Investigating the effects of calcium carbonate and benzoic acid, corn protein sources, and a dried fermentation product in the diets of nursery pigs

by

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Abstract

The 3 chapters of this thesis involve 1) effects of added calcium carbonate with and without benzoic acid on weanling pig growth performance, fecal dry matter, and blood Ca and P concentrations, 2) evaluation of different corn protein sources on nursery pig growth performance and fecal dry matter, and 3) evaluation of a dried fermentation product administered through drinking water on nursery pig growth performance, fecal *E. coli* characterization, antibiotic usage, and mortality. Chapter 1 utilized 1,055 pigs in two experiments. In Exp. 1, 695 pigs were used in two groups to evaluate increasing calcium carbonate ($CaCO_3$) levels from 0 to 1.80%. Experiment 2 utilized 350 pigs to investigate the interactive effects between $CaCO_3$ and benzoic acid. In Exp. 2, $CaCO_3$ was included at 0.45, 0.90, and 1.35% with and without 0.50% inclusion of benzoic acid. In both experiments, increasing $CaCO_3$ in the diet decreased G:F. In Exp. 2, there was no evidence for $CaCO_3$ × benzoic acid interactions, but providing benzoic acid improved ADG, ADFI, and tended to improve G:F. As well, the level $CaCO_3$ was directly reflective of serum Ca; as $CaCO_3$ decreased in the diet, so did serum Ca. Chapter 2 involved 670 nursery pigs in two experiments to investigate corn co-products as replacements to specialty protein sources in the swine industry. Experiment 1 utilized 315 pigs and observed decreased growth performance when feeding 5 or 10% of corn protein sources. The second experiment utilized 355 pigs and observed that a fourth corn protein source did not influence growth performance compared to a control. Increasing this fourth corn protein source increased daily gain and feed intake, with intermediate inclusion levels having the greatest ADG and ADFI. Gain-to-feed decreased linearly with increasing this fourth corn protein. Finally, chapter 3 utilized 34,749 pigs in two experiments to evaluate a dried fermentation product administered through drinking water on nursery pig growth performance, fecal *E. coli* characterization,

antibiotic usage, and mortality. Experiment 1 was conducted in a research setting utilizing 350 nursery pigs, where the dried fermentation product did not influence growth, antibiotic usage, fecal consistency, or *E. coli* presence. Experiment 2 was conducted in commercial nurseries utilizing 34,399 nursery pigs, where providing the dried fermentation product did not influence growth performance, reduced antibiotic injections, but increased nursery mortality.

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Chapter 1 - Effect of added calcium carbonate with and without benzoic acid on weanling pig growth performance, fecal dry matter, and blood Ca and P concentrations

Abstract

The objective of these studies was to determine the effects of increasing levels of $CaCO_3$ with and without benzoic acid on weanling pig growth performance, fecal dry matter (DM), and blood Ca and P concentration. In Exp. 1, 695 pigs (DNA Line 200 × 400, initially 5.9 ± 0.02 kg) were used in a 28-day study. Pigs were weaned at approximately 21 d of age and randomly assigned to pens and then pens were allotted to 1 of 5 dietary treatments. Treatment diets were fed from weaning (d 0) to d 14, with a common diet fed from d 14 to 28. Dietary treatments were formulated to provide 0, 0.45, 0.90, 1.35, and 1.80% added $CaCO_3$ at the expense of ground corn. From d 0 to 14 (treatment period), ADG and G:F decreased (linear, $P \leq 0.01$) as $CaCO_3$ increased. From d 14 to 28 (common period) and for the overall experiment (d 0 to 28), there was no evidence for differences in growth performance between treatments. For fecal DM, there was a trend (quadratic, $P = 0.091$) where pigs fed the highest and lowest $CaCO_3$ diets had the greatest fecal DM. Experiment 2 used 360 pigs (DNA Line 200 × 400, initially 6.2 ± 0.03 kg) in a 38-day study. Upon arrival to the nursery facility, pigs were randomly assigned to pens and then pens were allotted to 1 of 6 dietary treatments. Dietary treatments were fed in 3 phases with treatment diets fed from d 0 to 10 and d 10 to 24, and a common phase 3 diet fed from d 24 to 38. Dietary treatments were formulated to provide 0.45, 0.90, and 1.35% added $CaCO_3$ with or without 0.5% benzoic acid (VevoVitall, DSM Nutritional Products, Parsippany, NJ) added at the expense of ground corn. There was no evidence ($P > 0.05$) for a $CaCO_3$ by benzoic acid

interaction. For the experimental period (d 0 to 24), there was a tendency for benzoic acid to improve (P = 0.056) ADG and (P = 0.071) ADFI, and an improvement (linear, P = 0.014) in G:F as $CaCO_3$ decreased. During the common period (d 24 to 38), pigs previously fed benzoic acid had increased (P = 0.045) ADG and marginally increased (P = 0.091) ADFI. For the overall study, pigs fed benzoic acid had increased (P = 0.011) ADG and (P = 0.030) ADFI and marginally improved (P = 0.096) G:F and (P = 0.059) final BW. Serum Ca decreased (linear, P < 0.001) as $CaCO_3$ decreased in the diet. These data show that decreasing the $CaCO_3$ content in the nursery diet immediately after weaning may improve ADG and G:F. Dietary addition of benzoic acid may also provide beneficial effects on ADG and ADFI, regardless of dietary Ca level.

Key Words: acid binding capacity, benzoic acid, calcium, calcium carbonate, growth, nursery pig

Introduction

Acid binding capacity (ABC) is a feed ingredient's ability to resist a change in gastrointestinal (GIT) pH. A high ABC is associated with high pH when the ingredient is suspended within an aqueous solution. A high gastric pH has been observed to result in GIT challenges including increased intestinal bacteria such as *Escherichia coli* (Smith and Jones, 1963) whereas a low stomach pH improves protein digestion (Kil et al., 2011) and intestinal health (Li et al., 2008).

Although Ca is important to maximize bone mineralization (Lagos et al., 2019) and other physiological functions (Crenshaw, 2001), certain Ca containing ingredients have a high ABC. Calcium carbonate ($CaCO_3$) is one such ingredient with a relatively high ABC (Jasaitis et al., 1987; Lawlor et al., 2005). Calcium carbonate has an ABC of 12,932 mEq/kg, whereas other

ingredients such as corn, soybean meal, corn DDGS, and dicalcium phosphate have lower ABC (111, 642, 96, and 3,098 mEq/kg, respectively; Lawlor et al., 2005). An improvement in growth performance has been observed with lower dietary ABC – 4 (Lawlor et al., 2006).

To help lower gastric pH, dietary acidifiers have been extensively evaluated. Benzoic acid has been observed to decrease *E. coli* in the lower GIT (Guggenbuhl et al., 2007; Diao et al., 2021) and improve growth performance (Guggenbuhl et al., 2007; Chen et al., 2017; Bradley et al., 2020; Bergstrom et al., 2020). Benzoic acid has also been observed to lower the pH of the stomach and lower GIT organs of nursery pigs (Kluge et al., 2006; Halas et al., 2010). Our hypothesis was that short-term reduction in dietary $CaCO_3$, and the addition of benzoic acid would decrease dietary ABC resulting in increased pig performance. Therefore, the objective of this study was to investigate the effects of increasing levels of $CaCO_3$ and the interactive effects of $CaCO_3$ level with or without benzoic acid on the growth performance, fecal dry matter (DM), and blood Ca and P concentrations of nursery pigs.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocols (4035) used in these experiments. The studies were conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. The facility has two identical barns that are completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder, and a cup waterer to provide *ad libitum* access to feed and water. Pens (1.22 × 1.22 m) had metal tri-bar floors and allowed approximately 0.30 m^2/pig.

Animals and diets

Experiment 1

A total of 695 castrated male pigs (DNA Line 200 × 400, Columbus, NE; initially 5.9 ± 0.02 kg) were used in 2 groups in a 28-day study with 5 pigs per pen and 27 or 28 replications (pens) per treatment. There were 350 and 345 pigs in group 1 and 2, respectively. Upon arrival to the nursery research facility, pigs were randomly assigned to pens and then pens were allotted to 1 of 5 dietary treatments. Treatment diets were fed from d 0 to 14 and a common phase 2 diet was fed from d 14 to 28. Dietary treatments were formulated to provide 0, 0.45, 0.90, 1.35, or 1.80% added $CaCO_3$ at the expense of ground corn (Table 1). The calculated dietary Ca levels were 0.49, 0.66, 0.84, 1.01, and 1.18%, with corresponding dietary ABC – 4 values of 424, 481, 539, 597, and 655 mEq/kg, respectively. Treatment diets were fed in meal form for group 1 and pellet form for group 2, with the common phase 2 diet fed in meal form in both groups. At the time of manufacturing for each group, a single base diet was manufactured at Hubbard Feeds in Beloit, KS, packaged in 22.7 kg bags and then transported to Manhattan, KS, where $CaCO_3$ and corn additions were mixed with the base diet to form experimental treatments. In group 2, diets were subsequently pelleted at the O.H. Kruse Feed Technology Innovation Center at Kansas State University, Manhattan, KS.

Experiment 2

A total of 360 castrated male pigs (DNA Line 200 × 400, Columbus, NE; initially 6.2 ± 0.03 kg) were used in a 38-d study with 5 pigs per pen and 12 replications (pens) per treatment. Upon arrival to the nursery research facility, pigs were randomly assigned to pens and then pens were allotted to 1 of 6 dietary treatments. Dietary treatments were formulated similar to Exp. 1 (Tables 2 and 3) and fed in 3 phases with treatment diets fed from d 0 to 10 (phase 1) and d 10 to 24 (phase 2) with a common phase 3 diet fed from d 24 to 38. Dietary treatments were formulated to provide 0.45, 0.90, or 1.35% added $CaCO_3$ with or without 0.5% benzoic acid

(VevoVitall, DSM Nutritional Products, Parsippany, NJ) added at the expense of ground corn. In phase 1, the calculated dietary Ca levels were 0.66, 0.83, and 1.01% with corresponding dietary ABC – 4 values of 450, 508, and 566 mEq/kg, respectively, without benzoic acid included. In phase 2, the calculated dietary Ca levels were 0.54, 0.72, and 0.89% (387, 445, and 502 mEq/kg of ABC – 4 without benzoic acid, respectively). The addition of benzoic acid decreased ABC – 4 by approximately 30 mEq/kg when added to the phase 1 and phase 2 diets. Similar to Exp. 1, a single base diet was manufactured at Hubbard Feeds in Beloit, KS, and packaged in 22.7 kg bags and then transported to Manhattan, KS. Calcium carbonate, benzoic acid, and corn additions were then mixed at the O.H. Kruse Feed Technology Innovation Center at Kansas State University, Manhattan, KS. All treatment diets in Exp. 2 were fed in meal form.

Data collection

Pigs were weighed individually, and feed disappearance was measured for each pen on d 0, 14, and 28 in Exp. 1, and on d 0, 10, 24, and 38 in Exp. 2 to determine ADG, ADFI, and G:F. On d 10 of Exp. 1 and on d 7 of Exp. 2, ~40g of feces were pooled from 3 randomly selected pigs per pen and then dried at 55°C for 48 h to determine fecal DM. On d 21 of Exp. 2, blood was collected from the jugular region of 1 randomly selected pig per pen (72 pigs; 12 observations per treatment), centrifuged at 4°C and 1800 × g for 30 min, and 1.0 mL serum collected.

Diet sampling and chemical analysis

In both experiments, complete diet samples of each treatment were taken with a grain probe from every other bag (phase 1) and every 3rd bag (phase 2) upon completion of manufacturing. Samples were combined to obtain a homogenous sample of each diet and were stored at -20°C until further analysis.

Six sub-samples of each diet were collected by using a riffle splitter and ground with a food processor to create a homogeneous sample. After creating these homogenous samples, 3 subsamples were submitted to the K-State Research and Extension Soil Testing Laboratory in Manhattan, KS, for analysis of Ca (AOAC 985.01, 2006) and P (AOAC 985.01, 2006). In Exp. 1, ABC – 4 was determined as described by Lawlor et al. (2005). In Exp. 2, 0.5 mL of serum was submitted to the Kansas State University Veterinary Diagnostics Laboratory for analysis of Ca and P (Cobas c 501, Roche Diagnostics).

Statistical analysis

In both experiments, data were analyzed as a completely randomized design with pen serving as the experimental unit. Treatment was included in the statistical model as a fixed effect. In Exp. 1, block was incorporated in the model as a random effect to account for initial pen average body weight, barn, and group (group 1 and 2). In Exp. 2, barn was incorporated in the model as a random effect to account for pigs being housed in two identical nursery barns. Data were analyzed using R Studio (Version 3.5.2, R Core Team. Vienna, Austria). Contrasts were used to test for linear and quadratic responses to $CaCO_3$ (Exp. 1 and 2) and for the main effects of $CaCO_3$, benzoic acid, and their interaction (Exp. 2). Differences between treatments were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

The analyzed ABC – 4 values of the experimental diets in Exp. 1 were lower than calculated (Table 1) however increased with increasing $CaCO_3$ in the diet as expected.

Experiment 1

There was no evidence ($P > 0.05$) for treatment × group interactions, so data from both groups were combined. From day 0 to 14 (treatment period), ADG (linear, $P = 0.010$) and d 14

BW (linear, $P = 0.006$) decreased as $CaCO_3$ increased (Table 4). Likewise, G:F decreased (linear, $P < 0.001$) as $CaCO_3$ increased with no evidence for difference in ADFI observed ($P > 0.05$). For fecal DM collected on d 10, there was a trend (quadratic, $P = 0.091$) where pigs fed the highest $CaCO_3$ had the greatest DM where other treatments were relatively similar.

From d 14 to 28 (common period) and for the overall experiment (d 0 to 28), there was no evidence ($P > 0.10$) for differences in growth performance between treatments

Experiment 2

For all response criteria, there was no evidence for $CaCO_3$ × benzoic acid interactions observed ($P > 0.10$, Table 5). From d 0 to 10, pigs fed benzoic acid tended to have increased ($P = 0.092$) ADG and had increased ($P = 0.042$) ADFI (Table 6). From d 10 to 24, pigs fed decreasing $CaCO_3$ had improved (linear, $P = 0.022$) G:F but ADG, ADFI, and d 24 BW were not influenced by dietary treatment ($P > 0.10$) . For the experimental period (d 0 to 24), there was a tendency observed for benzoic acid to improve ($P = 0.056$) ADG and ($P = 0.071$) ADFI, and an improvement (linear, $P = 0.014$) was observed in G:F as $CaCO_3$ decreased in the diet.

During the common period (d 24 to 38), pigs previously fed benzoic acid had increased ($P = 0.045$) ADG and marginally increased ($P = 0.091$) ADFI. For the overall study, pigs fed benzoic acid had increased ADG ($P = 0.011$) and ADFI ($P = 0.030$) and marginally improved G:F ($P = 0.096$) and final BW ($P = 0.059$); however, no overall impact of $CaCO_3$ level was observed ($P > 0.10$) .

For fecal DM on d 7, there was no evidence ($P > 0.10$) for differences among treatments. For serum analysis on d 21, serum Ca increased (linear, $P < 0.001$) as the level of $CaCO_3$ in the diet increased, while no difference ($P > 0.05$) in serum P was observed.

Discussion

Calcium (Ca) is one of the most abundant macrominerals required by the pig and 0.8% of the animals' body is Ca (Hendriks and Moughan, 1993). Of that 0.8%, 96 to 99% is present in bone tissue in the form of hydroxyapatite. Calcium is therefore essential for bone formation and mineralization. In the present study, a reduction of calcium carbonate ($CaCO_3$) was used to decrease the ABC – 4 of the diet and as a consequence, the total dietary Ca percentage decreased. However, the analyzed Ca values were higher than the calculated values and revealed that the pigs fed the diets at or below 0.45% $CaCO_3$ in both experiments and the 0.90% $CaCO_3$ in Exp. 2 were below the NRC (2012) requirement estimates of 0.85 and 0.80% for 5 to 7 kg and 7 to 11 kg, respectively. However, if providing a 0.10% Ca release value for phytase inclusion, only the diet with no $CaCO_3$ used in Exp. 1 was Ca deficient. It can be assumed that difference in analyzed Ca is due to a discrepancy in a Ca concentration of one or more ingredients used in the experiments and/or analytical error. As well, analyzed ABC – 4 values were lower than the calculated values and could be attributed to variation in the ingredient values compared to those provided from Lawlor et al. (2005).

In the present study the Ca:P ratio increased as the concentration of $CaCO_3$ increased. The Ca:P ratio appears to be more critical to growth performance and bone mineralization at low P levels (Wu et al., 2018). Qian et al.(1996) observed decreased ADG when Ca:P increased from 1.2:1 to 2:1. This response was greater when diets were fed with deficient P levels. To avoid this concern, dietary STTD P in the present experiments were at least 0.58%, which is much greater than NRC (2012) requirement estimates of 0.45% and 0.40% for pigs weighing 5 to 7 and 7 to 11 kg, respectively. González-Vega et al. (2016) observed that increasing $CaCO_3$ in diets from 0.05 to 1.72% for 11 to 25 kg pigs while maintaining a constant STTD P (0.36%) affects

performance, with no change in ADG and G:F up to 0.77% analyzed Ca (1.05% added $CaCO_3$), but with reduced ADG and G:F with higher inclusions. A second experiment by González-Vega et al. (2016) evaluated constant dietary Ca (0.72%) at 0.33, 0.36, and 0.40% STTD P for 11 to 25 kg pigs, where a linear improvement in G:F was observed with increasing STTD P. In other studies, Wu et al. (2018) observed interactive effects of dietary Ca and P in the first 24 d of the nursery period. Where increasing Ca by increasing $CaCO_3$ significantly reduced G:F in diets fed at the NRC (2012) requirement estimate (0.45% STTD P) for 5 to 7 kg pigs, but no difference compared to the diets that were fed above the requirement estimate (0.56% STTD P). These data altogether show that pigs fed adequate dietary P can maintain performance when fed a wide range of Ca levels. Thus, improvements in performance when nursery pigs were fed lower Ca levels may be a result of reduced diet ABC.

