Fecal Numbers of Bifidobacteria Are Higher in Pigs Fed Bifidobacterium longum with a High Amylose Cornstarch Than with a Low Amylose Cornstarch1,2

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ABSTRACT Twelve young male pigs consumed a purified diet containing wheat bran as fiber source. Starch provided 50% of total daily energy either as a low amylose cornstarch or as a high amylose (amylo maize) starch. The pigs were given a supplement of a freeze-dried probiotic organism (Bifidobacterium longum CSCC 1941). A block crossover design was used so that at any one time two groups of three pigs consumed either the high or low amylose cornstarch without probiotic and a further two groups of three pigs consumed either high or low amylose cornstarch with probiotic. Neither food intake nor body weight gain was affected by diet. Fecal output was higher when pigs were fed the high amylose cornstarch, but moisture content was unaffected. Fecal concentrations and excretion of total volatile fatty acids were higher when pigs were fed the high amylose cornstarch. Concentrations of acetate were unaffected by dietary starch, but those of propionate and butyrate were higher when the high amylose cornstarch was consumed. Fecal excretion of all three acids was higher during high amylose cornstarch feeding. Bifidobacteria were detected in the feces only when pigs were fed Bifidobacterium longum. Fecal bifidobacteria counts (expressed per gram of wet feces) and their daily fecal excretion were higher when pigs were fed high amylose cornstarch. Feeding the probiotic did not alter fecal starch or volatile fatty acids. None of the variables studied was affected by the order of feeding of starch or probiotic. The data show that a high amylose starch acts as a prebiotic in promoting the fecal excretion of probiotic organisms. J. Nutr. 127: 1822–1827, 1997

KEY WORDS: • pigs • prebiotics • probiotics • starch • volatile fatty acids

Probiotic microorganisms (Lactobacillus sp., Bifidobacterium sp. and others) have been defined as live microbial food supplements that affect the host animal beneficially by improving its intestinal microbial balance (Fuller 1989). It has been suggested that probiotics may be of therapeutic or preventative benefit for a number of pathological states, including gastroenteritis, diarrhea, constipation and hypercholesterolemia (Goldin and Gorbach 1992). Consumption of yogurt containing Bifidobacterium longum lowers the frequency of antibiotic-induced gastrointestinal disorders (Colombel et al. 1987). Experimental studies in mice fed bifidobacteria have shown lower numbers of chemically induced tumors in the large bowel (Koo and Rao 1991), which is consistent with a possible reduction in the risk of cancer in that viscus. These and other observations indicate that probiotics have the potential to improve human colonic health (Playne 1995).

While these data are suggestive of benefit from the standpoint of disease reduction and health promotion, a number of criteria need to be met before increased consumption of probiotics can be recommended to the general population. One of these is survival of the live organisms in the gastrointestinal tract. The large bowel is the major site of bacterial colonization and metabolism in the human gut and contains a wide range of species. These include bacteria that metabolize dietary carbohydrates to volatile fatty acids (VFA)7 that are thought to mediate some of the health benefits normally ascribed to the carbohydrates themselves. (Annison and Topping 1994, Cummings and Macfarlane 1991) These actions include stimulation of electrolyte and fluid transport, enhancement of colonic muscular activity and promotion of a normal cell phenotype in colonocytes. Potentially pathogenic species such as

