). Only acetate, propionate and n-butyrate were reported as they accounted for ≥ 93% of the total SCFA produced.

**RESULTS**

**Collection and composition of pig ileal digesta.** Similar amounts of chromium from the two test meals were recovered in ileal digesta, 69.2 ± 5.4% from pigs fed the OB meals and 64.2 ± 5.4% from pigs fed the WB meals. The mean dry weight output, crude protein (N X 6.25) content and percent moisture of the digestas collected from swine fed OB, 46.4 g, 9.2 g and 93.2%, respectively, were not significantly different.
from ileal digesta from swine fed WB, 47.2 g, 8.6 g, 93.6%, respectively. No significant differences were observed in the compositions of the ileal digesta composites prepared from the 2-h collections from swine fed the two diets (Table 3).

For each test meal, the patterns of flow for chromium, wet and dry weights, and crude protein as the 2-h collections of ileal digesta were similar during the 16-h collections (Fig. 1). The moisture content of the collections remained constant at 93 to 95% (data not shown). The percentage of total dry weight of ileal digesta collected and the percentage of total wet weight, chromium and crude protein collected every 2 h during each test meal were significantly correlated, \( r = 0.87 \) (\( P < 0.05 \)). The percentage of the total chromium and crude protein collected at each 2 h following feeding of the OB and WB diets were also significantly correlated, \( r = 0.72 \) (\( P < 0.05 \)).

**Carbohydrate composition of 0 h fermentation flasks.** Glucose, xylose and arabinose accounted for 83% of the carbohydrate at 0 h fermentation of ileal digesta from swine fed either bran (Table 4). Fucose, galactose, glucosamine and galactosamine, the sugars in mucin (Monsma et al. 1992), represented 12–13% of the total carbohydrate.

**Carbohydrate fermentation.** After the initial 3 h, significantly more of the total carbohydrate in the OB ileal digesta disappeared than in the WB ileal digesta at every time point measured (Fig. 2). Carbohydrate disappearance did not increase significantly after the 12-h time point during WB fermentation or after 24 h during fermentation of OB ileal digesta.

Independent of the fiber source, both the initial rates and maximum disappearances of \( \beta \)-glucan and non-\( \beta \)-glucan glucose were significantly greater than those for arabinose and xylose (Table 5). Initial fermentation rates of arabinose and xylose were similar in the two digesta. The maximum disappearance of arabinose, but not of xylose, was significantly less from the WB sample than from the OB digesta.

Most (>95%) of the \( \beta \)-glucan-derived glucose had disappeared from the OB ileal digesta by 12 h. At 24 h ~60–70% of non-\( \beta \)-glucan glucose, arabinose and xylose had disappeared from the OB digesta, but only ~25–45% of the same polymers in the WB digesta had been apparently degraded (Fig. 3).

**SCFA production.** Significantly more total SCFA were produced at every time point during fermentation of the OB digesta compared to WB digesta, although SCFA production did not increase significantly after 48 h (Fig. 2).

Acetate production dominated all fermentations (Table 6). The initial rate of propionate production from OB ileal digesta was three times faster than that from WB digesta, whereas the initial production rates of n-butyrate from both digestas were similar. Maximum productions of propionate and n-butyrate were greater from the fermentation of OB, compared to WB digesta. A significantly greater proportion of propionate and significantly smaller proportion of acetate were produced during the first 24 to 48 h of fermentation of OB than was produced by fermentation of WB (Fig. 4). The source of fiber in the digesta had no effect on the proportions of total SCFA produced as n-butyrate. The molar proportion of individual SCFA did not change after 48 h of fermentation.

**Microbial mass and bacterial efficiency of carbohydrate utilization.** The mass of the bacteria, estimated by the measurement of muramic acid in the fermentation, was significantly greater at 6, 48 and 96 h during the fermentation of OB digesta, compared to WB digesta (Fig. 5). Bacterial mass increased to a maximum of 133% of the initial amount at 48 h in the OB fermentations, but to only 117% at 24 h in the WB fermentations. At 96 h, the muramic acid level in the OB fermentations remained at 125% of initial amount, compared to 86% of the initial level in the WB fermentations. However, bacterial efficiencies of carbohydrate utilization by bacteria...
TABLE 4
Carbohydrate composition of 0 h fermentations of ileal
digesta from swine fed oat bran or wheat bran