The use of ABC has been evaluated more extensively in European nursery diets (Blank et al., 1999; Mroz et al., 2000; Lawlor et al., 2006) compared to US based formulations. Lawlor et al. (2005) conducted acid/base titrations to develop ABC – 4 ingredient values that were used in diet formulation in the present study. With the purpose of evaluating ABC – 4 diet values on growth performance, Lawlor et al. (2006) fed different Ca and P levels with and without acidifiers. They observed that low Ca (0.28%) and P (0.51%) concentrations for the first 7 days post-weaning improved ADFI and tended to improve feed conversion through the first 14 days. The improvement in feed conversion they found agrees with the present experiments. The change in Ca investigated by Lawlor et al. (2006) was completed by removing limestone flour (1.2%) and dicalcium phosphate (1.0%), ultimately lowering ABC – 4 values from 315 to 207 mEq/kg. In a separate experiment however, Lawlor et al. (2006) observed no growth response to diet Ca level when feeding limestone flour from 0.03 to 1.19% (340 to 500 mEq/kg ABC – 4) of

the diet while maintaining constant total P. Finally, Lawlor et al. (2006) also examined keeping limestone flour and dicalcium phosphate constant and included 2.0% formic acid, which lowered the ABC – 4 from 315 to 180 mEq/kg. They reported a mineral level × acid interaction with increased ADFI when pigs were fed formic acid with adequate (NRC, 1998) Ca and P in the first 7 days, but no difference when formic acid was fed with low Ca and P levels. This interaction is in contrast with the present study, where no interaction was found between acid and Ca level, which could be attributed to the type or level of acidifier used or other ingredient combinations that made up the experimental diets.

Given the NRC (2012) requirement estimates of 0.85% and 0.80% Ca for pigs weighing 5 to 7 kg and 7 to 11 kg, respectively, the pigs in the present studies fed the low $CaCO_3$ levels were deficient in Ca on a calculated basis (≤ 0.66 and 0.72% in Exp. 1 and 2, respectively). However, diet Ca values from chemical analysis revealed that only the 0% added $CaCO_3$ diets were below while the 0.45% were only slightly below the NRC (2012) requirement estimates. Also, all diets contained added phytase which is shown to increase Ca availability (González-Vega et al., 2015). These calculations were not considered in the present studies, and therefore, even the diet without $CaCO_3$ would most likely been at or very close to the pigs Ca requirement. The concern of feeding below the pig's Ca requirement for an extended period is that bone mineralization would likely be limited. This is supported by Lagos et al. (2021) who fed weaned pigs' diets with 50% (NRC, 2012) estimated Ca and P levels for 16 d. These authors reported decreased bone mineralization at d 15 with no differences in growth performance. While we did not measure bone mineralization directly, in Exp. 2 there were statistical differences for serum Ca with serum Ca percentage decreasing as the level of dietary $CaCO_3$ decreased. However, the serum Ca levels for all treatments were within normal biological ranges 9 to 13 mg/dL (Puls,

1994). The response to serum Ca being reflective of diet Ca concentration agrees with previous studies (Nielsen et al., 1971; Mahan, 1982; Hall et al., 1991; Lagos et al., 2019).

The use of acidifiers in nursery diets and the proposed mechanism of action has been extensively researched and reviewed (Ravindran and Kornegay, 1993; Kim et al., 2005; Kil et al., 2011; Suiryanrayna and Ramana, 2015). Acidifiers added to feeds are used to decrease stomach and lower GIT pH, improve nutrient digestibility, and decrease pathogen proliferation. Newly weaned pigs are accustomed to a liquid milk-based diet. Through bacterial fermentation of lactose at a pH environment around 5.0, lactic acid is produced which inhibits hydrochloric acid (HCl) secretion (Kidder and Manners, 1978). A low stomach pH is required for adequate protein digestion because pepsinogen is more rapidly cleaved to produce pepsin at a pH 2.5 – 3.0 (Herriott, 1938). Pepsin is the primary proteolytic enzyme of the stomach. Although it was not the objective of this study to measure gastric pH, numerical changes in stomach pH with different acidifiers has been researched (Kil et al., 2011) where it was reported that statistical differences of stomach pH are variable due to other dietary inclusion levels with varying ABC estimates. Certain ingredients with high ABC – 4 may interact with acidifiers to lessen the response to acidifiers. Although we did not see an interaction between calcium carbonate and benzoic acid in the present study, an interaction with other ingredients or a combination of ingredients that have relatively high ABC – 4 values may exist, but this would require further investigation.

In the present study, it was hypothesized that an acidifier would further decrease the ABC of the diet and potentially provide additional benefit regardless of $CaCO_3$ level in the diet. Therefore, benzoic acid was used due to previous research evaluating early nursery diets where improvements in growth performance and nutrient utilization have been shown (Guggenbuhl et

al., 2007; Chen et al., 2017; Diao et al., 2021). Guggenbuhl et al. (2007) compared feeding 0.50% benzoic acid to a control diet and observed increased total digestibility of nitrogen, energy, and apparent ileal digestibility of Lys and Thr. Although it was not measured, the improvement of ADG and ADFI when benzoic acid was fed in the present study might be attributed to the increase in nutrient digestibility. The inclusion of benzoic acid in the present study decreased the calculated ABC – 4 by 30 mEq/kg. It is unclear whether the magnitude was sufficient to illicit a statistical growth response that could be attributed to lower ABC – 4.

In our diet formulation, $CaCO_3$ and benzoic acid were added at the expense of ground corn, and as a result the dietary energy decreased in these diets. Decreasing the dietary NE by 1% has been shown to decrease G:F by 1% (Nitikanchana et al., 2015). It was therefore expected in the present study that we could observe reductions in G:F as $CaCO_3$ increased in the diet. However, in Exp. 1 increasing $CaCO_3$ from 0 to 1.80% reduced diet NE by 2%, while we observed a 10% reduction in G:F when the experimental diets were fed. Similarly, in Exp. 2, increasing $CaCO_3$ reduced NE by 0.9 and 1% in phase 1 and 2, respectively, and yet we observed a 3% reduction in G:F when treatment diets were fed. From these observations we can conclude that the change in G:F may not be solely attributed to dietary NE.

In summary, increasing $CaCO_3$ in the early nursery diets with adequate dietary P decreased BW, ADG, and G:F. This response is potentially due to the increase in the ABC – 4 of the diet. The use of a benzoic acid showed an improvement in ADG and ADFI and marginal improvement in G:F, and the response was independent of the dietary level of $CaCO_3$. While not unexpected, serum Ca level decreased with reduced diet Ca levels. Additional research needs to be conducted to determine if the results were a direct result of changes in ABC – 4 from altering the diet $CaCO_3$ concentration or other mechanisms.

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Table 1-1 Experiment 1 phase 1 and 2 diet composition (as fed basis)[1]

	Calcium carbonate, %					
Item, %	0	0.45	0.90	1.35	1.80	Phase 2[3]
Ingredient						
Corn[2]	44.07	43.62	43.17	42.72	42.27	48.57
Soybean meal, 46.5% CP	17.65	17.65	17.65	17.65	17.65	23.73
Spray-dried whey	10.00	10.00	10.00	10.00	10.00	---
Whey permeate	10.00	10.00	10.00	10.00	10.00	10.00
Corn DDGS	5.00	5.00	5.00	5.00	5.00	7.50
ESBM[4]	5.00	5.00	5.00	5.00	5.00	5.00
Menhaden fish meal	2.50	2.50	2.50	2.50	2.50	---
Spray-dried bovine plasma	2.00	2.00	2.00	2.00	2.00	---
Choice white grease	1.00	1.00	1.00	1.00	1.00	1.00
Monocalcium P, 21.5% P	0.80	0.80	0.80	0.80	0.80	1.00
Calcium carbonate	---	0.45	0.90	1.35	1.80	0.90
Zinc oxide	0.40	0.40	0.40	0.40	0.40	0.25
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.50
Vitamin premix with phytase[5]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
L-Lys-HCL	0.40	0.40	0.40	0.40	0.40	0.55
DL-Met	0.19	0.19	0.19	0.19	0.19	0.22
L-Thr	0.18	0.18	0.18	0.18	0.18	0.23
L-Trp	0.03	0.03	0.03	0.03	0.03	0.04
L-Val	0.09	0.09	0.09	0.09	0.09	0.13
Total	100	100	100	100	100	100
Calculated analysis						
SID amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	56	56	56	56	56	55
Leu:Lys	118	118	118	118	117	116
Met:Lys	36	36	36	36	36	37
Met & cys:Lys	58	58	58	58	58	57
Thr:Lys	64	64	64	64	64	63
Trp:Lys	19.3	19.3	19.3	19.3	19.3	19.3
Val:Lys	71	71	70	70	70	70
Total Lys, %	1.51	1.51	1.51	1.52	1.52	1.51
NE, kcal/kg	2,558	2,545	2,534	2,520	2507	2,494
SID Lys:NE, g/Mcal	6.03	6.00	5.97	5.95	5.92	6.26
CP, %	21.5	21.5	21.4	21.4	21.4	21.4
Ca, %	0.49	0.66	0.84	1.01	1.18	0.72
P, %	0.68	0.68	0.68	0.68	0.68	0.63
STTD P, %	0.58	0.58	0.58	0.58	0.58	0.48
Analyzed Ca:P	0.72	0.97	1.23	1.48	1.74	1.21
ABC – 4[6]	424	481	539	597	655	475
Chemical analysis[7]						

Ca, %	0.61	0.80	0.99	1.15	1.37	0.89
P, %	0.75	0.75	0.77	0.70	0.71	0.60
ABC – 4, mEq/kg[8]	318	347	376	368	409	-

[1]Phase 1 experimental diets were fed from 5.9 to 8 kg.

[2] Corn level altered with increasing calcium carbonate inclusions.

[3]A common diet was fed following the experimental period.

[4] HP 300, Hamlet Protein, Findlay, Ohio.

[5] Ronozyme HiPhos 2700 (DSM Nutritional Products) provided a 0.13 release of STTD P % for 750 FYT/kg inclusion in the diet.

[6] Acid binding capacity (ABC) was calculated based on published ingredient values (Lawlor et al., 2005).

[7] Three representative samples were collected from each treatment diet and two representative samples were collected from the phase 2 diet, ground with a food processor, and submitted for analysis to the Kansas State University Soil Testing Laboratory for Ca and P analysis. ABC – 4 analysis was performed as described by Lawlor et al. (2005).

[8] Acid binding capacity (ABC) was measured and calculated as the amount of acid in milliequivalents required to bring 1.0 kg of ground feed to a pH of 4.0.

Table 1-2 Experiment 2 phase 1 diet composition (as-fed basis)[1]

Benzoic acid[2]:	Without			With		
Ingredient, % $CaCO_3$, %:	0.45	0.90	1.35	0.45	0.90	1.35
Corn	44.82	44.30	43.79	44.33	43.84	43.36
Soybean meal, 46.5% CP	17.47	17.55	17.60	17.47	17.50	17.54
Spray-dried whey	10.00	10.00	10.00	10.00	10.00	10.00
Whey permeate	10.00	10.00	10.00	10.00	10.00	10.00
Corn DDGS	5.00	5.00	5.00	5.00	5.00	5.00
Fermented soybean meal[3]	4.00	4.00	4.00	4.00	4.00	4.00
Menhaden fish meal	2.50	2.50	2.50	2.50	2.50	2.50
Spray-dried bovine plasma	2.00	2.00	2.00	2.00	2.00	2.50
Choice white grease	1.00	1.00	1.00	1.00	1.00	1.00
Monocalcium P 21.5% P	0.80	0.80	0.80	0.80	0.80	1.00
Calcium carbonate	0.45	0.90	1.35	0.45	0.90	1.35
Zinc oxide	0.40	0.40	0.40	0.40	0.40	0.40
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix with phytase[4]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
L-Lys-HCl	0.40	0.40	0.40	0.40	0.40	0.40
DL-Met	0.19	0.19	0.19	0.19	0.19	0.19
L-Thr	0.17	0.17	0.17	0.17	0.17	0.17
L-Trp	0.02	0.02	0.02	0.02	0.02	0.02
L-Val	0.08	0.08	0.08	0.08	0.08	0.08
Benzoic acid	---	---	---	0.50	0.50	0.50
Total	100	100	100	100	100	100
Calculated analysis						
SID amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	57	57	57	57	57	57
Leu:Lys	120	120	119	120	119	119
Met:Lys	36	36	36	36	36	36
Met & cys:Lys	59	59	59	59	59	59
Thr:Lys	64	64	64	64	64	64
Trp:Lys	19.2	19.2	19.2	19.2	19.2	19.2
Val:Lys	70	70	70	70	70	70
Total Lys, %	1.51	1.52	1.52	1.51	1.51	1.51
NE, kcal/kg	2,542	2,529	2,518	2,529	2,516	2,505
SID Lys:NE g/Mcal	5.87	5.91	5.94	5.90	5.93	5.96
CP, %	21.5	21.5	21.5	21.5	21.4	21.4
Ca, %	0.66	0.83	1.00	0.66	0.83	1.00
P, %	0.66	0.66	0.66	0.66	0.66	0.66
STTD P, %	0.58	0.58	0.58	0.58	0.58	0.58
Formulated analyzed Ca:P	0.99	1.26	1.52	1.00	1.26	1.53
ABC – 4, mEq/kg[5]	450	508	566	420	477	535
Chemical analysis, %[6]						
Ca	0.77	0.90	1.04	0.74	0.89	1.05

P	0.68	0.65	0.63	0.63	0.65	0.58

[1] Phase 1 experimental diets were fed for 10 days.

[2] VevoVitall, DSM Nutritional Products, Parsippany, NJ.

[3] ME Pro, Prairie Aquatech, Brookings, SD.

[4] Ronozyme HiPhos 2700 (DSM Nutritional Products) provided a 0.12 release of STTD P % for 1250 FYT/kg inclusion in the diet.

[5] Acid binding capacity (ABC) was calculated based on published or estimated ingredient values (Lawlor et al., 2005).

[6] Three representative samples were collected from each treatment diet, ground with a food processor, and submitted for analysis to the Kansas State University Soil Testing Laboratory.

Table 1-3 Experiment 2 phase 2 and 3 diet composition (as-fed basis)[1]

Benzoic acid[2]:	Without			With			
Ingredient, % $CaCO_3$, %:	0.45	0.90	1.35	0.45	0.90	1.35	Phase 3[3]
Corn	50.16	49.67	49.19	49.62	49.13	48.65	64.71
Soybean meal, 46.5% CP	23.75	23.79	23.82	23.79	23.83	23.86	31.30
Whey permeate	10.00	10.00	10.00	10.00	10.00	10.00	---
Corn DDGS	7.50	7.50	7.50	7.50	7.50	7.50	---
Fermented soybean meal[4]	3.85	3.85	3.85	3.85	3.85	3.85	---
Choice white grease	1.00	1.00	1.00	1.00	1.00	1.00	---
Monocalcium P, 21.5% P	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Calcium carbonate	0.45	0.90	1.35	0.45	0.90	1.35	0.85
Zinc oxide	0.25	0.25	0.25	0.25	0.25	0.25	---
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.60
Vitamin premix with phytase[5]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lys-HCL	0.55	0.55	0.55	0.55	0.55	0.55	0.52
DL-Met	0.22	0.22	0.22	0.22	0.22	0.22	0.23
L-Thr	0.22	0.22	0.22	0.22	0.22	0.22	0.22
L-Trp	0.04	0.04	0.04	0.04	0.04	0.04	---
L-Val	0.12	0.12	0.12	0.12	0.12	0.12	0.06
Benzoic acid	---	---	---	0.50	0.50	0.50	---
Total	100	100	100	100	100	100	100
Calculated analysis							
SID amino acids, %							
Lys	1.35	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	57	56	56	56	56	56	55
Leu:Lys	118	118	118	118	118	118	114
Met:Lys	37	37	37	37	37	37	37
Met & cys:Lys	58	58	58	58	58	58	58
Thr:Lys	63	63	63	63	63	63	63
Trp:Lys	19.3	19.3	19.3	19.3	19.3	19.3	20.3
Val:Lys	70	70	70	70	70	70	69
Total Lys, %	1.51	1.51	1.51	1.51	1.51	1.51	1.49
NE, kcal/kg	2,503	2,489	2,476	2,487	2,476	2,463	2,423
SID Lys:NE, g/Mcal	6.14	6.17	6.20	6.17	6.21	6.24	5.67
CP, %	21.5	21.4	21.4	21.4	21.4	21.4	21.2
Ca, %	0.54	0.72	0.89	0.54	0.72	0.89	0.68
P, %	0.61	0.61	0.61	0.61	0.61	0.60	0.61
STTD P, %	0.51	0.51	0.51	0.51	0.51	0.51	0.47
Formulated analyzed Ca:P	0.89	1.18	1.47	0.89	1.18	1.47	1.13
ABC – 4, mEq/kg[6]	387	445	502	357	415	473	412
Chemical analysis, %[7]							
Ca	0.58	0.74	1.04	0.61	0.79	1.07	---
P	0.52	0.51	0.53	0.58	0.59	0.54	---

[1] Phase 2 experimental diets were fed from d 10 to 24.

