Nonstarch Polysaccharide Hydrolysis Products of Soybean and Canola Meal Protect against Enterotoxigenic Escherichia coli in Piglets

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Abstract

Infectious diarrhea is a major problem in both children and piglets. Enterotoxigenic Escherichia coli (ETEC) infection results in fluid and electrolyte losses in the small intestine. We investigated the effect of nonstarch polysaccharide (NSP) hydrolysis products of soybean meal (SBM) and canola meal (CM) on net absorption of fluid and solutes during ETEC infection. Products were generated by incubating SBM and CM with a blend of carbohydrase enzymes. Following incubation, slurries were centrifuged and the supernatants mixed with absolute ethanol to produce 2 product types: 80% ethanol-soluble (ES) and 80% ethanol-insoluble (EI). Products from SBM and CM were studied in 2 independent experiments in which 2 factors were investigated: product type (EI vs. ES) and time of ETEC infection (before vs. after perfusion).

Pairs of small intestine segments, one noninfected and the other ETEC infected, were perfused simultaneously with different products for 7.5 h. Net absorption of fluid and solutes were determined. In both experiments, ETEC-infected segments perfused with saline control had lower (P ≤ 0.05) net fluid and solute absorption compared with SBM and CM products. The interaction (P ≤ 0.05) between product type and time of infection on fluid absorption was only evident for SBM, in which case perfusing ES products before infection resulted in higher fluid absorption (735 ± 22 μL/cm²) compared with ETEC infection before perfusion (428 ± 34 μL/cm²). In conclusion, NSP hydrolysis products of SBM and CM, particularly ES from SBM, were beneficial in maintaining fluid balance during ETEC infection, suggesting potential for controlling ETEC-induced diarrhea in piglets. J. Nutr. 138: 502–508, 2008.

Carbohydrase enzymes (CE) are routinely used in piglet diets to improve nutrient utilization, putatively by depolymerizing complex cell wall NSP in feedstuffs (12). In the process of depolymerizing NSP, supplemental CE may create NSP hydrolysis products in situ that might influence enteric bacterial infections in piglets (13,14). Indeed, it was previously reported that the addition of CE to piglet diets reduced the frequency and severity of nonspecific diarrhea (15,16). However, in no case was the cause of any episode of diarrhea determined. This is important because it is necessary to distinguish diarrhea of dietary origin from that of bacterial etiology, which might be influenced by the presence of NSP hydrolysis products (14).

We utilized a piglet small intestinal segment perfusion method to evaluate the efficacy of NSP hydrolysis products from soybean meal (SBM) and canola meal (CM) to attenuate ETEC-induced fluid and electrolyte losses. We used CE preparations to produce ethanol soluble (ES), low molecular weight and ethanol insoluble (EI), high molecular weight hydrolysis products.

Materials and Methods

NSP hydrolysis products. All products were produced from the same batch of SBM and CM, which were ground to pass through a 1-mm screen. Prior to incubation with CE, feedstuffs were subjected to ethanol extraction to remove free sugars and low molecular weight carbohydrate components (i.e. sucrose, oligosaccharides) (17). Briefly, each feedstuff...
was mixed with 80% ethanol in 1:5 (w:v) and the mixture was subjected to 4 cycles of ethanol extraction in an environmentally controlled incubator shaker set at 200 rotations per minute and 40°C (New Brunswick Scientific). Cycles were run one after another; the first and last cycles were run for 16 h and cycles 2 and 3 were run for 3 h. Every cycle was followed by centrifugation (1838 × g; 15 min). The supernatant was discarded and the retentate mixed with fresh 80% ethanol to start the next cycle. After cycle 4, the retentate was dried at room temperature under a fume hood and finely ground in a coffee grinder.

