Effects of standardized ileal digestible valine:lysine ratios, pharmacological levels of copper, and feed mitigants on swine growth

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Abstract

This thesis contains three chapters that include: 1) evaluation of increasing standardized ileal digestible valine:lysine (SID Val:Lys) ratio on growth performance and plasma urea nitrogen of 21- to 116-kg pigs, 2) evaluation of copper (Cu) feeding strategies and sources on nursery and finishing pig performance, and 3) effects of a formaldehyde-based product or a blend of phytochemicals and carboxylic acids on growth performance, serum haptoglobin, serum cytokine levels, and intestinal morphology in enterotoxigenic *Escherichia coli* challenged nursery pigs. Chapter 1 utilized 998 pigs across three experiments to determine the optimal Val:Lys ratio within grow-finish pigs. A standardized ileal digestible valine:lysine ratio of at least 69% appeared to maximize gain or feed efficiency for 21-to 116- kg pigs. Chapter 2 included 2,204 nursery pigs to evaluate the effect of Cu feeding strategies and 2,160 grow-finish pigs to evaluate the effect of Cu source. In nursery pigs, when pharmacological levels of Zn were used, the addition of pharmacological levels of 150 mg/kg added Cu did not influence the overall average daily gain, average daily feed intake, gain: feed, removals, or mortality. In grow-finish diets, Cu source did not influence growth performance. However, feeding pharmacological levels of 150 mg/kg added Cu, regardless of source, improved growth performance. Chapter 3 utilized 84 nursery pigs to evaluate the effect two feed additives, a formaldehyde-based product and a second product containing a blend of phytochemicals and carboxylic acids, in enterotoxigenic *Escherichia coli* F18 challenged nursery pigs. Neither feed additive improved overall growth performance, fecal dry matter, intestinal morphology, or serum haptoglobin, with only marginal changes in serum cytokine concentrations when fed to enterotoxigenic *Escherichia coli* F18-challenged nursery pigs. In summary, these experiments provide data on the optimal SID Val:Lys ratio for 21- to 116-kg pigs, the effect of tribasic copper chloride feeding strategies on

nursery pigs, the impact of Cu source on grow-finish pigs, and the effects of formaldehyde-based product or a blend of phytochemicals and carboxylic acids on growth performance, serum haptoglobin, serum cytokine levels, and intestinal morphology in enterotoxigenic *Escherichia coli* challenged pigs.

Table of Contents

List of Figures

List of Tables

Chapter 1 - Evaluation of increasing standardized ileal digestible valine: lysine ratio on growth performance and plasma urea nitrogen of 21 to 116 kg pigs

Abstract

Three experiments evaluated the effects of increasing standardized ileal digestible (SID) Val:Lys ratio on growth performance and plasma urea nitrogen (PUN) of pigs from 21 to 116 kg. In Exp. 1, a total of 351 pigs (initially 21.4 ± 0.35 kg) were used in a 21-d study. Pens of pigs were blocked by body weight (BW) and randomly allotted to one of six dietary treatments with 5 pigs per pen and 12 replications per treatment. Pigs were weighed on d 0, 10 and 21 to determine average daily gain (ADG), average daily feed intake (ADFI) and gain:feed ratio (G:F). A blood sample was collected from 2 pigs per pen on d 10 after a 5-h fasting period. Gain:feed ratio increased (linear, $P = 0.012$) as SID Val:Lys ratio increased, but with little improvement past 69% SID Val:Lys ratio as supported by broken-line analysis. Plasma urea nitrogen (PUN) increased (linear, $P = 0.029$) as SID Val:Lys ratio increased whereas no differences were observed for ADG or ADFI. In Exp. 2, a total of 647 pigs (initially 38.6 ± 0.90 kg) were used in a 27-d experiment. Pens of 8 or 9 pigs were blocked by BW and randomly allotted to one of six dietary treatments with 12 replications per treatment. Pigs were weighed on d 0, 14, and 27 to determine ADG, ADFI, and G:F. After a 10 h fasting period on d 15, blood was collected from 3 pigs per pen. Final BW, ADG, and ADFI increased (quadratic, $P < 0.05$) as SID Val:Lys ratio increased. A quadratic polynomial analysis indicated a SID Val:Lys ratio of near 69% was able to maximize ADG. As the SID Val:Lys ratio increased, both G:F and PUN decreased (linear, $P < 0.050$). For Exp. 3, after a 30-d common feeding period, the same 647 pigs (initially 90.0 ± 1.86

kg) from Exp. 2 were used in another 27-d experiment. Pigs were randomly reallotted to one of six dietary treatments and weighed on d 0, 14, and 27. On d 15, after a 15-h fasting period, blood was collected from 3 pigs per pen. Average daily gain and ADFI increased (linear, $P < 0.05$) as SID Val:Lys ratio increased. However, little improvement in ADG was observed when feeding greater than 69% SID Val:Lys ratio. Final BW also tended to increase (linear, $P = 0.078$) as SID Val:Lys ratio increased; however, there was no evidence of an effect on G:F or PUN. Collectively, these results indicate a SID Val:Lys ratio of 69% appears to maximize ADG or G:F for 21 to 40 and 38 to 67 kg pigs. In 90 to 116 kg pigs a ratio of at least 69% SID Val:Lys appeared to be sufficient for growth performance.

Key Words: finishing pig, lysine, valine

Introduction

Amino acid (AA) requirement estimates for growing–finishing pigs are commonly expressed relative to lysine on a standardized ileal digestible (SID) basis. This is because Lys is typically the first limiting AA in corn–soybean meal-based diets. Within corn-soybean meal diets, Val is recognized as the fifth limiting AA after Lys, Met, Thr, and Trp (Figueroa et al., 2003). Valine is a branched-chain amino acid (BCAA) that plays important roles in protein synthesis, muscle accretion, immune function, and gastrointestinal integrity (Wang et al., 2023). Historically, there has been limited research establishing Val requirement estimates across the growing-finishing period. The NRC (2012) estimates SID Val:Lys ratios between 63.4% and 67.2% for pigs from 11 to 135 kg BW. However**,** at the time of publication, no studies had evaluated Val requirements in pigs with an average BW greater than 27 kg.

Since the publication of NRC (2012), several studies have evaluated Val requirement estimates in pigs greater than 27 kg, although results are variable. Previous studies contain a variety of experimental methodologies, statistical models, and diet compositions. Gonçalves et al. (2018) observed that pigs weighing 25 to 45 kg required an SID Val:Lys ratio of 73% to maximize ADG, although a ratio of 68% achieved 99% of maximal ADG response and 69% fully maximized G:F. More recently, Wu et al. (2024) estimated SID Val:Lys requirements of 62 to 68% for pigs weighing 40 to 75 kg and 64 to 71% for pigs weighing 75 to 100 kg, depending on the statistical model used. Few studies have evaluated Val requirement estimates across the entire growing-finishing period. Therefore, the objective of this project was to determine the SID Val:Lys ratio requirement estimate for 21 to 116 kg pigs in three controlled experiments.

Materials And Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments (4942, 4485.42, and 4485.5).

General

These experiments were conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. In all experiments, the barns were enclosed and environmentally regulated. In Exp. 1, each pen (1.52 × 1.52 m) provided 0.46 m^2 per pig and contained a 4-hole stainless steel dry self-feeder (Thorp Equipment Inc., Thorp, WI) and nipple waterer to provide *ad libitum* access to feed and water. In Exp. 2 and 3, each pen (2.44 m × 3.05 m) provided 0.74 m^2 per pig and was equipped with a 2-hole dry, single-sided feeder (Farmweld, Teutopolis, IL) and 1-cup waterer to provide *ad libitum* access to feed and water. Pens in Exp. 1 had tri-bar metal flooring while pens in Exp. 2 and 3 had completely slatted concrete flooring

with a 1.21 m pit underneath for manure storage. In each experiment, each weight block had a similar number of barrows and gilts.

