Dietary Inclusion of Colicin E.: Effect on Pig Behavior

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Summary and Implications

In the US prophylactic antibiotics are frequently included in the diets of weanling pigs to prevent post-weaning diarrhea (PWD). Despite this, PWD still causes substantial losses to the swine industry through both mortalities and morbidly. Additionally, the use of prophylactic antibiotics has become a concern among some groups regarding their potential contribution to the spread and creation of antibiotic resistant bacteria.

The objective of this study was to determine if dietary inclusion of Colicin E1 (ColE1) altered a pigs behavior and postures when challenged with *E. coli*. Twenty-four newly weaned barrows; 21 days of age were placed into individual pens. Three dietary treatments were compared; treatment 1 had no ColE1 (C; n=8), treatment 2 added 1.1 mg of purified ColE1 (L; n=8), and treatment 3 added 16.5 mg (H; n=8) of purified ColE1. Two behaviors were collected; time spent at the feeder trough and drinking. Three postures were collected; active and inactive and other. There were no ($P < 0.05$) effects on the time engaged in active, drinking and other. For pigs in the H group they engaged in more ($P < 0.05$) eating and less time in inactive postures than C pigs. From this study, it can be concluded that pigs with the highest coverage of ColE1 displayed less inactive behaviors and spent more time at the feeder indicating that ColE1 may have offered some form of protection against the *E. coli* challenge and therefore improved the individual pig's well-being.

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 worldwide is attributable to the causative agent of PWD, enterotoxigenic *Escherichia coli* (ETEC).

The ETEC strains most commonly associated with PWD in pigs possess the F18 fimibrial type. As a result of the significant impact that F18 ETEC 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. Despite the use of antibiotic prophylaxis, 48 % of these farms reported an incidence of disease caused by *E. coli* infections.

With worldwide concern over the use of prophylactic antibiotics in animal agriculture and its contribution to the spread of antibiotic resistance, the development of alternatives to conventional antibiotics is urgently needed to protect swine from these *E. coli* infections.

Worldwide concern over this use of antibiotics and its contribution to the spread of antibiotic resistance has led to increased regulation over the use of antibiotics in animal agriculture, and will likely continue. Based on the experience of the Danish swine industry following the ban of all growth promoting and prophylactic antibiotics, it was estimated that the removal of these antibiotics increased the cost of production by $1.30 (7.75 DKK) per pig produced. Even with this increase in production cost, the overall use of antibiotics in pig production in Denmark resulted in only a very modest, if any, reduction in total antibiotic usage in Denmark's swine industry due to a dramatic increase in the use of veterinarian directed therapeutic antibiotic usage. It has been estimated that a complete ban on the use of antimicrobials in swine production in the U.S. would increase production costs by over $6 per pig.

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. These proteins are particularly attractive for use as an alternative to conventional antibiotics for the control of *E. coli* caused PWD for several reasons.

The objective of this study was to determine if dietary inclusion of Colicin E1 (ColE1) altered pigs behavior and postures when challenged with *E. coli*.

Materials and Methods

Housing and Animals: Twenty-four newly weaned barrows; 21 days of age were placed into individual pens (1.22 m length x 65 cm width x 77 height). Pens were grouped in sets of 4 and divided by piping so that each pig could see at least one other pig. Each group of 4 pens were in the same treatment. Pigs were housed indoors in a climate controlled room. A corn / soybean based pellet diet was formulated to meet or exceed all nutritional requirements (26% crude protein, 3.51 kcal/kg) and water was supplied via a nipple style waterer were for *ad libitum* consumption.

Treatments: Pigs were given 2d to adjust to individual housing before the experimental diets were fed. Three dietary treatments were compared; treatment 1 had no ColE1 (C; n=8), treatment 2 added 1.1 mg of purified ColE1 (L; n=8), and treatment 3 added 16.5 mg (H; n=8) of

purified ColE1. Colicin E1 was produced and purified to homogeneity according to the method of Stahl, et al.. Briefly, a Colicin E1 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 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).

Behavior: One 12 V CCTV camera (Model WV-CP484, Matsushita Co. Ltd., Japan) was positioned to record 4 pens in black and white mode. Continuous video was collected for 5 days following placement of piglets (Figure 1). Video was collected at 10 frames per second using a DVR (RECO-204 Darim Vision, USA). Behavior was scored by one experienced observer using Observer 5.0.25 (Noldus®), using a 5 minute scan sampling technique for each hour.

Behaviors of interest were; feeding, defined when a pig had its head over the feeding trough, and drinking, when the pig had its mouth wrapped around the nipple waterer in the pen. Postures included active (that combined standing and walking) and inactive (that combined sitting and lying).

Figure 1. Screen print for the behaviors and postures collected.

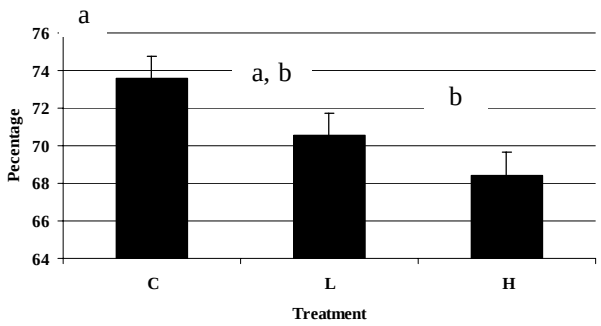
Statistical Analysis: Behavioral analysis was performed using the Proc Mix procedure in SAS® 2007; SAS® Inst. Inc., Cary, NC) software for parametric data. The design was a repeated measure over time. The model included the main effects of treatment and time. A random affect of date, date by treatment and pig nested within date by treatment was included.

Results and Discussion

There were no ($P < 0.05$) effects on the percentage of time engaged in active postures, drinking behavior and other between treatments.

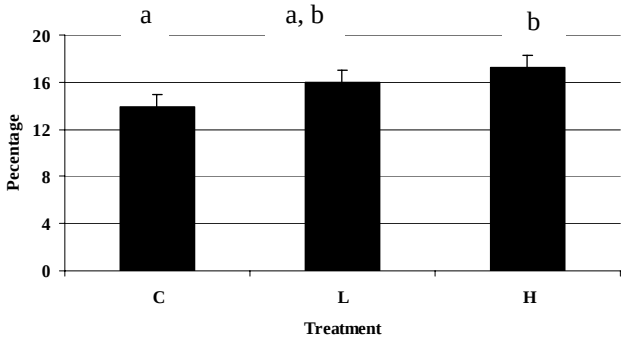
Time engaged in inactive related postures were less for H compared to C pigs (Figure 2)

Figure 2. Percentage of time pigs were inactive over a 5 d period by treatment ($P = 0.03$).



The H pigs spent more ($P = 0.028$) time at the feeder than the C pigs (Figure 3).

Figure 3. Percentage of time pigs were at the feeding trough over a 5 d period by treatment ($P = 0.02$).



From this study it can be concluded that pigs with the highest coverage of ColE1 displayed less inactive behaviors and spent more time at the feeder indicating that ColE1 may have offered some form of protection against the *E. coli* challenge and therefore improved the individual pigs' well-being.

Acknowledgements

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