NSP hydrolysis products were prepared by mixing 50 g of either ethanol-extracted SBM or CM with 0.5 g of a CE blend in distilled water. The CE blend contained pectinase (10,000 IU/g), cellulase (600 IU/g), mannanase (10,900 IU/g), xylanase (63,600 IU/g), glucanase (48,300 IU/g), galactanase (1000 IU/g), and other enzymes and activities. The enzymes were provided, along with the enzyme assay procedures, by Canadian Bio-System. The mixtures were incubated for 16 h in a shaker set at 200 rotations per min and 40°C. Following incubation, the mixtures were centrifuged at 1838 × g; 20 min. The supernatants were then mixed with absolute ethanol at 1:4 v:v and allowed to stand at room temperature for 1 h and then centrifuged at 1838 × g; 20 min. The supernatant was decanted and ethanol was evaporated in a rotary evaporator to yield 80% ES. The retentate was dissolved in a small amount of water to yield 80% EI. Hydrolysis products were then frozen at −80°C, freeze dried, finely ground, and stored in sealed containers at 4°C.

**Piglets and jejunal segment preparation.** The experimental protocol was approved by the University of Manitoba Animal Care Committee (protocol no. F06-025) and followed the principles established by the Canadian Council on Animal Care (18). Eight Genus (Yorkshire × Hampshire × Duroc; Keystone Pig Advancement) 3-wk-old piglets were obtained from the University of Manitoba Glenlea Swine Research Farm and fed a standard commercial starter diet (FeedRite) for 7 d before experimentation. At 37°C containing 5% CO2 (Pharmaceutical Partnership of Canada) and oxygen via intubation. The piglet was placed in dorsal recumbency on a heated surface to maintain body temperature.

The abdominal cavity was opened and the first intestinal segment was prepared (~300 cm caudal to the pylorus. A small cranial tube (inflow; 3 mm i.d., 5 mm o.d.) was placed and a wider tube (outflow; 5 mm i.d., 9 mm o.d.) was placed ~20 cm distal from the first. Caudal from and adjacent to this first segment 9 other segments were prepared in the same way, giving a total of 10 segments. Segments were labeled consecutively in numerals beginning from the first segment to the last. Between odd and even segments, 2-cm pieces of the intestine were removed during segment preparation for measurement of the circumference. The segments were completely isolated from each other and covered between 37 and 73% of the total length of the jejunum.

**Bacterial strain.** The ETEC strain was confirmed by PCR genotyping as possessing the genes for K88 fimbrial antigen, heat-labile, and heat-stable toxins (20). The cultures were grown in LB containing 5 μg ciprofloxacin/mL (Sigma). After overnight incubation at 37°C with shaking, cultures were centrifuged at 3000 × g; 10 min at 37°C and the pellet suspended in PBS to an OD value of 1.0 measured at 600 nm corresponding to 108 colony-forming units (CFU/mL).

**Test solutions, infection, and perfusion procedure.** All test solutions were made fresh before experiments. ES and EI (0.3 g/L) from SBM and CM were dissolved in sterile isotonic saline solution (0.85% NaCl; Fisher Scientific). Sterile isotonic saline solution was also prepared as a test solution to serve as an internal control to determine maximum response to infection (9). All test solutions were supplemented with 1 g/L of glucose and casamino acids (acid-hydrolyzed casein) to ensure bacteria growth. Test solutions were adjusted with 1 mol/L NaHCO3 to pH 6.6, the average pH of the piglet jejunum lumen (21).

**NSP hydrolysis product analysis.** Carbohydrate concentrations were determined by GLC (component neutral sugars) and by colorimetry (uronic acids) using the procedure for NSP analysis. The procedure for component neutral sugars was performed as described by Englyst and Cummings (22) with modifications (23). Briefly, 30 mg of hydrolysis product was mixed with 6 mL of water, 5 mL of myoinositol (standard solution), and 1 mL of 12 mol/L sulfuric acid and the mixture boiled for 2 h. The resulting hydrolyzate was used to determine uronic acids and component neutral sugars. For the component neutral sugars, 1 mL of the hydrolyzate was neutralized with 12 mol/L ammonium hydroxide, reduced with sodium borohydride, and acetylated with acetic anhydride in the presence of 1-methylimidazole. Component sugars were separated using SP-2340 column and Varian CP 3380 gas chromatograph (Varian Canada). Uronic acids were determined using the procedure described by Scott (24).