Treatment diets for Exp. 1 were manufactured at the O. H. Kruse Feed Technology Innovation Center (Table 1; Manhattan, KS). Experiment 2 and 3 diets were manufactured at Hubbard Feeds (Table 1; Beloit, KS). In Exp. 1 and 2, dietary treatments contained SID Val:Lys ratios of 60, 63, 66, 69, 72, and 75%. In Exp. 3, dietary treatments contained SID Val:Lys ratios of 63, 66, 69, 72, 75, and 78%. In each experiment, a basal diet was formulated with the lowest Val:Lys ratio, and L-Val was added to create a diet with the highest Val:Lys ratio. Intermediate SID Val:Lys ratio diets were created by blending the high and low diets. In Exp. 1, daily feed additions were performed manually. In Exp. 2 and 3, daily feed additions to each pen were accomplished using a robotic feeding system (FeedPro, Feedlogic Corp., Wilmar, MN) able to record feed amounts for individual pens. All experimental diets were corn-soybean meal-based and fed in meal form. Dietary nutrient composition and SID AA coefficients were derived from NRC (2012).

For all experiments, Val was estimated to be the second-limiting AA, with SID Lys formulated to be 1.10% in Exp. 1, 0.90% in Exp. 2, and 0.65% in Exp. 3. In amino acid ratio studies, dietary Lys must be below the pig's requirement to accurately determine the requirement for the test amino acid relative to Lys. The projected Lys requirement for pigs within this facility for Exp. 1, 2, and 3 was calculated to be 1.41% (20.4 g/d SID Lys intake), 1.12% (24.6 g/d SID Lys intake), and 0.71% (21.8 g/d SID Lys intake), respectively (Royall et al., 2022). All other AA ratios relative to Lys were maintained above requirement estimates (NRC, 2012).

Experiment 1

A total of 351 pigs (DNA 600 × 241; initially 21.4 ± 0.35 kg) were used in a 21-d experiment. Pens of pigs were blocked by body weight and randomly allotted to one of six dietary treatments in a randomized complete block design (RCBD) with 5 pigs per pen and 12 replications per treatment. Pigs were weighed on d 0, 10, and 21 for ADG, ADFI and G:F ratio. After a 5-h fasting period on d 10, a blood sample was collected from the jugular vein of 2 pigs per pen (one barrow and one gilt). Whole blood was centrifuged, and plasma was collected and stored at -20°C until analysis. Plasma was analyzed for urea N concentration using a Urea Nitrogen Colorimetric Detection Kit (Arbor Assays, Ann Arbor, MI). SID Valine and SID Lys intake per day was calculated using the overall ADFI and the SID Val and SID Lys concentration of the diets.

Experiment 2 and 3

A total of 647 pigs (DNA 600 × 241; Exp. 2, initially 38.6 ± 0.90 kg; Exp. 3 initially 90.0 ± 1.86 kg) were used in two 27-d experiments. Pens of 8 or 9 pigs were blocked by BW and randomly allotted to one of six dietary treatments in a RCBD with 12 replications per treatment. Pigs were weighed on d 0, 14, and 27 for ADG, ADFI, and G:F. After a 10 h fasting period on d 15, 10 mL of blood was collected from the jugular vein of 3 pigs per pen (two barrows and one gilt). Whole blood was centrifuged, and plasma was collected and stored at -20°C until analysis as described for Exp. 1. SID Valine and SID Lys intake per day was calculated as described for Exp. 1. Following the conclusion of Exp. 2, all pigs were fed a common diet for 30 d and then pens were randomly re-allotted to dietary treatments for Exp. 3.

Chemical Analysis

In all experiments, diet samples were collected. In Exp. 1, samples were taken from every 3rd bag during the diet manufacturing process. In Exp. 2 and 3, feed samples were collected from each feeder after the beginning of each trial. Diet samples were stored at -20°C until they were homogenized, subsampled, and submitted for analysis. The high and low diets that were used for blending in all experiments were sent to the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories (Columbia, MO) for the determination of amino acids (AOAC 982.30 E(a,b,c) and 988.15), moisture (AOAC 934.01), and ash (AOAC 942.05). Crude protein determination was performed in duplicate using the Dumas combustion method (AOAC 992.15) with a LECO Tru-Mac N Protein analyzer (LECO Corporation, St. Joseph, MI) at Kansas State University on low and high basal diets as well as all blended intermediate diets (Table 2).

Statistical analysis

Data were analyzed as a RCBD for a one-way ANOVA using R Studio version 4.3.1 (R Core Team., Vienna, Austria) with pen as the experimental unit. In Exp. 1, BW on d 0 was used as a covariate for analysis on all reported growth performance measures except for d 0 BW. In all experiments, linear and quadratic contrasts were used to evaluate the effects of increasing the SID Val:Lys ratio. For the analysis of plasma urea nitrogen (PUN), treatment was included as a fixed effect, and block and plate were included as random effects. Results from all experiments were considered significant at $P < 0.05$ and marginally significant at $P \leq 0.10$. Dose response curves were evaluated using the NLMIXED procedure within SAS (v. 9.4, SAS Institute, Inc., Cary, NC) using linear, quadratic polynomial (QP), and broken-line linear (BBL) models. The best-fitting model was selected using the Bayesian Information Criterion (BIC).

Results

Results of laboratory analysis indicated nutrient profiles were consistent with expected formulated values (Table 2).

Experiment 1

Gain:feed ratio increased (linear, $P = 0.012$; Table 3) as SID Val:Lys ratio increased, but with little additional improvement beyond 69% SID Val:Lys ratio. Broken-line analysis indicated overall G:F increased with increasing SID Val:Lys ratio, with a breakpoint observed at a ratio of 69% of Lys (Figure 1). The estimated mean minimum SID Val:Lys ratio required to achieve 99% of the maximum G:F was approximately 66% (Table 4). No other significant differences in growth performance were observed. As the SID Val:Lys ratio increased, there was an increase in PUN concentration (linear, $P = 0.029$). Valine intake (g/d and g/kg of gain) increased (linear, $P < 0.001$) as the SID Val:Lys ratio increased, while Lys intake g/d was similar across treatments. Lysine intake g/kg of gain decreased (linear, $P = 0.029$) as the SID Val:Lys ratio increased.

Experiment 2

Final BW, ADG, and ADFI increased (quadratic, $P < 0.05$) as the SID Val:Lys ratio increased (Table 5). Pigs fed the 66% SID Val:Lys ratio had the greatest final BW and ADG, whereas pigs fed 72% SID Val:Lys ratio had the greatest ADFI. A QP analysis indicated ADG was maximized near 69% SID Val:Lys (Figure 2), but 99% of maximum ADG was near 65% SID Val:Lys ratio (Table 4). However, as the SID Val:Lys increased, G:F decreased (linear, $P < 0.001$), primarily driven by the middle treatments of 63, 66, 69, and 72% SID Val:Lys. Plasma urea nitrogen decreased (linear, $P = 0.048$) with increasing SID Val:Lys ratio. Following the trend observed in ADFI, Val and Lys intake g/d increased (quadratic, $P < 0.05$) with increasing

SID Val:Lys ratio. As the SID Val:Lys ratio increased, Val and Lys intake g/kg of gain increased (linear, $P < 0.05$).

Experiment 3

Average daily gain and ADFI increased (linear, $P < 0.05$) as the SID Val:Lys ratio increased (Table 6). However, little additional improvement in ADG was observed when SID Val:Lys exceeded 69%. When dose response modeling was performed, the linear model was the best fit (Figure 3). Final BW also tended to increase (linear, $P = 0.078$) as SID Val:Lys ratio increased; however, there was no evidence of an effect on G:F or PUN. Valine intake (g/d and g/kg of gain) and Lys intake g/d increased (linear, $P < 0.05$) as SID Val:Lys ratio increased. There were no differences observed in Lys intake g/kg of gain.

