Arabinoxylan in Wheat Is More Responsible Than Cellulose for Promoting Intestinal Barrier Function in Weaned Male Piglets

Hong Chen,4,5 Wei Wang,5 Jeroen Degroote,5,7 Sam Possemiers,6 Daiwen Chen,4,* Stefaan De Smet,5 and Joris Michiels5,7

4Institute of Animal Nutrition, Sichuan Agriculture University, Ya’an, China; 5Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Melle, Belgium; 6ProDigest BVBA, Ghent, Belgium; and 7Department of Applied Biosciences, Ghent University, Ghent, Belgium

Abstract

Background: The effect of dietary fiber on intestinal function primarily has been ascribed to its interaction with intestinal bacteria in the hindgut, whereas changes in intestinal bacteria in the host have been considered to depend on fiber composition.

Objectives: The objectives of this study were to determine the contribution of the major fiber components to the health-promoting effects of wheat bran on intestinal mucosal barrier function and to elucidate the involvement of microbiota changes in weaned piglets.

Methods: Thirty freshly weaned male piglets were assigned to 5 dietary treatment groups (n = 6) according to litter and weight. The piglets consumed synthetic diets ad libitum for 30 d, including a basal control diet (CON) without fiber components, a wheat bran diet (WB) as reference diet (10% wheat bran), and 3 other diets containing amounts of fiber components equivalent to those in the WB, i.e., an arabinoxylan diet (AX), a cellulose diet (CEL), and a combined arabinoxylan and cellulose diet (CB).

Results: The groups consuming diets containing arabinoxylans (i.e., the WB, AX, and CB groups) had increased intestinal secretory immunoglobulin A concentrations, goblet cell number and cecal short-chain fatty acid concentrations, and reduced branched-chain fatty acid concentrations and pH values compared with the CON group. In the WB group, the stimulated secretion of Cl− was suppressed (60.8% and 47.5% change in short-circuit current caused by theophylline and carbachol, respectively) in the distal small intestine compared with the CON group. The AX and CB groups also had increased intestinal alkaline phosphatase activities and reduced intestinal transcellular permeability (by 77.3% and 67.2%, respectively) compared with the CON group. Meanwhile, in the WB group, cecal Bacteroidetes and Enterobacteriaceae populations were lower, and the growth of Lactobacillus was higher in the AX and CB groups than in the CON group, whereas no positive effect on intestinal barrier function was observed in the CEL group.

Conclusion: Arabinoxylan in wheat bran, and not cellulose, is mainly responsible for improving various functional components of the intestinal barrier function and the involvement of microbiota changes. J Nutr 2015;145:51–8.

Keywords: arabinoxylan, cellulose, intestinal permeability, chloride secretion, goblet cell

Introduction

Dietary fiber is closely associated with intestinal health in humans and animals because it enhances the intestinal epithelium barrier function (1–3). The fermentability of dietary fiber is often regarded as the dominant factor, because the fermentation end-products generated by intestinal bacteria, such as acetic acid, propionic acid, and butyric acid, supply energy to and stimulate the proliferation and differentiation of intestinal epithelial cells (4, 5). However, wheat bran, as a slowly fermentable fiber, could influence intestinal epithelial cell turnover and reduce intestinal permeability independent of changes in SCFAs (1, 6). Our previous study showed that wheat bran fiber exerted a positive effect on intestinal epithelium barrier function in piglets, which was linked to changes in intestinal epithelial cell turnover and reduced intestinal permeability.
microbiota composition (2). It thus remains equivocal to what extent microbiota and fermentative alterations are involved in the health-promoting effect of wheat bran. Moreover, Castillo et al. (7) showed that the changes in the host’s intestinal bacteria were dependent on the fiber components because of the specific substrate preferences of the bacteria. Wheat bran consists of 64–69% arabinoxylans and 15–31% cellulose as the major nonstarch polysaccharides. (7, 8).

Recently, studies in which these isolated fiber components were used determined whether increasing their amounts in a diet is beneficial to intestinal health (9, 10). Arabinoxylans extracted from wheat have been shown to exert positive effects on gut epithelial integrity, including protecting colonocyte DNA and modulating mucin degradation in animal models, and enhance fermentation and regulation of intestinal bacteria (10, 11). Although cellulose was regarded as a nonfermentable fiber, it is able to be degraded by cellulolytic bacteria in various animal inocula including pigs (12). Meanwhile, cellulose was reported to improve intestinal health through reducing intestinal permeability (1). We therefore hypothesized that the positive effect of wheat bran on intestinal barrier function was attributed to the main fiber components, i.e., cellulose and arabinoxylan, via interaction with intestinal microbiota.