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clostridia and coliforms also reside in the large bowel and may proliferate and produce adverse reactions (Tancrede 1992). The hind gut is the region where probiotics could be expected to colonize and where some of their beneficial actions could originate, e.g., through altering of VFA profiles. Studies of the effects of probiotics on VFA excretion have been inconclusive and Bartram et al. (1995) noted that the fecal flora and VFA excretion of normal humans were extremely stable with respect to dietary perturbation through ingestion of live B. longum. These authors attempted to enhance the survival of the probiotic culture by adding lactulose as a metabolic substrate for the bifidobacteria. This illustrates the concept of prebiotics, which are nondigestible food ingredients that affect the host beneficially by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improving host health (Gibson and Roberfroid 1995). Certain oligosaccharides are used selectively by probiotic microorganisms, and the fructo-oligosaccharides seem to act as prebiotics in humans (Gibson et al. 1995). Other, nutritionally important, complex carbohydrates including starches and non-starch polysaccharides (NSP) have been viewed as having a low potential as prebiotics (Gibson and Roberfroid 1995). In the case of starches, this is understandable because they may be digested completely by human digestive enzymes, in contrast to fructo- and galacto-oligosaccharides and NSP, which are resistant. However, it seems that this is an oversimplification because a large proportion of starch escapes small intestinal digestion and enters the large bowel, where it is fermented by the bacteria, yielding VFA (Cummings and MacFarlane 1991). This so-called resistant starch (RS) arises for a variety of reasons, including cooking, the presence of NSP and the degree of mastication (Annison and Topping 1994). Of particular interest is the fact that a high amylose content in corn lowers the small intestinal enzymic hydrolysis of the starch. Feeding trials with high amylose starch in humans have shown loss of starch from the ileum (Muir et al. 1994) and studies in pigs have shown increased starch concentrations in the proximal colon (Topping et al. 1997). Fecal VFA excretion is higher in humans consuming high amylose starch (Noakes et al., 1996) or another RS manufactured by a commercial process (van Munster et al. 1994). These changes are consistent with enhanced large bowel bacterial fermentation and we considered it possible that RS might act as a prebiotic. This hypothesis runs contrary to current opinion and was tested by examining the fecal excretion of bifidobacteria in pigs fed freeze-dried Bifidobacterium longum with a high amylose starch. In view of the fact that important health benefits of RS (and NSP) are total starch. During the experiment, one pig became lame and re-

Methods

Animals. Young adult male pigs of the Large White strain were used. All of the animals were purchased from a commercial piggery (Millwards' Piggery, Eulunda, SA, Australia) and were approximately 14 wk old at the start of the experiment. The pigs were housed in temperature-controlled individual pens and fed a standard pig production diet as described previously (Topping et al. 1993). All of the procedures described were approved formally by the Animal Care and Ethics Committee of the Division of Human Nutrition and conformed to published guidelines (National Health and Medical Research Council, CSIRO and Australian Agricultural Council 1985).

Diet and feeding procedures. Twelve pigs were fed a diet com-

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Analytical procedures. A fresh fecal sample (1.5–2.0 g) was weighed accurately into a 10-mL plastic centrifuge tube and diluted threefold with water containing 3.52 mmol/L oenanthic acid as internal standard. The sample was then centrifuged for 15 min at 3000 g and 5°C. An aliquot was taken, acidified with phosphoric acid and distilled in vacuo and the distillate was analyzed for VFA by gas-liquid chromatography (Topping et al. 1993). A sample of fresh feces was freeze-dried for total starch determination using the Megazyme total starch assay procedure (Amyloglucosidase/α-amylase method; Megazyme Ltd, Sydney, NSW, Australia). The bacteriological examination of feces was performed within 2 h of collection. A 10-g sample of feces was suspended homogeneously in 1% buffered peptone and was further diluted in 10-fold dilutions. Of the appropriate dilutions, 0.1 mL was plated in duplicate onto the surface of Biﬁdus Blood Agar (Pachenari et al. 1997, Reuter 1963) and spread evenly over the plate. The plates were incubated under anaerobic conditions using an Anaerocult A mini-procedure (Merck Pty Ltd, Kilsyth, VIC, Aus-

Bacterial supplementation. All bacterial counts are expressed as the log10 colony-forming units (cfu). The freeze-dried culture of Bifidobacterium longum (1941) was provided kindly by Gist Brocades Aus-

Sampling procedures. At the start of the experiment and during each period when the commercial ration was fed, fecal samples were taken for microbiological assessment to establish the baseline level of naturally occurring and residual bifidobacteria. Fresh feces were collected each morning after feeding and analyzed for VFA concentrations. Additional samples were taken aseptically after 6 d for micro-

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**TABLE 1**

Fecal concentrations and daily excretion of bifidobacteria of pigs fed either a low amylose or high amylose (amylomaize) cornstarch with live Bifidobacterium longum

<table>
<thead>
<tr>
<th>Starch type</th>
<th>Fecal concentration</th>
<th>Fecal excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log10 cfu/g wet wt</td>
<td>log10 cfu/d</td>
</tr>
<tr>
<td>Low amylose</td>
<td>8.12</td>
<td>10.76</td>
</tr>
<tr>
<td>High amylose</td>
<td>8.91</td>
<td>11.73</td>
</tr>
<tr>
<td>SED</td>
<td>0.20</td>
<td>0.19</td>
</tr>
<tr>
<td>Statistical analysis, (P) value</td>
<td>$&lt;0.01$</td>
<td>$&lt;0.01$</td>
</tr>
</tbody>
</table>

