Chito-oligosaccharide reduces diarrhea incidence and attenuates the immune response of weaned pigs challenged with *Escherichia coli* K88

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ABSTRACT: Seventy-two barrows (Landrace × Large White, initial BW of 4.9 ± 0.3 kg and 17 ± 3 d old) were used to determine if dietary chito-oligosaccharides can replace antibiotics as a means to reduce signs associated with infection in weaned pigs challenged with *Escherichia coli*. Pigs were assigned to 1 of 4 treatments in a randomized complete block design using 6 pens per treatment with 3 pigs per pen. The treatments consisted of pigs fed the unsupplemented corn-soybean meal diet challenged or unchallenged with *E. coli* K88 and pigs fed the same diet supplemented with 160 mg of chito-oligosaccharides or 100 mg of cyadox/kg and challenged with *E. coli* K88. On d 7, 1 group of pigs fed the unsupplemented diet, as well as all pigs fed diets containing chito-oligosaccharides or cyadox, were orally dosed with 30 mL of an alkaline broth containing *E. coli* K88. Another group of pigs fed the unsupplemented diet was orally dosed with 30 mL of sterilized alkaline broth. Fecal consistency was visually assessed each morning from d 7 to 14. Blood samples were collected at 0, 24, 48, and 168 h postinfection. On d 14 postchallenge, all pigs were killed to evaluate intestinal morphology and determine *E. coli* concentrations in the intestine. During the postchallenge period (wk 2), unsupplemented pigs challenged with *E. coli* had decreased (*P* < 0.05) BW gain, feed intake, fecal consistency, villus height, villus height:crypt depth ratio, and plasma IGF-1, and increased (*P* < 0.05) diarrhea incidence, *E. coli* counts in the intestine, plasma interleukin-1β, plasma IL-10, and IGA-positive cells in the jejunal and ileal lamina propria, compared with unchallenged pigs. Supplementation with cyadox largely mitigated these effects. Although chito-oligosaccharide reduced the incidence of diarrhea, the growth performance of *E. coli*-challenged pigs supplemented with chito-oligosaccharide was not better than that of unsupplemented pigs challenged with *E. coli*. Therefore, chito-oligosaccharide, at the amount used in this experiment, does not seem to be an effective substitute for antibiotics as a growth promoter for newly weaned pigs challenged with *E. coli*.

Key words: chito-oligosaccharide, cyadox, *Escherichia coli*, immune response, performance, pig

INTRODUCTION

Acute diarrhea caused by enteric diseases is a universal problem in newly weaned animals (Osek, 1999; Mao et al., 2005). Although antibiotic therapy has been used to control diarrhea for many years, issues with bacterial antibiotic resistance may cause problems for human health (Bach Knudsen, 2001; Smith et al., 2002).

Therefore, many functional substances are being tested as a means to control diarrhea in weaned animals (Correa-Matos et al., 2003; Wang et al., 2003, 2007).

Dietary oligosaccharides have been shown to improve performance and enhance host health status by modulating the intestinal microflora (Gibson and Roberfroid, 1995; LeMieux et al., 2003). Furthermore, dietary oligosaccharides, such as chito-oligosaccharides, serve not only as a growth promoter for helpful bacteria (Lee et al., 2002), but also effectively inhibit the growth and activity of pathogenic microorganisms (Tsai et al., 2000; Rhoades et al., 2006).