To further elaborate the change in intestinal barrier function, the Ussing chamber system was used in the present study. The Ussing chamber system, as ex vivo technology, provides a physiologic system to measure gastrointestinal mucosal permeability (paracellular or transcellular routes) and the capability of chloride secretion across epithelial cells (13). Intestinal barrier function disorder can result in an increase in intestinal permeability and excess secretion of chloride ion (1, 13). Additionally, piglets often suffer from major stress at weaning, accompanied by intestinal mucosal damage and diarrhea, in conventional pig production (14), so we chose the weaned piglet as the experimental animal. Our objective was to determine the contribution of the major fiber components, i.e., arabinoxylan and cellulose, to the health-promoting effects of wheat bran on intestinal barrier function in weaned piglets and to elucidate the involvement of microbiota changes.

Methods

The experiment was carried out according to the guidelines of the Ethics Committee of Ghent University (Belgium) for the humane care and use of animals in research.

Preparation of diets. The cellulose source (Alphacel; Biomedicals) was manufactured by a dry mechanical process from bleached and purified wood pulp. The arabinoxylan source (Naxus; BioActor) was mechanically extracted from wheat endosperm. The wheat bran (Panflower) was milled through a 0.8 mm sieve. The Alphacel, Naxus, and wheat bran were analyzed for their monomer composition in the nonstarch polysaccharide fraction using the method described by Theander et al. (15). The total amount of ß-glucose was taken as the amount of cellulose present in the Alphacel and wheat bran, and the total amount of ß-xylose and ß-arabinose was taken as the amount of arabinoxylan present in the Naxus and wheat bran. The proximate analysis of cellulose and arabinoxylan contents in the Alphacel, Naxus, and wheat bran are presented in Supplemental Table 1.

Experiment design. Thirty male piglets (Seghers Hybrid × Piétrain) weaned at age 26–28 d (body weight: 6.86 ± 0.22 kg) originated from 6 litters with 5 piglets per litter. One pig per litter was assigned to a different treatment and each treatment was replicated in 2 pens with 3 piglets per pen. Piglets were housed in units with conventional ventilation schemes, starting ambient temperature of 28°C, and a light-dark schedule of 18 h:6 h (dark from 2000 h until 0200 h). No medicine or antibiotics were used during the experimental period. Feed and water were consumed ad libitum throughout the whole experimental period (30 d).

Experiment diets. The basal diet was formulated to meet or exceed the piglets’ requirements according to the Centraal Veevoeder Bureau (16) (Supplemental Table 2). The 5 dietary treatments included 1 control diet (CON),8 which was a basal synthetic diet without fiber components, and 4 fibrous diets, including a wheat bran diet (WB), an arabinoxylans diet (AX), a cellulose diet (CEL), and a combined arabinoxylan and cellulose diet (CB). For the AX, the corn starch was replaced by 4.96% Naxus, providing amounts of arabinoxylan equivalent to those in the WB, taking into account the analyzed content of arabinoxylan in wheat bran and Naxus. For the CEL, the corn starch was replaced by 0.93% Alphacel, providing amounts of cellulose equivalent to those in the WB, taking into account the analyzed content of cellulose in wheat bran and Alphacel. For the CB, the corn starch was replaced by 4.96% Naxus and 0.93% Alphacel, making a combination of the 2 previous diets. Except for corn starch content and fiber inclusion, there were no differences across the diets in the contents of other ingredients.

Collecting samples. Piglets were sampled at the end of the trial, i.e., 30 d after weaning, without prior fasting. After electrical stunning and killing by exsanguination, the abdomen was immediately opened to collect the digesta and intestinal sections from the gastrointestinal tract. Segments measuring 20 cm at 90% of the length of the small intestine (90% SI) and midcolon were collected for immediate Ussing chambers measurements. Additionally, 4-cm segments from the same intestinal sections were fixed in 10% formaldehyde buffer for intestinal morphology analysis, and 10-cm segments were taken to collect mucosal scrapings that were immediately snap-frozen end stored at −80°C pending analysis for secretory IgA (SIgA) concentration and intestinal alkaline phosphatase (IAP) activities. The digesta of the cecum and midcolon were collected, and the pH was measured. The fresh digesta were stored at −20°C pending SCFA analysis. One gram digesta of the cecum was immediately snap-frozen in liquid nitrogen, and stored at −80°C for analysis of microbial DNA.

pH measurement. The pH of the digesta was measured by direct insertion of a pH electrode (Radiometer Analytical) in fresh samples.

Intestinal morphology and goblet cell analysis. Paraffin-embedded samples were sliced into ~4 μm sections by using a microtome, mounted on slides, stained with Periodic Acid Schiff, and counter-stained with hematoxylin. Villus height, midvillus width, and crypt depth were measured at 10× magnification using a Nikon microscope and Olympos software (Olympos) in 15–40 well-oriented villi and associated crypts. The villus cross-sectional area was calculated by multiplying villus height and midvillus width (17). A minimum of 15 complete villi or crypts per intestinal site were selected to determine the number of goblet cells per villus or crypt.

