Dietary inclusion of Colicin E1 is effective in preventing Escherichia coli F18 post-weaning diarrhea in pigs.

Running Title:
Colicin E1 prevents post-weaning diarrhea

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Abstract

With world-wide concern over the use of antibiotics in animal agriculture and their contribution to the spread of antibiotic resistance, alternatives to conventional antibiotics are needed. Previous research in our lab has shown that Colicin E1 (ColE1) is effective against some *E. coli* strains responsible for post-weaning diarrhea (PWD) in vitro. In this study we examined the efficacy of dietary inclusion of ColE1 in preventing experimentally induced F18-positive enterotoxigenic *E. coli* caused PWD in young pigs.

Twenty-four weaned pigs (23 days of age), identified by genotyping to be susceptible to F18-positive *E. coli* infections, were individually housed and fed diets containing 0, 11, or 16.5 mg ColE1/kg diet. Two days after the start of the trial, all animals were orally inoculated with $1 \times 10^9$ CFU each of 2 F18-positive *E. coli* strains isolated from pigs with PWD. The dietary inclusion of ColE1 decreased the incidence and severity of F18-positive enterotoxigenic *E. coli* caused PWD and improved the growth performance of the piglets. Additionally, the reduced incidence of PWD, due to dietary ColE1, lowered the gene expression of *IL1β* and *TNFβ* in ileal tissue from these animals. Dietary inclusion of ColE1 may be an effective alternative to conventional antibiotics in weaning pig diets for the prevention of PWD caused by F18-positive enterotoxigenic *E. coli*.
Introduction

Post-weaning diarrhea (PWD) is a serious threat to the economic success of the swine industry, due both to losses as a result of mortalities, as well as reduced growth performance of surviving pigs. It is estimated that 50% of piglet mortality due to diarrhea is attributable to the causative agent of PWD, enterotoxigenic *Escherichia coli* (ETEC) (18). The ETEC strains most commonly associated with PWD possess either the F4 or F18 fimbral type (11, 39). As a result of the significant impact that F18-positive ETEC and other bacterial infections can have on pig production, prophylactic antibiotics are frequently included in the diets of young pigs in an attempt to prevent ETEC colonization and the resulting PWD. An estimated 78% of large swine farms in the U.S. include subtherapeutic antibiotics in the diets for young pigs (37). Despite the use of antibiotic prophylaxis, 40.7% of these farms reported an incidence of diarrhea caused by *E. coli* infections (37). The lack of effective PWD prevention with the use of prophylactic antibiotics is not surprising, because of the frequency and spectrum of antibiotic resistance seen among ETEC strains (7, 23, 25). It is expected that antibiotic resistance will further increase among these strains, based on the overall increase in resistance to antibiotics by ETEC over the last 20 years (25).

With worldwide concern over the use of prophylactic antibiotics in animal agriculture and its contribution to the spread of antibiotic resistance (12, 34, 38), the development of alternatives to conventional antibiotics is urgently needed to protect swine from these *E. coli* infections. Public concerns surrounding the antibiotic resistance issue led to the elimination of prophylactic antibiotic use in animal agriculture in Denmark (20, 34). This cessation of the use of prophylactic antibiotics in pig production
caused increases in the rate of PWD and a 30% increase in piglet mortality (34). These infections led to a large increase in veterinarian prescribed antibiotic use in Denmark’s swine industry (35). The switch from growth promoting or prophylactic antibiotic usage to only veterinary prescribed therapeutic usage resulted in a very modest, if any, reduction in total antibiotic usage in Denmark’s swine industry (35). In order to realize a true reduction in antibiotic use in animal agriculture, effective alternative therapies must be developed.