Enterotoxigenic *Escherichia coli* K88 is a major cause of diarrhea and death in neonatal and weaned pigs (Francis et al., 1998). Enterotoxigenic *E. coli* K88 can not only colonize in the small intestine, but also release

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Animals and Experimental Design

Seventy-two barrows (Landrace × Large White, initial BW of 4.94 ± 0.30 kg and 17 ± 3 d of age) were obtained from a commercial pig farm. Pigs were assigned to 1 of 4 treatments in a randomized complete block design. The treatments consisted of pigs fed the unsupplemented corn-soybean meal diet (Table 1) challenged or unchallenged with E. coli K88 and pigs fed the same diet supplemented with 160 mg of chito-oligosaccharides or 100 mg of cyadox (Veterinary Research Institute of Huazhong Agricultural University, Wuhan, China)/kg of diet and challenged with E. coli K88 (Huabei Zhongmu Anda Limited, Jubei, China). The cyadox is a new derivative of quinoxaline-1,4-dioxide, which is similar to carbadox and olaquindox but safer to animals than carbadox and olaquindox, it has been shown to be effective in reducing pathogenic bacteria such like E. coli K88 in pigs (Ding et al., 2006). The supplementary amount of chito-oligosaccharide was selected based on the results of an earlier experiment where breakpoint analysis indicated that maximal BW gain could be obtained by supplementation with 158.8 mg of chito-oligosaccharide/kg of diet (Liu et al., 2008). All diets were formulated according to the nutrient requirements suggested by NRC (1998) for 3- to 5-kg nursery pigs.

At 0800 h of d 7, 1 group of pigs fed the unsupplemented diet and pigs fed the diets supplemented with chito-oligosaccharide or cyadox were orally dosed with 30 mL of an alkaline broth containing 10^10 cfu/mL of E. coli K88 culture using an orogastric tube (Sarmiento et al., 1988). The E. coli K88 (serotype O139:K88, resistant to oxytetracycline) was obtained from China Institute of Veterinary Drug Control (Beijing, China) and has been extensively used to create diarrhea in weaning pigs (Ding et al., 2006). The enterotoxigenic E. coli was confirmed by PCR genotyping as genes expressing K88 fimbrial antigen and primarily cultured in Luria broth medium. The enterotoxigenic E. coli K88 was grown overnight in Luria broth agar plate at 37°C using 0.3 mL of inoculum from stock. Cells were then washed twice with 30 mL of sterilized saline solution (0.9%, pH 7.2), and then the suspension containing 10^10 cfu of E. coli K88 (calculated based on the optical density established by serial dilution before viable bacterial count) was used for oral challenge. Another group of pigs fed the unsupplemented diet were orally dosed with 30 mL of sterilized alkaline broth.

The experimental pigs were housed 3 piglets per pen in 180 × 170 cm raised weaner decks equipped with a mesh floor, and 6 pens were assigned to each treatment.

### Table 1. Ingredient and chemical composition of the basal diet (as-fed basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Content</th>
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<tbody>
<tr>
<td>Ingredient, %</td>
<td></td>
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<tr>
<td>Corn, yellow</td>
<td>67.45</td>
</tr>
<tr>
<td>Soybean meal, dehulled, 45% CP</td>
<td>22.65</td>
</tr>
<tr>
<td>Fish meal, 65% CP</td>
<td>3.00</td>
</tr>
<tr>
<td>Spray-dried porcine plasma</td>
<td>3.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.58</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.67</td>
</tr>
<tr>
<td>l-Lysine·HCl, 98%</td>
<td>0.29</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.04</td>
</tr>
<tr>
<td>Salt</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamin and mineral premix</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient composition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ME, Mcal/kg</td>
<td>3.20</td>
</tr>
<tr>
<td>CP, %</td>
<td>20.00</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.80</td>
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<tr>
<td>Phosphorus, %</td>
<td>0.73</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.40</td>
</tr>
<tr>
<td>Methionine, %</td>
<td>0.40</td>
</tr>
</tbody>
</table>

1The basal diet was supplemented with 160 mg of chito-oligosaccharides or 100 mg of cyadox/kg.
2Mogao Sweetening = 15% sodium saccharin (Chongqing Mintai Spices Chemical Co. Ltd., Chongqing, China).
3Vitamin and mineral premix provided the following per kilogram of diet: vitamin A, 12,000 IU as vitamin A acetate; vitamin D, 2,500 IU as vitamin D_3; vitamin E, 30 IU as dl-α-tocopheryl acetate; 12 mg of vitamin B_12; vitamin K, 3 mg as menadione sodium bisulfate; B-pantothenic acid, 15 mg as calcium pantothenate; 40 mg of nicotinic acid; choline, 400 mg as choline chloride; Mn, 40 mg as manganous oxide; Zn, 100 mg as zinc oxide; Fe, 90 mg as iron sulfate; Cu, 8.8 mg as copper oxide; I, 0.35 mg as ethylenediamine dihydroiodide; and Se, 0.3 mg as sodium selenite.