Intestinal permeability and ion transport analysis using an Ussing chamber system. The mucosal layer of the small intestine was used to measure intestinal permeability and ion transport by using an Ussing chamber system (13). Permeability of the tissue was assessed by using 2 macromolecular probes: fluorescein isothiocyanate-dextran with molecular size 4 kDa (FD4), and HRP, a 40 kDa protein (2 chambers for each probe and intestinal section). Variables of intestinal ion transport and tissue integrity, including transepithelial electrical resistance, baseline short-circuit current, and Cl− secretion stimulated by agonists, were assessed in 2 other chambers: serosal 10 μmol/L carbachol (Ca2+)-mediated 8 Abbreviations used: AX, arabinoxylan diet; BCFA, branched-chain fatty acid; CB, combined arabinoxylan and cellulose diet; CEL, cellulose diet; cGMP, cyclic guanosine-5'-monophosphate; CON, control diet; FD4, fluorescein isothiocyanate-dextran, 4 kDa; IAP, intestinal alkaline phosphatase, SIgA, secretory IgA; WB, wheat bran diet; 90% SI, 90% of the length of the small intestine.
secretagogue) and bilateral 5 mM theophylline [cAMP/cyclic guanosine-
5’-monophosphate (cGMP)–mediated secretagogue].

**SCFA analysis.** Concentrations of SCFAs were determined by using GC according to the method described by Missotten et al. (18) with modifications. The total SCFA concentration in digesta was expressed as micromole per gram of fresh matter and individual FAs were expressed as percentages relative to the total SCFA content.

**Characterization of intestinal bacteria using qPCR.** Bacterial DNA was extracted from cecal digesta using the cetyltrimethylammonium bromide protocol (19). DNA concentration was determined by using a Nanodrop ND-1000 Spectrophotometer (Isogen Lifescience).

The primers of intestinal bacteria and references are presented in Supplemental Table 3. qPCR for total bacteria, Lactobacillus and Bifidobacterium were previously described by Possemiers et al. (20). Bacteroidetes and Firmicutes were determined as reported by Guo et al. (21), and Enterobacteriaceae as described by Nakato et al. (22). Standard curves were constructed with DNA from representative species in a concentration range from 1010 to 102 DNA copies/mL. All reactions were specific for the target species. The qPCR data were normalized based on the DNA concentrations. The total bacteria counts were expressed as log10 16S ribosomal RNA gene copies per gram of fresh matter.

**SlgA and IAP analysis.** Approximately 1 g of mucosal scrapings was homogenized by ultrasonic homogenizer after being suspended in 9 mL PBS. After centrifugation at 2500 × g for 15 min, the supernatant was removed and centrifuged at 12,000 × g for 5 min. The total protein content of the samples was measured by using the Bradford brilliant blue method. Then, SlgA was analyzed by using an antiswine ELISA kit (Bethyl laboratories). IAP was analyzed by using an alkaline phosphatase assay kit (Abacom), with p-nitrophenyl phosphate, which turns yellow when dephosphorylated by IAP, used as a phosphatase substrate. Absorbance value was measured at 405 nm. The concentration of SlgA and IAP was expressed as milligrams per gram of protein and U (μmol p-nitrophenol) phosphate · min⁻¹ · mg⁻¹ protein, respectively.

**Statistical analysis.** The piglet was considered the experimental unit for all analysis (n = 6). Normality of all variables was evaluated using a Kolmogorov–Smirnov normality test. Bacterial copies were transformed (log₁₀) before statistical analysis. All data were subjected to 1-factor ANOVA with the fixed effect of diet by using the general linear model procedure of SAS 9.0 (SAS Inst.). Statistical comparisons between treatments were performed by using Tukey’s multiple-range test. The α level used for significance was 0.05 (P < 0.05). All data were presented as means ± SEMs.

### Results

**Growth performance, diarrhea score, and general health.** Growth performance and diarrhea score were not significantly affected by group (data not shown), and growth performance was normal throughout the experimental period. However, digesta were very dry and looked like a string of beads in the colons of 4 piglets from the CON group at the moment of collection.

**Small intestinal length and pH value of digesta.** The length of the small intestine was not affected by diet (Table 1). Large differences in pH values among groups were observed in the cecal contents, whereas midcolonic pH was not significantly influenced by group (Table 1). A lower cecal pH was observed in

### Table 1: Effect of wheat bran fiber components on the length of small intestine and pH value of intestinal contents in male piglets