A potential alternative to conventional antibiotics that holds a great deal of promise are colicins. Colicins are a class of bacteriocins produced by, and effective against, *E. coli* and closely related bacteria (14). Pore forming colicins, such as Colicin E1 (ColE1), bind to their target bacteria and kill them by disrupting the ionic gradient of the cell (14). These proteins are particularly attractive for use as an alternative to conventional antibiotics for the control of *E. coli* caused PWD for several reasons. We have previously shown them to be effective against ETEC strains isolated from pigs with PWD *in vitro* (33), and other work has demonstrated that ColE1 is effective against a wide range of *E. coli* (22, 27, 30). They are also not related to any antibiotics that are currently being used in human medicine. Additionally, colicins would not be absorbed intact by the animals, thereby eliminating concerns over antibiotic residues in meat, and colicins could be effective at low enough concentrations so as not to significantly alter the nutrient density of the diet. The objective of this study was to determine the efficacy of dietary inclusion of ColE1 in preventing PWD due to F18-positive ETEC.

**Materials and Methods**
Colicin Production

Colicin E1 was produced and purified to homogeneity according to the method of Stahl, et al. (33). Briefly, a ColE1 producing strain of *E. coli* was grown in LB and colicin production was induced by the addition of Mitomycin C (EMD Biosciences, San Diego, CA) to the media. The ColE1 was purified from the cell free supernatant by ion exchange chromatography, first utilizing DEAE cellulose (Sigma-Aldrich, St. Louis, MO) and then further purifying the protein utilizing Q sepharose (GE Healthcare, Piscataway, NJ). The purity of the Colicin E1 used in this study has been visualized by SDS-PAGE and is estimated at over 95% purity (30).

ETEC challenge strains

*Escherichia coli* F18 strains 2144 (O147:NM) and S1191(O139) were used as challenge strains. Strain S1191 was isolated from a pig with edema disease, produces heat stable toxins STa, STb, and Shiga toxin 2e, and is chloramphenicol resistant. Strain 2144 was a field isolate that was made nalidixic acid resistant and produces the toxins STa and STb. Both strains were grown overnight as pure cultures in LB at 37°C with shaking. They were then individually diluted to an OD$_{600}$ = 0.1 in fresh LB and allowed to grow to an OD$_{600}$~1. The cultures were then centrifuged at 4,000 × g for 10 min at 4°C. The bacterial pellets were resuspended in 20% dextrose and 5% non-fat dry milk. The challenge dose consisted of an equal amount of each strain and was determined by serial diluting and plating to provide a total 2×10$^9$ CFU/0.5 ml oral dose.

 Animals

All of our protocols involving animals were approved by the Institutional Animal Care and Use Committee of Iowa State University. The 24 barrows (castrated pigs)
utilized were obtained from the Iowa State University Swine Nutrition Farm and were determined to be susceptible to F18-positive *E. coli* infections based on a restriction fragment length polymorphism test described by Frydendahl et al. (15). Briefly, genomic DNA was purified (DNeasy Kit, Qiagen, Valencia, CA) from pig tail clippings and primers (forward - TTGGGAACCAGATGGGACAGTATG and reverse - CCCGCCAAGGAGCGTGCTGTCTA) were used to amplify a 162 bp section of the 1,2*α* fucosyltransferase enzyme gene (*ECF18R*) by PCR (26). The 162 bp PCR product was then digested with *HhaI* (New England Biolabs, Ipswich, MD) and the polymorphism was determined by size comparison on a 3% agarose gel. Pigs were weaned at 17 d of age and allowed to adjust to solid food (TechStart 17-25, Kent Feeds, Muscatine, IA). At 21 d of age, the pigs were allotted into treatment groups based on body weight (n = 8) and transferred to individual pens. Pigs were given 2 days to adjust to individual housing before the experimental diets were fed. The basal diet for all of the experimental diets was corn and soy based and contained no animal products (26% crude protein, 3.51 kcal/kg). This diet met or exceeded the nutrient requirements of these pigs based on the NRC (1998) requirements (29). Either 0 (control), 11, or 16.5 mg of purified ColE1 (supplied at 10 mg/mL in 10 mM Tris, pH 7.6) was added per kg to the basal diet, and the diets were then pelleted at low temperature (Purina TestDiet, Richmond, IN). The pelleted rations were provided to the pigs twice daily at a rate which exceeded consumption for each animal (approximately 500 g/d). Unconsumed feed was weighed daily and feed intake was determined. After receiving the experimental diets for 2 days, all animals were orally inoculated with the two F18-positive ETEC strains and their fecal scores were recorded.
Fecal scores were determined (0 = dry, hard, and well formed feces; 1 = soft, but formed feces; 2 = pasty and green/brown in color; 3 = viscous and light in color, episodic; 4 = fluid and light in color, episodic; 5 = watery and continuous) twice daily after the bacterial challenge. Fecal samples were obtained 2 days prior to the ETEC challenge and daily after the challenge by inserting a 10 µl fecal loop (Fisher Scientific, Pittsburgh, PA) into the rectum. These samples were immediately diluted in 5 ml of sterile PBS. Serial 10 fold dilutions, (up to 1:100,000) were plated onto MacConkey’s (MAC, Difco, Franklin Lakes, NJ), MAC + chloramphenicol, and MAC + nalidixic acid agar plates for CFU determination. Our limit of detection for the fecal samples was 10,000 CFU/g.