### MATERIALS AND METHODS

The Animal Care and Use Protocol was approved by the China Agricultural University Animal Care and Use Committee.

#### Preparation and Composition of Chito-Oligosaccharide

The chito-oligosaccharide supplement (GlycoBio Company, Dalian, China) used in the current experiment was as described by Li et al. (2007). The chito-oligosaccharide supplement contained 40% chito-oligosaccharide and 60% cyclodextrin as a carrier. This chito-oligosaccharide was found to be composed of 5 oligomers identified as chitobiose, chitotriose, chitotetrose, chitopentose, and chitohexose, and the concentrations of these 5 oligomers were 2.90, 12.55, 22.45, 29.00, and 11.05% for chitobiose, chitotriose, chitotetrose, chitopentose, and chitohexose, respectively. The average molecular weight was 1,500 Da. Water solubility of the chito-oligosaccharide supplement was more than 99% (Li et al., 2007; Deng et al., 2008).

### Animals and Experimental Design

Seventy-two barrows (Landrace × Large White, initial BW of 4.94 ± 0.30 kg and 17 ± 3 d of age) were obtained from a commercial pig farm. Pigs were assigned to 1 of 4 treatments in a randomized complete block design. The treatments consisted of pigs fed the unsupplemented corn-soybean meal diet (Table 1) challenged or unchallenged with E. coli K88 and pigs fed the same diet supplemented with 160 mg of chito-oligosaccharides or 100 mg of cyadox (Veterinary Research Institute of Huazhong Agricultural University, Wuhan, China)/kg of diet and challenged with E. coli K88 (Huabei Zhongmu Anda Limited, Jubei, China). The cyadox is a new derivative of quinoxaline-1,4-dioxide, which is similar to carbadox and olaquindox but safer to animals than carbadox and olaquindox, it has been shown to be effective in reducing pathogenic bacteria such like E. coli K88 in pigs (Ding et al., 2006). The supplementary amount of chito-oligosaccharide was selected based on the results of an earlier experiment where breakpoint analysis indicated that maximal BW gain could be obtained by supplementation with 158.8 mg of chito-oligosaccharide/kg of diet (Liu et al., 2008). All diets were formulated according to the nutrient requirements suggested by NRC (1998) for 3- to 5-kg nursery pigs.

At 0800 h of d 7, 1 group of pigs fed the unsupplemented diet and pigs fed the diets supplemented with chito-oligosaccharide or cyadox were orally dosed with 30 mL of an alkaline broth containing 10^10 cfu/mL of E. coli K88 culture using an orogastric tube (Sarmiento et al., 1988). The E. coli K88 (serotype O139:K88, resistant to oxytetracycline) was obtained from China Institute of Veterinary Drug Control (Beijing, China) and has been extensively used to create diarrhea in weaning pigs (Ding et al., 2006). The enterotoxigenic E. coli was confirmed by PCR genotyping as genes expressing K88 fimbrial antigen and primarily cultured in Luria broth medium. The enterotoxigenic E. coli K88 was grown overnight in Luria broth agar plate at 37°C using 0.3 mL of inoculum from stock. Cells were then washed twice with 30 mL of sterilized saline solution (0.9%, pH 7.2), and then the suspension containing 10^10 cfu of E. coli K88 (calculated based on the optical density established by serial dilution before viable bacterial count) was used for oral challenge. Another group of pigs fed the unsupplemented diet were orally dosed with 30 mL of sterilized alkaline broth.