[2] VevoVitall, DSM Nutritional Products, Parsippany, NJ.

[3] Phase 3 common diet was fed from d 24 to 38.

[4] ME Pro, Prairie Aquatech, Brookings, SD.

[5] Ronozyme 2700 (DSM Nutritional Products) provided a 0.13 release of STTD P % for 1250 FYT/kg inclusion in the diet.

[6] Acid binding capacity (ABC) was calculated based on published or estimated ingredient values (Lawlor et al., 2005).

[7] Three representative samples were collected from each treatment diet, ground with a food processor, and submitted for analysis to the Kansas State University Soil Testing Laboratory.

Table 1-4 Effects of increasing calcium carbonate on weanling pig growth performance, Exp. 1[1]

	Calcium carbonate, %[2]						*P* =	
Item	0.00	0.45	0.90	1.35	1.80	SEM	Linear	Quadratic
BW, kg								
d 0	5.9	5.9	5.9	5.9	5.9	0.02	0.846	0.498
d 14	8.0	8.0	7.8	7.7	7.7	0.11	0.006	0.641
d 28	14.7	14.9	14.8	14.5	14.6	0.23	0.397	0.612
Experimental period (d 0 to 14)								
ADG, g	149	147	136	129	133	7.2	0.010	0.443
ADFI, g	171	170	170	164	171	7.4	0.676	0.627
G:F, g/kg	864	854	805	783	776	18.8	< 0.001	0.585
Common period (d 14 to 28)[3]								
ADG, g	477	489	498	485	494	14.9	0.331	0.470
ADFI, g	659	682	678	660	669	15.4	0.798	0.424
G:F, g/kg	723	725	730	734	735	10.4	0.182	0.914
Overall (d 0 to 28)								
ADG, g	312	314	312	304	311	9.7	0.569	0.954
ADFI, g	413	417	418	408	416	11.1	0.918	0.920
G:F, g/kg	753	751	745	744	745	7.1	0.246	0.736
Fecal DM, %[4]								
d 10	22.5	22.1	21.9	21.5	24.4	1.1	0.303	0.091

[1] A total of 695 weanling barrow pigs (DNA 200 × 400; initially 5.9 ± 0.02 kg) approximately 21 days of age were used in 2, 28-d experiments with 5 pigs per pen and 27 or 28 pens per treatment.

[2] Analyzed Ca of the treatment diets were 0.61, 0.80, 0.99, 1.15, and 1.37%, respectively.

[3] Analyzed Ca of the common phase 2 diet was 0.89%.

[4] Feces from three pigs from each pen were pooled, weighed, and dried to measure fecal dry matter.

Table 1-5 Effects of calcium carbonate with or without benzoic acid on nursery pig growth performance, fecal dry matter and blood calcium and phosphorus concentration, Exp. 2[1]

								P =	
	Benzoic acid[2]:	Without			With				Calcium carbonate × benzoic acid
Item	$CaCO_3$, %:	0.45	0.90	1.35	0.45	0.90	1.35	SEM	Linear	Quadratic
BW, kg										
d 0		6.2	6.2	6.2	6.2	6.2	6.2	0.03	0.948	0.636
d 10		7.5	7.4	7.5	7.5	7.6	7.6	0.12	0.451	0.534
d 24		13.2	13.1	12.9	13.3	13.2	13.4	0.31	0.535	0.784
d 38		21.0	20.8	20.8	21.5	21.3	21.5	0.46	0.847	0.871
Phase 1 period (d 0 to 10)										
ADG, g		130	117	128	132	140	146	13.9	0.451	0.471
ADFI, g		137	132	144	147	146	160	10.4	0.715	0.878
G:F, g/kg		940	846	855	891	954	913	57.4	0.241	0.188
Phase 2 period (d 10 to 24)										
ADG, g		401	399	385	416	402	410	25.1	0.677	0.446
ADFI, g		526	530	517	538	526	563	23.4	0.292	0.259
G:F, g/kg		762	753	743	773	763	729	20.4	0.359	0.657
Experimental Period (d 0 to 24)										
ADG, g		287	274	276	297	291	295	12.4	0.655	0.936
ADFI, g		362	355	359	375	365	387	13.2	0.516	0.602
G:F, g/kg		791	771	768	793	797	763	11.2	0.751	0.129
Common period (d 24 to 38)										
ADG, g		553	554	562	584	580	581	15.9	0.693	0.966
ADFI, g		829	829	827	851	840	859	16.7	0.766	0.561
G:F, g/kg		666	669	679	686	689	676	10.4	0.301	0.513
Overall (d 0 to 38)										

ADG, g	384	373	379	403	396	396	11.7	0.904	0.736
ADFI, g	531	522	527	550	539	554	13.1	0.736	0.775
G:F, g/kg	721	714	718	732	736	715	7.6	0.281	0.160
Fecal DM, %[3]									
d 7	27.5	30.4	29.8	28.1	27.3	28.3	1.31	0.303	0.159
Serum[4]									
Ca, mg/dL	10.7	11.2	11.6	11.0	11.3	11.6	0.21	0.355	0.942
P, mg/dL	11.0	10.6	11.0	10.9	11.3	10.8	0.32	0.897	0.156

[1] A total of 360 weanling barrows (DNA 200 × 400, initially 6.2 ± 0.03 kg) approximately 21 days of age were used in a 38-d experiment with 5 pigs per pen and 12 pens per treatment.

[2] DSM Nutritional Products, Parsippany, NJ.

[3] Feces from three pigs from each pen were pooled, weighed, and dried to measure fecal dry matter.

[4] Blood was collected from 1 pig per pen on d 21 and submitted to the Kansas State University Veterinary Diagnostic Lab for Ca and P analysis.

Table 1-6 Main effects of calcium carbonate and benzoic acid on nursery pig growth performance, fecal dry matter and blood calcium and phosphorus concentrations, Exp. 2[1]

	$CaCO_3$, %:				*P* =		Benzoic acid[2]:			
Item	0.45	0.90	1.35	SEM	Linear	Quadratic	Without	With	SEM	*P* =
BW, kg										
d 0	6.2	6.2	6.2	0.03	0.793	0.283	6.2	6.2	0.03	0.257
d 10	7.5	7.5	7.6	0.10	0.598	0.403	7.4	7.6	0.10	0.156
d 24	13.3	13.1	13.2	0.26	0.620	0.665	13.1	13.3	0.23	0.294
d 38	21.2	21.1	21.2	0.38	0.821	0.678	20.9	21.4	0.35	0.059
Phase 1 period (d 0 to 10)										
ADG, g	131	129	137	12.0	0.550	0.529	125	139	11.2	0.092
ADFI, g	142	139	152	8.8	0.228	0.245	138	151	8.2	0.042
G:F, g/kg	915	900	884	47.9	0.485	0.990	880	919	44.3	0.293
Phase 2 period (d 10 to 24)										
ADG, g	408	400	397	23.4	0.397	0.832	395	409	22.8	0.188
ADFI, g	532	528	540	20.4	0.595	0.566	524	542	19.4	0.174
G:F, g/kg	768	758	736	18.1	0.022	0.611	752	755	17.2	0.799
Experimental Period (d 0 to 24)										
ADG, g	292	282	286	10.3	0.519	0.451	279	295	9.4	0.056
ADFI, g	368	360	373	10.5	0.690	0.288	358	376	9.4	0.071
G:F, g/kg	792	784	766	8.5	0.014	0.595	777	784	7.3	0.374
Common period (d 24 to 38)										
ADG, g	569	567	572	11.7	0.846	0.839	557	582	10.0	0.045
ADFI, g	840	835	843	12.6	0.849	0.607	828	850	10.9	0.091
G:F, g/kg	676	679	678	7.3	0.883	0.851	672	684	6.0	0.159
Overall (d 0 to 38)										
ADG, g	393	384	387	9.5	0.531	0.485	378	398	8.7	0.011
ADFI, g	541	530	541	10.3	0.982	0.288	527	548	9.1	0.030

G:F, g/kg	726	725	716	5.8	0.163	0.550	718	727	5.1	0.096
Fecal DM, %[3]										
d 7	27.8	28.8	29.0	1.09	0.249	0.657	29.2	27.9	1.00	0.126
Serum[4]										
Ca, mg/dL	10.8	11.3	11.6	0.15	< 0.001	0.808	11.2	11.3	0.13	0.470
P, mg/dL	10.9	11.0	10.9	0.23	0.959	0.941	10.9	11.0	0.19	0.591

[1] A total of 360 weanling barrows (DNA 200 × 400, initially 6.2 ± 0.03 kg) approximately 21 days of age were used in a 38-d experiment with 5 pigs per pen and 24 pens per calcium carbonate treatment and 36 pens per benzoic acid treatment. There was no calcium carbonate × benzoic acid interactions observed ($P > 0.10$).

[2] DSM Nutritional Products, Parsippany, NJ.

[3] Feces from three pigs from each pen were pooled, weighed, and dried to measure fecal dry matter.

[4] Blood was collected from 1 pig per pen on d 21 and submitted to the Kansas State University Veterinary Diagnostic Lab for Ca and P analysis.

Chapter 2 - Evaluation of different corn protein sources on nursery pig growth performance and fecal dry matter

Abstract

The objective of these studies was to determine the effects of 4 different corn protein sources (CPS) compared to conventional specialty protein sources on weanling pig growth performance and fecal DM. In Exp. 1, 315 pigs (DNA 241 × 600; initially 5.5 ± 0.72 kg) were used in a 35-day study. The 7 experimental diets were a control diet that contained enzymatically treated soybean meal (ESBM) and select menhaden fish meal (MFM), or diets formulated with 5 or 10% of 3 different CPS sources (CPS1, CPS2 or CPS3) added at the expense of ESBM and MFM. Treatment diets were fed in pellet form in 2 phases from d 0 to 7 and d 7 to 21, with a common mash diet fed from d 21 to 35. There was no evidence for CPS × level interactions (P > 0.10) or differences (P > 0.05) between CPS2 and CPS3. From d 0 to 21 (experimental period), feeding 10% of any CPS decreased (P < 0.05) ADG compared to pigs fed the control. Feeding 10% CPS2 also decreased (P < 0.05) G:F compared to the control. Additionally, compared to the control, feeding 5% CPS1 decreased (P < 0.05) ADG, and 5 or 10% CPS1 (P < 0.05) decreased ADFI. Feeding CPS1 decreased (P < 0.05) ADG and ADFI compared to CPS2 or CPS3. For the overall experiment, feeding CPS1 decreased (P < 0.05) ADFI compared to CPS2 or CPS3, decreased ADG compared to CPS3, and increased G:F compared to CPS2. In addition, CPS1 decreased (P < 0.05) ADFI compared to the control, while CPS2 and CPS3 did not affect overall growth performance. In Exp. 2, 355 pigs (DNA 200 × 400, initially 6.1 ± 0.05 kg) were used in a 38-day study. The 6 experimental diets were a control diet containing 6% ESBM or 5 diets with increasing levels (3, 6, 9, 12, and 15% in phase 1 with half of those in phase 2) of a fourth CPS (CPS4). Treatment diets were fed from d 0 to 10, d 10 to 25, with a common diet from d 25 to

38. From d 0 to 25 (experimental period), ADG and ADFI increased then decreased (quadratic, $P < 0.05$), and G:F decreased (linear, $P = 0.006$) with increasing CPS4. For the overall experiment, d 38 BW, ADG and ADFI increased then decreased (quadratic, $P < 0.05$) with increasing levels of CPS4. Fecal DM on d 25 increased then decreased (quadratic, $P < 0.05$), and was greater ($P < 0.05$) on d 25 than on d 10. These data indicate that CPS4 may be an alternative specialty protein source to ESBM. In summary, the CPS used in Exp. 1 reduced growth performance when included at 10%, but CPS4 used in Exp. 2 may be useful when fed at 12 and 6% in phase 1 and 2, respectively.

Key Words: amino acid, corn, corn protein, growth, nursery pig

Introduction

In order to transition piglets to a plant protein diet, either fermented or enzymatically treated soybean meal (ESBM) are often utilized in weaned pig diets of to provide digestible AA without the negative effects of anti-nutritional factors such as glycinin and β-conglycinin present in conventional soybean meal (Zhao et al., 2010). Along with soybean-based ingredients, corn-derived protein ingredients are also being evaluated as alternative specialty protein sources. In the past, corn co-products that have been evaluated in swine nursery diets include high protein dried distillers grains (HP DDG; Cemin et al., 2021) and corn gluten meal (Mahan, 1993).

Through the wet corn-milling process, acid removes the germ from the corn kernel and the germ is then pressurized to extract corn oil. The remaining portion of the germ is approximately 60 to 65% CP. The portion of the kernel not removed by acid is finely ground, fiber removed, and the starch and sugar (high fructose corn syrup) are separated. A mixture of co-products from these production streams can then be used in animal feeds. Although further

processed corn co-products can be high in CP in comparison to soybean meal, fish meal, and ESBM, the AA composition and digestibility may cause AA imbalances that lead to poor growth or feed intake.

Little data is available to describe the effects of corn protein sources derived as co-products from the wet milling industry, Thus, two experiments were conducted to evaluate different corn protein sources (CPS) as replacements to specialty protein sources in nursery pig diets. The objective of the first experiment was to investigate the effects of diets containing 5 or 10% of 3 different CPS on nursery pig growth performance. The objective of the second experiment was to investigate the effects of increasing levels of a fourth CPS (CPS4) on nursery pig growth performance and fecal dry matter.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols (4036 and 4506) used in these experiments. The studies were conducted at the Kansas State University Swine Teaching and Research Center and the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. Both facilities are completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder and a cup waterer to provide *ad libitum* access to feed and water. Pens (1.22 × 1.22 m) had metal-slatted floors and allowed approximately 0.30 m^2/pig. The corn protein sources utilized in both experiments were provided by Cargill Inc., Blaire, NE.

Animals and diets

Experiment 1

A total of 315 mixed gender pigs (DNA Line 241 × 600; initially 5.5 ± 0.72 kg) were used in a 35-day study with 5 pigs per pen and 9 replications per treatment. After weaning, pigs were randomly assigned to pens and pens were allotted to treatments. There was a total of 7 dietary treatments which consisted of 6 treatments containing 5 or 10% of one of the 3 different CPS (Cargill Inc., Blair, NE; Tables 2 and 3). A control diet was also fed that contained 5% ESBM (HP300, Hamlet Protein, Findlay, OH) and 5% select menhaden fish meal (MFM) in phase 1 and 5% MFM in phase 2. In phase 1, the CPS were added at the expense of MFM in the 5% diets and both MFM and ESBM in the 10% diets. In phase 2, the CPS were added at the expense of MFM in the 5% diets, and MFM and ground corn in the 10% diets. Feed grade AAs were included to create similar ratios of Met and Cys, Thr, Trp, and Val to SID Lys and similar SBM levels in all diets. Nutrient loading values for the 3 corn protein sources (CPS) were obtained from analysis and previous digestibility studies conducted at the University of Illinois (unpublished data) that determined the standardized ileal digestibility (SID) AA and standardized total tract digestibility (STTD) P coefficients (Table 1), while the nutrient values for the other ingredients were provided by the supplier (HP300, Hamlet Protein, Findlay, OH) or obtained from the NRC (2012). Treatment diets were fed from d 0 to 21 (d 0 to 7 and 7 to 21 for phase 1 and 2, respectively). A common phase 3 diet was fed from d 21 to 35 (Table 4). All the experimental diets were fed in pellet form. Phase 1 and 2 diets were manufactured at Cargill-Provimi, Brookville, OH, with the phase 3 common diet manufactured at Hubbard Feeds, Beloit, KS.

Experiment 2

A total of 355 castrated male pigs (DNA 200 × 400, initially 6.1 ± 0.05 kg) were used in a 38-day study with 5 pigs per pen and 11 or 12 replications per treatment. Upon arrival to the

nursery research facility, pigs were randomly assigned to pens and pens were allotted to treatments. There was a total of 6 dietary treatments which consisted of 5 treatments with increasing levels of an additional corn protein source (CPS4; Cargill Inc., Blair, NE). The CPS4 was added at 3, 6, 9, 12, and 15% of the diet in phase 1 and 1.5, 3, 4.5, 6, and 7.5% in phase 2, respectively (Tables 5 and 6). The sixth experimental diet was a control containing ESBM (HP300, Hamlet Protein, Findlay, OH) at 6 and 3% inclusion in phase 1 and 2, respectively. The nutrient concentrations for minerals and AA (total and SID coefficients) were provided by the supplier and the energy was calculated using equations (EvaPig) based on its nutrient profile (Table 1). Diets were balanced to provide similar SID AA and STTD P levels, but not balanced for energy. Diets were also formulated to maximize L-Lys HCl while keeping the SID Lys:CP constant (6.42) between treatments. Soybean meal levels decreased with increasing CPS4.

Treatment diets were fed from d 0 to 10 (pelleted) and 10 to 25 (meal) for phase 1 and 2, respectively. A common phase 3 diet was fed in meal form from d 25 to 38 (Table 7). A single base diet (meal) was manufactured at Hubbard Feeds in Beloit, KS. Hand additions of ESBM, CPS4, feed grade AA, calcium carbonate, and monocalcium phosphate were mixed with the base diet and then pelleted (phase 1) at the O.H. Kruse Feed Technology Innovation Center at Kansas State University, Manhattan, KS.