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>WB</th>
<th>AX</th>
<th>CEL</th>
<th>CB</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of SI, m</td>
<td>11.1 ± 0.75</td>
<td>11.2 ± 0.53</td>
<td>11.0 ± 0.53</td>
<td>10.7 ± 0.37</td>
<td>11.8 ± 0.70</td>
<td>0.73</td>
</tr>
<tr>
<td>90% SI pH</td>
<td>6.86 ± 0.20</td>
<td>6.86 ± 0.08</td>
<td>6.74 ± 0.18</td>
<td>6.69 ± 0.22</td>
<td>6.66 ± 0.19</td>
<td>0.91</td>
</tr>
<tr>
<td>Cecum pH</td>
<td>6.43 ± 0.05</td>
<td>6.07 ± 0.05</td>
<td>5.81 ± 0.12</td>
<td>6.25 ± 0.07</td>
<td>5.72 ± 0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Midcolonic pH</td>
<td>6.78 ± 0.05</td>
<td>6.42 ± 0.23</td>
<td>6.55 ± 0.19</td>
<td>6.66 ± 0.08</td>
<td>6.31 ± 0.17</td>
<td>0.27</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs, n = 6 (2 pens of 3 piglets). Labeled means in a row without a common letter differ, P < 0.05. AX, arabinoxylan diet; CB, combined arabinoxylan and cellulose diet; CEL, cellulose diet; CON, control diet; FD4, fluorescein isothiocyanate-dextran; 4, 40 kDa; HRP, horseradish peroxidase; Papp, apparent permeability coefficient; R(basal) transepithelial electrical resistance; WB, wheat bran diet; 90% SI, 90% of the length of the small intestine.

### Table 2: Effect of wheat bran fiber components on intestinal permeability and electrophysiological parameters in male piglets

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>WB</th>
<th>AX</th>
<th>CEL</th>
<th>CB</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>90% SI Papp HRP, 10⁻⁶ cm/s</td>
<td>19.4 ± 1.6⁵</td>
<td>13.3 ± 1.5⁶</td>
<td>4.7 ± 0.7⁵</td>
<td>15.0 ± 2.3⁴</td>
<td>11.6 ± 1.7⁵</td>
<td>0.046</td>
</tr>
<tr>
<td>90% FB4, 10⁻⁶ cm/s</td>
<td>18.3 ± 6.4</td>
<td>24.5 ± 3.7</td>
<td>20.6 ± 4.3</td>
<td>24.1 ± 1.5</td>
<td>18.9 ± 3.8</td>
<td>0.81</td>
</tr>
<tr>
<td>R(basal), Ωcm²</td>
<td>30.0 ± 3.4</td>
<td>33.7 ± 5.4</td>
<td>32.2 ± 5.3</td>
<td>35.1 ± 2.6</td>
<td>36.8 ± 5.5</td>
<td>0.90</td>
</tr>
<tr>
<td>ΔIsc(arabino), μA/cm²</td>
<td>53.0 ± 5.7³</td>
<td>27.8 ± 2.2³</td>
<td>45.3 ± 4.6³</td>
<td>35.5 ± 4.6³</td>
<td>32.2 ± 2.1³</td>
<td>0.048</td>
</tr>
<tr>
<td>ΔIsc(celulose), μA/cm²</td>
<td>49.7 ± 6.2³</td>
<td>19.5 ± 1.5³</td>
<td>35.4 ± 3.0³</td>
<td>35.8 ± 4.7³</td>
<td>28.4 ± 2.5³</td>
<td>0.019</td>
</tr>
<tr>
<td>Papp HRP, 10⁻⁶ cm/s</td>
<td>14.8 ± 2.8</td>
<td>8.0 ± 1.5⁴</td>
<td>3.4 ± 0.4⁴</td>
<td>10.6 ± 2.0⁴</td>
<td>4.9 ± 0.7⁴</td>
<td>0.047</td>
</tr>
<tr>
<td>90% SI Papp HRP, 10⁻⁶ cm/s</td>
<td>10.0 ± 1.8</td>
<td>10.7 ± 2.4</td>
<td>5.7 ± 1.2</td>
<td>11.7 ± 1.4</td>
<td>7.2 ± 1.8</td>
<td>0.40</td>
</tr>
<tr>
<td>R(basal), Ωcm²</td>
<td>46.3 ± 6.2</td>
<td>37.8 ± 4.0</td>
<td>46.3 ± 4.6</td>
<td>47.3 ± 2.5</td>
<td>39.9 ± 3.7</td>
<td>0.40</td>
</tr>
<tr>
<td>ΔIsc(arabino), μA/cm²</td>
<td>52.6 ± 9.7</td>
<td>32.8 ± 7.8</td>
<td>45.5 ± 7.7</td>
<td>52.7 ± 8.2</td>
<td>31.0 ± 6.1</td>
<td>0.47</td>
</tr>
<tr>
<td>ΔIsc(celulose), μA/cm²</td>
<td>66.8 ± 8.5</td>
<td>46.3 ± 7.1</td>
<td>68.0 ± 9.8</td>
<td>60.2 ± 6.6</td>
<td>60.4 ± 8.2</td>
<td>0.60</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs, n = 6 (2 pens of 3 piglets). Labeled means in a row without a common letter differ, P < 0.05. AX, arabinoxylan diet; CB, combined arabinoxylan and cellulose diet; CEL, cellulose diet; CON, control diet; FD4, fluorescein isothiocyanate-dextran, 4, 40 kDa; HRP, horseradish peroxidase; Papp, apparent permeability coefficient; R(basal) transepithelial electrical resistance; WB, wheat bran diet; 90% SI, 90% of the length of the small intestine; ΔIsc, change in short-circuit current.
midcolon, HRP flux was reduced by 77.3% in the AX group compared with the CON group. In the midcolon, this effect was seen by theophylline (60.8%, \( P < 0.05 \)) and carbachol (47.5%,
\( P < 0.05 \)).