The experimental pigs were housed 3 piglets per pen in 180 × 170 cm raised weaner decks equipped with a mesh floor, and 6 pens were assigned to each treatment.
The temperature of pig barn was controlled between 26 and 28°C with a 12-h light:dark cycle. A 1-way traffic path was implemented to avoid nonchallenged pigs coming in contact with challenged pigs. All pigs had free access to feed and water throughout the 2-wk feeding trial. Pigs and feeders were weighed at 0750 h on d 0, 7, and 14 to calculate BW gain, feed intake, and BW gain efficiency. Fecal consistency was visually assessed at 0800 h each morning from d 7 to 14, and diarrhea incidence was calculated based on the fecal consistency.

A 5-mL blood sample was obtained by vena cava puncture from 1 randomly selected pig per pen using a 9-mL clot activator tube (Greiner Bio-One GmbH, Kremsmunster, Austria) immediately before challenge, as well as at 24, 48, and 168 h postchallenge. Blood samples were centrifuged at 1,342 × g (Heraeus Biofuge 22R Centrifuge, Hanau, Germany) for 5 min at 4°C, and the serum samples were frozen immediately and stored at −20°C for later analysis for IL-1β, IL-10, and IGF-I.

At 0830 h on d 14, all pigs were humanely killed by exsanguination after electrical stunning. The small intestine was divided into 3 parts by cutting from the ligament of Treitz to the ileocecal valve. The contents of the jejunum, ileum, cecum, and colon were aseptically collected, pooled within pigs, and immediately immersed in liquid nitrogen and preserved at −80°C to −100°C until modification. In brief, the frozen samples were thawed to 4°C for a 10-h incubation before enumeration. Thereafter, 1 g of digesta was taken from each sample and serially diluted 10-fold with sterile physiological saline, resulting in dilutions ranging from 10⁻¹ to 10⁻³. Escherichia coli were cultivated on MacConkey Agar (Beijing Haidian Microbiological Culture Factory, Beijing, China). Each dilution was counted in triplicate, and the result was expressed as the mean of the 3 replicates. All plates were incubated at 37°C for 36 h. The microbial enumerations were expressed as log₁₀ cfu per gram. Bacteria were enumerated by a visual count of colonies using the best replicate set from dilutions that resulted in 30 to 300 colonies per plate or tube.

Statistical Analyses

All the data were subjected to ANOVA suited for a randomized complete block design by using the GLM procedure (SAS Inst. Inc., Cary, NC). The pen was the experimental unit. Repeated measures analysis was conducted for fecal consistency, plasma IL-1β, IL-10, and IGF-I to account for differences over time postchallenge. Statistical differences among treatments were separated by Duncan’s multiple-range tests. Results were expressed as least squares means and SEM. Probability values less than 0.05 were used as the criterion for statistical significance.

RESULTS

Pig Performance

During wk 1 (prechallenge), there were no differences in BW gain, feed intake, or gain efficiency among the
treatments (Table 2). During wk 2 (postchallenge), daily BW gain ($P = 0.01$) and feed intake ($P < 0.05$) were reduced in the unsupplemented-challenged pigs compared with the unchallenged pigs, whereas cyadox supplementation overcame this depression. Chito-oligosaccharide failed to improve BW gain or feed intake compared with the unsupplemented-challenged group. No treatment effects were observed for BW gain efficiency during wk 1 or 2.

**Fecal Consistency and Diarrhea Incidence**

*Escherichia coli* challenge decreased ($P < 0.05$) fecal consistency (Figure 1). However, pigs fed chito-oligosaccharides and cyadox had improved fecal consistency compared with the unsupplemented *E. coli*-challenged pigs but not to the same extent as unchallenged pigs. The effects of treatment on diarrhea incidence from d 7 to 14 postchallenge are presented in Figure 2. During wk 2 postinfection, unsupplemented pigs with *E. coli* challenge had greater ($P < 0.05$) diarrhea incidence than unchallenged pigs. Supplementation with chito-oligosaccharide or cyadox reduced diarrhea incidence of challenged pigs compared with unsupplemented-challenged pigs but not to the same extent as unchallenged pigs ($P < 0.05$).