Data collection

Pigs were individually weighed, and feed disappearances were measured for each pen on d 0, 7, 21, and 35 in Exp. 1, and on d 0, 10, 25, and 38 in Exp. 2 to determine ADG, ADFI, and G:F. In Exp. 2 on d 10 and 25, ~ 40 g of feces were pooled from three randomly selected pigs per pen and dried at 55°C for 48 h to determine fecal DM.

Diet samples

In Exp. 1, diet samples were collected from the feeders of each pen during the middle of each dietary phase. Samples were thoroughly mixed within treatment and phase. In Exp. 2, complete diet samples of each treatment were taken with a grain probe from every other bag (phase 1) and every 4th bag (phase 2) upon completion of manufacturing. Phase 3 diet samples were taken with a grain probe from four random bags per 907 kg of feed delivered. All diet samples were stored at -20°C.

Statistical analysis

Data were analyzed using the lmer function in R Studio (Version 3.5.2, R Core Team. Vienna, Austria) with pen serving as the experimental unit. In Exp. 1, data were analyzed in a randomized complete block design with pen initial BW incorporated in the model as a blocking factor. Contrasts were used to test for differences between each CPS. Pairwise comparisons were used to test for differences between all treatments. In Exp. 2, data were analyzed in a completely randomized design with barn included in the model as a random effect. Contrasts were used to test linear and quadratic responses to increasing CPS4 source excluding the control treatment. Similar to Exp. 1, pairwise comparisons were used to test for differences between all treatments. Fecal DM data were analyzed as repeated measures to evaluate the fixed effects of treatment, day, and the associated interaction. A Tukey-Kramer multiple comparison adjustment was incorporated when evaluating pairwise differences between treatments. In both experiments, results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Experiment 1

In Exp. 1, there was no evidence ($P > 0.05$) for a source × level interaction for any of the growth periods (Table 8). From d 0 to 7, there was no evidence ($P > 0.05$) for differences in BW, ADG, or ADFI between any CPS at 5 or 10% compared to pigs fed the control diet. There was, however, evidence for CPS level to affect G:F, with pigs fed diets containing 10% CPS having decreased ($P = 0.018$) G:F compared to 5% inclusion.

From d 7 to 21, pigs fed 10% CPS had decreased ADG ($P = 0.003$), ADFI ($P = 0.066$), and G:F ($P = 0.003$) and were lighter ($P = 0.006$) on d 21 compared to those fed 5% CPS. Compared to the control, pigs fed 10% of any CPS were lighter ($P < 0.05$) and had decreased ADG. Feeding 5% inclusion of CPS1 also decreased ($P < 0.05$) BW and ADG compared to the control. Also compared to the control, feeding both levels of CPS1 and 10% of CPS2 decreased ($P < 0.05$) ADFI. For main effects of CPS source, pigs fed CPS1 had decreased ($P < 0.05$) BW and ADFI, and a tendency ($P < 0.10$) for decreased ADG compared to pigs fed diets containing CPS2. Pigs fed CPS1 also had decreased ($P < 0.05$) BW, ADG, and ADFI compared to those fed CPS3.

From d 0 to 21 (experimental feeding period), there was a CPS level main effect for ADG ($P = 0.003$) and G:F ($P < 0.001$) where pigs fed 10% CPS had worse performance than those fed 5%. Pigs fed 5 or 10% of CPS1 had decreased ADG and ADFI ($P < 0.05$) compared to control fed pigs, with no differences in G:F. Pigs fed 10% CPS2 had decreased ADG and G:F ($P < 0.05$) compared to pigs fed the control diet. Feeding 10% CPS3 decreased ADG ($P < 0.05$) compared to the control diet. For the main effects for CPS source, pigs fed CPS1 had lower ADG and ADFI ($P < 0.05$) compared to either CPS2 or CPS3.

From d 21 to 35 (common period), there was a marginal source × level interaction for ADG ($P = 0.056$), with pigs previously fed 10% CPS1 or CPS3 having numerically increased ADG while those fed CPS2 had numerically decreased ADG compared to pigs previously fed 5% within each source. There was a main effect of CPS level for G:F ($P = 0.021$), where pigs previously fed 10% had greater G:F than pigs previously fed 5% CPS. Also, pigs previously fed 10% CPS1 had improved G:F ($P < 0.05$) compared to pigs previously fed the control diet or the diet with 5% CPS3.

For the overall experiment (d 0 to 35), there was a main effect for CPS source for ADFI ($P = 0.018$) which was primarily the result of pigs fed CPS1 having lower ADFI ($P < 0.05$) than those fed either CPS2 or CPS3. There was no difference ($P > 0.05$) between CPS2 and CPS3 sources. For CPS levels, pigs fed 5 or 10% CPS1 had lower ADFI ($P < 0.05$) compared to the control.

Experiment 2

There was no evidence (P > 0.05) for differences between the control and any diets that contained CPS4 for any growth periods (Table 9). From d 0 to 10, CPS4 did not impact ADG, ADFI, or G:F; however, there was a tendency for decreased d 10 BW (linear, $P = 0.092$) as CPS4 increased. From d 10 to 25, there was evidence for ADG and ADFI to increase (quadratic, $P < 0.05$) up to 3 and 6% of the diet, respectively, with no improvements thereafter. There was a tendency for G:F to decrease as CPS4 source increased (linear, $P = 0.063$). On d 25, BW increased then decreased (quadratic, $P = 0.037$) with increasing CPS4 in the diet.

From d 0 to 25 (experimental feeding period), ADG increased (quadratic, $P = 0.030$) up to the intermediate level (6.0 and 3.0% in phase 1 and phase 2, respectively) of CPS4. Similarly, ADFI increased (quadratic, $P = 0.036$) up to 12.0 and 6.0% inclusion of CPS4 in phase 1 and

phase 2, respectively, with a reduction observed at the highest inclusion rate. Feed efficiency worsened (linear, $P = 0.006$) as CPS4 increased. From d 25 to 38 (common period), there was no evidence ($P > 0.10$) for differences in growth performance; however, d 38 BW was maximized (quadratic, $P = 0.041$) in the pigs fed 6.0 and 3.0% CPS4 in phase 1 and phase 2, respectively.

For the overall experiment, pigs fed increasing CPS4 had improved ADG (quadratic, $P = 0.028$) and ADFI (quadratic, $P = 0.032$) with pigs fed intermediate levels (6 to 12% CPS4 in phase 1 and 3 to 6% CPS4 in phase 2) having the greatest performance. There was also a tendency for G:F to decrease as CPS4 increased (linear, $P = 0.066$).

For fecal DM, there was a marginally significant treatment × day interaction (quadratic, $P = 0.064$). There was no evidence of a treatment effect on fecal DM when samples were collected on d 10 ($P > 0.10$). However, there was a marginally significant quadratic ($P = 0.051$) response in fecal DM where increasing CPS4 up to 12% in phase 1 and 6% in phase 2 increased fecal DM but when pigs were fed the highest level of CPS4 they had reduced fecal DM. Fecal DM was also greater ($P = 0.004$) on d 25 than on d 10.

Discussion

Corn is typically used to supply energy in swine diets. However, corn co-products of the ethanol and wet corn milling industries such as high protein dried distillers grain (HP DDG) and corn gluten meal provide high CP ingredients that could be used in formulations for nursery pig diets. Cemin et al. (2021) evaluated increasing levels of HP DDG in the diets of nursery pigs, where it was observed that increasing HP DDG decreased d 21 BW, ADG, and ADFI. Other co-products of the wet milling industry such as corn germ meal and corn gluten feed, have also been studied as protein sources for nursery pig diets (Mahan, 1993; Hastad et al., 2001; Wiltafsky et al., 2009; Almeida et al., 2011). Almeida et al. (2011) evaluated the digestibility of corn and

different co-products and reported that wet milling co-products can have greater AA digestibility than corn or other co-products of corn milling such as HP DDG and DDGS.

Based on a meta-analysis by Cemin et al. (2019), there is reason to believe that if an abundance of corn protein is included in the diet, a decrease in growth performance will occur. This could be due to excess of the branch chain AA (BCAA) leucine (Leu) when corn and corn co-products are provided in the diet with insufficient supplementation of the other BCAA, Val and Ile (Cemin et al., 2019). Kwon et al. (2019) observed that increasing SID Leu in the diet from 100 to 300% of the NRC (2012) requirement estimate decreased ADFI and G:F in 30 to 40 kg growing pigs possibly due to lower serotonin transport in the brain. This observation agrees with the findings of Exp. 1 where CPS1 contained higher Leu:Lys than the other CPS and had reduced ADG and ADFI from d 0 to 21. As well, increasing Leu:Lys ratios from 123% in the control diet to 172 to 196% in 10% CPS diets in phase 2 decreased ADG from d 0 to 21. In other studies, Htoo et al. (2017) observed that increasing Leu:Lys in 8 to 20 kg pigs required an increase in the Ile concentration to maximize growth performance. The demand for Ile is attributed to excess Leu stimulating catabolism of Ile, as they both share similar chemical properties in their AA geometry (Harper et al., 1984). In the present study, feed grade AAs were included in the diets to maintain similar SID AA ratios for most AA compared to Lys across the dietary treatments, but Leu increased as a result of increasing CPS. According to a meta-analysis by Cemin et al. (2019), the negative responses to increased Leu are alleviated by increased Val. Gloaguen et al. (2011) observed that Val would lessen the decreased growth performance response to increasing Leu levels in nursery pigs. In Exp. 2, the Val:Lys ratio was maintained (> 74%) above the NRC (2012) requirement estimate (63%). Similarly, SID Ile:Lys in the present study (> 57%) was higher than the NRC (2012) requirement estimates (51%) and increased as

CPS increased in the diet. Therefore, we would conclude that Ile and Val should not be limiting with increasing Leu in the diet.

In our present research, the corn protein sources were compared to ESBM. Acosta et al. (2021) compared feeding corn protein with a control diet that contained ESBM and spray-dried plasma protein. The CP of the corn protein (47.98%) was similar to the CPS4 used in Exp. 2. These authors observed that the corn protein did not influence growth performance of weaned pigs through 35 days when included up to 10% at the expense of ESBM and spray-dried plasma protein. This agrees with the present study where similar responses to growth performance were observed when replacing ESBM with increasing CPS4 in Exp. 2.

In both experiments, the corn protein sources were formulated based on the ingredient analysis and previously determined digestibility values. The energy value of CPS4 (1,759 kcal NE/kg) was estimated from nutrient composition using EvaPig. The energy value may have been overestimated, which would explain the linear reduction in G:F. Nitikanchana et al. (2015) observed a 1% improvement in G:F with every 1% increase in diet NE. This is in contrast to the results in Exp. 2. As CPS4 increased, the NE decreased by 1.27% in phase 1 and 0.77% in phase 2, respectively. Therefore, we expected a reduction in G:F as CPS4 increased, however our results showed a reduction by 5% from d 0 to 25, which is greater than the reduction in NE. It could be that the NE of the CPS4 was overestimated, or the AA digestibility coefficients were lower than anticipated. If the diets in Exp. 2 had been formulated with a lower NE value for CPS4, we would have observed a greater reduction in NE as CPS4 increased, which would agree with the reduction in G:F.

In conclusion, the 3 different CPS used in Exp. 1 decreased nursery pig growth performance as the level of each source increased from 5 to 10%. The results may be due to the

Leu levels in the diets. In Exp. 2, feeding increasing amounts of CPS4 decreased G:F, but there were no negative effects in comparison to the ESBM control. There were quadratic effects of increasing the CPS4 on ADG and ADFI with levels of 6 to 12% in phase 1 and 3 to 6% in phase 2 providing similar performance. In summary, these data suggest that the CPS sources evaluated in Exp. 1 were not equal replacements for ESBM and MFM, while the CPS4 source in Exp. 2 elicited similar responses as diets containing ESBM when included at levels up to 12% in phase 1 and 6% in phase 2 without reducing performance.

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Table 2-1 Chemical analysis of corn protein sources[1]

	Protein source[2]			
Item, %	CPS1	CPS2	CPS3	CPS4
Dry matter	97.5	91.2	91.2	74.4
Starch	5.3	1.7	1.7	1.5
Crude protein	77.3	69.0	69.0	47.1
Crude fiber	0.7	1.0	1.0	0.7
Ether extract	4.7	3.0	3.0	1.8
Total AA				
Indispensable AA				
Lys	1.1	2.8	2.7	3.6
Ile	3.3	2.7	2.4	1.7
Leu	13.0	10.6	9.4	6.7
Met	1.9	1.6	1.5	0.9
Met & Cys	3.4	2.9	2.6	1.7
Thr	2.5	2.2	2.1	1.6
Trp	0.5	0.4	0.3	0.2
Val	3.5	3.0	3.0	2.1
His	1.6	1.3	1.2	1.0
Dispensable AA				
Arg	2.3	2.1	1.8	1.6
Phe	5.0	4.2	3.8	2.5
Ala	---	---	---	3.8
Asn	---	---	---	2.9
Cys	1.4	1.3	1.1	0.8
Glu	---	---	---	10.3
Gly	---	---	---	1.4
Pro	---	---	---	4.1
Ser	---	---	---	2.2
Tyr	4.0	3.4	3.1	2.3
ME, kcal/kg[3]	4,276	3,757	3,757	2,847
NE, kcal/kg[3]	2,643	2,285	2,285	1,759
Calcium	0.01	0.01	0.01	0.01
Phosphorus	0.18	0.58	0.58	0.78
STTD P	0.13	0.55	0.55	0.74

[1] Four corn protein sources were analyzed at the University of Illinois (CPS1 – 3; unpublished data) or supplied by the supplier (CPS4; Cargill Inc., Blaire, NE) for nutrient composition and then used for diet formulation for the respective experiments.

[2] Corn protein sources (CPS) 1, 2, and 3 were used in Exp. 1, and CPS4 was used in Exp. 2.

[3] Metabolizable and net energy were estimated based on nutrient analysis (EvaPig 020 version 2.0.3.2).

Table 2-2 Phase 1 diet composition, Exp. 1 (as-fed basis)[1]

		Corn protein source[2]					
		CPS1		CPS2		CPS3	
Item, %	ESBM[3]	5%	10%	5%	10%	5%	10%
Ingredients							
Corn	38.75	37.68	37.58	37.78	37.78	37.79	37.76
Soybean meal	16.14	16.16	16.15	16.17	16.16	16.14	16.15
Dried whey	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Fish meal	5.00	--	--	--	--	--	--
ESBM	5.00	5.00	--	5.00	--	5.00	--
CPS1	--	5.00	10.00	--	--	--	--
CPS2	--	--	--	5.00	10.00	--	--
CPS3	--	--	--	--	--	5.00	10.00
Corn DDGS, 7.5% oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calcium carbonate	0.50	0.80	0.78	0.85	0.90	0.85	0.90
Monocalcium phosphate	0.50	1.10	1.20	1.00	0.95	1.00	0.95
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lys HCl	0.40	0.60	0.75	0.50	0.55	0.51	0.56
DL-Met	0.19	0.14	0.06	0.16	0.10	0.17	0.12
L-Thr	0.17	0.17	0.16	0.18	0.18	0.18	0.19
L-Trp	0.04	0.04	0.05	0.04	0.06	0.05	0.07
L-Val	0.16	0.13	0.10	0.15	0.15	0.15	0.14
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Phytase[4]	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Zinc oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis, %							
SID amino acids							
Lys	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Ile:Lys	58	61	63	59	60	58	58
Leu:Lys	116	146	175	138	160	134	152

Met:Lys	37	34	32	34	33	35	34
Met & cys:Lys	57	57	57	57	57	57	57
Thr:Lys	64	64	64	64	64	64	64
Trp:Lys	19.0	19.0	19.0	19.0	19.0	19.0	19.0
Val:Lys	74	74	74	74	74	74	74
His:Lys	33	34	34	33	33	33	32
Net energy, kcal/kg	2,586	2,575	2,591	2,560	2,560	2,560	2,560
CP	21.9	22.7	23.8	22.2	22.9	22.2	22.9
Ca	0.80	0.80	0.80	0.80	0.80	0.80	0.80
STTD P	0.63	0.63	0.63	0.63	0.63	0.63	0.63

[1] Phase 1 diets were fed from approx. 5.5 to 6 kg.

[2] Corn protein sources (CPS) were provided by Cargill Inc., Blair, NE.

[3] Enzymatically treated soybean meal (HP300, Hamlet Protein, Findlay, OH)

[4] Ronozyme HiPhos GT 2700 (DSM Nutritional Products, Basel, Switzerland) provided a 0.14% STTD P release with 2,027 FYT/kg inclusion in the diet.