Intestinal permeability and electrophysiological parameters at 90% SI and midcolon. FD4 flux from the mucosal to the serosal side was not different among treatments, either at 90% SI or midcolon (Table 2). At 90% SI, supplemental arabinoxylans in the AX group reduced the HRP flux by 75.6% compared with the CON group, and in the midcolon, a higher number of goblet cells per villus and crypt (\( P < 0.05 \)) at 90% SI than did the CON group, and in the midcolon, a higher number of goblet cells per crypt (\( P < 0.05 \)) was observed in the AX and CB groups than in the CON group.

Intestinal morphology and goblet cells at 90% SI and midcolon. Morphologic traits were not affected by treatment, except that the WB group had a higher ratio of villus height to crypt depth (\( P < 0.05 \)) at 90% SI than did the CON group (Table 3). Furthermore, piglets in the WB group had a higher number of goblet cells per villus and crypt (\( P < 0.05 \)) at 90% SI than did the CON group, and in the midcolon, a higher number of goblet cells per crypt (\( P < 0.05 \)) was observed in the AX and CB groups than in the CON group.

SCFA production and profiles in digesta of the cecum and midcolon. The production and profile of SCFAs in the cecum and midcolon are presented in Table 4. Alterations upon including wheat bran fiber components in the diet were marked and very comparable in cecal and midcolon digesta, but more pronounced in the former. In the cecal digesta, total SCFA content was higher (\( P < 0.05 \)) in all groups of piglets fed fibrous diets except the CEL group than in the CON group; and the AX group had a higher butyrate proportion (\( P < 0.05 \)) in the cecal digesta compared with the CON group, and in the midcolon, a higher number of goblet cells per crypt (\( P < 0.05 \)) was observed in the AX and CB groups than in the CON group.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>WB</th>
<th>AX</th>
<th>CEL</th>
<th>CB</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>90% SI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villus height, ( \mu m )</td>
<td>355 ± 20</td>
<td>415 ± 11</td>
<td>405 ± 33</td>
<td>392 ± 24</td>
<td>358 ± 41</td>
<td>0.45</td>
</tr>
<tr>
<td>Midvillus width, ( \mu m )</td>
<td>148 ± 8</td>
<td>152 ± 7</td>
<td>133 ± 7</td>
<td>155 ± 10</td>
<td>139 ± 6</td>
<td>0.25</td>
</tr>
<tr>
<td>Villus area, ( 10^{-4} \text{mm}^2 )</td>
<td>5.31 ± 0.54</td>
<td>6.30 ± 0.32</td>
<td>4.95 ± 0.27</td>
<td>6.07 ± 0.99</td>
<td>4.73 ± 0.61</td>
<td>0.17</td>
</tr>
<tr>
<td>Crypt depth, ( \mu m )</td>
<td>270 ± 9</td>
<td>259 ± 10</td>
<td>262 ± 15</td>
<td>272 ± 11</td>
<td>251 ± 9</td>
<td>0.67</td>
</tr>
<tr>
<td>Villus height:crypt depth</td>
<td>1.29 ± 0.08</td>
<td>1.71 ± 0.06</td>
<td>1.57 ± 0.03a,b</td>
<td>1.41 ± 0.06ab</td>
<td>1.53 ± 0.15ab</td>
<td>0.041</td>
</tr>
<tr>
<td>Goblet cells, n/villus</td>
<td>6.8 ± 0.7b</td>
<td>14.3 ± 1.6a</td>
<td>10.0 ± 0.9b</td>
<td>10.0 ± 1.1ab</td>
<td>10.8 ± 1.1b</td>
<td>0.044</td>
</tr>
<tr>
<td>Goblet cells, n/crypt</td>
<td>17.8 ± 1.0b</td>
<td>22.0 ± 1.6a</td>
<td>20.4 ± 1.0ab</td>
<td>18.9 ± 1.1ab</td>
<td>20.6 ± 0.6ab</td>
<td>0.047</td>
</tr>
<tr>
<td>Midcolon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crypt depth, ( \mu m )</td>
<td>515 ± 37</td>
<td>522 ± 19</td>
<td>465 ± 27</td>
<td>530 ± 22</td>
<td>469 ± 31</td>
<td>0.33</td>
</tr>
<tr>
<td>Goblet cells, n/crypt</td>
<td>40.5 ± 1.1b</td>
<td>47.4 ± 1.5ab</td>
<td>50.6 ± 1.7a</td>
<td>44.0 ± 1.8b</td>
<td>53.1 ± 1.8ab</td>
<td>0.009</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs, \( n = 6 \) (2 pens of 3 piglets). Labeled means in a row without a common letter differ, \( P < 0.05 \). AX, arabinobioxyan diet; CB, combined arabinoxylan and cellulose diet; CEL, cellulose diet; CON, control diet; WB, wheat bran diet; 90% SI, 90% of the length of the small intestine.