**Histomorphology**

Villus height and villus height:crypt depth ratio in the mid-jejunum and ileum were decreased ($P < 0.05$) by *E. coli* challenge, whereas dietary supplementation with cyadox mitigated the challenge-induced damage to the intestinal epithelium (Table 2). Chito-oligosaccharide supplementation had no effect on intestinal morphology in the mid-jejunum but increased ($P < 0.05$) the villus-height-to-crypt-depth ratio in the ileum compared with the challenged, unsupplemented pigs. No difference was found in crypt depth among the treatments in the mid-jejunum or ileum.

**Quantification of Hormones and Cytokines in Plasma**

The effects of dietary treatment on plasma IL-1β, IL-10, and IGF-I concentrations at 0, 24, 48, and 168 h postchallenge are presented in Figure 3. At 24 and 48 h postinfection, *E. coli* challenged-unsupplemented pigs had greater ($P < 0.05$) plasma IL-1β concentrations than unchallenged pigs. The plasma IL-1β concentrations for chito-oligosaccharide-supplemented pigs were intermediate to the challenged and unchallenged piglets, whereas values for cyadox-supplemented pigs did not differ from the unchallenged pigs. At 24 and 48 h postinfection, plasma IL-10 concentrations of all 3 *E. coli* challenged groups were greater ($P < 0.05$) than the unchallenged pigs, but there was no difference in the plasma IL-10 concentrations among the 3 challenged groups (Figure 3). At 24 h postchallenge, plasma IGF-I concentrations of pigs from all 3 challenged groups were less ($P < 0.05$) than unchallenged pigs. Values for pigs in the cyadox-supplemented treatment were greater ($P < 0.05$) than challenged-unsupplemented pigs but still less than unchallenged pigs. At 48 h postinfection, plasma IGF-I concentrations of the chito-oligosaccharide and cyadox pigs increased to the concentration of unchallenged pigs and were greater ($P < 0.05$) than unsupplemented-challenged pigs.

**IgA-Positive Cells**

Pigs in the challenged-unsupplemented group had greater ($P < 0.05$) IgA-positive cell numbers in the jejunal and ileal lamina propria than pigs assigned to the other 3 treatments (Figure 4). There were no differences in IgA-positive cell numbers between the unchallenged pigs and the *E. coli*-challenged pigs fed diets supplemented with chito-oligosaccharide and cyadox.

**Microbiological Analysis**

Treatment effects on concentrations of *E. coli* in the jejunum, ileum, cecum, and colon contents are presented in Table 3. *Escherichia coli* challenge increased ($P < 0.05$) *E. coli* concentrations in the jejunum, ileum, cecum, and colon compared with nonchallenged pigs. Cyadox supplementation prevented the increase ($P < 0.05$) in *E. coli* concentration in the cecum and colon, but not the jejunal or ileum. Chito-oligosaccharide reduced ($P < 0.05$) *E. coli* concentrations in the cecum but not the other parts of the intestine compared with unsupplemented-challenged pigs.

**DISCUSSION**

The results of the current experiment are in close agreement with previous observations showing that the performance of weaned pigs is impaired by enterotoxigenic *E. coli* K88 challenge (Bosi et al., 2004; Ding et al., 2006; Jensen et al., 2006). However, supplementation of the diet of early-weaned pigs challenged with *E. coli* with cyadox reduced recovery time and improved challenge-induced signs as indicated by attenuated growth depression, decreased diarrhea incidence, reduced intestinal mucosal damage, decreased jejunal, and ileum IgA-positive cell numbers, and increased plasma hormone and cytokine concentrations.