Table 2-3 Phase 2 diet composition, Exp. 1 (as-fed basis)[1]

		Corn protein source[2]					
		CPS1		CPS2		CPS3	
Item, %	ESBM[3]	5%	10%	5%	10%	5%	10%
Ingredients							
Corn	47.86	46.86	42.24	46.91	42.51	46.90	42.45
Soybean meal	21.90	21.90	21.88	21.92	21.88	21.90	21.88
Corn DDGS, 7.5% oil	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Dried whey	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal	5.00	--	--	--	--	--	--
CPS1	--	5.00	10.00	--	--	--	--
CPS2	--	--	--	5.00	10.00	--	--
CPS3	--	--	--	--	--	5.00	10.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calcium carbonate	0.55	0.83	0.83	0.88	0.93	0.88	0.93
Monocalcium phosphate	0.40	1.00	1.00	0.93	0.80	0.93	0.80
Sodium chloride	0.55	0.55	0.55	0.55	0.55	0.55	0.55
L-Lys HCl	0.45	0.65	0.61	0.55	0.41	0.56	0.43
DL-Met	0.15	0.10	0.00	0.12	0.00	0.13	0.03
L-Thr	0.18	0.18	0.09	0.19	0.11	0.19	0.11
L-Trp	0.04	0.05	0.03	0.05	0.03	0.05	0.04
L-Val	0.14	0.12	0.00	0.14	0.01	0.14	0.01
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Phytase[4]	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Zinc oxide	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis, %							
SID amino acids							
Lys	1.35	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	57	60	70	59	67	57	65
Leu:Lys	123	154	196	146	180	142	172
Met:Lys	36	33	32	34	30	34	31

Met & cys:Lys	57	57	59	57	57	57	57
Thr:Lys	64	64	64	64	64	64	64
Trp:Lys	19.0	19.0	19.0	19.0	19.0	19.0	19.0
Val:Lys	74	74	76	74	74	74	74
His:Lys	36	36	40	36	40	35	39
Net energy, kcal/kg	2,549	2,540	2,536	2,523	2,500	2,523	2,500
CP	22.3	23.1	26.3	22.6	25.3	22.6	25.4
Ca	0.72	0.72	0.72	0.72	0.72	0.72	0.72
STTD P	0.55	0.55	0.55	0.55	0.55	0.55	0.55

[1] Phase 2 diets were fed from approx. 6 to 10 kg.

[2] Corn protein sources (CPS) were provided by Cargill Inc., Blair, NE.

[3] Enzymatically treated soybean meal (HP300, Hamlet Protein, Findlay, OH)

[4] Ronozyme HiPhos GT 2700 (DSM Nutritional Products, Basel, Switzerland) provided a 0.14% STTD P release with 2,027 FYT/kg inclusion in the diet.

Table 2-4 Phase 3 common diet composition, Exp. 1 (as-fed basis)[1]

Item, %	
Ingredients	
Corn	65.47
Soybean meal	28.30
Fat	2.00
Calcium carbonate	0.75
Monocalcium phosphate	1.10
Sodium chloride	0.60
L-Lys HCl	0.55
DL-Met	0.25
L-Thr	0.23
L-Trp	0.05
L-Val	0.16
Trace mineral premix	0.15
Vitamin premix with phytase[2]	0.25
Alltech All-Bind HD[3]	0.15
Total	100.00
Calculated analysis, %	
SID amino acids	
Lys	1.30
Ile:Lys	53
Leu:Lys	111
Met:Lys	39
Met & cys:Lys	60
Thr:Lys	63
Trp:Lys	19.3
Val:Lys	70
His:Lys	35
NE, kcal/kg	2,540
CP	19.9
Ca	0.65
STTD P	0.48

[1] Phase 3 common diets were fed from approx. 10 to 18 kg.

[2] Ronozyme HiPhos GT 2700 (DSM Nutritional Products, Basel, Switzerland) provided a 0.11% STTD P release with 1,226 FYT/kg inclusion in the diet.

[3] Alltech, Lexington, KY.

Table 2-5 Phase 1 diet composition, Exp. 2 (as-fed basis)[1]

	ESBM, %[2]	CPS4, %[3]				
Ingredient, %	6.0	3.0	6.0	9.0	12.0	15.0
Corn	38.29	36.96	37.03	37.11	37.04	37.10
Soybean meal, 46.5% CP	23.52	27.90	24.85	21.80	18.91	15.86
Spray-dried whey	12.50	12.50	12.50	12.50	12.50	12.50
Whey permeate	11.25	11.25	11.25	11.25	11.25	11.25
ESBM	6.00	---	---	---	---	---
CPS4	---	3.00	6.00	9.00	12.00	15.00
Corn oil	3.00	3.00	3.00	3.00	3.00	3.00
Spray-dried bovine plasma	2.00	2.00	2.00	2.00	2.00	2.00
Calcium carbonate	0.73	0.73	0.75	0.75	0.78	0.80
Monocalcium phosphate	0.75	0.68	0.60	0.55	0.48	0.43
Zinc oxide	0.39	0.39	0.39	0.39	0.39	0.39
Sodium chloride	0.33	0.33	0.33	0.33	0.33	0.33
Vitamin premix with phytase[4]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
L-Lys HCl	0.35	0.34	0.33	0.33	0.32	0.31
DL-Met	0.20	0.19	0.19	0.18	0.17	0.16
L-Thr	0.16	0.16	0.17	0.18	0.18	0.19
L-Trp	0.01	0.02	0.03	0.05	0.06	0.07
L-Val	0.11	0.13	0.13	0.13	0.13	0.13
L-Ile	0.03	0.05	0.06	0.07	0.08	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis, %						
SID amino acids						
Lys	1.40	1.40	1.40	1.40	1.40	1.40
Ile:Lys	60	61	60	60	60	61
Leu:Lys	114	119	125	131	138	144
Met:Lys	34	34	34	34	34	33
Met & cys:Lys	56	57	57	57	57	57
Thr:Lys	63	63	63	63	63	63
Trp:Lys	19.7	19.6	19.7	19.6	19.6	19.6
Val:Lys	72	72	72	72	72	72
Total Lys	1.54	1.56	1.57	1.59	1.60	1.62
Net energy, kcal/kg	2,639	2,600	2,591	2,584	2,575	2,567
SID Lys:NE g/Mcal	5.31	5.39	5.40	5.42	5.44	5.46
CP	21.7	21.8	21.8	21.8	21.8	21.8
Ca	0.70	0.68	0.67	0.65	0.64	0.63
P	0.63	0.61	0.60	0.59	0.58	0.57
STTD P	0.54	0.54	0.53	0.54	0.53	0.54
Analyzed Ca:P	1.11	1.12	1.12	1.10	1.11	1.11

[1] Phase 1 experimental diets were fed from approx. 6.1 to 7.4 kg.

[2] Enzymatically treated soybean meal (HP300, Hamlet Protein, Findlay, OH).

[3] Corn protein source (CPS4) was provided by Cargill, Inc, Blair, NE.

[4] Ronozyme HiPhos 2700 (DSM Nutritional Products) provided a 0.13% release of STTD P with 1,250 FYT/kg inclusion in the diet.

Table 2-6 Phase 2 diet composition, Exp. 2 (as-fed basis)[1]

	ESBM, %[2]	CPS4, %[3]				
Ingredient, %	3.00	1.50	3.00	4.50	6.00	7.50
Corn	55.55	54.95	54.91	55.05	54.96	54.90
Soybean meal, 46.5% CP	26.38	28.49	27.05	25.44	24.00	22.56
Spray-dried whey	10.00	10.00	10.00	10.00	10.00	10.00
ESBM	3.00	---	---	---	---	---
CPS4	---	1.50	3.00	4.50	6.00	7.50
Corn oil	1.00	1.00	1.00	1.00	1.00	1.00
Calcium carbonate	0.88	0.88	0.88	0.88	0.90	0.93
Monocalcium phosphate	0.88	0.85	0.83	0.78	0.78	0.75
Zinc oxide	0.25	0.25	0.25	0.25	0.25	0.25
Sodium chloride	0.55	0.55	0.55	0.55	0.55	0.55
Vitamin premix with phytase[4]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
L-Lys HCl	0.48	0.47	0.47	0.47	0.46	0.46
DL-Met	0.21	0.21	0.20	0.20	0.19	0.19
L-Thr	0.20	0.20	0.21	0.21	0.22	0.22
L-Trp	0.04	0.05	0.05	0.06	0.07	0.07
L-Val	0.16	0.16	0.16	0.17	0.17	0.17
L-Ile	0.05	0.06	0.06	0.07	0.07	0.08
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis, %						
SID amino acids						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	60	60	60	60	60	60
Leu:Lys	113	116	119	122	126	129
Met:Lys	36	36	36	36	35	36
Met & cys:Lys	57	57	57	57	57	57
Thr:Lys	63	63	63	63	63	63
Trp:Lys	19.6	19.6	19.6	19.6	19.6	19.6
Val:Lys	72	71	72	72	72	72
Total Lys, %	1.48	1.49	1.50	1.51	1.51	1.52
NE, kcal/kg	2,498	2,478	2,474	2,470	2,465	2,459
SID Lys:NE g/Mcal	5.41	5.45	5.46	5.47	5.48	5.49
CP	20.9	20.9	20.9	20.9	20.9	20.9
Ca	0.74	0.73	0.72	0.71	0.71	0.71
P	0.61	0.61	0.60	0.59	0.60	0.59
STTD P	0.50	0.50	0.50	0.50	0.50	0.50
Analyzed Ca:P	1.20	1.20	1.19	1.19	1.20	1.20

[1] Phase 2 experimental diets were fed from approx. 7.4 to 13.4 kg.

[2] Enzymatically treated soybean meal (HP300, Hamlet Protein, Findlay, OH).

[3] Corn protein source (CPS4) was provided by Cargill, Inc, Blair, NE.

[4] Ronozyme HiPhos 2700 (DSM Nutritional Products) provided a 0.13% release of STTD P with 1,250 FYT/kg inclusion in the diet.

Table 2-7 Phase 3 diet composition, Exp. 2 (as-fed basis)[1]

Ingredient, %	
Corn	64.71
Soybean meal, 46.5% CP	31.30
Monocalcium phosphate	1.00
Calcium carbonate	0.85
Sodium chloride	0.60
L-Lys HCl	0.52
Vitamin premix with phytase[2]	0.25
DL-Met	0.23
L-Thr	0.22
Trace mineral premix	0.15
L-Val	0.13
L-Trp	0.06
Total	100.00
Calculated analysis	
SID amino acids, %	
Lys	1.35
Ile:Lys	55
Leu:Lys	114
Met:Lys	37
Met & cys:Lys	58
Thr:Lys	63
Trp:Lys	20.3
Val:Lys	69
Total Lys, %	1.49
NE, kcal/kg	2,423
SID Lys:NE, g/Mcal	5.67
CP, %	21.2
Ca, %	0.68
P, %	0.61
STTD P, %	0.47
Analyzed Ca:P	1.13

[1] Phase 3 common diets were fed from approx. 13.4 to 20.0 kg.

[2] Ronozyme HiPhos 2700 (DSM Nutritional Products) provided a 0.13% release of STTD P with 1,250 FYT/kg inclusion in the diet.

Table 2-8 Effects of 5% or 10% inclusion of 3 corn protein sources on nursery pig growth performance and feed efficiency, Exp. 1[1]

		Corn protein source[2]									
		CPS1		CPS2		CPS3			P =[3]		
Item	ESBM	5%	10%	5%	10%	5%	10%	SEM	Interaction	Source	Level
BW, kg											
d 0	5.5	5.5	5.5	5.4	5.5	5.5	5.5	0.72	0.442	0.407	0.141
d 7	6.3	6.1	6.2	6.3	6.2	6.2	6.1	0.75	0.334	0.280	0.535
d 21[5,6]	11.1^{a}	9.9^{bc}	10.0^{c}	10.6^{ab}	10.0^{bc}	10.6^{abc}	10.1^{bc}	1.18	0.524	0.044	0.006
d 35	19.1	17.6	17.8	18.8	17.7	18.7	18.3	1.78	0.181	0.095	0.124
d 0 to 7											
ADG, g	105	83	92	126	93	100	89	12.0	0.216	0.170	0.237
ADFI, g	124	104	123	134	127	121	112	11.4	0.289	0.206	0.944
G:F, g/kg	845^{ab}	755^{ab}	719^{ab}	936^{a}	689^{b}	826^{ab}	781^{ab}	56.6	0.103	0.638	0.018
d 7 to 21[5]											
ADG, g[4,6]	343^{a}	275^{bc}	249^{c}	308^{ab}	264^{bc}	313^{ab}	283^{bc}	32.1	0.776	0.024	0.003
ADFI, g[5,6]	438^{a}	354^{bc}	342^{c}	407^{ab}	370^{bc}	405^{abc}	368^{abc}	39.3	0.670	0.005	0.066
G:F, g/kg	782	776	722	755	710	772	736	17.7	0.872	0.454	0.003
d 0 to 21 (Treatment period)											
ADG, g[5,6]	264^{a}	211^{bc}	197^{c}	248^{ab}	207^{bc}	242^{ab}	218^{bc}	22.9	0.448	0.025	0.003
ADFI, g[5,6]	333^{a}	270^{b}	269^{b}	316^{ab}	289^{ab}	311^{ab}	295^{ab}	28.7	0.535	0.009	0.122
G:F, g/kg	791^{a}	779^{ab}	723^{ab}	782^{a}	712^{b}	781^{ab}	744^{ab}	16.0	0.601	0.628	< 0.001
d 21 to 35 (Common period)											
ADG, g	576	550	583	586	560	569	587	45.1	0.056	0.656	0.408
ADFI, g	795	733	739	794	745	779	773	59.1	0.299	0.072	0.292
G:F, g/kg	726^{b}	755^{ab}	790^{a}	738^{ab}	752^{ab}	730^{b}	761^{ab}	13.8	0.683	0.085	0.021
d 0 to 35 (Overall)											
ADG, g	389	247	351	283	348	272	366	31.4	0.135	0.103	0.138
ADFI, g[5,6]	518^{a}	455^{b}	457^{b}	507^{ab}	471^{ab}	497^{ab}	486^{ab}	40.7	0.366	0.018	0.176
G:F, g/kg	751	764	768	754	737	748	755	9.8	0.415	0.130	0.806

[1] A total of 315 pigs (initially 5.5 ± 0.72 kg) were used with 5 pigs/pen and 9 replicates/treatment.

[2] Corn protein sources (CPS) were provided by Cargill Inc., Blair, NE.

[3] Source: CPS1 vs CPS2 vs CPS3. Level: 5% vs 10%.

[4] CPS1 vs CPS2: $P < 0.10$
[5] CPS1 vs CPS2: $P < 0.05$
[6] CPS1 vs CPS3: $P < 0.05$
[abc] Means with different superscripts within a row differ ($P < 0.05$).

Table 2-9 Effects of increasing a corn protein source on nursery pig growth performance, Exp. 2[1]

	ESBM, %[2]	CPS4, %[3]							
d 0 to 10:	6.00	3.0	6.0	9.0	12.0	15.0		*P* =[4]	
Item d 10 to 25:	3.00	1.5	3.0	4.5	6.0	7.5	SEM	Linear	Quadratic
BW, kg									
d 0	6.1	6.1	6.1	6.1	6.1	6.1	0.05	0.532	0.113
d 10	7.3	7.4	7.5	7.3	7.3	7.2	0.09	0.092	0.278
d 25	13.2	13.2	13.7	13.3	13.6	12.9	0.30	0.210	0.037
d 38	19.7	19.7	20.6	19.9	20.3	19.6	0.37	0.569	0.043
Day 0 to 10									
ADG, g	121	126	135	120	123	111	9.1	0.124	0.430
ADFI, g	121	123	133	121	125	118	5.8	0.245	0.408
G:F, g/kg	988	1,005	1,011	996	983	924	42.0	0.139	0.396
Day 10 to 25									
ADG, g	395	386	417	400	415	381	16.3	0.723	0.026
ADFI, g	534	517	554	548	570	532	15.7	0.338	0.034
G:F, g/kg	743	749	753	730	727	717	21.3	0.063	0.902
Day 0 to 25 (Experimental period)									
ADG, g	283	281	302	286	296	270	10.5	0.293	0.030
ADFI, g	363	358	383	375	389	361	11.1	0.703	0.036
G:F, g/kg	781	786	791	763	761	748	15.1	0.006	0.784
Day 25 to 38 (Common period)									
ADG, g	501	497	525	510	517	514	10.6	0.444	0.299
ADFI, g	795	94	834	818	820	813	19.2	0.631	0.232
G:F, g/kg	633	627	630	624	630	632	10.5	0.734	0.742
Day 0 to 38 (Overall)									
ADG, g	356	353	378	362	371	351	9.3	0.671	0.028
ADFI, g	508	504	536	526	536	512	12.7	0.638	0.032

G:F, g/kg	703	701	706	689	693	686	7.7	0.066	0.951
Fecal DM, %[5]									
d 10	19.5	23.1	21.8	21.0	23.3	21.8	1.13	0.715	0.488
d 25	23.7	23.0	24.0	24.2	24.4	21.7	1.13	0.493	0.051

[1] A total of 355 weanling barrows (DNA 200 × 400, initially 6.1 ± 0.05 kg) approximately 21 days of age were used in a 38-d experiment with 5 pigs per pen and 11 or 12 pens per treatment.

[2] Enzymatically treated soybean meal (HP300, Hamlet protein, Findlay, OH).

[3] Corn protein source (CPS4) was provided by Cargill, Inc, Blair, NE.

[4] Contrasts were used to test for linear and quadratic differences between treatments with the CPS4. Pairwise comparisons were used to test for differences between all treatments, and all pairwise comparisons ($P > 0.05$).

[5] Feces from three pigs from each pen were pooled, weighed, and dried to measure fecal dry matter. Treatment × day, quadratic $P = 0.064$. Day, $P = 0.004$.

