

# Dietary Inclusion of Colicin E1.: Effect on Behavior Over Time

## A.S. Leaflet R2465

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### Summary and Implications

In the U.S., 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 morbidity. 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 pigs behavior and postures when challenged with *E. coli* over a 24 h period. Two postures (active and inactive) and two behaviors (drinking and feeding) were compared over three dietary treatments; 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. Regardless of treatment, nursery aged pigs generally engaged in higher levels of active postures, drinking behaviors, and feeding behaviors during the afternoon and night (13:00 to 22:00). Inversely, the pigs were generally inactive during the late night and morning hours (22:00 to 13:00). Nursery pigs in the H groups displayed slightly higher levels of active postures and feeding behaviors over a 24 h period indicating that ColE1 had some positive effects on the well-being of pigs infected with *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 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 fimbrial 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. A potential alternative to conventional antibiotics, which hold 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 ColE1 altered pigs behavior and postures when challenged with *E. coli* over a 24 h period.

### Materials and Methods

**Housing and Animals:** Twenty-four weaned barrows; 21 d of age were grouped by body weight and transferred to individual pens (1.22 m length x 65 cm width x 77 cm 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 was 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 % CP, 3.51 kcal / kg) and water was supplied via a nipple style waterer. Pigs were provided ad libitum access to feed and water throughout the trial.

**Treatments:** Pigs were given 2 d adjustment to their 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. (2007). 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).

**Postures and Behaviors:** 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 d following placement of the pigs (Figure 1). Day one was termed base line where the pigs received their dietary treatments

only. On day two pigs were challenged with *E. coli*. The effects of Col E1 and the diets were observed over d two through five. Video was collected at 10 frames per second using a DVR (RECO-204 Darim Vision, USA). Behavior and postures were scored by one experienced observer using the Observer 5.0.25 (Noldus®), using a 5 min scan sampling technique. The first time interval scored was at 0600 and thus the first observation day ran from 06:00 to 05:55 the following day. Postures included *active* (that combined standing and walking) and *inactive* (that combined sitting and lying). Behaviors of interest were *drinking* (when the pig had its mouth wrapped around the nipple waterer) and *feeding* (defined when a pig had its head over the feeding trough).

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



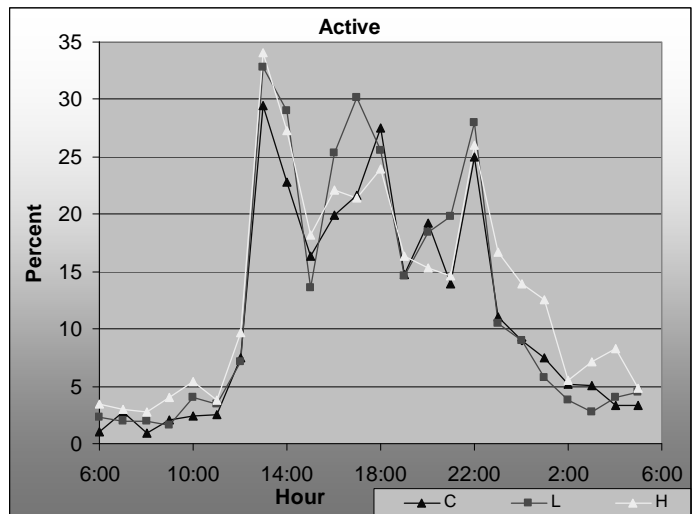
Data was entered into Microsoft Excel®. Data from the 5 min scans for each individual pig were calculated into percentages of individual behaviors and postures on an hourly basis. Means were then calculated for each hour. The information collected will be presented in a descriptive form.

### Results and Discussion

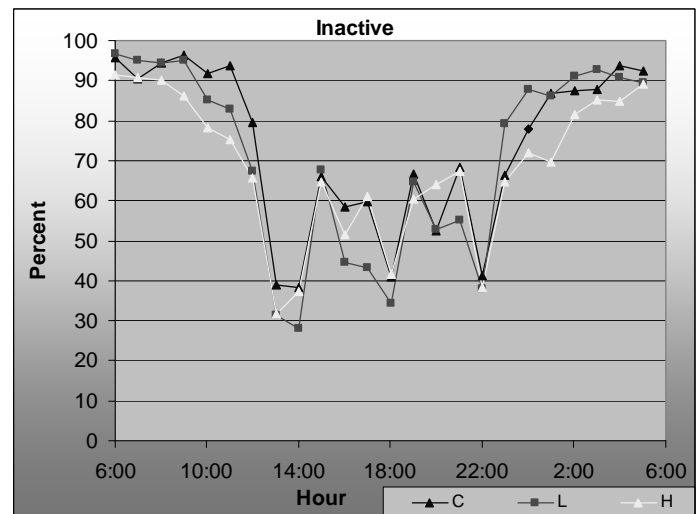
Between the treatments (C, L and H) pigs followed similar behavioral patterns throughout the day. Independent of treatment groups all pigs displayed two noticeable patterns of activity at 1300 (~ 32 %) and 2200 (~ 26 %), respectively. At 1700, the H pigs displayed another peak of activity (~ 30 %), however, C and L pigs' activity levels increased from 18 % to 29 % an hour later (1800). Overall, pigs that received the highest coverage of ColE1 were more active (~ 21 %) from 1300 to 2200 than the C and L treatment groups (~ 18 %), who followed very similar activity patterns over a 24 h cycle (Figure 2). Inactive followed an inverse pattern compared to activity levels for pigs in all treatment groups showing higher levels of inactivity from 0600 to 1200 and 2300 to 0600 (Figure 3). During the levels of increased activity the pigs