Oligosaccharides, mainly fructooligosaccharides and mannanoligosaccharides, have previously been added to pig diets with varying degrees of success (White et al., 2002; Smiricky-Tjardes et al., 2003; Yuan et al., 2006). In the present experiment, dietary supplementation with chito-oligosaccharide alleviated some, but not all, of the signs associated with infection of weaned pigs challenged with *E. coli*.

The most obvious change was an improvement in fecal consistency and reduced incidence of diarrhea. Diarrhea caused by infectious disease is a serious problem...
Table 2. Performance and intestinal morphology of nonchallenged control pigs or pigs orally inoculated with *Escherichia coli* K88 and fed an unsupplemented control diet or diets supplemented with 160 mg of chito-oligosaccharide or 100 mg of cyadox/kg of diet.

<table>
<thead>
<tr>
<th>Item</th>
<th>Unchallenged + unsupplemented</th>
<th>Challenged + unsupplemented</th>
<th>Challenged + chito-oligosaccharide</th>
<th>Challenged + cyadox</th>
<th>SEM</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Wk 1 (prechallenge)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Daily BW gain, g</td>
<td>158.6</td>
<td>161.0</td>
<td>160.2</td>
<td>165.2</td>
<td>11.8</td>
<td>0.79</td>
</tr>
<tr>
<td>Daily intake, g</td>
<td>223.3</td>
<td>226.8</td>
<td>224.0</td>
<td>229.7</td>
<td>12.1</td>
<td>0.82</td>
</tr>
<tr>
<td>BW gain efficiency (G:F)</td>
<td>0.71</td>
<td>0.71</td>
<td>0.71</td>
<td>0.72</td>
<td>0.02</td>
<td>0.83</td>
</tr>
<tr>
<td>Wk 2 (postchallenge)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily BW gain, g</td>
<td>287.2a</td>
<td>242.4b</td>
<td>270.0ab</td>
<td>283.3a</td>
<td>28.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Daily intake, g</td>
<td>407.8a</td>
<td>380.7b</td>
<td>391.0ab</td>
<td>402.9a</td>
<td>18.1</td>
<td>0.03</td>
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<tr>
<td>BW gain efficiency (G:F)</td>
<td>0.71</td>
<td>0.63</td>
<td>0.69</td>
<td>0.70</td>
<td>0.05</td>
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<tr>
<td>Mid-jejunum</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Villus height, μm</td>
<td>364.2a</td>
<td>321.6b</td>
<td>341.3ab</td>
<td>358.6a</td>
<td>25.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Crypt depth, μm</td>
<td>108.7</td>
<td>116.6b</td>
<td>113.8</td>
<td>112.4</td>
<td>9.3</td>
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<tr>
<td>Villus height: crypt depth</td>
<td>3.38a</td>
<td>2.77b</td>
<td>3.02ab</td>
<td>3.20b</td>
<td>0.39</td>
<td>0.03</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villus height, μm</td>
<td>332.2a</td>
<td>295.6b</td>
<td>312.4ab</td>
<td>322.6a</td>
<td>21.3</td>
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<tr>
<td>Crypt depth, μm</td>
<td>102.5</td>
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<tr>
<td>Villus height: crypt depth</td>
<td>3.25a</td>
<td>2.70b</td>
<td>2.99a</td>
<td>3.09a</td>
<td>0.29</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Means in the same row with different superscripts differ (P < 0.05).