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Oat bran</th>
<th>Wheat bran</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/flask</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>604 ± 61</td>
<td>ND</td>
</tr>
<tr>
<td>Non-β-glucan derived</td>
<td>580 ± 48</td>
<td>771 ± 34</td>
</tr>
<tr>
<td>Xylose</td>
<td>382 ± 36</td>
<td>654 ± 59</td>
</tr>
<tr>
<td>Arabinose</td>
<td>296 ± 22</td>
<td>451 ± 32</td>
</tr>
<tr>
<td>Galactose</td>
<td>140 ± 24</td>
<td>146 ± 26</td>
</tr>
<tr>
<td>Fucose</td>
<td>25 ± 9</td>
<td>28 ± 9</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>61 ± 14</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>46 ± 18</td>
<td>53 ± 16</td>
</tr>
<tr>
<td>Mannose</td>
<td>62 ± 6</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>19 ± 2</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Ribose</td>
<td>38 ± 3</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Muramic acid</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Total</td>
<td>2256 ± 5</td>
<td>2253 ± 8</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 5 pigs. Carbohydrates, except as noted, were analyzed in duplicate by gas chromatography. β-glucan glucose was measured in duplicate by an enzymatic-colorimetric assay (Shinnick et al. 1988).
2 ND = not determined.
3 Non-β-glucan-derived glucose calculated as the difference between total glucose and β-glucan-derived glucose.

fermenting digesta containing either bran were not significantly different; the change in the percentage of muramic acid was 3.6 ± 1.2%/100 μmol carbohydrate disappeared in the OB fermentations and 5.2 ± 1.1%/100 μmol carbohydrate disappeared in the WB fermentation.

DISCUSSION

This experiment was designed with the intent of using animals to model fermentation in the human large intestine. Ileal digesta was used because it is the substrate for microflora in the large intestine; protein, fat and other carbohydrates, besides the dietary fiber, in ileal digesta are rarely considered in in vitro fermentation studies, even though they are considered important sources of fermentable material (Cummings 1981). For example, we found that ileal digesta from both rats (Monsma et al. 1992) and swine (Monsma and Marlett, unpublished data) fed purified fiber-free diets contained ~500 μmol carbohydrate/g of dry weight ileal digesta. Further, as much as 20% of the total SCFA generated during microbial fermentation of the glycoprotein mucin, produced endogenously by the gastrointestinal tract, were propionate and n-butyrate (Monsma and Marlett, unpublished data). Ileal digesta was collected from pigs because the digested remnants of defined meals could be collected in sufficient quantity to analyze and ferment from this animal model.

The similarity among the proximate compositions of the swine ileal digesta used in this study, other swine ileal digesta we have analyzed and ileal effluent from humans supports our decision to use swine ileal digesta as the substrate to model human ileal digesta. McBurney et al. (1988) fed a human ileostomate a basal diet containing 13 g/d of fiber and the same diet supplemented with white bread, OB, kidney beans or red lentils. In these studies, the mean (±SEM) content of the ileal effluent was (g/kg dry digesta) 217 ± 18 protein, 27 ± 6 fat, 143 ± 17 ash and 611 ± 37 carbohydrate by difference. Lia et al. (1996) fed nine ileostomy subjects a basal diet supplemented with bread containing only white flour or supplemented with OB, OB and β-glucanase or a barley fraction. In these studies, the mean content of the ileal effluent was (g/kg dry digesta) 236 ± 9 protein, 39 ± 12 fat and 725 ± 18 carbohydrate by difference and ash combined. The mean content of ileal digesta collected from pigs fed a fiber-free test meal or one containing 5% dietary fiber from canned peas was (g/kg dry digesta) 215 protein, 45 fat, 264 ash and 476 carbohydrate by difference (Marlett and Longacre, unpublished), similar to the composition of the swine ileal digesta containing WB or OB used in this study.