Four days post challenge, all pigs were euthanized by captive bolt and tissue samples were collected. Ileal sections (approximately 10cm each) were collected from each pig for RNA extraction and *E. coli* enumeration. Additionally, rectal and cecal contents were collected for *E. coli* enumeration.

**Gene expression**

Isolation of RNA from the ileum was performed utilizing a whole ileal homogenate and the RNeasy kit (Qiagen, Valencia, CA). Genomic DNA was eliminated from the extracted total RNA using the DNA-free kit from Ambion (Austin, TX) according to the manufacturer’s instructions. The RNA was reverse transcribed using Superscript III (Invitrogen Life Technologies, Carlsbad, CA), and the RNA was removed from the resulting cDNA by incubation with *E. coli* RNase H (Invitrogen) according to the manufacturer’s instructions. Concentrations of cDNA were determined utilizing the Quant-it kit (Invitrogen), and the cDNA samples were then stored at -80°C until analysis by real-time PCR. The levels of interferon (*IFN*), interleukin 10 (*IL10*), interleukin 8
(IL8), tumor necrosis factor α (TNF α), tumor necrosis factor β (TNF β), interleukin1β (IL1β), and inducible nitric oxide synthase (iNOS) mRNA were semi-quantitatively determined by real-time PCR using the MyiQ™ Single Color Real-Time PCR Detection System and Sybr Green (Bio-Rad Laboratories; Hercules, CA). Thermal cycling conditions included 45 cycles of 30 s of melting at 95°C followed by 30 s of annealing and extension at 65°C. Following amplification, all samples were subjected to a melt curve analysis to insure that only a single product was formed. Primer oligonucleotides (Table 1) were designed using “PrimerQuest” software available from Integrated DNA Technologies (Coralville, IA). All primer sets were validated to amplify only the sequence of interest and to do so in a linear fashion over a 2 log range of cDNA concentrations. The data from all samples were normalized to cDNA concentration prior to statistical analysis.

**Bacterial enumeration**

Enumeration of *E. coli* from the ileal mucosa, cecal content, and rectal content samples was performed as follows. Samples were weighed, diluted 1:2 with buffered peptone water and placed in a stomacher blender (Seward Stomacher 80, Worthing, UK) for 30 s. The samples were then serially diluted over a 4 log range at a 1:200 starting dilution. Ten µl of each dilution was plated in duplicate on MAC, MAC + chloramphenicol (30µg/ml), and MAC + nalidixic acid (50 µg/ml) agar plates. Plates were incubated for 16 hours at 37°C and the countable dilution was then recorded. Our limit of detection for these samples was 1000 CFU/g.

**Statistical analysis**
Statistical analysis of the data was performed with Statistical Analysis Software version 9.1 (SAS, Cary, North Carolina) utilizing the GLM procedure for comparison of least squares means. Treatment was considered a fixed effect, and for growth parameters initial body weight was used as a covariate in the analysis. For bacterial enumeration data, when counts were below our limit of detection, a value of 1 CFU/g less than our limit of detection was ascribed prior to analysis. Statistical significance was set at an $\alpha = 0.05$ and trends are discussed at an $\alpha = 0.1$.