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Figure 1. Fecal consistency (experimental d 7 to 14) of nonchallenged control pigs (NONC) or pigs orally challenged with *Escherichia coli* K88 and fed either an unsupplemented control diet (CHAC) or diets supplemented with 160 mg of chito-oligosaccharide (COS) or 100 mg of cyadox (Veterinary Research Institute of Huazhong Agricultural University, Wuhan, China)/kg of diet (CYX). Fecal consistency was assessed each morning at 0800 h and recorded as 0 = solid; 1 = semi-solid; 2 = semi-liquid; and 3 = liquid. Data are means ± SEM. a–cWithin each day, means without the same letter differ, P < 0.05.
in weaned animals, which usually leads to an increased mortality rate (Glass et al., 1991; Osek, 1999). The rationale behind the present experiment originated from the observation that dietary oligosaccharides improved animal performance and enhanced host health status by modulating the types of intestinal microbiota (Gibson and Roberfroid, 1995). Oligosaccharides have been shown to reduce the number of pathogenic bacteria such as _Escherichia coli_ and _Salmonella typhimurium_ (LeMieux et al., 2003; Wang et al., 2003) and increased the numbers of beneficial bacteria such as _Lactobacilli_ (Oli et al., 1998). These changes in the intestinal bacterial population are associated with decreased incidence of diarrhea (Oli et al., 1998). In the present experiment, reductions in _E. coli_ were observed in the cecum of chito-oligosaccharide treated pigs, but _E. coli_ numbers in other parts of the intestine were not different from untreated-challenged pigs or untreated-unchallenged pigs.

The decreased intestinal _E. coli_ concentration in the cecum of weaned pigs fed with the chito-oligosaccharide might be due to 1 or more of the following reasons. First, _N_ -acetyl glucosamine is a basic component of the structure of chito-oligosaccharide (Kim and Rajapakse, 2005), and is also a component of intestinal mucus (Podolsky, 1985) that serve as a receptor when bacteria bind to the gut of the host (Klemm and Schembri, 2000; Ofek et al., 2003). Therefore, chito-oligosaccharide comprised of _N_ -acetyl glucosamine may bind to certain types of bacteria (Klemm and Schembri, 2000; Ofek et al., 2003) and possibly interfere with their adhesion in mammalian cells (Stanley et al., 2000; Rhoades et al., 2006). Second, chito-oligosaccharide can serve as a fermentable substrate for beneficial intestinal bacteria (Lee et al., 2002; Huang et al., 2005; Li et al., 2007), which may induce increased organic acid production to reduce intestinal pH (Gidenne, 1996; Mikkelsen et al., 2003). Therefore, the decreased intestinal pH will minimize intestinal pathogen concentration and alleviate postweaning diarrhea in young animals (Peeters et al., 1995; Mourão et al., 2006).

Villus:crypt ratio represents the nutrient digestion and absorption capacity of the small intestine (Pluske et al., 1996; Montagne et al., 2003). In the current experiment, dietary supplementation of chito-oligosaccharide increased the villus:crypt ratio in the ileum overcoming the damage caused by the _E. coli_-challenge. These results are consistent with previous studies in broilers (Wang et al., 2003) and rats (He et al., 2006). Other oligosaccharides, including mannan-oligosaccharides and fructo-oligosaccharide, have also been effective in improving gut morphology in turkeys (Savage et al., 1996) and pigs (Spencer et al., 1997).

Proinflammatory cytokine response to immunological challenge is an important criterion reflecting the extent of cellular immunity (Johnson, 1997). Overproduction of cytokines such as IL-1β not only causes redistribution of nutrients away from the growth processes in support of the immune system (Wannemacher, 1977), but also inhibits IGF-1 mRNA expression (Thissen and Verniers, 1997), which reduces feed intake (Liu et al., 2003). In the present study, it was observed that dietary supplementation of chito-oligosaccharide attenuated the challenge-induced changes in plasma IL-1 and IGF-1. Tang et al. (2005) previously reported increases in IGF in pigs treated with oligosaccharides.

The concentrations of IgA-positive cells have been used as a sensitive indicator of intestinal immune responses after 1 wk of _E. coli_ oral challenge (Ahren et al., 1998). Pathogenic bacteria increase production of IgA-positive cells (Ding et al., 2006). In the current experiment, we found that _E. coli_-challenged pigs had increased jejumum and ileum IgA-positive cells compared with nonchallenged pigs, but chito-oligosaccharide prevented this increase.