Chapter 3 - Evaluation of a dried fermentation product administered through drinking water on nursery pig growth performance, fecal *E. coli* characterization, antibiotic usage, and mortality

Abstract

A total of 34,749 pigs were used in 2 experiments to evaluate the effects of a dried fermentation product (DFP) administered through drinking water on nursery pig growth performance, antibiotic injection frequency, morbidity, mortality, fecal consistency, and characterization of fecal *E. coli*. The DFP is an intermediate bioactive molecule obtained from *Lactobacillus acidophilus.* In Exp. 1, 350 barrows (DNA Line 200 × 400; initially 6.1 ± 0.01 kg) were used in a 42-d study with 5 pigs per pen and 35 pens per treatment. The DFP was supplied for 14 d at a target dosage of 24 mg/kg BW using a water medicator at a 1:128 dilution. On d 7 and 14, fecal samples were collected and analyzed using multiplex PCR for *E. coli* gene typing and fecal DM. There was no evidence ($P > 0.10$) for differences for growth performance, incidence of diarrhea, number of antibiotic injections, removals, or fecal DM. On both fecal collection days, *E. coli* was present within the population with d 7 samples positive for *hlyA*, *exhA*, *eae*, and *astA* genes. Only 1 sample was positive for enterotoxigenic *E. coli* (ETEC) in the control, and only F18 was found for the fimbriae genes tested. Presence of *E. coli* genes increased on d 14. On d 14, pigs administered either treatment had positive samples for *elt*, *estA*, *estB astA*, *ehxA* and *eae* genes. There were positive samples for F4, F18, ETEC, EPEC, as well as ETEC and EPEC with fimbriae genes present in both treatments. In Exp. 2, 34,399 nursery pigs (initially 5.6 kg) were used in 20 nursery barns with 10 barns per treatment (control or

DFP). The target dosage of the DFP for the first 14 d was 35 mg/kg BW. Following the 14-d supplementation period, pigs continued to be monitored for approximately 31 d. There was no evidence ($P > 0.05$) for the DFP to influence the overall percentage of pigs that died or growth performance. From d 0 to 14, providing the DFP reduced ($P < 0.05$) the percentage of pigs that were euthanized. However, providing the DFP increased ($P < 0.05$) the overall percentage of pigs that were euthanized and total mortality. For the number of injections (treatment interventions), providing the DFP reduced the number of injections for the common period ($P < 0.001$) and overall ($P = 0.002$). These results indicate that the DFP did not influence growth performance but providing the DFP in Exp. 2 led to increased total nursery pig mortality.

Key Words: antibiotic, diarrhea, *E. coli*, euthanasia, injections, mortality, nursery pig

Introduction

Post-weaning diarrhea (PWD) associated with diet change, stress, and environmental bacteria continues to be an issue for the swine industry. Stressors associated with weaning pigs amplify the impact of PWD and increase the prevalence of toxigenic bacteria. Enteric infections caused by *Escherichia coli* (*E. coli*), including subclinical infections, particularly in nursery pigs, are of significant economic importance to the swine industry (Moxley and Duhamel, 1999). Enteric colibacillosis in nursery pigs includes three diseases caused by different pathotypes of *E. coli*: neonatal enteritis, post-weaning diarrhea, and edema disease. The pathotypes involved in enteric colibacillosis in animals and humans are enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), Shiga-toxigenic (STEC) and hybrid pathotypes (STEC/ETEC and EAEC/STEC). The major virulence factors carried by the pathotypes responsible for the enteric infections common in the swine industry are fimbriae F4, F5, F6, F18,

and F41. The economic impact is due to decreased weight gain and feed efficiency, costs associated with treatment and prevention, and increased mortality (García-Meniño et al., 2018).

There have been various studies looking at feeding probiotics to balance the digestive tract microflora and alleviate the stressors associated with PWD and ETEC. More recently, probiotic bacteria have been found to produce bioactive molecules through fermentation, which when delivered, act through inhibition of quorum sensing signals of bacteria. Inhibition of these signals reduces the induction of virulence genes and reduces the ability of pathogens to cause infection. Importantly, these signals do not affect the bacterial populations through growth inhibition or bactericidal activity. Fermentation products affect pathogenicity by changing the gene expression profile, reducing genes associated with quorum sensing signals, toxins, and adhesion (Zeinhom et al., 2012). Nordeste et al. (2017) looked at the *in vivo* effects of these bioactive molecules produced by *Lactobacillus acidophilus*, termed dried fermentation product (DFP). They observed that after an *E. coli* ETEC challenge in swine, there were improvements in fecal scores when the DFP was provided. However, the DFP has not been experimented in larger research facilities or in commercial settings within the US. It was hypothesized that including the DFP in a nursery research facility with recent *E. coli* presence would decrease mortalities. Therefore, the objective of the first experiment was to investigate the effects of a DFP produced by lactic acid bacteria fermentation and provided through the drinking water to affect growth performance, antibiotic injection frequency, mortality, fecal consistency, and characterization of pathogenic *E. coli* of nursery pigs. The second experiment was conducted to evaluate the DFP administration in a commercial nursery setting on antibiotic injection frequency, mortality, and growth performance.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocols (IACUC #4506 and #4435) used in this study.

Experiment 1

General

Experiment 1 was conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. The facility has two identical barns that are completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder and a cup waterer to provide *ad libitum* access to feed and water. Barns were equipped with a water medicator (Dosatron D128R, Dosatron International, LLC., Clearwater, FL) so that water treatment could be applied independently to each pen. Pens (1.22 × 1.22 m) had metal tri-bar floors that allowed ~ 0.30 m^2/pig.

Animals and diets

A total of 350 weanling barrows (DNA Line 200 × 400; initially 6.1 ± 0.01 kg) were used in a 42-d study with 5 pigs per pen and 35 pens per treatment (17 replications in one barn and 18 replications in a second barn). Upon arrival to the nursery research facility, pigs were randomly assigned to pens and then pens were allotted to 1 of 2 water treatments. Pigs received normal drinking water (municipal source) or a dilution rating of 1:128 of the DFP (Nuvio, MicroSintesis Inc., Victoria, CA) from weaning until d 14. The DFP was formulated to be provided at 24 mg/kg BW. Water usage was measured with a water meter (Recordall, Badger Meter Inc., Milwaukee, WI) for pigs on each treatment within each barn (n = 2). Product usage was measured by weighing stock solution on a daily basis to determine disappearance. Medicator accuracy was used to calculate actual product usage: weight of product disappearance divided by

weight of water consumed divided by 1:128. The same common diets were fed to all pigs and were fed in 3 phases with 1.8 kg of phase 1 and 6.8 kg of phase 2 diet provided per pig with phase 3 diet fed until d 42. Diets were manufactured and delivered by Hubbard Feeds in Beloit, KS. Pig weights and feed disappearances were measured on d 0, 14, and 42 of the experiment to determine ADG, ADFI, and G:F. Growth performance was also calculated on a close-out basis as the total gain and intake per pen divided by the number of pigs within pen at the start of the trial.

Fecal scores and collection

From d 0 to 14 of the experiment, fecal scores were assigned to each pen every other day by the same two trained observers. Fecal scores were assigned based on a 1 to 5 numerical scale, with 1 indicating fully formed feces, 2 – moist, firm feces, 3 – mild diarrhea, 4 – severe diarrhea, and 5 – watery diarrhea. For data analysis, fecal scores were further assigned as either diarrhea present within the pen (scores of 3, 4, or 5) or not (scores of 1, 2, or no feces).

On d 7 and 14 of the experiment, ~40 g of feces were collected from the same 3 pigs per pen. Fecal samples were sub-divided with some of the feces used for *E. coli* gene typing at the Kansas State University Preharvest Food Safety Laboratory. The remaining fecal sample was dried at 55°C for 48 h to determine fecal DM.

Detection of major virulence genes of *E. coli* pathotypes

Approximately, 1 g of feces was suspended in *E. coli* broth (Difco, BD, Waltham, MA; Paddock et al., 2012), vortexed for 1 min, and incubated at 40°C for 6 h. After incubation, 1 mL was pipetted into a 2 mL centrifuge tube, boiled for 10 min, centrifuged at 9,400 × g for 5 min and DNA in the supernatant was purified by GeneClean Turbo Kit (MP Biomedicals, Solon,

OH). The purified DNA was subjected to a multiplex PCR assay to detect genes that encode for 11 major virulence factors associated with intestinal *E. coli* pathotypes in swine (Table 1).

Isolation and identification of *E. coli* pathotypes by culture method

Enriched fecal samples were spot inoculated with a sterile cotton swab onto MacConkey agar (MAC; Remel, Lenexa, KS) and then sterile loops were used to streak from the swabbed area for isolation of *E. coli*. Also, samples were diluted (1:100 dilution) in EC broth, and 25 µL of the diluted inoculum were spread-plated onto MAC plates. Inoculated plates were incubated at 37°C for 18 to 24 h. A total of 10 putative colonies presumptive for *E. coli* (pink, round, smooth colonies) for each sample were streaked onto BAP plates and incubated at 37°C for 18 to 24 h. The colonies were tested for spot indole production and confirmed as *E. coli* by PCR assay for *clpB/uidA/ybbW* genes, which encode for beta-glucuronidase, caseinolytic peptidase B, putative allantoin receptor, respectively (Walker et al., 2017). The 10 colonies obtained for each sample were pooled in 50 µL of distilled water, boiled for 10 min, and centrifuged at 2,200 × g for 2 min. The boiled lysate was subjected to the 11-plex PCR assay to detect virulence genes associated with swine enteric colibacillosis. If pooled lysates were positive for any of the 11 virulence genes, then each of the 10 colonies was tested individually by the 11-plex PCR assay to identify virulence gene-positive *E. coli*. All virulence gene-positive isolates were stored at -80°C in cryogenic beads.

Identification of animals requiring treatment

Pigs were removed for welfare concerns when they were observed to continually lose weight or were unthrifty by a trained animal caretaker. Pigs that required injectable antibiotics received penicillin G (Pro-Pen-G, Bimeda, Dublin, IE; 33,069 U/kg BW administered intramuscularly once daily for 3 to 5 days) or enrofloxacin (Baytril 100; Bayer HealthCare LLC,

Shawnee Mission, KS; 7.5 mg/kg BW administered intramuscularly once) as clinically indicated. Injection regimens were recorded by pen and treatment to be used for data analysis. If a pig was treated with penicillin G, a treatment regimen consisting of 3 to 5 d of therapy was considered a single injection regimen.

Experiment 2

General

Experiment 2 was conducted at commercial nursery facilities located in the Midwest. At the commercial nurseries, each barn was completely curtain sided with 80 pens that contained between 25 and 30 pigs per pen. Each pen contained a 5-hole, dry self-feeder, and a cup waterer to provide *ad libitum* access to feed and water. Pens (2.00 × 3.05 m) had fully slatted, plastic flooring that allowed ~ 0.22 m^2/pig. Barns were equipped with a water medicator (Dosatron DM11F, Dosatron International, LLC., Clearwater, FL) to administer the DFP. Room temperatures started at ~30°C when pigs arrived and decreased according to the animals' comfort.

Animals and diets

A total of 34,399 mixed gender and genetic source pigs (initially 5.6 kg) were used in 2 identical production sites with 4 barns on each site. One site was used for 3 consecutive turns with the other site used for 2 consecutive turns (simultaneously) for 20 total groups of pigs. Thus, there were 10 groups (barns) per treatment. Upon arrival to the nursery facility, pigs on each truck from each originating sow source were divided evenly between pairs of barns, with one barn provided the DFP and the other barn serving as the control. Weights of pigs delivered were obtained for each barn by dividing the net weight of each semi-tractor trailer by the number of barns delivered to (n = 2). For the first 8 pairs of barns (blocks), ending weights of pigs were

obtained from each barn by weighing each semi tractor-trailer upon shipment from the nursery facility. Feed intake was determined by the difference between the amount of feed delivered that was reported from the feed mill and the feed remaining upon completion of the nursery group that was visually estimated by a trained swine producer. Mortalities were classified as animals that died without intervention or animals that were euthanized for reasons of animal welfare, injury, or continued illness with no signs of recovery. Mortality was recorded from d 0 to 14 (treatment period), d 14 to the end of the nursery (common period), and the overall nursery period.

The target dosage for the DFP was 35 mg/kg BW. The amount of DFP provided to each treatment barn was recorded and used to determine an estimated dosage based on initial BW and estimated average weight through d 14. Diets were provided in a 4-phase nursery feeding program. In the initial 6 blocks used in the commercial research facility, DFP was the only water additive used for the first 14 d. Due to the concern of herd health, blocks 7 through 10 used water-soluble medications and/or electrolytes with the DFP at the discretion of the herd veterinarian, field service personnel, and farm staff. Each pair of barns (replicate) was treated similarly when water-soluble medications and/or electrolytes were provided.

Identification of animals requiring treatment

Pigs that required injectable antibiotics received ceftiofur (Excede, Zoestis, Parsippany, NJ; 5.0 mg/kg BW administered intramuscularly) or enrofloxacin (Baytril 100; Bayer HealthCare LLC, Shawnee Mission, KS; 7.5 mg/kg BW administered intramuscularly) as clinically indicated. Injections were recorded by barn and day as the number of individual pigs receiving an injection on that day.

Statistical analysis

In both experiments, data were analyzed using R Studio (Version 3.5.2, R Core Team. Vienna, Austria) unless otherwise specified. In Exp. 1, data were analyzed as a completely randomized design with pen serving as the experimental unit. For growth performance data, treatment was included in the statistical model as a fixed effect. Fecal dry matter data were analyzed using repeated measures analysis with fixed effects of treatment, day, and the associated interaction with barn incorporated as a random effect. Injection frequency data were analyzed using a Poisson distribution using a log link function for count of antimicrobial injections and analyzed using a binomial distribution using a logit link function for proportion of pigs per pen receiving an antimicrobial injection. Statistical models for growth, fecal DM, and injection frequency incorporated barn as a random intercept. Characterization of diarrhea presence was conducted using a logistic regression model fit with the GLIMMIX procedure of SAS (version 9.4, Cary, NC) using a logit link function using a Kenward-Rodgers denominator degrees of freedom adjustment. Treatment, day of evaluation, and the associated interaction were considered fixed effects, and pen nested within treatment and the cross product of pen, treatment, and day was considered a random intercept to account for multiple observations on each day of evaluation. Data were analyzed as repeated measures over time and reported as percentage of pens having diarrhea.

In Exp. 2, data were analyzed as a completely randomized block design with barn serving as the experimental unit and pair of 2 barns used as a blocking factor. Data were analyzed using treatment as a fixed effect and block as a random effect. Morbidity and mortality data were analyzed assuming a binomial distribution with a logit link function. Injection data were analyzed and reported as count of injections per day per 1,000 pigs. Injection data were analyzed

using a Poisson distribution with an offset function using the log transformed number of days at risk for each experimental unit using the GLIMMIX procedure of SAS. Treatment was included in the model as a fixed effect, block as a random effect, and a Kenward-Rodgers denominator degrees of freedom adjustment was incorporated. All results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Experiment 1

The actual concentration of test product delivered was greater than the targeted level of 24 mg/kg BW due to the water medicator dosing at a higher rate than expected, likely because of low water intake immediately after weaning (Table 2). Water leakage during d 4 in one barn resulted in higher water and product usage. Thus, a portion of this day was removed from the analysis. The average of daily product disappearance, which includes water that may be wasted by the pigs, was 35.7 mg/kg which was 149% of the targeted dose.

During the experimental period, there was no evidence ($P > 0.10$) for differences in growth performance (Table 3). There was, however, a tendency ($P = 0.070$) for pigs provided the DFP to have higher ADFI when analyzed on a close-out basis. There was no evidence for differences in the percentage of pigs injected with antibiotics between the control group and those receiving the fermentation product ($P > 0.10$; Table 4). During the common period, there was no evidence ($P > 0.10$) for differences in growth performance; however, there was a tendency ($P < 0.10$) for pigs that were previously provided DFP in drinking water to have more injections per pen and greater percentage of pigs receiving an injection compared to the control treatment. For the overall experiment, there was no evidence ($P > 0.10$) for differences in growth performance or injection criteria. There was no evidence for differences ($P > 0.10$) in removals,

mortalities, or total removals between treatments. For fecal DM, there was no evidence for differences between treatments ($P > 0.10$).

For fecal scores, there was no evidence ($P > 0.10$) for a treatment × day interaction or a treatment effect (Table 5). There was evidence ($P < 0.05$) for a day effect where the proportion of pens with diarrhea increased from d 0 to 6, then decreased from d 6 to 12 and increased again to d 14.

E. coli pathotype specific virulence genes were present within the population of both the control and DFP treatment, with a numerical increase on d 14 when compared to d 7 (Table 6). The most common virulence gene detected on d 7 was the *hlyA* gene (11 of 18 and 9 of 18 for the control and the DFP, respectively). Other genes present on d 7 in both treatment groups were *exhA*, *eae* (3 of 18 samples each, respectively), and 1 sample was positive for *astA* gene in the control group. One fecal sample from the control group was found positive for the ETEC pathotype. For the fimbriae genes, 1 fecal sample tested positive for detection of F18 gene. There was a numerical increase in the presence of virulence genes on d 14. The occurrence of EPEC virulence genes, *elt*, *estA*, *estB* and *astA*, did show increase in trend but, remained below 50% throughout. The detection of *hlyA* gene on d 14 was similar to that on d 7 with 10 of 18 and 11 of 18 positive samples for the control and the DFP, respectively. The positive samples of *ehxA* and *eae* increased numerically for both treatment groups (6 of 18 samples each, respectively). The number of tested samples positive for fimbriae genes F4 and F18 as well as ETEC and EPEC increased numerically on d 14. The number of samples with positive F4 fimbriae genes were 5 of 18 and 7 of 18 for control and DFP treatment, respectively, and F18 had 1 positive sample from each treatment. Positive ETEC gene samples were 3 of 18 for the control and 7 of 18 for the DFP

treatment, with 3 of 18 and 2 of 18 EPEC positive samples for the control and the DFP treatment, respectively.