The rat was the inoculum source as it was more cost-effective and justifiable than using swine for this purpose. The cecum was the inoculum source because that is the microbial population that is first exposed to ileal residue in vivo. Our experience indicates that rat fecal inoculum does not ferment...
Our in vitro fermentation results are similar to net fiber digestibility in vivo in humans and rats. The apparent digestibilities of WB fiber in rats (Hansen et al. 1992, Nyman et al. 1986), of 41 and 49%, are similar to digestibilities reported in humans, of 34% (Nyman et al. 1986) and 56% (Chen et al. 1998), and to the disappearance of WB-derived sugars, of 47%, in our in vitro fermentation system. Likewise, the disappearance of OB-derived sugars in our in vitro system, of 84%, is similar to apparent digestibility of OB in the rat (Hansen et al. 1992), of 93% and humans (Chen et al. 1998), of 96%. These in vitro and in vivo results of WB and OB fibers across species are remarkably similar, in light of the facts that they were conducted by different laboratories using different substrates. In humans, OB dietary fiber increases fecal bacterial mass (Chen et al. 1998). Changes in the muramic acid content of the in vitro system we used also are consistent with these in vivo observations.

The initial increase in propionate in our study corresponded with the rapid disappearance of β-glucan in the OB digesta fermentation, suggesting that fermentation of β-glucan is at least one source of the increase in propionate. This observation supports those by Bach Knudsen et al. (1993) who reported in vivo increases in propionate in the ceca of swine fed diets containing either OB or β-glucan-enriched fractions, compared to diets containing the insoluble residue of OB. The larger molar proportion of propionate we measured during in vitro OB fermentation is consistent with the data of Jackson and Topping (1993). They observed a larger proportion of

to the same extent as cecal inocula from the same animal prefed the test material to be fermented (Monsma and Marlett 1995, and 1996). Savage (1983) views feces as a waste product and proposed that determining the effect of diet on biochemical activities in the proximal colon lumen from biochemical activities in feces may be misleading. Actual measurements made by MacFarlane et al. (1992) using human colonic contents support Savage’s contention. MacFarlane et al. (1992) reported that bacteria from the ascending colon of two sudden death victims generated five to eight times more SCFA than did bacteria from the sigmoid-rectum region of the same subjects. However, rat cecal inocula production of SCFA from pectin and purified soybean fiber was not different than what was produced when human fecal inocula was used to ferment the same substrates (Barry et al., 1995). In vitro fermentation using human fecal inocula of 11 of 12 dietary fiber concentrates or fiber extracted from mixed diets were generally similar to the net digestibilities of the sugars from the same fibers in the same humans from which the fecal inocula were collected (Daniel et al. 1997, Wisser et al. 1998). The one fiber source that was fermented much more extensively in vitro vs. in vivo was a barley concentrate that contained a high proportion of total fiber as cellulose.

<table>
<thead>
<tr>
<th>Ileal digesta</th>
<th>Carbohydrate</th>
<th>Initial rate</th>
<th>Maximum disappearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/h</td>
<td>µmol</td>
<td></td>
</tr>
<tr>
<td>Oat bran</td>
<td>β-glucan glucose</td>
<td>119 ± 10A</td>
<td>613 ± 61A</td>
</tr>
<tr>
<td></td>
<td>Non-β-glucan</td>
<td>25 ± 3B</td>
<td>474 ± 14B</td>
</tr>
<tr>
<td></td>
<td>glucose</td>
<td>15 ± 2C</td>
<td>265 ± 14D</td>
</tr>
<tr>
<td></td>
<td>Xylose</td>
<td>14 ± 2C</td>
<td>346 ± 25C</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>Non-β-glucan</td>
<td>23 ± 4B</td>
<td>431 ± 14B</td>
</tr>
<tr>
<td></td>
<td>glucose</td>
<td>9 ± 3C</td>
<td>160 ± 25E</td>
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<tr>
<td></td>
<td>Xylose</td>
<td>14 ± 4C</td>
<td>312 ± 48C</td>
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Main effects

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>OB &gt; WB</th>
<th>B &gt; N &gt; X = A</th>
<th>B &gt; N &gt; X &gt; A</th>
</tr>
</thead>
</table>

1 Carbohydrate disappearance data during in vitro fermentation of ileal digesta from pigs fed test meals containing wheat bran or oat bran were fitted to a first-order exponential curve to determine initial rate and maximum disappearance; see Methods and Materials section for complete details. Values are means ± SEM, n = 5 pig ileal digesta; different superscripts in columns denote significant differences (P < 0.05).