1 Values are the means of 11 observations per treatment; cfu = colony-forming units.

VIC, Australia). Plates were incubated at 37°C for a period of 5–6 d and counted. The numbers of bifidobacteria per gram of wet feces were calculated. Bifidobacteria were distinguished from other bacteria present on the plates by their distinctive raised, copper-pigmented colonies (Pachenari et al. 1997). These isolates were then Gram-stained and examined for Gram-positive slightly bifurcated club-shaped rods, indicative of bifidobacteria species (Wood and Holzapfel 1995).

**Statistical methods.** The data were analyzed by ANOVA using the residual maximum likelihood procedure of Genstat 5 (release 3.1; Genstat 5 committee 1993) on a Sun Ultra workstation. This procedure accounted for random variation due to time, experimental group and animal. The significance of the fixed effects of starch type and the feeding of Bifidobacterium longum in the model then was assessed by testing the deviance change with and without the treatment effects in the model against a chi-square distribution. Data are shown as the means of 11 observations for each of the main treatments (cornstarch, amylomaize starch, no Bifidobacterium longum or feeding of Bifidobacterium) with the standard error of the difference. A value of \(P < 0.05\) was taken as the criterion of significance.

**RESULTS**

**Fecal output and moisture.** Fecal output was unaffected by the feeding of the probiotic but was raised significantly (\(P < 0.01\)) from 451 g/d when pigs were fed low amylose cornstarch (with or without Bifidobacterium) to 648 g/d when they were fed high amylose cornstarch (SED 28, \(n = 22\)). Fecal moisture averaged 72.3% when the pigs were fed low amylose cornstarch and 75.4% when they were fed this starch with the probiotic. Moisture was unaffected by the feeding of the high amylose cornstarch (75.8%). However, there was a significant (\(P < 0.05\)) interaction when they were fed this starch with the Bifidobacterium, with a mean of 73.0% (SED 1.5, \(n = 11\)). There was no effect of the order of feeding on either of these variables.

**Bacteriological results.** The analysis of fecal specimens showed high counts of bifidobacteria when pigs were fed the experimental high amylose cornstarch diet with the bacterial supplementation. No bifidobacteria were detected in the absence of the supplement (at a detection limit of 4 cfu/g), and counts were significantly higher when the pigs were fed the high amylose cornstarch diet than when they were fed the low amylose cornstarch diet. The difference was significant when the data were expressed either on a wet weight basis or as total bifidobacteria excreted per day (Table 1). Average fecal concentrations and total fecal excretion were 0.79 log10 cfu/g wet wt and 0.97 log10 cfu/d higher, respectively, when the pigs were fed the high amylose cornstarch (Table 1). There was no effect of the order of feeding on either bacterial concentration or excretion. In a small number of pigs, samples were collected throughout the morning, and bacteria were enumerated. There was minimal variation within pigs (data not shown).

**Fecal volatile fatty acids.** Fecal short-chain fatty acid concentrations were averaged over the last 5 d of the test period for each pig and showed a significant increase in total acids when the diet contained high amylose cornstarch (Table 2). This difference was due principally to an increase in propionate and butyrate because acetate was unaffected. The probiotic treatment had no independent effect on fecal VFA concentrations, which were unaffected by the order of feeding. Calculation of the excretion of total and individual VFA (i.e., fecal concentration × daily stool output) showed that output was significantly higher when high amylose cornstarch was consumed (Table 3). There was no effect of probiotic ingestion or of the order of feeding. Fecal excretions of all three major acids (acetate, propionate and butyrate) were higher when high amylose cornstarch was fed.

**Fecal starch.** The concentration of starch in feces was very low in pigs when they consumed the low amylose cornstarch diet and was greater when they were fed high amylose cornstarch. Total excretion was unaffected by order of feeding or by the probiotic and averaged 3.66 g starch/d when pigs were fed low amylose cornstarch. Excretion rose to 8.85 g starch/d when pigs were fed high amylose cornstarch (\(P < 0.001\), SED 0.89, \(n = 22\)).