**Calculations and statistical analysis.** Net fluid, total solutes, Na+, K+, and Cl− absorption were calculated from the difference between the volume and concentration of inflow and outflow divided by the surface area (length × circumference) of the segment. We determined net fluid loss upon ETEC infection by subtracting net fluid absorption in ETEC-infected segments from net fluid absorption in noninfected segments perfused with the same test solutions. Bacterial enumeration data were transformed to log10 CFU/mL or mL before statistical analysis.

Data were analyzed using the SAS statistical package (SAS 9.1, SAS Institute). Data from the noninfected and ETEC-infected segments
performed with the same perfusion solution were compared using the Student’s paired t test for equal and unequal variances. The effects of the product type, time of infection, and interaction were analyzed as a Latin square design with piglet and segments within a pair as random effects (25). Comparison between saline and test treatment combinations was performed using the Student’s paired t test for equal and unequal variances. The effects of the product type (HP), time of infection (time), and their interactions (HP × time) were analyzed as a Latin square design with piglet and segments within a pair as random effects (25). Comparison between saline and test treatment combinations was performed using the Student’s paired t test for equal and unequal variances. The effects of the product type (HP), time of infection (time), and their interactions (HP × time) were analyzed as a Latin square design with piglet and segments within a pair as random effects (25). Comparison between saline and test treatment combinations was performed using the Student’s paired t test for equal and unequal variances. The effects of the product type (HP), time of infection (time), and their interactions (HP × time) were analyzed as a Latin square design with piglet and segments within a pair as random effects (25).

Results

Carbohydrate concentration of NSP hydrolysis products and characteristics of the test solutions. Total component sugar concentrations of the ES and EI hydrolysis products derived from SBM and CM are presented in Table 1. Within the feedstuff, electrolytes and total solute concentrations were comparable in ES and EI (Table 2).

### Table 1

Carbohydrate concentrations of products derived from hydrolysis of SBM and CM NSP by CE1,2

<table>
<thead>
<tr>
<th>Item</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Uronic acids</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBM</td>
<td>ES</td>
<td>14.7</td>
<td>nd</td>
<td>5.2</td>
<td>95.6</td>
<td>29.9</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>EI</td>
<td>2.6</td>
<td>2.9</td>
<td>3.2</td>
<td>33.0</td>
<td>2.8</td>
<td>85.0</td>
</tr>
<tr>
<td>CM</td>
<td>ES</td>
<td>65.8</td>
<td>36.3</td>
<td>nd</td>
<td>11.0</td>
<td>39.8</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>EI</td>
<td>15.0</td>
<td>3.1</td>
<td>13.8</td>
<td>2.7</td>
<td>18.3</td>
<td>52.9</td>
</tr>
</tbody>
</table>

1 Average of duplicate analysis. Hydrolysis products were generated by incubating 50 g of ethanol extracted SBM or CM in distilled water medium with 0.5 g of carbohydrase blend supplying pectinase, cellulase, mannanase, xylanase, gluconase, galactanase, and other activities.

2 Supernatant of enzyme hydrolyzed meals were mixed with absolute ethanol to yield both 80% ES and 80% EI products.

3 nd, Not detected (minimum detection limit < 1 mg/g).

Effect of SBM NSP hydrolysis products on net absorption.

ETEC infection reduced \( P < 0.05 \) net fluid and electrolyte (Na\(^+\), Cl\(^-\), and K\(^+\)) absorption upon perfusion with saline over the 7.5-h period compared with noninfected segments perfused with saline (Table 3). There was an interaction \( P < 0.05 \) between hydrolysis product type and time of infection on net fluid and Cl\(^-\) absorption in infected segments; infecting segments after perfusion of ES hydrolysis products resulted in higher net fluid and Cl\(^-\) absorption compared with segments infected before perfusion. In noninfected segments, perfusing saline resulted in lower \( P < 0.05 \) net fluid and Na\(^+\) absorption compared with perfusing ES and EI. There was no interaction between hydrolysis product type and time of infection on the net absorption of fluid and electrolytes in noninfected segments.

Effect of CM NSP hydrolysis products on net absorption.