**Results**

**Fecal Scores**

Prior to bacterial challenge, there was no indication of diarrhea or loose stool among any of the pigs. There were no differences in fecal scores between the treatment groups until 48h post-challenge. At this point the group fed the control diet had a mean fecal score of 2.38 which tended to be higher ($P < 0.06$) than that of the group fed the 16.5 mg ColE1/kg diet (mean score of .5) (Figure 1). Over the entire study, the control group had a significantly higher ($P < 0.05$) average fecal score than the high dose (16.5 mg/kg) ColE1 group (2.1 vs. 0.59, respectively). The low dose (11mg/kg) ColE1 group also had higher ($P < 0.05$) average fecal scores than the high dose group, and these were not significantly different from those of the control pigs. These differences in group fecal scores were caused by the incidence of diarrhea (fecal scores $\geq 4$) in 1, 4, and 5 out of the 8 control group pigs after 24, 48, and 72h post-ETEC inoculation, respectively. Among the pigs fed the low dose of ColE1, no animals had diarrhea 24h post-inoculation, however 3 and 4 pigs had diarrhea at 48 and 72h post-challenge, respectively. None of
the pigs fed the high dose of ColE1 had any incidence of diarrhea at any time during the experiment.

**Growth Performance**

Dietary inclusion of ColE1 had a significant effect (P < 0.05) on the growth performance of the pigs in this study (Table 2). From the time of *E. coli* challenge until the completion of the study, pigs fed the control diet gained an average of 380 g while the pigs receiving the low and high doses of ColE1 in their diets gained 540 and 940 g, respectively (Table 2). Although the animals fed the control diet gained the least body weight over the course of the study, they consumed significantly (P < 0.05) more feed than either of the groups fed diets containing ColE1. The control group animals averaged a total consumption of 1.54 kg of diet, while the ColE1 treated pigs ate 1.22 kg and 1.44 kg, for the low and high dose ColE1 diets, respectively. Although there were significant differences in both body weight gain and feed consumption, there was not a significant difference in feed conversion efficiency (body weight gain/feed intake, P < 0.19) among any of the treatment groups. This was due to 2 of the pigs in the control group losing weight (approximately 400 g each) and 1 pig gaining virtually no weight (less than 10 g) over the length of the study while still consuming feed. This negative body weight gain resulted in a negative feed conversion efficiency, which resulted in tremendous variation in the feed conversion efficiency values for this group.

**Bacterial enumeration**

**Fecal cultures**

There were no colonies isolated on the agar plates containing either chloramphenicol or nalidixic acid from any samples prior to the ETEC challenge. At 24
h post-inoculation, levels of both total coliforms and ETEC challenge strain 2144
recovered in the feces were significantly higher (P < 0.05) in the control animals
compared with both groups of ColE1 fed pigs (Table 3). There were no significant
differences between groups in the recovery of the ETEC S1191 strain at the first day post
challenge, but in the following day there was a reduction in the high dose ColE1 group as
compared to the controls (P<0.05). By the last day of fecal sampling, the levels of the
S1191 strain had dropped below our detection limit for most of the animals regardless of
dietary treatment (16/24 pigs) and there were no significant differences in the amount of
the 2144 strain recovered.

Tissues

In the ileum, both ColE1 fed groups averaged lower levels (P < 0.05) of the
S1191 challenge strains and coliforms as compared to the controls (Table 4). There were
no significant differences in the recovery of bacteria from the cecal samples among the
groups. The S1191 strain was only recovered from samples from 3 of the 8 animals in
the high dose ColE1 group compared with 6 and 4 of the 8 animals in the control and low
dose ColE1 treatment, respectively. In the rectum, similar levels of total coliforms and
2144, and S1191 were recovered for animals in all groups.

Gene Expression

The concentration of TNFβ mRNA in ileal tissue was higher (P < 0.06) in the
control animals than in either of the ColE1 treated groups (Figure 2). The amount of
IL1β mRNA was highest in the low dose ColE1 supplemented group (6 fold higher (P <
.01) than in the controls), whereas the high dose ColE1 group had nearly undetectable
levels (Figure 2). The levels of expression of both TNFα and iNOS tended (P < 0.1) to be
greater in control animals than in animals receiving the diets with ColE1 supplementation. There were no significant differences in the concentration of IFNγ, IL8, or IL10 message in the ileum among any of the groups.