Discussion

Valine plays an important role in protein synthesis, muscle accretion, immune function, and gastrointestinal integrity, and the SID Val:Lys ratio is important for optimizing growth performance (Wang et al., 2023). After Lys, Met, Thr, and Trp, Val is generally considered the 5th limiting AA in corn-soybean meal diets (Figueroa et al., 2003). Current NRC (2012) recommendations for SID Val:Lys ratios range from 63.4 to 67.2% for pigs from 11 to 135 kg BW. However, these estimates are largely based on nursery pig data and generated based on models, as the NRC does not cite any studies evaluating Val requirements in pigs with an average BW above 27 kg. Since the publication of NRC (2012), several studies have evaluated Val requirements. However, reported requirement estimates vary considerably depending on BW range, response criteria, and statistical model used (Liu et al., 2015; Gonçalves et al., 2018; Wu et al., 2024). Results from the present study suggest that NRC (2012) recommendations may underestimate the SID Val:Lys requirement for 21 to 116 kg pigs. However, results are in

agreement with other published research reporting optimal SID Val:Lys ratios between 65 and 73% in 20 to 45 kg pigs (Wiltafsky et al., 2009; Waguespack et al., 2012; Goncalves et al., 2018). In 45 to 130 kg pigs the SID Val:Lys ratio reported in the literature was optimized at a ratio ranging from 62 to 71% (Liu et al., 2015; Clizer et al., 2022; Wu et al., 2024).

In pigs of similar body weight to Exp. 1, Waguespack et al. (2012) and Goncalves et al. (2018) observed an increase in ADG as the SID Val:Lys ratio increased from 60 to 75% and 59 to 75.5%, respectively. Although insignificant, the ADG in the current study followed this pattern, numerically increasing as Val was increased in the diet. In Exp. 2, a quadratic polynomial-derived optimum of 69% was observed for ADG. Wu et al. (2024) observed optimal ADG at a SID Val:Lys between 63 and 68% in pigs weighing 40 to 75 kg. Goncalves et al. (2018) observed similar results to the present study, with 68% SID Val:Lys achieving 99% of maximal ADG. In diets with 30% DDGS, Clizer et al. (2022) reported similar results to the present study where a SID Val:Lys ratio of 68% would yield more than 99% maximum performance for ADG. However, Liu et al. (2015) estimated a wider range, with a SID Val:Lys requirement of 67% using standard broken line or 72% for the QP for ADG in 49 to 70 kg pigs. In heavier pigs with a similar body weight to Exp. 3, previous publications found minimal ADG response when increasing the SID Val:lys ratio (Wu et al., 2024).

The response in ADFI in pigs weighing from 38 to 116 kg was similar to other published data, with greater ADFI as the SID Val:Lys ratio increased (Clark et al., 2017; Gonçalves et al., 2018; Clizer et al., 2022). This may be partially attributed to reduced feed intake observed in pigs fed Val deficient diets (Gloaguen et al., 2011). However, this response was not observed for Exp. 1.

Feed efficiency responses varied across BW phases within these experiments. Gonçalves et al. (2018) observed maximum G:F near 69% SID Val:Lys in pigs of similar BW as used in Exp. 1, which is similar to the response observed in the present study. In contrast to most available literature, G:F worsened as SID Val:Lys increased in 38 to 67 kg pigs, possibly due to disproportionate increases in feed intake relative to ADG. Unlike Exp. 1 and 2, there was a lack of G:F response observed in Exp. 3 similar to that observed by Wu et al. (2024). Wu et al. (2024) theorized that the lack of response was in part attributed to the Leu level in the diet. Similar to the Wu et al. (2024) study, higher dietary Leu concentrations were present in the current study for Exp. 3. The excess Leu may increase the nutritional requirement for Val, as Leu may enhance oxidation and catabolism of BCAA (Wiltafsky et al., 2010). However, the response observed in ADG does not support this, as there was little numerical improvement in ADG after an SID Val:Lys ratio of 69%, whereas Wu et al. (2024) observed 73% SID Val:Lys to have the greatest ADG and BW.

Plasma urea nitrogen reflects the breakdown of excess amino acids and is commonly used as an indicator of nitrogen utilization (Coma et al., 1955; Knowles, 1997; Whang and Easter, 2000). In Exp. 2, PUN decreased as SID Val:Lys increased, indicating enhanced nitrogen utilization as Val supply approached adequacy. In contrast, PUN increased in Exp. 1 as Val increased, suggesting a potential oversupply of Val at the higher titration levels, particularly at 72 and 75%, where PUN increased 1.03 mg/dL from 69% to 72%. The SID Val:Lys ratio did not appear to impact PUN in Exp. 3, which may be a function of the total Lys intake as described below.

For SID Val:Lys ratio experiments, Lys must be limiting so that changes in Val supply alter growth performance responses. If Lys is supplied in excess, the optimal SID Val:Lys ratio

requirement may be underestimated. In the present study, this condition was met as SID Lys intake/kg gain was below predicted requirements for pigs in this facility based on previous research (Royall et al., 2022). In Exp. 1, SID Lys intake ranged from approximately 18.7 to 19.2 g/kg gain compared with a projected requirement of 22.6 g/kg gain, while in Exp. 2, SID Lys intake ranged from 17.9 to 19.2 g/kg gain relative to a predicted requirement of 24.6 g/kg gain. In Exp. 3, SID Lys intake averaged approximately 22 g/kg gain, which is slightly lower than the predicted requirement of 23 g/kg gain for pigs of this body weight (Royall et al., 2022).

In conclusion, results from the present study demonstrate in Exp. 1 a SID Val:Lys ratio near 69% optimizes feed efficiency in pigs weighing 21 to 40 kg. Results from Exp. 2 align closely with existing literature and indicate that a SID Val:Lys ratio of approximately 69% is sufficient to maximize ADG in pigs weighing 38 to 67 kg, which is slightly greater than NRC (2012) estimates. In heavier pigs, Exp. 3 suggests that a SID Val:Lys ratio of at least 69% may support maximal growth, although responses are modest. Collectively, these results indicate a SID Val:Lys ratio of 69% appears to maximize ADG or G:F for 21 to 40 and 38 to 67 kg pigs, with 99% of maximum performance achieved around 65 to 66% SID Val:Lys. In 90 to 116 kg pigs, a ratio of at least 69% SID Val:Lys appeared to be sufficient for growth performance.

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Table 1.1. Diet composition (as-fed basis)

	Exp. 1[1]		Exp. 2[2]		Exp. 3[3]	
Item SID Val:Lys, %[4]:	60	75	60	75	63	78
Ingredient, %						
Corn	74.28	74.10	81.90	81.75	90.20	90.05
Soybean meal, 47.73% CP	22.18	22.19	14.65	14.65	6.65	6.65
Calcium carbonate	0.90	0.90	0.90	0.90	0.75	0.75
Monocalcium P, 21% P	0.75	0.75	0.85	0.85	0.90	0.90
Salt	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys-HCl	0.48	0.48	0.46	0.46	0.39	0.39
DL-Met	0.17	0.17	0.12	0.12	0.04	0.04
L-Thr	0.21	0.21	0.18	0.18	0.14	0.14
L-Trp	0.05	0.05	0.05	0.05	0.04	0.04
L-Ile	0.07	0.07	0.07	0.07	0.06	0.06
L-Val	---	0.17	---	0.15	---	0.15
Vitamin premix[5]	0.20	0.20	0.15	0.15	0.15	0.15
Trace mineral premix[6]	0.15	0.15	0.15	0.15	0.15	0.15
Phytase[7]	0.06	0.06	---	---	---	---
Standardized ileal digestible amino acids, %						
Lys	1.10	1.10	0.90	0.90	0.65	0.65
Ile:Lys	60	60	60	60	60	60
Leu:Lys	121	121	128	128	149	149
Met:Lys	37	37	36	36	33	33
Met and Cys:Lys	59	59	60	60	60	60
Thr:Lys	65	65	65	65	67	67
Trp:Lys	19.4	19.4	19.0	19.0	18.9	18.9
Val:Lys	60	75	60	75	63	78
His:Lys	37	37	37	37	39	39
NE, kcal/kg	2,482	2,485	2,612	2,615	2,612	2,615
CP, %	17.4	17.4	14.4	14.5	11.2	11.2
Ca, %	0.63	0.63	0.61	0.61	0.54	0.54
STTD P, %[8]	0.40	0.40	0.39	0.39	0.36	0.36
Ca:P	1.24	1.24	1.22	1.22	1.13	1.13

[1] Diets were fed from 21.4 to 38.8 kg BW. The two diets were blended to create intermediate treatment diets containing 60, 63, 66, 69, 72, and 75% SID Val:Lys ratio.