ETEC infection reduced \( P < 0.05 \) net fluid and electrolytes absorption upon perfusion with saline over the 7.5-h period compared with noninfected segments perfused with saline (Table 4). There was an interaction \( P < 0.05 \) between hydrolysis product type and time of infection on net Na\(^+\) and Cl\(^-\) absorption in infected segments; infecting segments after perfusing with ES hydrolysis product resulted in lower net Na\(^+\) and Cl\(^-\) absorption compared with noninfected segments.

### Table 3

Net absorption of fluid, Na\(^+\), Cl\(^-\), and K\(^+\) in noninfected and ETEC-infected piglet jejunal segments after perfusion of saline or products derived from hydrolysis of SBM NSP by CE1,2

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Na(^+)</th>
<th>Cl(^-)</th>
<th>K(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Noninfected</td>
<td>Infected</td>
<td>Noninfected</td>
</tr>
<tr>
<td></td>
<td>(\mu L/cm^2)</td>
<td>(\mu mol/cm^2)</td>
<td>(\mu L/cm^2)</td>
</tr>
<tr>
<td>Saline</td>
<td>451 ± 50*</td>
<td>170 ± 20**</td>
<td>96 ± 7.29*</td>
</tr>
<tr>
<td>ES-0 min</td>
<td>692 ± 59*</td>
<td>428 ± 51**</td>
<td>135 ± 12.3*</td>
</tr>
<tr>
<td>ES-30 min</td>
<td>665 ± 43*</td>
<td>735 ± 22**</td>
<td>142 ± 12.0*</td>
</tr>
<tr>
<td>EI-0 min</td>
<td>640 ± 31*</td>
<td>536 ± 57**</td>
<td>150 ± 8.56*</td>
</tr>
<tr>
<td>EI-30 min</td>
<td>578 ± 33*</td>
<td>546 ± 13**</td>
<td>135 ± 8.37*</td>
</tr>
<tr>
<td>(P) value</td>
<td>HP 0.245</td>
<td>0.821</td>
<td>0.684</td>
</tr>
<tr>
<td></td>
<td>Time 0.481</td>
<td>0.023</td>
<td>0.701</td>
</tr>
<tr>
<td></td>
<td>HP × time 0.203</td>
<td>0.010</td>
<td>0.315</td>
</tr>
</tbody>
</table>

1 Average of duplicate analysis. A total of 0.3 g of respective product type was dissolved in 1 L isotonic saline solution.

2 Positive or negative values represent net absorption and secretion, respectively. * Different from noninfected segments, \( P < 0.05 \). ** \( P < 0.01 \) analyzed using Student’s t test for equal and unequal variances. Means in a column with superscripts without a common letter differ, \( P < 0.05 \), and denote comparison between saline and treatment combinations. \( P\) value represents the effects of the product type (HP), time of infection (time), and their interactions (HP × time), and excludes segments perfused with saline.

2 Segments infected before perfusion (ES-0 min) and (EI-0 min) or 30 min postperfusion (ES-30 min) and (EI-30 min).
absorption compared with segments infected before perfusion. Except for K\(^+\), perfusing saline resulted in higher (P ≤ 0.05) net fluid and electrolyte absorption compared with perfusing ES and EI in noninfected segments. There was no interaction between hydrolysis product type and time of infection on net absorption of fluid and electrolytes in noninfected segments.

**Net fluid loss and total solutes absorption.** ETEC infection in saline-perfused segments resulted in higher net fluid loss for SBM and CM experiments (281 and 252 \(\mu\)L/cm\(^2\), respectively) compared with other test solutions except for ES from SBM infected before perfusion (Fig. 1). In the SBM experiment, there was an interaction (P ≤ 0.05) of hydrolysis product type and time of infection on net fluid loss; infecting segments after perfusion of ES protected against fluid loss compared with infection before perfusion. Net fluid loss in ETEC-infected segments was not affected by perfusion with CM hydrolysis products and was independent of the time of infection.