Discussion

We examined the efficacy of ColE1 in preventing E. coli F18 caused post-weaning diarrhea (PWD), because this disease has been estimated to be responsible for as much as 50% of the economic losses seen in the production of weaned pigs (19, 36). In herds with PWD, up to 2% mortality (21, 36) in weaning pigs can be seen, but of greater economical significance is the morbidity and reduction in growth performance in the pigs that survive these infections. Although there is a need for alternatives to conventional antibiotics, the development of compounds to combat ETEC associated PWD is beset with difficulties including having an adequate experimental model of ETEC infection. Establishing a good challenge model for this disease has proven to be difficult. Madec et al. (24) utilized four strains of ETEC and several different dosing methods in six trials utilizing a total of 168 specific pathogen free piglets and was only able to generate clinically significant, although transient, diarrhea in 50% of animals (24). Using a viral co-infection such as transmissible gastroenteritis (TGEV) or rotavirus that leads to immunosuppression and intestinal membrane disruption can also increase experimental ETEC infection rates (1, 9, 16, 28). While a co-challenge model can increase the success rate of experimentally reproducing PWD, it also adds a potential confounding effect particularly if the viral infection alone causes reduced growth performance. An alternative to the viral co-infection model that has also been shown to increase the
success of an ETEC bacterial challenge is modification of the post-weaning diet. The removal of all animal based protein sources from the post-weaning diet can increase the susceptibility of the pigs to ETEC infection due to a transient intestinal inflammatory response (2, 10). Diets designed to be fed to pigs immediately post-weaning typically provide over 40% of the total protein in the diet as protein from animal sources in part to help prevent this temporary inflammatory response. However, due to the higher cost of animal based protein sources there is a constant push to minimize the amount of animal protein included in pig diets. While both viral co-challenges and dietary alterations can increase infection rates in experimentally induced PWD models, identifying the genetic susceptibility of the animals may offer the most efficient way to increase infection rates in a challenge model. With the use of pre-screening for a F18 receptor polymorphism, the rate of infection can be increased from 5.9% in the F18 resistant genotype pigs to 87% in those that are genetically susceptible (15). In our study, we utilized only genetically susceptible animals, as well as a weaning diet that contained no animal protein. With this model we achieved a 75% infection rate in control animals with no mortality after 4 days, no viral co-infection, and an easily-performed one-time oral challenge.

Although our high dose of ColE1 (16.5mg/kg of diet) was able to eliminate all clinical signs of PWD, our low dose of ColE1 appeared to only be able to slightly delay the onset of PWD. The level of reduction of the challenge strains of E. coli that reached the ileum as a result of dietary ColE1 inclusion may have been the determining factor in the development of clinical disease. Colicin fed animals had significantly lower (P < 0.04) fecal shedding of the 2144 challenge bacteria at 1 day post-challenge. While this
suggests that the addition of colicin to the feed significantly reduced the amount of viable bacteria that reached the distal end of the small intestine, it also demonstrates that our colicin dose was not sufficient to completely eliminate the challenge strains. By the end of the study there were no significant differences based on colicin treatment on fecal shedding of either challenge strain. We would not anticipate that the feeding of ColE1 would have an effect on reducing the colonization of the ETEC strains if they reached the ileum in a viable state, since ColE1 is highly sensitive to proteolysis (3, 4, 6). It is likely that not all of the *E. coli* in our large challenge dose would be killed by the ColE1 present in the digesta prior to the inactivation of the ColE1 by proteolysis, but it appears that enough was eliminated as a result of high dose ColE1 supplementation to prevent disease.