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>WB</th>
<th>AX</th>
<th>CEL</th>
<th>CB</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cecum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total SCFAs, ( \mu mol/g )</td>
<td>102 ± 5a</td>
<td>124 ± 5b</td>
<td>150 ± 8a</td>
<td>117 ± 5ab</td>
<td>128 ± 8a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acetate, % of total SCFAs</td>
<td>60.9 ± 2.0a</td>
<td>58.5 ± 3.0a</td>
<td>51.2 ± 1.8ab</td>
<td>57.1 ± 4.2b</td>
<td>46.3 ± 0.8c</td>
<td>0.007</td>
</tr>
<tr>
<td>Propionate, % of total SCFAs</td>
<td>25.5 ± 1.3a</td>
<td>26.6 ± 1.3a</td>
<td>36.1 ± 2.5a</td>
<td>28.0 ± 2.3b</td>
<td>35.9 ± 2.2a</td>
<td>0.003</td>
</tr>
<tr>
<td>Butyrate, % of total SCFAs</td>
<td>4.68 ± 0.2a,b</td>
<td>7.52 ± 0.6ab</td>
<td>6.24 ± 0.55ab</td>
<td>4.62 ± 0.47b</td>
<td>8.71 ± 0.70a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BCFAs, % of total SCFAs</td>
<td>6.11 ± 0.3a</td>
<td>4.65 ± 0.16b</td>
<td>3.44 ± 0.39b</td>
<td>4.83 ± 0.21b</td>
<td>4.12 ± 0.22bc</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Midcolon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total SCFAs, ( \mu mol/g )</td>
<td>101 ± 7a</td>
<td>118 ± 8ab</td>
<td>125 ± 7a</td>
<td>96 ± 8b</td>
<td>127 ± 6a</td>
<td>0.028</td>
</tr>
<tr>
<td>Acetate, % of total SCFAs</td>
<td>56.2 ± 0.6a</td>
<td>54.2 ± 2.1ab</td>
<td>47.2 ± 1.3a</td>
<td>50.1 ± 2.1ab</td>
<td>50.2 ± 2.0ab</td>
<td>0.008</td>
</tr>
<tr>
<td>Propionate, % of total SCFAs</td>
<td>22.8 ± 0.7a</td>
<td>22.2 ± 0.9b</td>
<td>28.5 ± 2.3a</td>
<td>23.9 ± 1.5ab</td>
<td>26.3 ± 1.7ab</td>
<td>0.037</td>
</tr>
<tr>
<td>Butyrate, % of total SCFAs</td>
<td>6.7 ± 0.6a</td>
<td>12.0 ± 1.8a</td>
<td>9.9 ± 1.3ab</td>
<td>10.5 ± 1.8b</td>
<td>10.3 ± 0.9ab</td>
<td>0.047</td>
</tr>
<tr>
<td>BCFAs, % of total SCFAs</td>
<td>10.7 ± 0.3a</td>
<td>8.2 ± 0.5b</td>
<td>8.0 ± 0.7b</td>
<td>11.3 ± 1.0a</td>
<td>8.8 ± 0.9ab</td>
<td>0.008</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs, \( n = 6 \) (2 pens of 3 piglets). Labeled means in a row without a common letter differ, \( P < 0.05 \). AX, arabinobioxyan diet; BCFAs, branched-chain fatty acid (i.e., isobutyric acid and isovaleric acid); CB, combined arabinoxylan and cellulose diet; CEL, cellulose diet; CON, control diet; SCFA, short-chain fatty acid; WB, wheat bran diet.
branched-chain fatty acids (BCFAs) was lower \((P < 0.05)\) in all groups consuming fibrous diets than in the CON group, and lower BCFA proportion \((P < 0.05)\) was observed in the AX group than in the WB and CEL groups. In midcolon digesta, higher total SCFA production \((P < 0.05)\) was obtained in the AX and CB groups than in the CON and CEL groups. In contrast, the CON and CEL groups had a higher BCFA proportion \((P < 0.05)\) than did the WB, AX, and CB groups. The AX group had a lower acetate proportion \((P < 0.05)\) and higher propionate proportion \((P < 0.05)\) than the CON group, whereas the WB group had a higher butyrate proportion than the CON group \((P < 0.05)\).