Infected segments perfused with saline had lower total solute absorption than those perfused with all SBM and EI CM hydrolysis products (Fig. 2). In the SBM experiment, there was an interaction (P ≤ 0.05) between product type and time of infection on total solute absorption; perfusing ES before ETEC infection resulted in higher net solute absorption compared with perfusion after ETEC infection.

**ETEC counts in outflow fluid and mucosal scrapings of ETEC-infected segments.** Hydrolysis product type and time of infection did not interact to affect ETEC numbers in either experiment (Table 5). The ETEC numbers in the outflow fluid and mucosal scrapings of SBM hydrolysis products did not differ from those of saline segments. In the CM experiment, segments infected at time 0 and perfused with EI had higher (P ≤ 0.05) ETEC counts on mucosal scrapings than segments perfused with saline.

**Discussion**

ETEC is among the most common enteric pathogens causing acute secretory diarrhea in neonatal and weaned piglets (1). Moreover, ETEC is among the most common enteric pathogens causing acute secretory diarrhea in young children in developing countries (2) and in travelers to these regions. The pig gastrointestinal tract physiologically and anatomically resembles that of humans (27).

To test the hypothesis that products derived from hydrolysis of SBM and CM NSP by CE could influence secretory diarrhea caused by ETEC K88, we used an in situ model of secretory diarrhea. This model is considered suitable to quantitatively assess the effect of enterotoxin-producing pathogens on net absorption in the small intestine (19). ES and EI hydrolysis products differed in that the former were assumed to contain simple sugars, oligosaccharides, and low molecular weight polysaccharides, whereas the latter represented high molecular weight polysaccharides (17).

Challenge of the jejunal segments with ETEC strain resulted in reduced net fluid and total solutes absorption in segments

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**TABLE 4** Net absorption of fluid, \(\text{Na}^+\), \(\text{Cl}^-\), and \(\text{K}^+\) in noninfected and enterotoxigenic E. coli-infected piglet jejunal segments after perfusion of saline or products derived from hydrolysis of CM NSP by CE\(^{1,2}\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Fluid</th>
<th>Noninfected</th>
<th>Infected</th>
<th>Noninfected</th>
<th>Infected</th>
<th>Noninfected</th>
<th>Infected</th>
<th>Noninfected</th>
<th>Infected</th>
<th>Noninfected</th>
<th>Infected</th>
<th>Noninfected</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu)L/cm(^2)</td>
<td>(\mu)mol/cm(^2)</td>
<td>(\mu)mol/cm(^2)</td>
<td>(\mu)mol/cm(^2)</td>
<td>(\mu)mol/cm(^2)</td>
<td>(\mu)mol/cm(^2)</td>
<td>(\mu)mol/cm(^2)</td>
<td>(\mu)mol/cm(^2)</td>
<td>(\mu)mol/cm(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>517 ± 46(^a)</td>
<td>265 ± 15(^b)</td>
<td>108 ± 15(^c)</td>
<td>101 ± 17(^d)</td>
<td>54 ± 16(^e)</td>
<td>−1.24 ± 0.10</td>
<td>−2.37 ± 0.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES-0 min</td>
<td>378 ± 22(^b)</td>
<td>381 ± 31(^a)</td>
<td>68 ± 13(^d)</td>
<td>79 ± 14(^f)</td>
<td>64 ± 5.3(^e)</td>
<td>−1.22 ± 0.15</td>
<td>−1.96 ± 0.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES-30 min</td>
<td>378 ± 36(^b)</td>
<td>430 ± 40(^c)</td>
<td>63 ± 10(^e)</td>
<td>87 ± 10(^d)</td>
<td>40 ± 4.2(^e)</td>
<td>−1.32 ± 0.52</td>
<td>−1.53 ± 0.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI-0 min</td>
<td>341 ± 18(^b)</td>
<td>370 ± 11(^a)</td>
<td>63 ± 0.3(^c)</td>
<td>74 ± 16(^d)</td>
<td>61 ± 4.1(^b)</td>
<td>−1.28 ± 0.24</td>
<td>−1.34 ± 0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI-30 min</td>
<td>381 ± 26(^b)</td>
<td>389 ± 38(^b)</td>
<td>66 ± 9.2(^e)</td>
<td>77 ± 8.0(^d)</td>
<td>81 ± 6.6(^e)</td>
<td>−0.97 ± 0.39</td>
<td>−1.02 ± 0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SEM, n = 8. Positive or negative values represent net absorption and secretion, respectively. \(^2\) Different from noninfected segments, P ≤ 0.05, \(*\)P ≤ 0.01 analyzed using Student’s t tests for equal and unequal variances. Means in a column with superscripts without a common letter differ, P ≤ 0.05, and denote comparison between saline and treatment combinations. P-value represents the effects of the product type (HP), time of infection (time), and their interactions (HP × time), and exclude segments perfused with saline.