[2] Diets were fed from 38.6 to 66.3 kg BW. The two diets were blended to create intermediate treatment diets containing 60, 63, 66, 69, 72, and 75% SID Val:Lys ratio.

[3] Diets were fed from 90.0 to 116.1 kg BW. The two diets were blended to create intermediate treatment diets containing 63, 66, 69, 72, 75 and 78% SID Val:Lys ratio.

[4] Standardized ileal digestibility (SID) Val:Lys ratio.

[5] Provided per kg of premix: 1,653,465 IU of vitamin A as retinyl acetate; 661,387 IU of vitamin D_3 as cholecalciferol; 17,637 IU vitamin E as dl-α-tocopherol acetate; 1,323 mg of vitamin K as menadione; 13.23 mg of vitamin B_{12} as cyanocobalamin; 19,800 mg of niacin; 11,023 mg of pantothenic acid as d-calcium pantothenate; 3,307 mg of riboflavin.

[6] Provided per kg of premix: 11,000 mg of Cu as copper sulfate; 198 mg I as ethylenediamine dihydriodide; 73,413 mg Fe as ferrous sulfate; 22,046 mg Mn as manganese oxide; 198 mg Se as sodium selenite; 73,413 mg of Zn as zinc sulfate.

[7] Exp. 1, HiPhorius 2,400 (DSM-Firmenich, Maastricht, Netherlands) was added at 1,500 FYT/kg of feed and provided an estimated release of 0.12% STTD P. Exp. 2 and 3, phytase was included in the vitamin premix at 499,898 FTU/kg of premix and provided an estimated release of 0.12% STTD P.

[8] Standardized total tract digestible phosphorus.

Table 1.2. Analyzed nutrient composition of experimental diet (as-fed basis)[1]

		Exp. 1[2]		Exp. 2[3]		Exp. 3[4]	
Item, %	SID Val:Lys, %[5]:	60	75	60	75	63	78
DM[6]		87.72	87.84	88.16	87.28	88.01	88.20
CP[7]		17.28	16.84	13.68	14.01	11.06	11.01
Ash		3.71	4.15	3.57	3.53	3.16	3.07
Amino acids							
Lys		1.15	1.16	1.02	0.98	0.80	0.84
Ile		0.78	0.76	0.64	0.61	0.48	0.48
Leu		1.54	1.46	1.28	1.27	1.12	1.10
Met		0.37	0.39	0.35	0.32	0.22	0.23
Cys		0.31	0.30	0.26	0.26	0.21	0.22
Thr		0.74	0.75	0.66	0.67	0.51	0.50
Trp		0.21	0.21	0.17	0.17	0.14	0.16
Val		0.83	0.90	0.64	0.79	0.51	0.60
His		0.46	0.43	0.36	0.35	0.29	0.29

[1] Diet samples were taken directly after diet manufacturing and stored at -20°C. Values are reported on a total analyzed basis. Composite samples were submitted to University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories (Columbia, MO) for dry matter, ash and amino acid analysis. Crude protein analysis was performed at Kansas State University.

[2] Diets were fed from 21.4 to 38.8 kg BW. The two diets were blended to create intermediate treatment diets containing 60, 63, 66, 69, 72, and 75% standardized ileal digestibility (SID) Val:Lys ratio.

[3] Diets were fed from 38.6 to 66.3 kg BW. The two diets were blended to create intermediate treatment diets containing 60, 63, 66, 69, 72, and 75% SID Val:Lys ratio.

[4] Diets were fed from 90.0 to 116.1 kg BW. The two diets were blended to create intermediate treatment diets containing 63, 66, 69, 72, 75 and 78% SID Val:Lys ratio.

[5] Standardized ileal digestibility (SID) Val:Lys ratio.

[6] DM = dry matter.

[7] CP = crude protein.

Table 1.3. Effects of increasing standardized ileal digestible (SID) Val:Lys ratio on growth performance and plasma urea nitrogen of 21- to 40-kg pigs (Exp. 1)[1]

	SID Val:Lys ratio, %							*P* =	
Item	60	63	66	69	72	75	SEM	Linear	Quadratic
BW, kg									
d 0	21.5	21.5	21.3	21.2	21.6	21.2	0.35	0.380	0.832
d 21	39.8	39.7	39.7	39.9	40.0	40.2	0.31	0.162	0.481
Growth performance									
ADG, g	920	916	921	927	931	936	13.9	0.242	0.739
ADFI, kg	1.61	1.60	1.60	1.58	1.58	1.61	0.025	0.815	0.346
G:F, g/kg	572	574	577	588	589	582	5.0	0.012	0.252
Plasma urea N, mg/dL[2]	5.12	5.56	5.37	5.45	6.48	6.05	0.400	0.029	0.872
Val intake, g/d	10.61	11.07	11.59	12.00	12.52	13.29	0.194	< 0.001	0.376
Val intake, g/kg gain	11.53	12.08	12.59	12.93	13.45	14.20	0.114	< 0.001	0.301
Lys intake, g/d	17.69	17.57	17.56	17.40	17.38	17.73	0.279	0.815	0.346
Lys intake, g/kg gain	19.22	19.18	19.07	18.74	18.67	18.94	0.163	0.016	0.243

[1] A total of 351 pigs (DNA 600 × 241) were used in a 21-d trial with 5 pigs per pen and 12 replications per treatment.
[2] After a 6-h fasting period, blood samples were taken on d 10 from 2 pigs per pen (1 barrow and 1 gilt).

Table 1.4. Estimated mean lowest standardized ileal digestible (SID) Val:Lys ratio for selected target mean performance levels

	Percent of maximum performance, %			
	95%	98%	99%	100%
Exp. 1, G:F[1]	52.7	62.5	65.7	69.0
Exp. 2, ADG[2]	59.7	63.0	64.7	68.7
Exp. 3, ADG[3]	61.4	71.4	74.7	78.0

[1] Broken line linear question for predicted G:F, g/kg = 585.9 + 179 (SID Val:Lys ratio – 0.69) if SID Val:Lys ratio < 0.69, G:F = 585.9 if SID Val:Lys ratio ≥ 0.69.

[2] QP equation for predicted ADG, g = -6,323.89 × (SID Val:Lys ratio)^2 + 8,689.46 × (SID Val:Lys ratio) – 1965.16.

[3] Linear equation for predicted ADG, g = 729.11 + 286.92 × (SID Val:Lys ratio).

Table 1.5. Effects of increasing standardized ileal digestible (SID) Val:Lys ratio on growth performance and plasma urea nitrogen of 38- to 67-kg pigs (Exp. 2)[1]

	SID Val:Lys ratio, %							*P* =	
Item	60	63	66	69	72	75	SEM	Linear	Quadratic
BW, kg									
d 0	38.6	38.6	38.6	38.6	38.7	38.6	0.90	0.871	0.666
d 27	65.3	66.0	66.7	66.5	66.6	66.9	0.83	0.189	0.008
Growth performance									
ADG, g	972	997	1,022	1,014	1,014	995	12.0	0.118	0.005
ADFI, kg	2.09	2.07	2.16	2.20	2.26	2.14	0.023	<0.001	0.002
G:F, g/kg	465	482	474	460	449	465	4.1	<0.001	0.721
Plasma urea N, mg/dL[2]	8.14	8.16	7.62	7.95	7.16	7.30	1.181	0.048	0.918
Val intake, g/d	11.12	11.47	12.53	13.38	14.27	14.06	0.576	<0.001	0.011
Val intake, kg gain	11.40	11.50	12.26	13.21	14.10	14.14	0.550	<0.001	0.874
Lys intake, g/d	18.14	17.83	18.61	19.02	19.44	18.40	0.960	<0.001	0.007
Lys intake, kg gain	18.59	17.87	18.20	18.77	19.20	18.49	0.913	0.008	0.803

[1] A total of 647 pigs (DNA 600 × 241) were used in a 27-d trial with eight to nine pigs per pen and 12 replications per treatment.
[2] After a 10-h fasting period, blood samples were taken on d 15 from 3 pigs per pen (2 barrows and 1 gilt).