We have previously studied the effects of chito-oligosaccharides on the performance of unchallenged weaned pigs (Liu et al., 2008). Diets fed to weaned pigs were supplemented with 0, 100, 200, and 400 mg of chito-oligosaccharides/kg and 100 and 200 mg of chito-oligosaccharides/kg improved both growth and BW gain efficiency. Broken-line analysis indicated that the chito-oligosaccharide quantity that maximized BW gain was 158.8 mg/kg, and that was the basis for choosing the amount of chito-oligosaccharide used in the present study. However, using _E. coli_-challenged pigs, 160 mg of oligosaccharide/kg did not improve growth or BW gain efficiency. Whether larger amounts would effectively...
improve performance in E. coli-challenged pigs is not known and requires further study.

In conclusion, dietary supplementation with 160 mg of chito-oligosaccharide/kg reduced incidence of diarrhea and alleviated many of the signs associated with infection in weaned pigs challenged with E. coli. However, the performance of E. coli-challenged pigs supplemented with chito-oligosaccharide was not better than that of unsupplemented pigs challenged with E. coli. Therefore, chito-oligosaccharide, at the amounts used in the present experiment, does not seem to be an effective substitute for antibiotics as a growth promoter for recently weaned pigs when subjected to bacterial challenge.

Figure 3. Plasma IL-1β, IL-10, and IGF-I concentration of nonchallenged control pigs (NONC) or pigs orally challenged with Escherichia coli K88 and fed either an unsupplemented control diet (CHAC) or diets supplemented with 160 mg of chito-oligosaccharide (COS) or 100 mg of cyadox (Veterinary Research Institute of Huazhong Agricultural University, Wuhan, China)/kg of diet (CYX). Data are means ± SEM. *Within each hour, means without common letters differ, \( P < 0.05 \).

Table 3. Intestinal Escherichia coli K88 (log cfu/g) counts 7 d postchallenge of nonchallenged control pigs or pigs orally inoculated with E. coli K88 and fed either an unsupplemented control diet or diets supplemented with 160 mg of chito-oligosaccharide or 100 mg of cyadox\(^1\)/kg of diet

<table>
<thead>
<tr>
<th>Location in intestine</th>
<th>Unchallenged + unsupplemented</th>
<th>Challenged + unsupplemented</th>
<th>Challenged + chito-oligosaccharide</th>
<th>Challenged + cyadox</th>
<th>SEM</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>2.00(^b)</td>
<td>2.32(^a)</td>
<td>2.20(^ab)</td>
<td>2.13(^ab)</td>
<td>0.20</td>
<td>0.02</td>
</tr>
<tr>
<td>Ileum</td>
<td>1.95(^b)</td>
<td>2.20(^a)</td>
<td>2.12(^ab)</td>
<td>2.04(^ab)</td>
<td>0.17</td>
<td>0.04</td>
</tr>
<tr>
<td>Cecum</td>
<td>3.09(^b)</td>
<td>3.49(^a)</td>
<td>3.26(^b)</td>
<td>3.18(^b)</td>
<td>0.22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Colon</td>
<td>3.31(^b)</td>
<td>3.70(^a)</td>
<td>3.54(^ab)</td>
<td>3.43(^a)</td>
<td>0.26</td>
<td>0.04</td>
</tr>
</tbody>
</table>

\(^a\)Means in the same row with different superscripts differ (\( P < 0.05 \)).

\(^1\)Veterinary Research Institute of Huazhong Agricultural University, Wuhan, China.
Figure 4. Immunoglobulin A-positive cells in jejunum and ileum lamina propria of nonchallenged control pigs (NONC) or pigs orally inoculated with *Escherichia coli* K88 and fed either an unsupplemented control diet (CHAC) or diets supplemented with 160 mg of chito-oligosaccharide (COS) or 100 mg of cyadox (Veterinary Research Institute of Huazhong Agricultural University, Wuhan, China)/kg of diet (CYX) 7 d postchallenge. Data are means ± SEM. **a** Means without common letters differ, $P < 0.05.$

LITERATURE CITED