There were no positive samples on d 7 for ETEC + fimbriae genes or EPEC + fimbriae genes. On d 14 however, the number of tested samples positive for ETEC + fimbriae were 3 of 18 and 5 of 18 for the control and the DFP treatment group, respectively, and 2 of 18 samples positive for EPEC + fimbriae in both treatments. None of nursery pigs used in Exp. 1 shed STEC, EAEC or any of the hybrid pathotypes in the feces.

Experiment 2

Although diagnostic testing was not conducted on all groups, *E. coli* was confirmed from intestinal tissue samples and *Salmonella* was confirmed from colonic tissue samples during the beginning of blocks 3 and 4. There was no evidence ($P > 0.05$) for the DFP to influence water intake (Table 7). Pigs provided the DFP consumed approximately 44.7 mg/kg of initial BW and 37.5 mg/kg BW with an assumed 1.0 kg total gain from d 0 to 14 (Batson et al., 2021). For responses in growth performance, providing the DFP had no effect ($P > 0.05$) on growth performance. During the treatment period (d 0 to 14), there were no differences ($P > 0.10$) in the percentage of pigs that died or total mortality; however, a lower ($P = 0.018$) percentage of pigs were euthanized when providing the DFP in drinking water (Figure 3.1).

During the common period (d 14 to end) after the DFP was removed, there was significant evidence for the percentage of pigs requiring euthanasia ($P < 0.001$) and total mortality ($P = 0.009$) to be greater in pigs previously provided the DFP. This was driven by the later experimental replications (Figure 3.2).

Overall, euthanasia frequency ($P = 0.010$) and total morality ($P = 0.049$) was greater for groups provided the DFP from d 0 to 14. This was again driven by the later replications of the experiment (Figure 3.3). For injection criteria, from d 0 to 14 there was no evidence ($P > 0.05$) for the DFP to affect the rate of injections. From d 14 to the end of the nursery, providing the DFP decreased injections ($P < 0.001$) compared to the control. Therefore, there was a significant reduction ($P = 0.002$) in the overall injections per day per 1,000 pigs at risk when providing the DFP.

Discussion

Bioactive molecules are produced as intermediates of probiotic growth. These bioactive molecules have observed positive effects on reducing *E. coli* (Medellin-Peña, 2007; Medellin-Peña and Griffiths, 2009; Zeinhom et al., 2012). The dried fermentation product (DFP) utilized in our experiments was obtained by *Lactobacillus acidophilus* growth as described by Nordeste et al. (2017). Post-weaning diarrhea (PWD) is a common occurrence of the swine industry and has often been attributed to the presence of virulence factors isolated from enterotoxigenic *E. coli* (Luppi et al., 2016). The DFP used in the present experiment is designed to interfere with the signaling molecule autoinducer 2 (AI-2) and the *lux*S gene of *E. coli* 0157:H7 (Medellin-Peña, 2007). The research facility used in Exp. 1 had a recent history of increased morbidity and mortality attributed to K88 ($F4^+$) *E. coli* based on veterinary diagnostic testing. Similarly, there were positive *E. coli* tests from intestinal tissue samples of pig populations in the initial periods of Exp. 2 of the present study. The positive laboratory results of *E. coli* may provide a greater understanding of the DFP and the effectiveness on injections in *E. coli* challenged populations of a commercial nursery (Figures 1 to 3). However, specific gene typing of *E. coli* was not

performed in Exp. 2 of the present study. Therefore, it is unclear if K88 ($F4^+$) *E. coli* that was present in Exp. 1 was also present in Exp. 2.

Enterotoxigenic K88 ($F4^+$) *E. coli* is known to cause infectious diarrhea in swine and increase the inflammatory response of the immune system (Devriendt et al., 2010). In the swine industry, *E. coli* can be treated with the use of water-soluble antibiotics such as gentamicin or injectable antibiotics (Ex. enrofloxacin or other injectable antimicrobials) based on culture and antimicrobial sensitivity results. Antibiotics are an effective way to decrease *E. coli* in swine (Pan et al., 2017; Kim et al., 2021) and visual scours (Bhandari et al., 2008). However, the concern for antimicrobial resistance (AMR) has increased the pressure and interest of swine producers to seek alternatives to antibiotics. Pan et al. (2017) fed a probiotic blend and observed similar responses to antibiotics after pigs were challenged with *E. coli*. In other studies, Bhandari et al. (2008) observed that after an *E. coli* challenge, pigs provided direct fed microbials (DFM) or antibiotics had lower mortality and decreased incidence of scours compared to animals without any supplementation. With the use of the DFP at intermediate levels, Nordeste et al. (2017) observed lower diarrhea scores. This is in contrast with the results of Exp. 1 where we observed no difference in diarrhea presence as measured by fecal scores nor did we see any difference in fecal DM. These conflicting results may be due to the experimental designed health challenge used by Nordeste et al. (2017) compared to the present study. Nordeste et al. (2017) challenged pigs with K88 *E. coli* and although *E. coli* presence was documented in Exp. 1 of the present study, clinical signs of disease were not observed. Fecal scores were not assigned in Exp. 2 of the present study. Therefore, additional research would be needed to understand to if the DFP would lower the percentage of pigs that have diarrhea and visual fecal scores in a commercial environment.

The DFP used in the present study is produced from *Lactobacillus acidophilus* and is associated with less *E. coli* expression (Medellin-Peña, 2007) and colonization (Medellin-Peña and Griffiths, 2009). Other bacteria such as *Saccharomyces cerevisiae* have also been researched to understand the impact on *E. coli* (Kiarie et al., 2011). Kiarie et al. (2011) fed weaned pigs with *Saccharomyces cerevisiae* and observed increased ADFI compared to a negative control after an ETEC challenge. This is in contrast with the present study where we did not observe any differences in ADFI. This could be due to the different products provided by Kiarie et al. (2011), as the DFP used in the present study was produced from *Lactobacillus acidophilus*, not *Saccharomyces cerevisiae*. It is unclear if there are intermediates of *Saccharomyces cerevisiae* that interfere with *E. coli* infection in the GIT of the pig. Similarly, Becker et al. (2020) fed a DFM from *Bacillus amyloliquefaciens* and *Bacillus subtilis* and observed improvements in growth performance, whereas the DFP used in the present study was from *Lactobacillus acidophilus* and observed no effect.

During Exp. 1, the increase in enterotoxins (*astA*, *elt*, *estA*, and *estB*) present within feces indicated that the presence of *E. coli* was greater on d 14 than on d 7. However, clinical disease was not present within the population. All tested fecal samples were negative for the presence of *stx*1 and *stx*2, which encode for Shiga toxins 1 and 2, respectively. Shiga toxin 2 is the major virulence factor involved in the edema disease. This indicates that none of pig fecal samples harbored or shed STEC at the time of sampling. The absence of the *aggA* gene in our study, which encodes for a protein responsible aggregation of *E. coli* cells, indicates that there is no EAEC pathotype among these pigs. Strains of EPEC carrying *bfpA* gene, which encodes for a protein, are called typical EPEC. The absence of *bfpA* in all samples indicates that none of the pigs carried typical EPEC pathotype. Strains of EPEC produce a characteristic adherence, called

local adherence, in which bacterial cells form microcolonies or clusters. This type of adherence is associated with the presence of a plasmid, called EAF (EPEC adherence factor), which also has a cluster of genes that encode bundle-forming pili (BFP; Nataro and Kaper, 1998). In contrast to typical EPEC, certain strains that carry *eae* gene and one or more of the four enterotoxin genes, but do not have the EAF plasmid encoding *bfpA,* are called atypical EPEC (aEPEC; Chen and Frankel, 2005). Of the four genes that encode for enterotoxins, *astA* was the most dominant and the prevalence increased with age. Among the three genes, *elt, estA,* and *estB,* which encode for heat labile, heat stable A and heat stable B, respectively, and characteristic of ETEC pathotype and involved in neonatal enteritis and PWD, heat stable B (*estB*) was the most dominant and the prevalence increased with age. The *eae* gene, which encodes for intimin, a protein that mediates attachment of *E. coli* to enterocytes and is characteristic of both STEC and EPEC, was prevalent in the feces of both treatments. The genes *hlyA* and *ehxA,* which encode for two different hemolysins, were prevalent in almost all samples. Hemolysins are produced by a number of *E. coli* pathotypes, including non-pathogenic strains. The prevalence of any of the 11 virulence genes specific to *E. coli* did not appear to be affected by inclusion of the DFP in the drinking water of weanling pigs.

On d 14, 12 fecal samples tested carried the fimbriae genes F4 and 2 samples carried F18. The fimbriae mediate attachment of *E. coli* to enterocytes before enterotoxins are secreted that induce secretory diarrhea. The increase in in the fimbriae genes from d 7 to 14 indicate that fimbriae *E. coli* attachment was increased as the pigs grew older. However, fewer samples in the DFP treatment carried the *eae* gene with enterotoxins compared to the control and may indicate less attachment when providing the DFP. However, the DFP did not appear to have any effect on

the fecal prevalence of the ETEC or aEPEC pathotypes, and the analysis does demonstrate the presence of *E coli* in both treatments.

In Exp. 2, mortality percentages were increased when providing the DFP, which was driven by the percentage of pigs that were euthanized post-treatment. It is unclear why the percentage of pigs euthanized was increased, and more research should be conducted to explain the responses observed in the present study. The reason for euthanasia was not documented in Exp. 2 but providing the DFP did reduce the percentage of pigs that were euthanized from placement until d 14 after placement. Reasons for euthanasia for the first 14 days in the nursery can be attributed to lack of growth performance or increased illness with no signs of recovery. Given that this is the first experiment evaluating the DFP produced from *Lactobacillus acidophilus* in commercial nurseries, more research should be conducted to explain this response. However, in our study, the DFP did not influence the growth of nursery pigs and increased the mortalities of nursery close-outs.

Implications

In summary, providing a dried fermentation product through the drinking water for 14 d after weaning did not influence growth performance, antibiotic injection, fecal consistency, or mortality. In Exp. 1, all four genes that code for enterotoxins were found in the feces of nursery pigs. The dominant enterotoxin gene was *astA*. The two pathotypes of *E. coli* detected were ETEC and aEPEC. These results indicate that although virulence genes or pathotypes associated with enteric colibacillosis were present, the DFP did not influence diarrhea presence or performance. Similarly, in Exp. 2, *E. coli* presence was documented in the production system, but the DFP did not influence growth performance, reduced total injections, but total mortality increased which was not anticipated and unclear why this occurred.

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Table 3-1 Virulence genes and virulence factors associated with *E. coli* pathotypes involved in swine colibacillosis

Genes	Function/Product	Pathotypes
estA	Heat stable enterotoxin A	Enterotoxigenic *E. coli* (ETEC)
estB	Heat stable enterotoxin B	Enterotoxigenic *E. coli* (ETEC)
elt	Heat labile enterotoxin	Enterotoxigenic *E. coli* (ETEC)
hlyA	Hemolysin	Enteropathogenic *E. coli* (EPEC)
bfpA	Bundle-forming pilus protein	Enteropathogenic *E. coli* (EPEC)
aggA	Pilin subunit of enteroaggregative fimbriae	Enteroaggregative *E. coli* (EAEC)
astA	Enteroaggregative heat stable enterotoxin (EAST)	Enteroaggregative *E. coli* (EAEC)
*stx*1	Shiga toxin 1	Shigatoxigenic *E. coli* (STEC)
*stx*2	Shiga toxin 2	Shigatoxigenic *E. coli* (STEC)
eae	Intimin	Shigatoxigenic *E. coli* (STEC)
ehxA	Enterohemolysin	Shigatoxigenic *E. coli* (STEC)

Table 3-2 Dosage of a dried fermentation product administered through the water to post weaning piglets, Exp. 1

Day	Product usage, mg/kg BW[1]	Percentage of targeted usage[2]
1	43.3	181
2	39.8	166
3	40.5	169
4	37.4	156
5	35.6	148
6	50.3	209
7	45.6	190
8	42.3	176
9	34.6	144
10	32.7	136
11	29.4	122
12	26.1	109
13	21.3	89
14	21.3	89

[1] Dried fermentation product (provided by MicroSintesis, Victoria, Canada) was administered through water lines at a dilution rate of 1:28 and a targeted inclusion rate of 24 mg/kg BW from d 0 to 14 after weaning. Body weight was estimated based on 10% BW consumption in water for nursery pigs.

[2] Percentage of targeted dose was calculated based on stock solution water disappearance and water intake for each day.

Table 3-3 Effect of a dried fermentation product provided through the drinking water on nursery pig growth performance and fecal consistency, Exp. 1[1]

Item	Control	DFP[2]	SEM	*P* =
Initial BW, kg	6.1	6.1	0.01	0.377
d 14	8.07	8.03	0.06	0.651
d 42	21.7	21.9	0.37	0.410
Experimental period (d 0 to 14)				
ADG, g	135	135	4.2	0.925
ADFI, g	179	185	5.3	0.337
G:F, g/kg	749	731	12.8	0.233
Common period (d 14 to 42)				
ADG, g	485	493	10.2	0.320
ADFI, g	727	738	19.4	0.367
G:F, g/kg	667	668	5.1	0.897
Overall (d 0 to 42)				
ADG, g	364	371	6.1	0.300
ADFI, g	539	550	12.2	0.220
G:F, g/kg	677	675	5.4	0.743
Close-out data[3]				
Gain, kg/pig	14.40	15.16	0.341	0.120
Intake, kg/pig	21.79	22.75	0.367	0.070
G:F, g/kg	657	665	11.3	0.455
Removals and mortalities, %				
Mortality	1.71	0.57	0.981	0.338
Removal	3.43	2.29	1.375	0.524
Total removal	5.14	2.86	1.670	0.282
Fecal DM, %[4]				
d 7	19.75	20.08	1.004	0.770
d 14	20.59	20.47	1.004	0.912

[1] A total of 350 weaned pigs were used in a 42-day study with 5 pigs per pen and 35 pens per treatment.

[2] Dried fermentation product (provided by MicroSintesis, Victoria, Canada) was administered through water lines in a dilution rate of 1:128 from d 0 to 14 after weaning.

[3] Close out data is calculated on a pig placed basis: total gain and intake per pen divided by the number of pigs within pen at the start of the trial.

[4] Fecal samples from the same 3 piglets were pooled and dried for 48 h in 55°C. Treatment × day, $P = 0.775$; Treatment, $P = 0.898$; Day, $P = 0.432$.

Table 3-4 Effect of a dried fermentation product provided through the drinking water on individual postweaning antibiotic injection regimens, Exp. 1[1]

Item	Control	DFP[2]	SEM	*P* =
Experimental period (d 0 to 14)				
Injections/pen[3]	1.14	0.89	0.697	0.769
Injections, %[4]	20.57	14.86	0.031	0.163
Common period (d 14 to 42)				
Injections/pen	0.60	1.03	0.172	0.051
Injections, %	12.28	19.43	0.030	0.071
Overall (d 0 to 42)				
Injections/pen	1.74	1.91	0.700	0.856
Injections, %	26.29	29.71	0.035	0.475

[1] A total of 350 weaned pigs were used in a 42-d study with 5 pigs per pen and 35 pens per treatment.

[2] Dried fermentation product (provided by MicroSintesis, Victoria, Canada) was administered through water lines in a dilution rate of 1:128 from d 0 to 14 after weaning.

[3] Count of pigs per pen that had a treatment regimen started during the period.

[4] Percentage of pigs within pen that had a treatment regimen started.

Table 3-5 Effect of a dried fermentation product provided through the drinking water on percentage of pens with postweaning diarrhea, Exp. 1[1]

	Day								
Item	0	2	4	6	8	10	12	14	*P* =
Diarrhea presence[2]									
Treatment × day									
Control	2.3 ± 1.48	0.9 ± 0.78	36.6 ± 12.27	52.7 ± 13.1	7.0 ± 3.86	1.0 ± 0.75	0.6 ± 0.54	10.2 ± 5.12	0.343
DFP[3]	0.6 ± 0.54	0.8 ± 0.66	70.8 ± 11.04	39.1 ± 12.65	17.1 ± 7.71	1.0 ± 0.76	2.3 ± 1.49	10.8 ± 5.28	
Day	1.2 ± 0.65^{c}	0.9 ± 0.51^{c}	54.2 ± 9.33^{a}	45.8 ± 9.26^{a}	11.1 ± 3.96^{b}	1.0 ± 0.53^{c}	1.2 ± 0.66^{c}	10.5 ± 3.68^{b}	< 0.001

[1] A total of 350 weaned pigs were used in a 42-day study with 5 pigs per pen and 35 pens per treatment. Two observers individually classified pens of pigs as either having diarrhea present (score ≥ 2) within a pen or no diarrhea (score ≤ 3) or feces observed. Values represent percentage of pigs within treatment that had diarrhea (score ≥ 3). There was no main effect of treatment (*P* = 0.540).

[2] Fecal scores were categorized as: 1 – fully formed, 2 – moist, firm, 3 – mild diarrhea, 4 – severe diarrhea, and 5 – watery diarrhea. Values represent average fecal score per treatment.