2 Significantly different main effects (P < 0.05) are indicated by a greater than sign; nonsignificant effects by an equal sign. Abbreviations: B = β-glucan glucose, N = non-β-glucan glucose, X = xylose, A = arabinose.

Figure 3: Disappearance of individual carbohydrates during in vitro fermentations of ileal digesta collected from cannulated pigs fed test meals containing 50 g/kg of dietary fiber (DF) from either OB or WB. Each point represents mean ± SEM, n = 5. Fermentations were initiated with cecal contents of rats fed purified diets containing 50 g/kg of DF from the same fibers ingested by the pigs. The least significant difference test was used following two-way ANOVA to identify significant differences (P < 0.05) at each time point. For each digesta, carbohydrates at a time point marked with a different letter are significantly different.
propionate was generated in the ceca of rats consuming OB, compared to cecal SCFA composition of the group fed WB. Thus, some in vitro and in vivo data are consistent with the proposal that a component of the hypocholesterolemic effect of some dietary fibers could be caused by propionate inhibition of hepatic cholesterol synthesis (Anderson et al. 1990). However, other studies (McBurney and Thompson 1990, McIntyre et al. 1993) did not observe elevated molar proportions of propionate when OB was fermented, relative to when WB was fermented in vitro. As comprehensively reviewed by Bugaut and Bentejac (1993), data to support this hypothesis are not compelling. Rather, the hypocholesterolemic action of some fibers appears to be related to their effects on sterol balance (Marlett 1997). Viscous soluble fibers decrease bile acid absorption in the terminal small bowel (Marlett et al. 1994) which stimulates hepatic bile acid synthesis that uses LDL-cholesterol as its primary substrate (Schwartz et al. 1982).

Our observation that fermentation of WB ileal digesta did not produce a greater proportion of SCFA as n-butyrate than OB digesta, but rather a smaller absolute amount of n-butyrate, agrees with those of Bourquin et al. (1992), who fermented dietary fiber either isolated from WB or OB, or the fiber-derived polysaccharides extracted and subsequently recombined. Neither our findings or those of Bourquin et al. (1992) support the hypothesis of McIntyre et al. (1993) that n-butyrate from WB fermentation is a significant protective mechanism against tumor formation. Others (McBurney and Thompson 1990, Salvador et al. 1993) who observed increased n-butyrate production during in vitro fermentation of WB used WB that contained residual starch. In vitro fermentation of starch (Englyst and Macfarlane 1986) produces a significant proportion of SCFA as n-butyrate, and it is possible that fermentation of the starch contaminating the WB, and not the WB fiber, was responsible for the production of more butyrate in these studies.

The greater microbial efficiency of carbohydrate utilization...
we observed during fermentation of WB ileal digesta, compared to OB ileal digesta, in conjunction with the less complete fermentation of WB carbohydrate, suggests that the microflora used additional sources of carbon for growth. Protein fermentation has been estimated to account for 17% of the SCFA in the cecum to 38% in the sigmoid/rectum of humans (Macfarlane et al. 1986), and it likely occurred during our studies. At every time point in our study at least 1.5 to 2 times more SCFA were being produced than what was predicted by the stoichiometric equation for carbohydrate fermentation developed by Miller and Wolin (1979).

In summary, although we observed a larger proportion of propionate produced during OB fermentation, the majority of the evidence suggests that the hypocholesterolemic mechanism for viscous, soluble dietary fibers is not propionate inhibition of cholesterol synthesis (Bugaut and Bentjecac 1993, Marlett 1997). The lack of increase in butyrate production during WB fermentation in our studies supports the contention that poorly-fermented WB is protective against colon cancer because it dilutes luminal contents, not because it provides butyrate for the colonic mucosa (Bugaut and Bentjecac 1993, Klurfeld 1997, Lupton 1995).

ACKNOWLEDGMENTS

We acknowledge with appreciation the donation of these diet ingredients: cornstarch (A.E. Staley Manufacturing, Decatur, IL); OB (Quaker Oats, Barrington, IL); soy oil (Central Soya, Decatur, IA); and WB (Kellogg, Battle Creek, MI); and the technical assistance of Khristen J. Carlson, Corey W. Janecky and David Jensen.

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