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**FIGURE 1** Fluid loss upon ETEC infection in piglet jejunal segments after perfusion with saline or products derived from hydrolysis of SBM (A) and CM (B) NSP by CE. Values are means ± SEM. Labeled means within a panel without a common letter differ, P ≤ 0.05, and denotes comparison of saline and treatment combinations. P-value represents the effects of the product type (HP), time of infection (time), and their interactions (HP × time) and exclude segments perfused with saline. Hydrolysis products description presented in Table 1. Segments infected before perfusion (ES-0 min) and (EI-0 min) or 30 min postperfusion (ES-30 min) and (EI-30 min). Values are means ± SEM, n = 4.
perfused with saline in both experiments. This may be because of the inhibition of NaCl absorption by the villus cells as well as accelerated Cl⁻ secretion by the crypt cells (28). It is noteworthy that segments showed net K⁺ secretion in both experiments. This may be explained by the fact that K⁺ transport in the small intestine is a passive process influenced by the luminal K⁺ concentration (29). All perfusion solutions had lower K⁺ concentrations (<1 mmol/L) than those in plasma of 3-wk-old piglets (>4 mmol/L) (30).

It has previously been demonstrated that products of fermented SBM reduced ETEC-induced fluid and electrolyte losses in piglet small intestinal segments, whereas this was not the case for nonfermented SBM (9,10). Moreover, hydrolyzed guar gum has been shown to enhance net fluid absorption in models for secretory diarrhea (5). It has been suggested that the action of CE is mediated by degrading high molecular weight polysaccharides to simple sugars, oligosaccharides, and low molecular weight polysaccharides (12,17). Evidence for possible involvement of hydrolysis products derived from hydrolysis of SBM and CM by CE in the protective effect described in this study could be the presence of oligosaccharides and short chain polysaccharides.

ETEC-induced diarrhea involves a complex interplay between the host intestine and luminal bacteria; ETEC must attach to the intestinal epithelium to initiate colonization (1). We hypothesized that varying the time of infection relative to perfusion would give an indication of how hydrolysis products would influence establishment of ETEC on the intestinal mucosa and subsequent infection as measured by fluid and electrolytes kinetics. This hypothesis was only confirmed in segments perfused with ES hydrolysis products from SBM, which showed higher net fluid and solutes absorption when infected 30 min after perfusion compared with before perfusion. However, because these segments had a similar number of ETEC attached to the mucosa scrapings, it is not clear as to what may have mediated this observation. In contrast, perfusing ES hydrolysis products from CM 30 min before infection resulted in lower Na⁺ and Cl⁻ absorption compared with segments infected prior to perfusion. It is rather hard to explain this observation. Nevertheless, it is noteworthy that when compared with saline, CM products appeared to suppress fluid absorption in noninfected segments through mechanisms we could not establish.