[3] Dried fermentation product provided by MicroSintesis, Victoria, Canada was administered through water lines in a dilution rate of 1:128 from d 0 to 14 after weaning.

[a,b,c] Means with different superscripts differ, *P* < 0.05.

Table 3-6 Effect of a dried fermentation product provided through the drinking water and sampling day on probability of detection of virulence genes and pathotype within fecal *E. coli*, Exp. 1[1]

	Day 7		Day 14		*P* =		
Item:	Control	DFP[2]	Control	DFP	Treatment × day	Treatment	Day
Virulence gene							
elt	0/18	0/18	5/18	7/18	---	---	---
estA	0/18	0/18	6/18	7/18	---	---	---
estB	0/18	0/18	6/18	9/18	---	---	---
hlyA	61 [37,81]	50 [28,73]	56 [32,77]	61 [37,81]	0.423	0.842	0.792
bfpA	0/18	0/18	0/18	0/18	---	---	---
aggA	0/18	0/18	0/18	0/18			
astA	1/18	0/18	5/18	7/18	---	---	---
*stx*1	0/18	0/18	0/18	0/18	---	---	---
*stx*2	0/18	0/18	0/18	0/18	---	---	---
eae	17 [5,42]	17 [5,42]	33 [15,58]	33 [15,58]	1.000	1.000	0.069
ehxA	17 [5,42]	17 [5,42]	33 [15,58]	33 [15,58]	1.000	1.000	0.069
F4	0/18	0/18	5/18	7/18	---	---	---
F5	0/18	0/18	0/18	0/18	---	---	---
F6	0/18	0/18	0/18	0/18	---	---	---
F18	1/18	0/18	1/18	1/18	---	---	---
F41	0/18	0/18	0/18	0/18	---	---	---
Pathotype							
ETEC[3]	1/18	0/18	3/18	7/18	---	---	---
EPEC[4]	0/18	0/18	3/18	2/18	---	---	---
ETEC + fimbriae[5]	0/18	0/18	3/18	5/18	---	---	---
EPEC + fimbriae[6]	0/18	0/18	2/18	2/18	---	---	---

[1] A total of 350 weaned pigs were used in a 42-day study with 5 pigs per pen and 35 pens per treatment. Fecal samples from the same 3 piglets from 18 pens per treatment were pooled and submitted to Preharvest Food Safety Laboratory for *E. coli* gene typing by PCR. Values represent the probability of detection of *E. coli* virulence genes and pathotype [and 95% confidence intervals] of 18 *E. coli* isolates per sampling day (day 7 and 14). In the event that a statistical model representing the interaction of water treatment and sampling day could not be fit, data are reported as the number of samples PCR positive for the virulence gene over the total number of samples for treatment on sampling day.

[2] Dried fermentation product provided by MicroSintesis, Victoria, Canada was administered through water lines in a dilution rate of 1:128 from d 0 to 14 after weaning.

[3] *E. coli* strains positive for one or more of the four enterotoxin (elt, estA, estB, astA) genes.

[4] *E. coli* strains positive for eae gene and one or more of the four enterotoxin (elt, estA, estB, astA) genes.

[5] ETEC strain with any of the five fimbriae genes tested, F4, F5, F6, F18, and F41.

[6] Atypical EPEC strains with any of the five fimbriae genes tested, F4, F5, F6, F18, and F41.

Table 3-7 Effect of a dried fermentation product provided through the drinking water on nursery pig growth performance, mortality, euthanasia, and antibiotic frequency, Exp. 2[1]

Item	Control	DFP[2]	SEM	*P* =
Intake, d 0 to 14				
Water, L/pig/day	0.06	0.05	0.012	0.162
DFP, mg/kg[3]	---	44.7	---	---
DFP, mg/kg[4]	---	37.5	---	---
Growth performance[5]				
ADG, g	347	352	15.9	0.714
ADFI, g	544	546	26.8	0.915
G:F, g/kg	641	647	14.3	0.725
Treatment period (d 0 to 14)				
Mortality, %	1.14	1.08	0.271	0.575
Died, %	0.58	0.66	0.189	0.266
Euthanized, %	0.41	0.29	0.143	0.018
Injections[6]	10.74	10.79	2.673	0.857
Common period (d 14 to end)				
Mortality, %	3.42	3.94	0.442	0.009
Died, %	0.86	0.72	0.111	0.118
Euthanized, %	2.51	3.17	0.417	< 0.001
Injections[6]	1.76	1.18	0.257	< 0.001
Overall				
Mortality, %	4.67	5.16	0.615	0.049
Died, %	1.51	1.47	0.255	0.766
Euthanized, %	3.07	3.55	0.442	0.010
Injections[6]	4.86	4.48	0.864	0.002

[1] A total of 34,399 weaned pigs were used with 10 barns per treatment and approximately 1,700 pigs per barn.

[2] Treatment provided by Microsintesis, Victoria, Canada, was administered through water lines in a dilution rate of 1:128 from d 0 to 14 after weaning.

[3] Dosage was calculated by (# of packets delivered × 460 g/packet) / initial BW, kg / 14 d.

[4] Dosage was calculate based on ~ 1 kg gain from d 0 to 14 (# of packets delivered × 460 g/packet) / (initial BW, kg + 1 kg) / 14 d.

[5] Growth performance was obtained for the initial 8 blocks.

[6] Values represent count of injections per day per 1,000 pigs.

Figure 3.1. Difference in died, euthanized, and total mortality between the DFP and the control for blocks 1 to 10 (left to right) during the experimental treatment period (d 0 to 14).

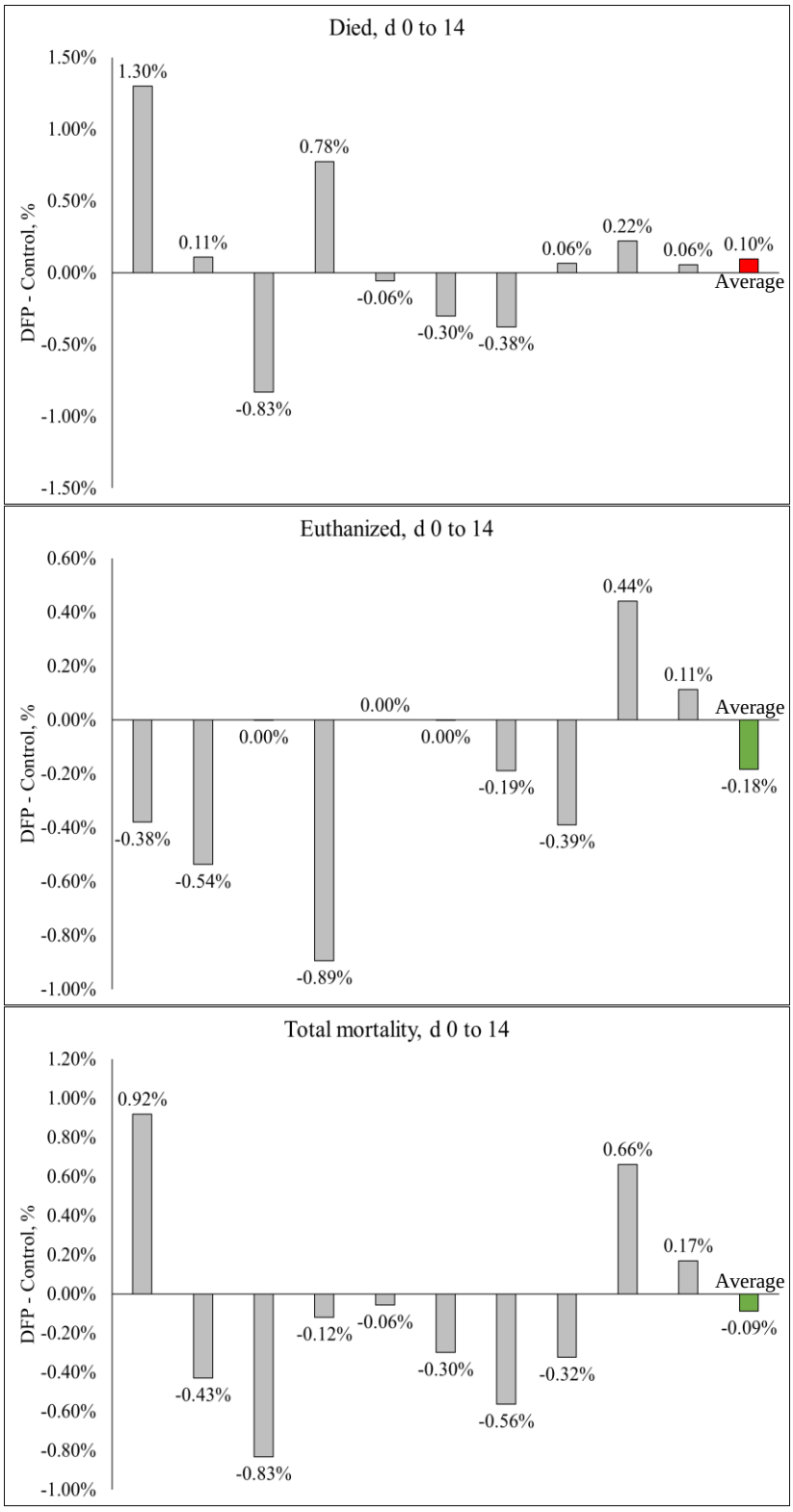

Figure 3.2 Difference in died, euthanized, and total mortality between the DFP and the control for blocks 1 to 10 (left to right) during the common period (d 14 to end).

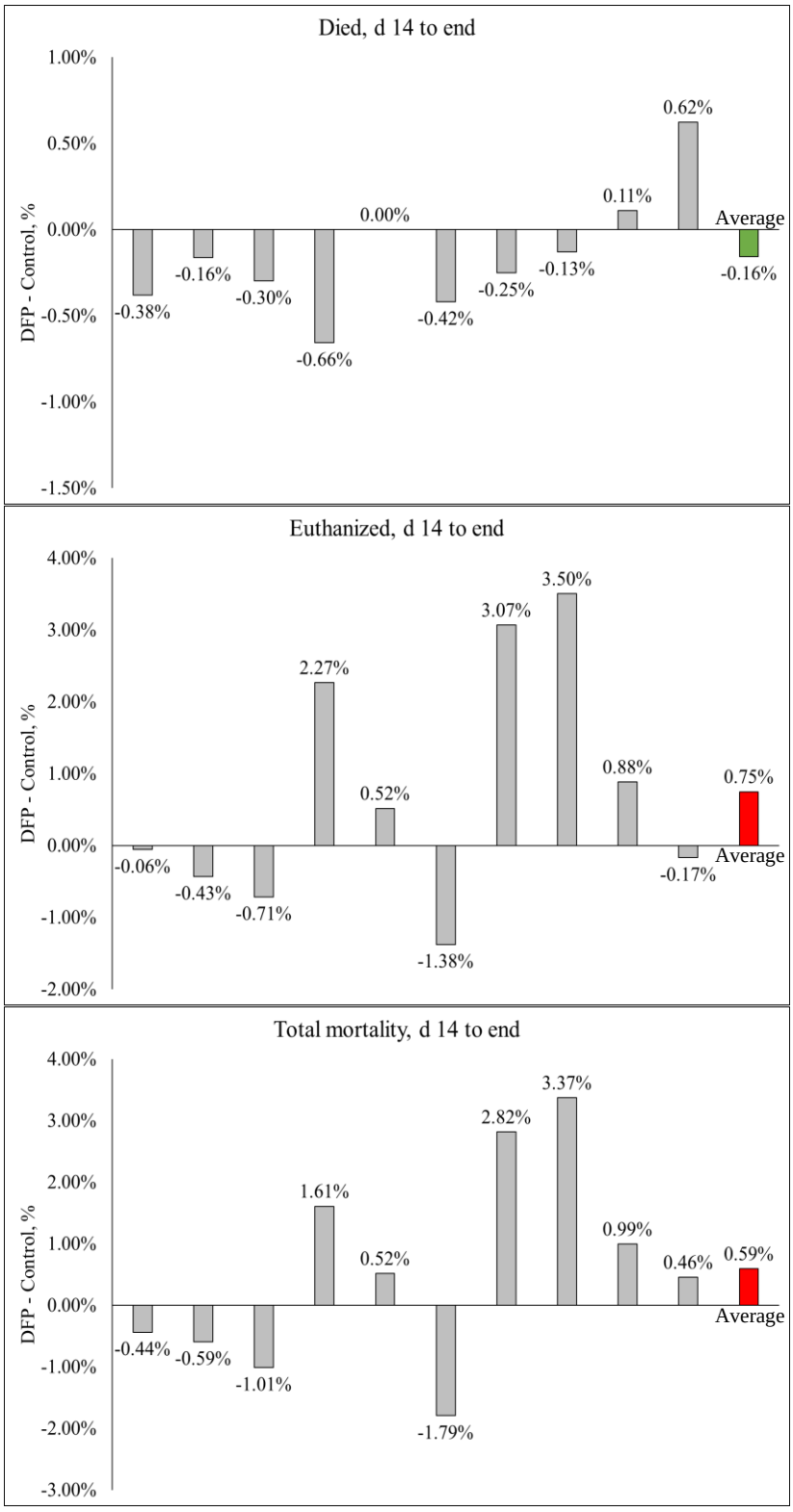

Figure 3.3 Difference in died, euthanized, and total mortality between the DFP and the control for blocks 1 to 10 (left to right) during the overall nursery period.

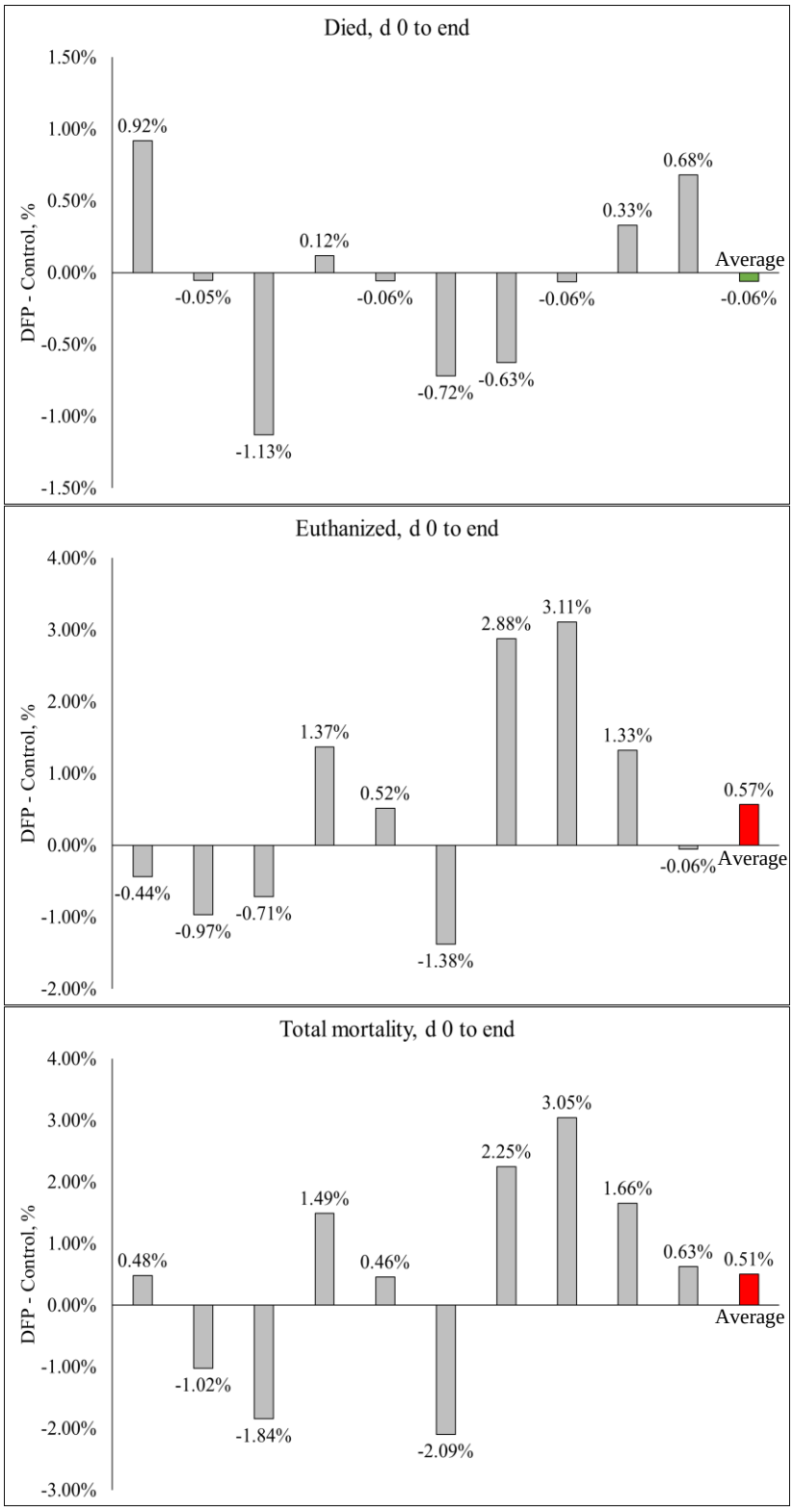

Figure 3.4 Difference in antibiotic injections per day per 1,000 hd between the DFP and the control for blocks 1 to 10 (left to right) during the treatment period (d 0 to 14), common period (d 14 to end), and the overall nursery period (d 0 to end).