**Bacterial populations in the cecum.** Fiber inclusion in the diet did not affect total bacteria and *Firmicutes* populations in the ceca of the piglets (data not shown). Lower *Bacteroidetes* and *Enterobacteriaceae* populations \((P < 0.05)\) in the WB group and higher *Lactobacillus* populations \((P < 0.05)\) in the CB and AX groups were observed compared with the CON group (Figure 1). Also, piglets in the CB and AX groups had higher *Lactobacillus* populations \((P < 0.05)\) than those in the WB group. Higher *Bacteroidetes* and *Bifidobacteria* populations \((P < 0.05)\) were observed in the CB group than in the WB group.

**SIgA concentration and IAP activities at 90% SI and midcolon.** Supplemental wheat bran and arabinoxylan resulted in higher mucosal SIgA concentrations \((P < 0.05)\) at 90% SI (Figure 2A) and midcolon (Figure 2B) of piglets than in the CON group. An increase in SIgA concentration in midcolon \((P < 0.05)\) was also observed in the CB group. Meanwhile, piglets in the AX group had higher IAP activities \((P < 0.05)\) than those in the CON and CEL groups at 90% SI (Figure 2C) and midcolon (Figure 2D). Compared with the CON and CEL groups, higher midcolonic IAP activities \((P < 0.05)\) occurred also in the CB group.

**Discussion**

In the current trial, wheat bran ameliorated the intestinal barrier function through interaction with intestinal bacteria, in agreement with the previous studies (1, 2). Also, fibrous diets containing arabinoxylan (WB, AX, and CB groups) increased the number of goblet cells, which play an important protective role in the intestine by synthesizing and secreting several mucins and protein barrier factors (23). The study from Piekarska et al. (24) showed that SCFAs, especially butyrate, stimulate the proliferation of goblet cells in the mouse intestine, in agreement with the higher number of goblet cells and SCFA production in the groups receiving wheat bran or arabinoxylan in the present study. Moreover, the growth of probiotics (e.g., *Lactobacillus* or *Bifidobacteria*) could partially explain the increase in goblet cells in the midcolon in the AX and CB groups (25, 26). Additionally, SIgA in the mucosal epithelia plays an important role in preventing pathogens and other antigens from adhering to host cells (27). Providing supplemental dietary fiber to rats resulted in a high intestinal concentration of SIgA (28), in line with our results in piglets fed wheat bran and arabinoxylan, which could be ascribed to a low concentration of toxic protein degradation products and better intestinal microflora (29).

However, a higher villus height: crypt depth was found only in the WB group compared with the CON group, which seemed to result from higher butyrate production (30). Huang et al. (31) concluded that an increase in the villus height: crypt depth ratio was in favor of intestinal barrier function, invasion present in the

**FIGURE 1** Effect of wheat bran fiber components on *Bacteroidetes* (A), *Lactobacillus* (B), *Enterobacteriaceae* (C), and *Bifidobacterium* (D) in the cecum of male piglets. Values are means ± SEs, \(n = 6\) (2 pens of 3 piglets). Means without a common letter differ, \(P < 0.05\). AX, arabinoxylan diet; CB, combined arabinoxylan and cellulose diet; CEL, cellulose diet; CON, control diet; rRNA, ribosomal RNA; WB, wheat bran diet.
feed. Meanwhile, wheat bran also regulated Cl\textsuperscript{−} secretion by the suppressed cAMP/cGMP- and Ca\textsuperscript{2+}-mediated Cl\textsuperscript{−} secretion pathway, which is beneficial in case of excessive chloride secretion, e.g., in response to toxins such as Escherichia coli enterotoxins (13) or cholera toxin (29). It is known that Cl\textsuperscript{−} secretion across the intestinal epithelium plays a crucial role in regulating water secretion into the intestinal lumen and closely regulates hormonal, neuronal, and paracrine mediators. A disturbed regulation of Cl\textsuperscript{−} secretion can change the water balance and cause pathophysiologic diseases, in which excessive water secretion occurs (32). Theophylline, a cAMP/cGMP−mediated secretagogue, was used to study the Cl\textsuperscript{−} secretory pathway in the intestinal epithelium (33). Carbachol could regulate the Cl\textsuperscript{−} secretion in the intestinal epithelium as Ca\textsuperscript{2+}-mediated secretagogue (34). The toxins secreted from E. coli were believed to stimulate excess chloride secretion by phosphorylating and activating cystic fibrosis transmembrane conductance regulator involved in cAMP/cGMP (35, 36), which is the partial reason that excess chloride secretion was suppressed by wheat bran with reduced Enterobacteriaceae populations in the present study.