The gene expression data also suggests that fewer of the challenge bacteria were able to cause the inflammatory response leading to diarrhea in the pigs. This is supported by the lower mRNA expression (P < 0.05) of *IL1β* and *TNFβ* in the ileal tissue of pigs fed the high ColE1 diet compared with the control. Interleukin 1β is primarily secreted by macrophages and activates lymphocytes during an inflammatory response and increases have been associated with *E. coli* toxin production (11, 17). While the concentration of *IL1β* message was significantly lower in the ileal tissue of the pigs fed the high dose of ColE1 compared with the control pigs, pigs in the low ColE1 dose group had mRNA levels that were over 6 fold higher than those of the control. At the time of tissue collection there was no longer any significant difference in fecal score between the low dose and the control groups and this elevated expression may indicate a delay in the inflammatory response as a result of the challenge bacteria. This would be reasonable since there appeared to be a delay in the onset of PWD with the low dose ColE1 fed pigs.
compared with the control animals. An increase in IL1β gene expression in the intestinal mucosa has been correlated with enteropathogenic E. coli challenge (16), although the associated increase in IL6, IL8, and IL10 seen by Girard et al. (16) was not noted in our study. Regardless of the dose, ColE1 in the diet caused reduced levels (P < 0.06) of expression of TNFβ. TNFβ, also known as lymphotoxin, is a primary effector of NO production and is associated with inflammatory responses related to T cell recruitment (13). The lower levels of TNFβ and IL1β in the ileal tissue of pigs fed the high dose of ColE1 compared with the control animals suggests that ColE1 was able to significantly reduce the bacterial load that initially reached the ileum in these animals; thereby, reducing the inflammatory response to the ETEC challenge.

While other researchers have examined the efficacy utilizing colicin producing bacterial cultures as probiotics for cattle in order to reduce E. coli 0157 contamination (32), we are the first, to our knowledge, to examine a purified colicin as a feed component for the prevention of an ETEC infection. We have demonstrated with growth performance data, clinical indicators of PWD, and intestinal gene expression that the inclusion of ColE1 can prevent experimentally induced PWD. The efficacy in preventing PWD at a 16.5mg/kg diet level of dietary inclusion, suggests that ColE1 warrants further evaluation as a potential alternative to conventional antibiotics for use in weaning pig diets. This protein may also have significant implications for human food safety as well, since the efficacy of ColE1 against many ETEC of concern for human food safety has been well-documented (5, 8, 31).

Acknowledgements
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References


22. Jordi, B. J., K. Boutaga, C. M. van Heeswijk, F. van Knapen, and L. J.


Stranzinger, and P. Vogeli. 2000. A DNA polymorphism influencing alpha(1,2)fucosyltransferase activity of the pig FUT1 enzyme determines
susceptibility of small intestinal epithelium to Escherichia coli F18 adhesion. Immunogenetics. 52:129-36.


Table 1. Primer sequences for semi-quantitative real-time PCR analysis of intestinal gene expression.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inducible Nitric Oxide Synthase (<em>iNOS</em>)</td>
<td>F: GGACGTACGAAAGTGACCAACC</td>
</tr>
<tr>
<td></td>
<td>R: GAACGTACGAAAGTGACCAACC</td>
</tr>
<tr>
<td>Interleukin 1 β (<em>IL1β</em>)</td>
<td>F: TGAAGAAGAGCCCATCGTCCT TGA</td>
</tr>
<tr>
<td></td>
<td>R: TGCAAAAGCTCATGCAGAACACC</td>
</tr>
<tr>
<td>Tumor Growth Factor β (<em>TGFβ</em>)</td>
<td>F: AGGCCGTACTGGCTTTTACAACA</td>
</tr>
<tr>
<td></td>
<td>R: TTGGTTGCCTTTCCACCATTAG</td>
</tr>
<tr>
<td>Interleukin 10 (<em>IL10</em>)</td>
<td>F: AAGACGTAATGCGCGAGGACAGAGA</td>
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<td></td>
<td>R: TGCTAAAGGGCACTCTTCACCTTCCT</td>
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<tr>
<td>Interferon γ (<em>IFN</em>)</td>
<td>F: ATGACTTCCAAAAGCTGGCTGTTGCC</td>
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<tr>
<td></td>
<td>R: TGCACTCGGATCGAAGTCTCTGC</td>
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<tr>
<td>Interleukin 8 (<em>IL8</em>)</td>
<td>F: ATGACTTCCAAAAGCTGGCTGTTGCC</td>
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<td>R: TGCACTCGGATCGAAGTCTCTGC</td>
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<tr>
<td>Tumor necrosis factor α (<em>TNFα</em>)</td>
<td>F: GCCCACGTGTAGCCAATGTCAAA</td>
</tr>
<tr>
<td></td>
<td>R: GTGTCTTTTCAGCTTCACGCGGT</td>
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<tr>
<td>Tumor necrosis factor β (<em>TNFβ</em>)</td>
<td>F: AGATCGCTGTCCAGACACACAGA</td>
</tr>
<tr>
<td></td>
<td>R: TAGAGCGAAAGGCTCCAAAAGGAGAC</td>
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Table 2. Effect of dietary inclusion of Colicin E1 on growth performance of weaning pigs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight gain, kg</th>
<th>SEM</th>
<th>Feed intake, kg</th>
<th>SEM</th>
<th>Feed Efficiency</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13</td>
<td>1.539&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.544</td>
<td>27.01</td>
<td>9.55</td>
</tr>
<tr>
<td>Low Dose</td>
<td>0.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.19</td>
<td>1.217&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.424</td>
<td>2.83</td>
<td>1.00</td>
</tr>
<tr>
<td>High Dose</td>
<td>0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33</td>
<td>1.442&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.510</td>
<td>1.94</td>
<td>0.69</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> values within a column not sharing a common superscript are different (P < 0.05)

