Dietary Inclusion of Colicin E1 Prevents Post-weaning Diarrhea in Pigs

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Summary and Implications
Including a purified antimicrobial peptide, Colicin E1, at a rate of 22mg/kg diet in a corn-soy based diet for weanling pigs prevented experimentally induced (E. coli F18) post-weaning diarrhea. Colicins may be an effective alternative to conventional antibiotics for use in animal feed.

Introduction
Post-weaning diarrhea (PWD), caused by E. coli infections, is one of the most prevalent disease problems in pigs in the U.S. (USDA, 2001). More than 43% of the large swine facilities in the U.S. reported E. coli infections among weaned pigs in 2000, and in an attempt to prevent the spread of these infections more than 78% of these facilities reported using prophylactic antibiotic treatments (USDA, 2001). Despite the use of traditional antibiotic prophylaxis, these infections still cause substantial losses to producers, due to both mortality and morbidity. The E. coli strains considered primarily responsible for these diseases in pigs are F4 (K88) and F18 (Fryndal, 2002; Bertshinger, 1999), and the lack of effective disease prevention with the use of prophylactic antibiotics is not surprising, due to the frequency and spectrum of antibiotic resistance seen in these strains (Choi et al., 2002; Lanz et al., 2003). With worldwide concern over the use of prophylactic antibiotics in animal agriculture, and their contribution to the spread of antibiotic resistance (WHO, 2000; FDA Guidance #78, 1999; #152, 2002), the development of alternatives to conventional antibiotics is urgently needed to protect swine from these E. coli infections.

Materials and Methods
Twenty-four barrows, all genetically susceptible to E. coli F18 infection, were weaned at 17d of age on to a commercial weaning diet that contained no antibiotics. All pigs were given 4d to adjust to their new diet and then were then moved to individual pens and fed corn-soy pelleted diets that contained either 0 (Control), 11 (Low Dose), or 22 (High Dose) mg Colicin E1/kg diet. After 2d of being fed the colicin containing diets, all pigs were orally inoculated with 9 log CFU of two E. coli F18 strains that were isolated from pigs with PWD. Body weight and feed intake were recorded for each individual pig daily for 5 days post-inoculation. Fecal scores were collected twice daily (scores ranged from 0 – 5 with a score of 4 indicating episodic, unformed diarrhea), and fecal swabs for bacterial enumeration were collected daily. Sections of ileum were collected for histopathological analysis as well as RNA extraction and bacterial culture.

Results and Discussion
Within 24h post-challenge, one pig in the Control group had severe diarrhea. By the end of the second day post-challenge, 3 of the 8 Control pigs had fecal scores of 4-5, indicating episodic or persistent diarrhea. Forty-eight hours post-challenge, 2 Low Dose pigs had diarrhea. Average fecal scores for the second day after bacterial challenge were 2.4 for the Control, 1.5 for the Low Dose, and 0.5 for the High Dose groups. On the third day post-challenge, 5/8 Control animals had persistent diarrhea that remained until the end of the 5 day study. An additional pig in the Control group demonstrated slight diarrhea on day 5. A similar pattern was seen among the Low Dose group, although their fecal scores tended to be lower and elevated later after bacterial challenge (Figure 1). Throughout the study, only 1 pig in the High Dose group had any indication of diarrhea, and that was seen only on the last day of the study.

In the Control group (see Table 1), 5 of the 8 pigs failed to gain greater than 200g over the length of the study, compared to only 1 in the High Dose group and 2 in the Low Dose group. Average weight gain over the 5 days post-challenge was 380g for Control, 540g for the Low Dose, and 940g for the High Dose pigs. Feed intake was greatest (p<0.05) among the Control group (1,578g/pig) as compared with either of the Colicin E1 fed groups (1222 and 1398g for the Low Dose and High Dose groups, respectively). Feed conversion was dismal for the Control group as compared to the high group (3.6 vs 1.98), though not statistically significant due to the enormous standard error caused by having negative values for individual body weight gain in two animals in this group.

Fecal swab bacterial recovery was higher among Control pigs compared to both the Low and High Dose groups. Total coliforms as well as challenge strain recovery (as indicated by selective media) from the ileum were higher in the Control group compared with either treatment group. Based on these results, the addition of Colicin E1 to the post-weaning swine diet may be an effective alternative to conventional antibiotics.
Table 1. Growth performance of pigs after oral *E. coli* F18 challenge.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BW gain, g</th>
<th>SEM</th>
<th>Feed intake, g</th>
<th>SEM</th>
<th>Feed intake:BW gain</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16</td>
<td>1578&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62</td>
<td>3.6</td>
<td>0.94</td>
</tr>
<tr>
<td>Low Dose</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16</td>
<td>1222&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62</td>
<td>2.83</td>
<td>0.74</td>
</tr>
<tr>
<td>High Dose</td>
<td>0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.16</td>
<td>1398&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62</td>
<td>1.98</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Values presented are means and standard errors of the means (SEM). Means within a column not sharing a common superscript are significantly different (P < 0.05).

Figure 1. Effect of dietary Colicin E1 inclusion on the incidence of post-weaning diarrhea in young pigs.