Table 1.6. Effects of increasing standardized ileal digestible (SID) Val:Lys ratio on the growth performance and plasma urea nitrogen of 90- to 116-kg pigs (Exp. 3)[1]

	SID Val:Lys ratio, %							*P* =	
Item	63	66	69	72	75	78	SEM	Linear	Quadratic
BW, kg									
d 0	89.9	90.0	90.0	90.0	89.9	90.0	1.86	0.975	0.912
d 27	115.5	115.6	116.5	116.1	116.2	116.7	2.01	0.078	0.867
Growth performance									
ADG, g	907	915	946	922	943	956	15.1	0.019	0.910
ADFI, kg	2.89	2.90	2.95	3.00	3.05	2.99	0.058	<0.001	0.224
G:F, g/kg	314	317	321	309	310	321	5.5	0.956	0.288
Plasma urea N, mg/dL[2]	7.95	8.52	8.17	8.19	7.89	9.57	1.014	0.105	0.177
Val intake, g/d	13.07	13.55	14.38	15.34	16.19	16.54	0.267	<0.001	0.665
Val intake, kg gain	14.41	14.82	15.22	16.67	17.23	17.32	0.231	<0.001	0.587
Lys intake, g/d	20.74	20.53	20.84	21.31	21.59	21.21	0.319	<0.001	0.611
Lys intake, kg gain	22.88	22.46	22.06	23.15	22.97	22.20	0.321	0.742	0.721

[1] A total of 647 pigs (DNA 600 × 241) were used in a 27-d trial with eight to nine pigs per pen and 12 replications per treatment.
[2] After a 10-h fasting period, blood samples were taken on d 15 from 3 pigs per pen (2 barrows and 1 gilt).

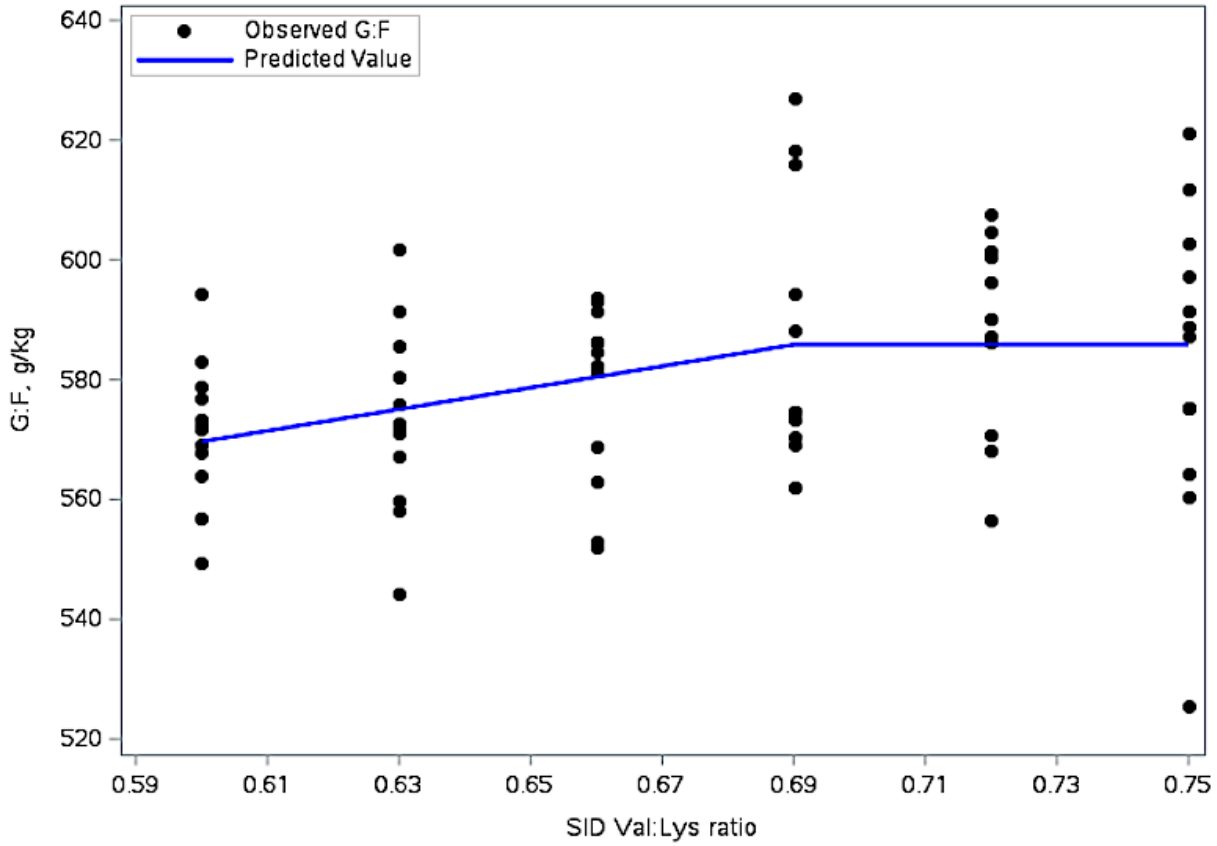

Figure 1.1. Estimation of standardized ileal digestibility (SID) Val:Lys ratio requirement to maximize G:F of 21- to 40-kg pigs, Exp. 1. A total of 351 pigs (DNA 600 × 241) were used in a 21-d trial. The broken-line linear model (BLL) resulted in the best fit based on the Bayesian Information Criterion. The BLL model predicted no further improvement beyond 69% SID Val:Lys ratio.