**DISCUSSION**

The present findings confirm earlier data from human feeding trials (van Munster et al. 1994) showing that ingestion of a resistant starch increased total fecal bulk. In the present experiment, fecal wet weight was approximately 45% higher in pigs fed high amylose cornstarch than when they were fed low amylose cornstarch (Table 1). There was no effect of the order of feeding on either bacterial concentration or excretion. In a small number of pigs, samples were collected throughout the morning, and bacteria were enumerated. There was minimal variation within pigs (data not shown).

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TABLE 2

Fecal concentrations of volatile fatty acids (VFA) of pigs fed either a low amylose or high amylose (amylomaize) cornstarch with or without live Bifidobacterium longum

<table>
<thead>
<tr>
<th>Starch type</th>
<th>Bifidobacterium longum</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Total VFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Low amylose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>48.1</td>
<td>21.2</td>
<td>10.4</td>
<td>83.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>49.2</td>
<td>21.1</td>
<td>10.0</td>
<td>84.0</td>
<td></td>
</tr>
<tr>
<td>High amylose</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>61.5</td>
<td>39.8</td>
<td>19.8</td>
<td>130.4</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>54.1</td>
<td>33.5</td>
<td>16.2</td>
<td>111.8</td>
<td></td>
</tr>
<tr>
<td>SED</td>
<td>5.1</td>
<td>4.0</td>
<td>2.8</td>
<td>13.6</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis, P value

- Starch type: NS NS NS NS
- Probiotic: NS NS NS NS
- Starch × probiotic interaction: NS NS NS NS

1 Values are the means of 11 observations per treatment. NS = not significant (P > 0.05).

experiment, the fecal bifidobacteria numbers and total excretion were comparable to those found in pigs fed the low and high amylose cornstarches in this study (Bird, A. R., Warhurst, M., Crittenden, R., Hayakawa, T., Playne, M. J., Illman, R. J., Brown, I. L. and Topping, D. L., unpublished observations). Other variables (including stool mass and fecal VFA) were similar, indicating that the inclusion of olaquindox did not influence the outcome. In that experiment, fecal bifidobacteria declined within a few days of stopping the feeding of live organisms when the diet contained low amylose starch, but the decline was much slower when high amylose starch was fed.

Total fecal excretion (cfu/g of feces × fecal output) of bifidobacteria was higher in pigs when the high amylose cornstarch was fed. Sampling of fresh feces throughout the morning in selected animals showed that the number of bacteria was stable, therefore the difference between the two treatments was not due to a diurnal effect. These data show that by the criteria of Gibson and Roberfroid (1995), the high amylose starch acted as a prebiotic even though these authors concluded that starches were unlikely to perform this function. However, the increase in fecal mass noted in this experiment was of such a magnitude as to suggest that the feeding of high amylose cornstarch increased the numbers of other bacterial species as well as the probiotic organisms.

The mechanism whereby high amylose cornstarch raises fecal probiotic numbers remains to be established, but there are a number of possibilities. The first is that by acting as a diluent in the upper gut, RS protected the bacteria against the bile acids, free fatty acids, partial glycerides and other products of digestion that have bactericidal actions. This is entirely feasible because high amylose starches raise the mass of starch reaching the large bowel (Mazur et al. 1990, Muir et al. 1994, Topping et al. 1997). This is supported by the observation that alginate, which is not degraded by human small intestinal enzymes, raises fecal bifidobacteria in humans (Terada et al. 1996). However, the fact that other NSP, which also affect the dynamics of the small intestine, do not seem to act as prebiotics tends to make this possibility less likely. Secondly, the bacteria may have been protected by adhesion to undigested starch or through entry into the pits formed in the starch granules during small intestinal amylolysis (Topping et al. 1997). A final possible mechanism is that the high amylose starch was simply a substrate for the bifidobacteria. Generally, it is thought that bifidobacteria do not metabolize starches efficiently (Sgorbati et al. 1995). This is supported by the lack

TABLE 3

Fecal pools of volatile fatty acids (VFA) of pigs fed either a low amylose or high amylose (amylomaize) cornstarch with or without live Bifidobacterium longum