Various modes of action may be involved in the protective effect of NSP hydrolysis products in this study. First, compounds in the products may interfere with attachment of ETEC to the enterocytes, as has been shown for fermented SBM and piglet brush borders in vitro (31). Second, certain plant polysaccharides are now recognized as having a prebiotic effect (32). Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selective stimulation of growth or activity of 1 or a limited number or activity of bacterial species especially lactic acid-producing bacteria (33). In this context, lactic acid bacteria have been shown to have antibacterial effects on E. coli and salmonella species (34–36). However, because the number of ETEC attached to the mucosa scrapings were similar for SBM and higher for CM hydrolysis products compared with segments perfused with saline, probably no interference with the adhesion of ETEC occurred. The lack of differences in the

### TABLE 5

Number of ETEC in the outflow fluid and mucosal scrapings of ETEC-infected jejunal segments after perfusion with saline or products derived from hydrolysis of SBM and CM NSP by CE

<table>
<thead>
<tr>
<th>Item</th>
<th>Outflow fluid</th>
<th>Mucosal scrapings</th>
<th>Outflow fluid</th>
<th>Mucosal scrapings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBM</td>
<td>CM</td>
<td>SBM</td>
<td>CM</td>
</tr>
<tr>
<td>Log₁₀CFU/mL</td>
<td>Log₁₀CFU/g</td>
<td>Log₁₀CFU/mL</td>
<td>Log₁₀CFU/g</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>7.0 ± 0.30</td>
<td>7.3 ± 0.24</td>
<td>5.7 ± 0.48</td>
<td>4.8 ± 0.49</td>
</tr>
<tr>
<td>ES-0 min</td>
<td>7.5 ± 0.29</td>
<td>7.0 ± 0.32</td>
<td>6.2 ± 0.35ab</td>
<td>6.1 ± 0.47</td>
</tr>
<tr>
<td>ES-30 min</td>
<td>7.7 ± 0.23</td>
<td>7.1 ± 0.25</td>
<td>6.8 ± 0.48ab</td>
<td>6.2 ± 0.48</td>
</tr>
<tr>
<td>EI-0 min</td>
<td>7.4 ± 0.49</td>
<td>7.3 ± 0.33</td>
<td>6.3 ± 0.36ab</td>
<td>6.8 ± 0.30</td>
</tr>
<tr>
<td>EI-30 min</td>
<td>7.5 ± 0.39</td>
<td>7.3 ± 0.31</td>
<td>6.5 ± 0.39ab</td>
<td>6.4 ± 0.42</td>
</tr>
</tbody>
</table>

P-value

| HP          | 0.616         | 0.710             | 0.431         | 0.277             |
| Time        | 0.727         | 0.614             | 0.862         | 0.729             |
| HP * time   | 0.967         | 0.813             | 0.136         | 0.582             |

1 Values are means ± SEM, n = 4. Means in a column with superscripts without a common letter differ, P ≤ 0.05 and denote comparison between saline and treatment combinations. P-value represents the effects of the product type (HP), time of infection (time), and their interactions (HP * time), and exclude segments perfused with saline.

2 Segments infected before perfusion (ES-0 min) and (EI-0 min) or 30 min postperfusion (ES-30 min) and (EI-30 min).
number of ETEC attached to the mucosal scrapings of segments perfused with saline and NSP hydrolysis products, albeit evidence of infection in the former segments, constitutes a known phenomenon associated with the present model of secretory diarrhea (9,21). Bruin et al. (21) suggested that the time frame used for the model may be too short to observe changes in mucosal ETEC count. Third, soluble fibers have been shown to enhance intestinal water and electrolyte absorption in normal and secreting rat small intestine (4). Fourth, the protective effect could have resulted from components in the products that could also interfere with the (binding of) enterotoxin, as was shown elsewhere for certain toxin receptor analogs (37). Alternatively, such components may have interfered with the cascade of events leading to increased Cl\(^-\) secretion and inhibited NaCl absorption, as was shown for polyphenolic compounds in plant extracts and boiled rice (7,38,39). This could be mediated through stimulated Na\(^+\)-solute cotransport, which is the basis for traditional WHO oral rehydration therapy (40).

In conclusion, NSP hydrolysis products of SBM and CM, particularly ES from SBM administered before infection, were beneficial in maintaining fluid balance during ETEC infection. In relation to piglet nutrition, this finding may aid in designing enzyme systems tailored for maximizing dietary nutrient utilization as well as controlling enteric infections such as ETEC-secretory diarrhea. Further research is required to identify the component(s) in the products that are responsible for the protective effect and to evaluate the specific mechanism(s) underlying the improved net fluid balance.

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Literature Cited