also engaged in increased drinking and feeding behaviors. Peaks in drinking behavior occurred at 1600 (~ 3 %) and remained high until 2200 (~ 2 %). Overall, the pigs engaged in roughly the same amount of drinking, at approximately ~1 % for the H, L, and C groups (Figure 4). Two feeding peaks were noted at 1400 (37 %) and 2200 (33 %). Overall the H pigs engaged in more feeding behavior (17 %), compared to L (16 %) or C (14 %) pigs respectively (Figure 5).

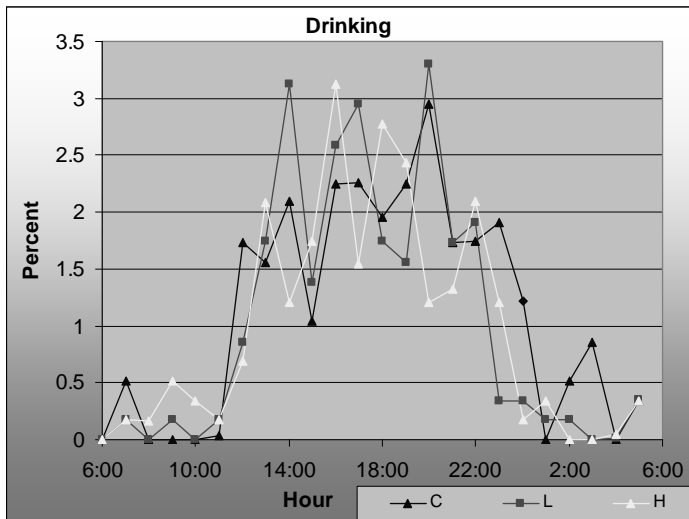
**Figure 2. Hourly averages of pigs by treatment engaged in ACTIVE postures.**



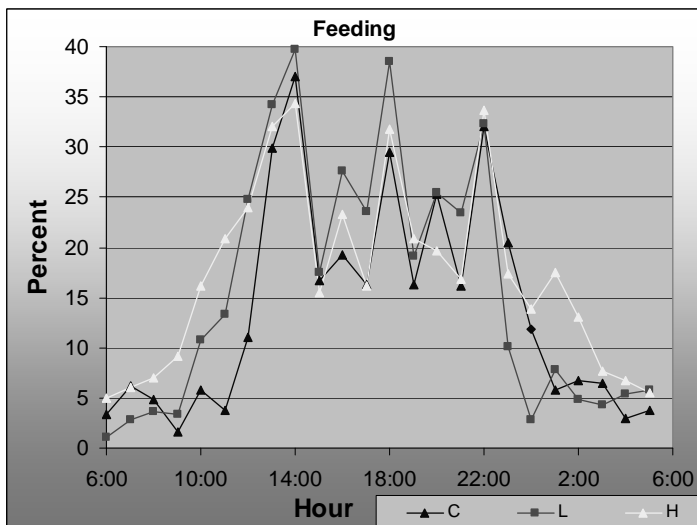
**Figure 3. Hourly averages of pigs by treatment engaged in INACTIVE postures.**



**Figure 4. Hourly averages of pigs by treatment engaged in DRINKING behaviors.**



**Figure 5. Hourly averages of pigs by treatment engaged in FEEDING behaviors.**



The prophylactic and growth promotant use of antibiotics in animal agriculture has been greatly scrutinized in recent years, due to concerns regarding its role in contributing to antibiotic resistance. 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.

Considering the current regulatory milieu, it is essential for the sustainability of the swine industry to examine alternatives to conventional antibiotics to improve animal health and production efficiency. In herds with PWD, up to 2% mortality in weaning pigs can be seen, but of greater economical significance is the morbidity, reduction in growth performance and overall pig well-being in the pigs that survive these infections. From this study it can be seen that regardless of treatment, pigs followed similar behavior and posture patterns throughout the day. In general, the pigs engaged in the highest levels of active postures, drinking behaviors and feeding behaviors during the afternoon into the night, with low level occurring during the morning hours. However, it should be noted that pigs receiving the highest doses of ColE1 engaged in slightly higher levels of feeding behaviors and active postures over a 24 h period indicating that ColE1 had some positive effects on the well-being of pigs infected with *E. coli*.

**Acknowledgements**

This work was supported in part by the Biotechnology Research and Development Corporation (Peoria, IL) and Iowa State University Animal Science start up funds.