<table>
<thead>
<tr>
<th>Starch type</th>
<th>Bifidobacterium longum</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Total VFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mmol</td>
<td>mmol</td>
<td>mmol</td>
<td>mmol</td>
</tr>
<tr>
<td>Low amylose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>14.8</td>
<td>6.4</td>
<td>3.2</td>
<td>25.4</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15.3</td>
<td>6.7</td>
<td>3.2</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td>High amylose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>29.4</td>
<td>18.0</td>
<td>9.1</td>
<td>60.7</td>
<td></td>
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<tr>
<td>Yes</td>
<td>24.7</td>
<td>15.2</td>
<td>7.3</td>
<td>50.7</td>
<td></td>
</tr>
<tr>
<td>SED</td>
<td>3.1</td>
<td>1.2</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis, P value

- Starch type: <0.01 <0.01 <0.01 <0.01
- Probiotic: NS NS NS NS
- Starch × probiotic interaction: NS NS NS NS

1 Values are the means of 11 observations per treatment. NS = not significant (P > 0.05).
of difference in fecal starch excretion between pigs fed RS alone and those fed RS with probiotic.

As in previous experiments with rats (Mazur et al. 1990) and pigs (Topping et al. 1997), the fecal concentrations of VFA were higher in pigs fed high amylose cornstarch. Also as in other previous studies, VFA excretion was higher during the feeding of a high amylose starch (Mazur et al. 1990, Noakes et al. 1996) and another RS (van Munster et al. 1994). These differences are consistent with enhanced large bowel bacterial fermentation through provision of extra substrate. As we have noted recently in pigs (Topping et al., 1997), fecal acetate, propionate and butyrate were raised during consumption of high amylose starch. This differs from findings in humans and rats in which feeding of RS raises fecal butyrate more than the other acids (Mazur et al. 1990, Noakes et al. 1996, van Munster et al. 1994), apparently through the preferential production of this acid during bacterial starch fermentation (Weaver et al. 1992). Butyrate is thought to exert protective effects on the colonic mucosa, including a diminished risk of neoplasms (Kruh et al. 1994). Epidemiologic data suggest a protective role for greater starch consumption in colorectal carcinogenesis (Cassidy et al. 1994) and one attractive possibility is throughfermentation of starch to butyrate. However, there seems to be a species difference between pigs on the one hand and humans and rats on the other. The reason for the difference may lie in the bacterial population present in the gut or in the physiology of the gut itself. The cecum and colon are a larger proportion of the total gastrointestinal tract in pigs compared with humans and other model species such as rats and dogs (van Soest 1995). Given that butyrate is used preferentially by colonocytes and that its supply may be limiting to utilization (Illman and Topping 1986), it is possible that any increase in its production could be offset by increased utilization. Alternatively, it may be an effect of the type of starch, because the feeding of brown rice to pigs increases the large bowel pool of butyrate, apparently through the fermentation of starch (Marsono et al. 1993).

As also reported by Bartram et al. (1994), there was no effect of probiotic ingestion on either total or individual VFA excretion when fed with either starch even though bifidobacteria numbers were higher when pigs consumed RS. However, it must be noted that fecal excretion is a net process and represents a balance between production and utilization. Studies in pigs have shown that the distribution of digesta and VFA along the large bowel cannot be predicted from values in the distal colon (Topping et al. 1993). More recently, Bartram and co-workers (1994) made a similar suggestion and proposed that there may be changes in VFA in the proximal bowel that might not be detected in the distal colon or feces. However, most degenerative bowel disease is expressed in the distal colon, suggesting that, if probiotic organisms were to be protective against them, VFA are not the likely mediators.

Resistant starches resemble nonstarch polysaccharides in their capacity to enhance large bowel bacterial fermentation. In this way, RS may be regarded as functionally similar to NSP. A recent meta-analysis of dietary influences on colorectal cancer risk has supported this view with a negative correlation between RS and NSP and risk that was higher than the correlation with NSP alone (Cassidy et al. 1994). In addition to this health benefit it has been noted that RS may offer a technological advantage in that their presence in human foods raises their content of “fiber” without necessarily altering their organoleptic properties (Annison and Topping 1994). In the case of high amylose starches, their capacity to act as prebiotics seems to extend the scope for their use in food so as to enhance delivery of probiotic microorganisms to the distal bowel.

ACKNOWLEDGMENTS

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LITERATURE CITED


raises proximal large bowel starch and increases colon length in pigs. J. Nutr. (in press.)