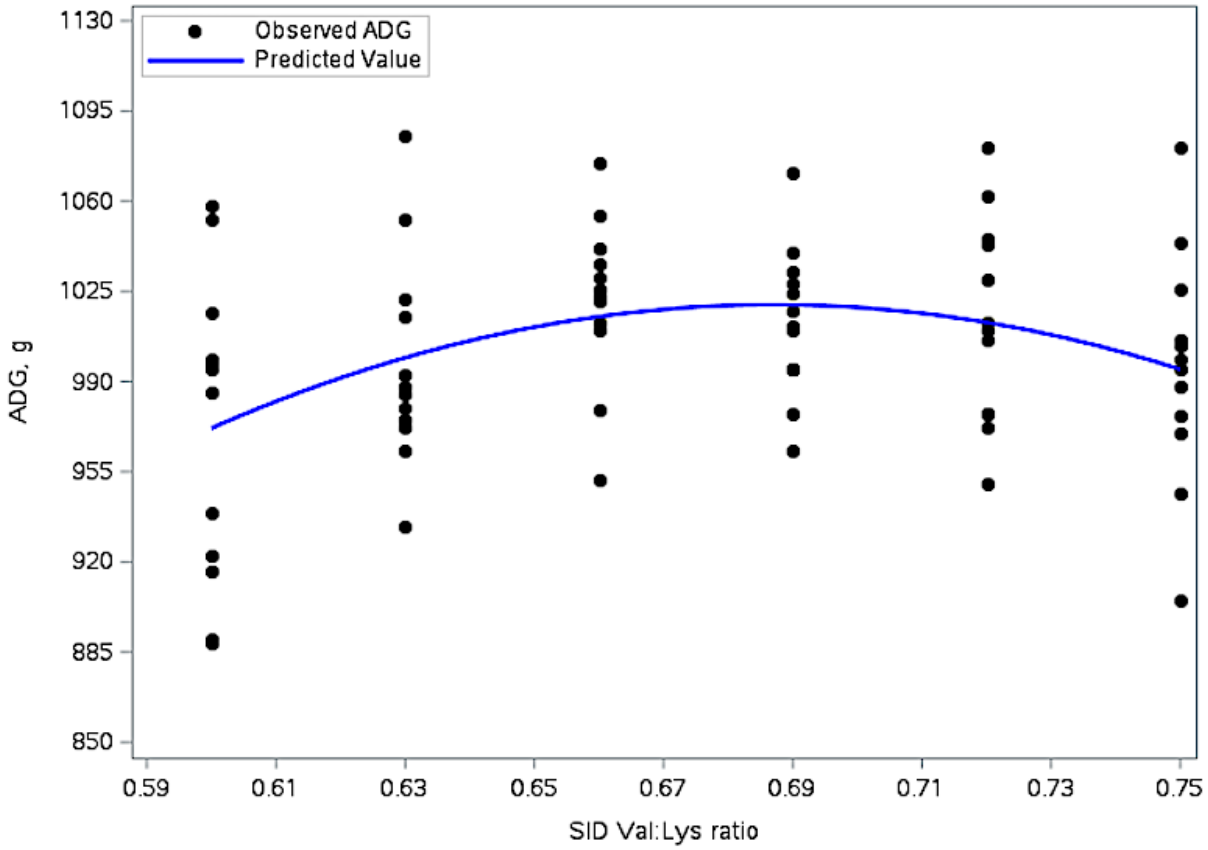

Figure 1.2. Estimation of standardized ileal digestibility (SID) Val:Lys ratio requirement to maximize ADG of 38- to 67-kg pigs, Exp. 2. A total of 647 pigs (DNA 600 × 241) were used in a 27-d trial. The quadratic polynomial model resulted in the best fit based on the Bayesian Information Criterion. ADG, g = -6,323.89 × (SID Val:Lys ratio)^2 + 8,689.46 × (SID Val:Lys ratio) – 1965.16. Maximum ADG was calculated to be 68.7% SID Val:Lys.

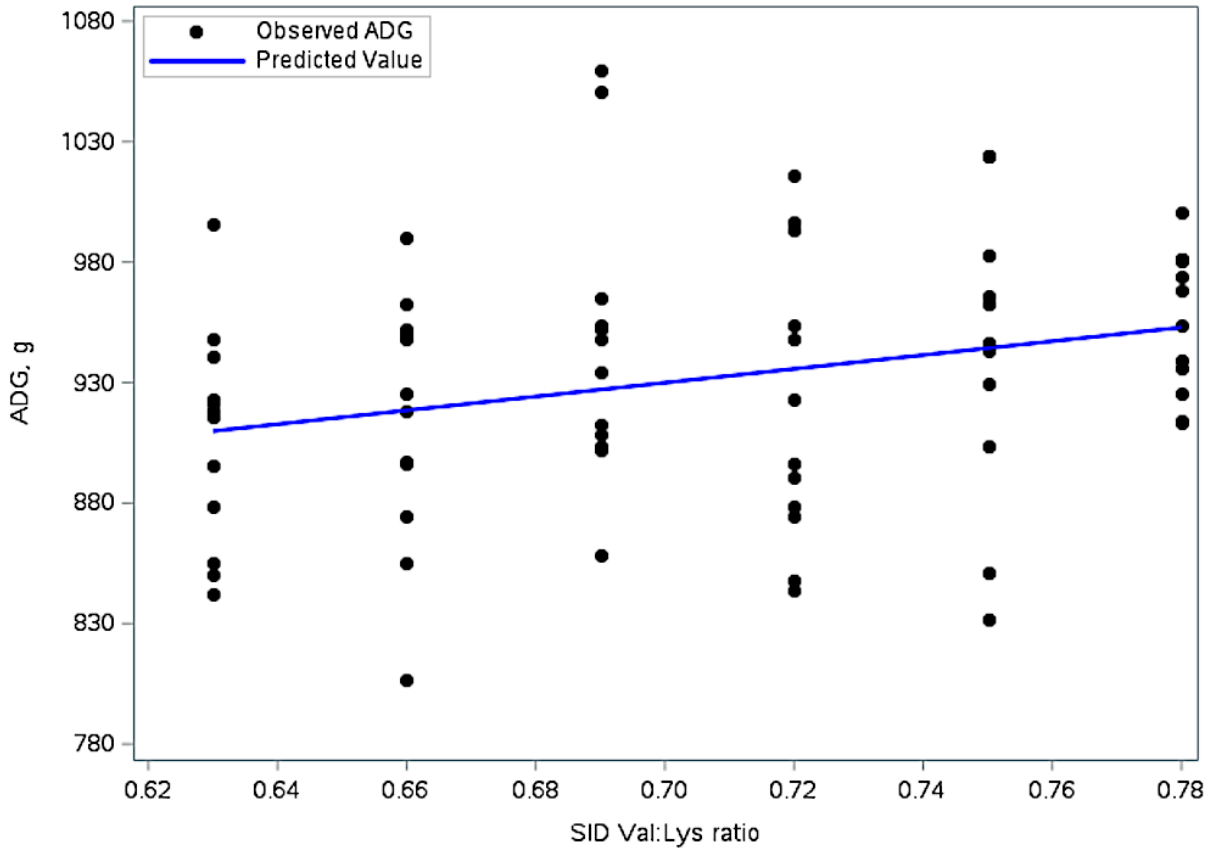

Figure 1.3. Estimation of standardized ileal digestibility (SID) Val:Lys ratio requirement to maximize ADG of 90- to 116-kg pigs, Exp. 3. A total of 647 pigs (DNA 600 × 241) were used in a 27-d trial. The linear model resulted in the best fit based on the Bayesian Information Criterion. ADG, g = 729.11 + 286.92 × (SID Val:Lys ratio).

Chapter 2 - Evaluation of pharmacological copper feeding strategies and sources on nursery and finishing pig performance

Abstract

Two experiments evaluated the use of pharmacological levels of Cu in nursery and finishing pig diets. In Exp. 1, 2,204 pigs (initially 5.3 ± 0.09 kg) were used in a 38-d study with 29 pigs/pen and 19 pens/treatment. Pens of pigs were allotted to four feeding strategies in a randomized complete block design. Strategies consisted of feeding duration of pharmacological Cu: 1) control with no pharmacological Cu, 2) pharmacological Cu in phase 3, 3) pharmacological Cu in phases 2 and 3, and 4) pharmacological Cu in all phases. Treatment diets were fed based on feed budgets of 1.8 kg/pig for phase 1 (d 0 to approximately 10), 5.4 kg/pig for phase 2 (approximately d 10 to 24), and phase 3 was fed for the remainder of the trial (approximately d 24 to 38). Diets within each phase contained either 17 mg/kg (base) or 167 mg/kg (pharmacological) Cu from tribasic copper chloride (NexTrace TBCC, 58%, SAM Nutrition, Bloomington, MN). All diets contained 3,000 mg/kg Zn in phase 1 and 2,000 mg/kg Zn in phase 2. In phase 2, pigs fed pharmacological Cu had greater average daily gain (ADG; $P = 0.012$) and average daily feed intake (ADFI; $P = 0.021$) than pigs fed the control diet. However, no differences were observed among strategies for overall performance. In Exp. 2, 2,160 pigs (initially 24.1 ± 0.60 kg) were used in a 110-d study with 27 pigs/pen and 20 pens/treatment. Pens of pigs were allotted to one of four treatments: base level of Cu or pharmacological Cu from TBCC source 1 (NexTrace TBCC, 58%, SAM Nutrition, Bloomington, MN), TBCC source 2 (Intellibond C, 54%, Selko, Tilburg, Netherlands), and $CuSO_4$ (25.2%, Phibro Animal Health Corp., Teaneck, NJ). All diets contained basal Cu from

the premix with those containing pharmacological Cu containing an additional 150 mg/kg Cu from treatment sources. From d 0 to 56, pigs fed pharmacological Cu had greater ADG ($P <$ 0.001) and a tendency for increased ADFI and G:F ($P \leq 0.059$) with no source differences. From d 56 to 110, no differences in growth were observed. Overall, pharmacological Cu increased ADG ($P = 0.018$) and final weight ($P = 0.008$) and a tendency for increased ADFI ($P = 0.072$); however, there were no differences observed between Cu source. In summary, pharmacological Cu provided limited overall benefit in the nursery when pharmacological levels of Zn were fed. In grow-finish pig diets, 150 mg/kg of Cu improved growth performance regardless of source, with the greatest response during the early growth phase.