1 Values presented are means and standard error of the means (SEM)

2 The Control, Low Dose, and High Dose treatments received 0, 11, and 16.5 mg Colicin E1/kg of diet, respectively.

3 Feed Efficiency is defined as feed intake/weight gain.
Table 3. Effect of dietary inclusion of Colicin E1 on *E. coli* levels recovered from the feces of weaning pigs\(^1\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total coliforms</th>
<th>SEM</th>
<th><em>E. coli</em> 2144(^3)</th>
<th>SEM</th>
<th><em>E. coli</em> S1191(^3)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1 day post-challenge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.1×10(^9)^a</td>
<td>4.6×10(^8)</td>
<td>1.7×10(^8)^a</td>
<td>8.8×10(^7)</td>
<td>4.9×10(^7)</td>
<td>4.3×10(^7)</td>
</tr>
<tr>
<td>Low Dose</td>
<td>1.5×10(^7)^b</td>
<td>5.6×10(^6)</td>
<td>1.2×10(^6)^b</td>
<td>3.7×10(^5)</td>
<td>7.4×10(^4)</td>
<td>2.3×10(^4)</td>
</tr>
<tr>
<td>High Dose</td>
<td>1.3×10(^7)^b</td>
<td>5.4×10(^6)</td>
<td>3.7×10(^3)^b</td>
<td>2.5×10(^6)</td>
<td>4.8×10(^4)</td>
<td>7.5×10(^3)</td>
</tr>
<tr>
<td><strong>2 days post-challenge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.6×10(^8)</td>
<td>2.9×10(^8)</td>
<td>1.6×10(^8)</td>
<td>9.8×10(^7)</td>
<td>4.7×10(^6)</td>
<td>2.7×10(^6)</td>
</tr>
<tr>
<td>Low Dose</td>
<td>5.0×10(^8)</td>
<td>2.9×10(^8)</td>
<td>9.1×10(^7)</td>
<td>6.7×10(^7)</td>
<td>3.2×10(^6)</td>
<td>3.1×10(^6)</td>
</tr>
<tr>
<td>High Dose</td>
<td>6.0×10(^8)</td>
<td>5.6×10(^8)</td>
<td>1.9×10(^8)</td>
<td>1.9×10(^8)</td>
<td>2.4×10(^5)</td>
<td>1.4×10(^5)</td>
</tr>
<tr>
<td><strong>3 days post-challenge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.0×10(^9)</td>
<td>5.3×10(^9)</td>
<td>6.3×10(^9)</td>
<td>6.2×10(^9)</td>
<td>6.6×10(^4)</td>
<td>2.6×10(^4)</td>
</tr>
<tr>
<td>Low Dose</td>
<td>9.4×10(^9)</td>
<td>7.3×10(^9)</td>
<td>2.9×10(^9)</td>
<td>2.5×10(^9)</td>
<td>3.6×10(^5)</td>
<td>3.6×10(^5)</td>
</tr>
<tr>
<td>High Dose</td>
<td>2.3×10(^8)</td>
<td>1.1×10(^8)</td>
<td>3.9×10(^7)</td>
<td>3.1×10(^7)</td>
<td>7.5×10(^5)</td>
<td>3.1×10(^5)</td>
</tr>
</tbody>
</table>