In contrast, our ex vitro technology showed that the AX and CB reduced macromolecular protein permeability using HRP, with unchanged paracellular permeability (assessed by FD4) (37) in the midcolon. HRP, a 40 kDa protein, is widely used as a tracer to examine mucosal permeability to foreign protein antigens with immunogenic properties via the transcellular route (38, 39). In accordance with changes in HRP flux, the AX and CB prevented the translocation of foreign protein antigens from the mucosal to the serosal side by increasing mucosal IAP activities (40). IAP, as a brush-border enzyme, has the ability to detoxify lipopolysaccharide and to prevent bacterial invasion across the gut mucosal barrier in vitro and in vivo (40, 41). IAP activity in the distal colonic mucosa was increased when rats were fed the AX (42). In addition, the coincubation of differentiating Caco-2 cells and the Lactobacillus strain restored IAP activity when Caco-2 cells were challenged with the potent mycotoxin deoxynivalenol in vitro (43). These results indicate that increased IAP activity in the AX and CB groups was associated with the stimulation of Lactobacillus populations.

Generally, the effect of dietary fiber on intestinal function is primarily ascribed to the changes in intestinal microflora composition (44) and SCFAs (45). In the present study, feeding diets containing arabinoxylans increased total SCFA production and reduced BCFA production in the large intestine of piglets. Although cellulose is considered to be a nonfermentable fiber, only a slight change was observed in SCFAs between the CON and CEL groups. Similar studies showed that a WB or AX increased total SCFA production in the large intestine of rats or piglets compared with a fiber-free group (42, 46, 47). In addition, fibrous diets were reported to alleviate putative fermentation patterns in the large intestine of pigs (46), because proteolytic activities were reduced with an increase in carbohydrate sources as fermentable substrates (48), and inhibition of the production of BCFA and toxic protein degradation products, such as phenols and cresols (46, 49). However, higher butyrate proportion was observed only in the WB and CB groups in the current experiment, suggesting that high butyrate production may result from the combination of arabinoxylans and cellulose in wheat bran, and not from their separate inclusion, which might be related to stimulation of specific butyrate-producing bacteria (50).

An increase in SCFA production as organic acids reduces the pH value in the intestinal tract (49). The present study showed

![FIGURE 2](image-url)  
**FIGURE 2** Effect of wheat bran fiber components on mucosal SIgA concentration at 90% SI (A) and midcolon (B) and IAP activity at 90% SI (C) and midcolon (D) of male piglets. Values are means ± SEs, n = 6 (2 pens of 3 piglets). Means without a common letter differ, P < 0.05. AX, arabinoxylan diet; CB, combined arabinoxylan and cellulose diet; CEL, cellulose diet; CON, control diet; IAP, intestinal alkaline phosphatase; SIgA, secretory IgA; WB, wheat bran diet; 90% SI, 90% of the length of the small intestine.
that diets containing arabinoxylans reduced the cecal pH value and increased SCFA production. A lower pH value is considered to be beneficial because it has been shown to suppress the numbers of pathogenic bacteria (49). We found that the WB, along with the lower pH in cecal contents, inhibited the growth of Enterobacteriaceae compared with the CON, which is supported by studies from Molist et al. (51) and Gasa et al. (52) demonstrating that supplemental wheat bran in piglets’ diets reduced enterobacteria and E. coli counts in the intestinal digesta. In addition, arabinoxylan fractions from wheat were reported to stimulate the growth of Lactobacillus and Bifidobacterium species, but are not used by E. coli (53, 54), in line with an increase in Lactobacillus and/or Bifidobacteria populations in hindgut contents in the CB and AX groups. However, Bacteroidetes and Bifidobacteria are implicated in the degradation of arabinoxylans in the intestinal tract (55). After degradation of arabinoxylans to arabinoxylan-oligosaccharides and xylo-oligosaccharides via endoxylanases secreted by Bacteroidetes, Bifidobacteria were able to degrade and utilize arabinoxylan-oligosaccharides and xylo-oligosaccharides by secreting arabinofuranosidases and xylosidas. Therefore, a lower number of Bifidobacteria from the WB might be due to a decrease in Bacteroidetes populations compared with the CB in the current study.

In sum, the present study shows that fiber-containing diets that include arabinoxylans promote intestinal barrier function as well as a change in bacteria profiles and metabolites, whereas supplementation with cellulose alone does not affect intestinal barrier function. Therefore, we conclude that the arabinoxylan content in wheat bran, not cellulose, improves intestinal mucosal barrier function in the weaned piglet. However, quantitative differences in the intestinal barrier function between piglets fed either the wheat bran or arabinoxylan diet alone appear to result from the combined effect of multi-components in wheat bran, which needs further investigation.

Acknowledgments

We thank the following technical personnel for their kind assistance: Tessa Van Der Eecken, Anneke Ovyn, Sabine Coolsaet, Daisy Baeyens, Erik Claeyts, and Charlotte Melis, who assisted in animal care-taking, sampling, and lab analysis. HC, DC, SDS, and JM designed the experiment; HC, JD, and JM conducted the experiment; HC, WW, and JM analyzed the data; HC wrote the paper; and SP, DC, SDS, and JM revised the paper. All authors read and approved the final manuscript.

References