Key Words: copper, copper sulfate, tribasic copper chloride, pharmacological copper, pigs

Introduction

Copper (Cu) is generally added to swine diets because it is needed for processes involving growth, development, and maintenance (Gaetke and Chow, 2003). There are a variety of sources of Cu available for use in swine diets. Common sources used include copper sulfate ($CuSO_4$) and tribasic copper chloride (TBCC). The NRC (2012) describes the dietary copper requirement estimate for pigs weighing between 5 and 130 kg as 3 to 6 mg/kg. However, previous research has observed improvements in growth performance when pharmacological levels (100 to 250 mg/kg) of Cu are included in the diet (Cromwell et al., 1998; Hill et al., 2000; Hastad et al., 2001).

A review of different levels and sources of added Cu in nursery diets identified that the inclusion of pharmacological levels of dietary Cu, independent of source, increased average daily

gain (ADG) and gain:feed ratio (G:F; Galiotto Miranda et al., 2024). Within commercial production, it is common for pharmacological levels of Cu to be added in late nursery diets (Faccin et al., 2023). However, limited research has been conducted in recent years evaluating the most beneficial phase feeding strategies for pharmacological levels of Cu when fed in combination with pharmacological levels of Zinc (Zn). Some research indicates there are no additive effects when pharmacological levels of Zn and Cu are used together (Smith et al., 1997). Whereas Pérez et al. (2011) indicated that pharmacological Zn and pharmacological Cu were additive in promoting the growth of weaned pigs. Therefore, the objective of Exp. 1 was to compare the effects of feeding pharmacological levels of Cu, provided by TBCC, throughout three nursery phases on growth performance when diets included pharmacological levels of Zn.

Within growing-finishing pigs, the benefits from pharmacological levels of Cu are typically observed in the early growing period (NCR-42, 1974; Hastad et al., 2001), although some research has also observed positive effects in later finishing stages. Coble et al. (2017) demonstrated a linear improvement in growth performance and hot carcass weight (HCW) in finishing pigs when supplementing diets with 75 mg/kg or 150 mg/kg Cu from either $CuSO_4$ or TBCC. Primarily, previous research has compared TBCC to $CuSO_4$, with little research directly comparing different sources of TBCC. As more Cu sources become available, further research is needed to characterize their response in swine diets. Therefore, the objective of Exp. 2 was to compare the effects of three Cu sources fed at a pharmacological level (TBCC source 1, TBCC source 2, or $CuSO_4$) compared to a control diet containing a base level of Cu provided from the trace mineral premix on growth performance and carcass characteristics in finishing pigs.

Materials And Methods

Animal facilities

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments (IACUC # 4847). Experiment 1 was conducted in two rooms at a commercial nursery research site (Hord Farms West, Pipestone, MN). Each pen (2.3 × 3.7 m) provided approximately 0.32 m^2 per pig and was equipped with a 6-hole dry feeder and a drinker pan. Experiment 2 was conducted in two independent barns at a commercial finishing research site (Hord Farms West, Pipestone, MN). Finishing barns were naturally ventilated and double-curtain-sided with totally slatted floors. Each pen (3.0 × 5.6 m) provided approximately 0.63 m^2 per pig and was equipped with a 5-hole dry feeder and a cup drinker. In both facilities, daily feed additions were recorded using a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN). Pigs were provided ad libitum access to feed and water throughout both experiments.

Animals and diets

Experiment 1

A total of 2,204 weanling pigs (PIC 337 × 1050, initially 5.3 ± 0.09 kg) were used in a 38-d study with 29 pigs per pen and 19 pens per treatment. Pigs were weaned at approximately 20 d of age and randomly allotted to pens. Pens of pigs were blocked by initial body weight (BW) and allotted to one of four feeding strategies in a randomized complete block design.

The four Cu feeding strategies evaluated consisted of pharmacological levels of Cu in the different nursery phases. Treatment diets were formulated in three phases and fed based on feed budgets of 1.8 kg/pig for phase 1 (d 0 to approximately 10), 5.4 kg/pig for phase 2 (approximately d 10 to 24), and phase 3 was fed for the remainder of the trial (approximately d

24 to 38). Feeding strategy 1 served as the control with no pharmacological levels of Cu, strategy 2 included pharmacological Cu in only phase 3, strategy 3 consisted of pharmacological Cu in phases 2 and 3, and strategy 4 included pharmacological Cu in all three phases (Table 1). Within each experimental phase, there were two diets provided, either a base level of Cu (17 mg/kg) or pharmacological levels (167 mg/kg) of Cu. The base level of Cu was provided from $CuSO_4$ in the vitamin/trace mineral premix. In phases where pharmacological levels of Cu were fed, 150 mg/kg of TBCC (NexTrace TBCC, 58%, SAM Nutrition, Bloomington, MN) was added to diets as a separate ingredient. Complete feed samples were analyzed for Cu in duplicate (Table 2; AOAC 2000, method 985.01; Cumberland Valley Analytical Services, Inc., Waynesbro, PA).

Diets included pharmacological levels of Zn from zinc oxide in phases 1 and 2 to provide Zn inclusion above basal nutritional levels (approximately 3,000 mg/kg Zn in phase 1 and approximately 2,000 mg/kg Zn in phase 2). Phase 3 contained 110 mg/kg Zn. Phase 1 diets were manufactured at Hubbard Feeds in Mankato, MN. Phase 2 and 3 diets were manufactured at the Hord Farms West feed mill located in Pipestone, MN. The first phase was fed in pellet form, and the remaining two phases were fed in meal form. Pen weights and feed disappearance were recorded on d 10, 17, 24, 31, and 38 to determine ADG, average daily feed intake (ADFI), and G:F. Feed delivered was recorded for each pen, with any feed remaining in feeders recorded on each weigh day by a feeder measurement. Mortality and removals were recorded for the duration of the trial. Under the circumstance a pig died or was removed from the study due to inability to overcome sickness or injury, the weight of the pig and feed consumption up until that date was recorded.

Experiment 2

A total of 2,160 pigs (PIC 337 × 1050; initially 24.1 ± 0.60 kg) were used in a 110-day study with 27 pigs per pen and 20 pens per treatment. Pigs were randomly assigned to a pen upon arrival in the barn and fed a common diet until they reached approximately 23 kg. Pens of pigs were then blocked by weight and randomly allotted to one of four dietary treatments.

Dietary treatments consisted of a control diet containing a base level of Cu, or a pharmacological level of one of three sources: TBCC source 1 (NexTrace TBCC, 58%, SAM Nutrition, Bloomington, MN), TBCC source 2 (Intellibond C, 54%, Selko, Tilburg, Netherlands), and $CuSO_4$ (25.2%, Phibro Animal Health Corp., Teaneck, NJ; Table 3). All treatment diets contained a base level of $CuSO_4$ provided by the trace mineral premix, which provided 16 mg/kg Cu in phases 1 and 2, 13 mg/kg Cu in phase 3, and 11 mg/kg Cu in phase 4 based on a tapered inclusion of the trace mineral premix throughout the experiment. An additional 150 mg/kg Cu was added from the other three sources for their respective dietary treatment. Complete feed samples were analyzed for Cu in duplicate identical as outlined for Exp. 1 (Table 4).