\(^{a,b}\) values within a day and column not sharing a common superscript are significantly different (P < 0.05).

\(^1\) Values presented are least square means and standard error of the means (SEM).

\(^2\) The Control, Low Dose, and High Dose treatments received 0, 11, and 16.5 mg Colicin E1/kg of diet, respectively.

\(^3\) *Escherichia coli* strain 2144 is F18-positive, produces the toxins STa and STb, and was made nalidixic acid resistant.
Escherichia coli strain S1191 is F18-positive, produces the toxins STa, STb, and Shiga toxin 2e, and is chloramphenicol resistant.
Table 4. Dietary inclusion of Colicin E1 affected bacterial recovery in the ileum of weaning pigs\textsuperscript{1}.

<table>
<thead>
<tr>
<th>Treatment\textsuperscript{2}</th>
<th>Total coliforms</th>
<th>SEM</th>
<th>\textit{E. coli} 2144\textsuperscript{3}</th>
<th>SEM</th>
<th>\textit{E. coli} S1191\textsuperscript{3}</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.7×10\textsuperscript{8a}</td>
<td>5.3×10\textsuperscript{7}</td>
<td>3.2×10\textsuperscript{8}</td>
<td>1.8×10\textsuperscript{6}</td>
<td>2.7×10\textsuperscript{5a}</td>
<td>1.0×10\textsuperscript{3}</td>
</tr>
<tr>
<td>Low Dose</td>
<td>1.1×10\textsuperscript{8b}</td>
<td>9.9×10\textsuperscript{7}</td>
<td>5.6×10\textsuperscript{7}</td>
<td>5.3×10\textsuperscript{7}</td>
<td>1.0×10\textsuperscript{3b}</td>
<td>0</td>
</tr>
<tr>
<td>High Dose</td>
<td>3.8×10\textsuperscript{7b}</td>
<td>1.7×10\textsuperscript{7}</td>
<td>2.7×10\textsuperscript{6}</td>
<td>1.8×10\textsuperscript{6}</td>
<td>2.5×10\textsuperscript{3b}</td>
<td>1.0×10\textsuperscript{3}</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b} value within a column not sharing a common superscript are significantly different (P < 0.05).

\textsuperscript{1} Values presented are least square means and standard error of the means (SEM).

\textsuperscript{2} The Control, Low Dose, and High Dose treatments received 0, 11, and 16.5 mg Colicin E1/kg of diet, respectively.

\textsuperscript{3} \textit{Escherichia coli} strain 2144 is F18-positive, produces the toxins STa and STb, and was made nalidixic acid resistant.

\textsuperscript{3} \textit{Escherichia coli} strain S1191 is F18-positive, produces the toxins STa, STb, and Shiga toxin 2e, and is chloramphenicol resistant.
Figure Legends

FIG. 1. Effect of dietary inclusion of Colicin E1 on incidence of diarrhea in weaning pigs following an F18-positive enteropathogenic *E. coli* challenge. Asterisks represent significant differences (P < 0.05) between the high dose group and both the low dose and control groups.

FIG. 2. Normalized gene expression of *TNFβ* and *IL1β* in ileal tissue samples 4 days post oral F18-positive enteropathogenic *E. coli* challenge. Gene expression was determined by semi-quantitative real-time PCR and was normalized to cDNA concentration. Values presented are least square means and the error bars represent the standard error of the mean. a,b values not sharing a common superscript are different (P < 0.06).
Control
Low Dose, 11 mg/kg
High Dose, 16.5 mg/kg

Incidence of Diarrhea, %

0 50 100

Days

1 2 3 4

* 

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