Test ingredients were mixed into hand-add packs using approximately 0.9 kg of ground corn per ton of feed. All diets were fed in meal form and manufactured at the Hord Farms West feed mill located in Pipestone, MN. Treatment diets were formulated in four phases and fed based on average barn weight: phase one, approximately 23 to 50 kg, phase two from 50 to 75 kg, phase three from 75 to 100 kg, and phase four from 100 kg to marketing at approximately 127 kg. Phases 1 and 2 were combined to form the grower phase (d 0 to 56) and phases 3 and 4 were combined to represent the finisher phase (d 56 to 110). Pen weights and feed disappearance were recorded approximately every 14 d to determine ADG, ADFI, and G:F. Feed deliveries

were recorded by pen, and residual feed was measured on each weigh day. Mortality and removals were recorded throughout the trial. For pigs that died or were removed due to illness or injury, body weight and feed intake were recorded up to the time of removal.

Carcass data

In Exp. 2, when the barn average pig weight reached approximately 110 kg, the three heaviest pigs per pen were selected and marketed at a United States Department of Agriculture (USDA) inspected processing facility (JBS Foods, Worthington, MN). No carcass data were collected during this first marketing event. At the end of the study, approximately two to three weeks after the first marketing event, depending on barn, the remaining pigs were marketed. Standard carcass measurements of backfat depth, loin depth, percentage lean, and hot carcass weight (HCW) were collected for each individual carcass and summarized by pen. The percentage lean calculations were obtained from a proprietary equation used by the packer. Carcass yield was calculated by dividing the pen average HCW by the pen average final live weight obtained at the farm.

Statistical Analysis

Data were analyzed in R studio (Version 4.3.1 R Core Team, Vienna, Austria) as a one-way ANOVA using the lmer function, including treatment as a fixed effect and weight block as a random effect with the addition of barn serving as a random effect in Exp. 2. Hot carcass weight was used as a covariate for carcass characteristics. In Exp. 1, contrasts were used to test for the main effects of Cu level within each phase. In Exp. 2, contrasts were used to test for the effect of base level Cu vs pharmacological levels of Cu and between Cu sources. *P*-values were considered significant at $P < 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Analysis of complete diets for both experiments indicated that analyzed Cu levels were within the expected range of formulated levels (Tables 2 and 4). No meaningful deviations from the expected Cu levels were observed when accounting for analytical variation and Cu levels coming from basal ingredients, nor were any observed for other nutrients.

Experiment 1

In phase 1 (approximately d 0 to 10), there was no evidence of a difference in performance observed between pigs fed the base level of Cu and pigs fed a diet containing pharmacological levels of Cu (Table 5). In phase 2 (approximately d 10 to 24), pigs fed pharmacological levels of Cu had greater ADG ($P = 0.012$) and ADFI ($P = 0.021$) compared to pigs fed the base level of Cu, with no evidence of a difference for G:F. Additionally, pigs fed pharmacological levels of Cu in phases 1 and 2 had greater ADG during phase 2 than those fed a base level of Cu in phase 1 and 2 ($P = 0.047$), with pigs fed pharmacological Cu levels in only phase 3 and pigs fed pharmacological Cu in phases 2 and 3 intermediate.

In phase 3 (approximately d 24 to 38), there were no differences observed in growth performance between pigs fed the base level of Cu and pharmacological level of Cu, nor any differences in any of the feeding strategies. There were no differences between Cu feeding strategies in the overall growth performance (d 0 to 38) or removals and mortality.

Experiment 2

There was no evidence of difference observed among Cu sources when fed at pharmacological levels for any response criteria. In the grower period (d 0 to 56), pigs fed pharmacological levels of Cu had a greater ($P < 0.001$) BW and ADG compared to pigs fed the control diet containing the basal level of Cu (Table 6). A tendency ($P \leq 0.059$) was observed in

pigs fed pharmacological levels of Cu for increased ADFI and G:F when compared to pigs fed the control diet. In the finishing period (d 56 to the end of the trial), there was no evidence of differences observed in ADG, ADFI or G:F when comparing pigs fed pharmacological levels of Cu to those fed the control diet.

Overall, pigs fed pharmacological levels of Cu had greater ($P = 0.018$) ADG and final BW ($P = 0.008$) when compared to pigs fed the base level of Cu. There was a tendency observed for greater ($P = 0.072$) ADFI for pigs fed pharmacological levels of Cu compared to pigs fed the base level of Cu. Hot carcass weight was greater ($P = 0.031$) in pigs fed pharmacological levels of Cu than pigs fed the base level of Cu. No evidence of any differences was observed for other carcass characteristics or removals and mortality.

Discussion

The NRC (2012) estimated Cu requirement for pigs is 3 to 6 mg/kg. The control diets without pharmacological levels of Cu for both of our experiments contained levels above this to ensure the basic requirement was met. Additional levels of Cu of 100 to 250 mg/kg have been known to improve growth performance (Hastad et al., 2001; Huang et al., 2015; Espinosa et al., 2017). Some researchers have attributed the improvement in performance to antimicrobial effects (Shurson et al., 1990). Jensen (2016) concluded that Cu supplementation, even in small amounts (less than 50 mg/kg), seemed to reduce the population of clostridia and coliform bacteria, whereas high levels (greater than 170 mg/kg) seem to reduce the presence of lactobacillus. However, others have suggested Cu impacts growth and metabolism in other ways. Pharmacological levels of Cu often increase ADG and ADFI. Zhou et al. (1994b) observed greater concentrations of growth hormones in the pituitary gland of pigs fed 215 mg/kg added Cu. Dove (1995) suggested that pharmacological levels of Cu may improve fat digestibility. Li et

al. (2008) observed that high dietary Cu increased neuropeptide Y concentrations and neuropeptide Y mRNA expression in the hypothalamus, which may be responsible for the increased feed intake and gain observed with pharmacological Cu addition.

Within swine diets, commonly used Cu sources include $CuSO_4$ and TBCC. Copper sulfate is manufactured using Cu metal and sulfuric acid and is highly soluble in water (Miles et al., 1998). Tribasic copper chloride is a by-product of the manufacturing process of circuit boards, where acidic cupric chloride and alkaline cupramine chloride solution are neutralized to form a green purified source of Cu (Coble et al., 2017). This source is less than 1% soluble in water (Miles et al., 1998). Due to the differences in manufacturing, the concentration of Cu in TBCC is greater than that of $CuSO_4$, with concentrations of 54 to 58% Cu in TBCC compared to 25.2% Cu in $CuSO_4$. Tribasic copper chloride has also been shown to have less oxidative instability during storage than $CuSO_4$ which may offer benefits in vitamin stability when included in a vitamin-trace mineral premix (Luo et al., 2005).

Previous findings have observed increases in growth performance of nursery pigs with additions of 100 to 250 mg/kg Cu, regardless of source (Fry et al., 2012; Huang et al., 2015; Espinosa et al., 2017). Cromwell et al. (1989) observed that feeding 150 mg/kg Cu was 75% as effective at increasing growth as feeding 250 mg/kg. However, other literature indicates that 125 mg/kg was equally effective in stimulating growth as 250 mg/kg Cu (Stahly et al., 1980). More recent research has observed that feeding 160 mg/kg or 200 mg/kg of TBCC resulted in similar weight gain and G:F but greater performance than pigs fed less than 80 mg/kg Cu (Lin et al., 2020). Therefore, 150 mg/kg of Cu was added to obtain pharmacological levels of Cu within the diet for the respective feeding strategy within the current trial.

In nursery diets, it is common in some countries to feed pharmacological levels of Zn during the first two or three weeks after weaning as it has been shown to promote growth rate and increase stool firmness (Bonetti et al., 2021; Faccin et al., 2023; Tang et al., 2024). However, it is important to evaluate Cu effects when pharmacological levels of Zn are included within the diet because of possible Cu and Zn interactions. Some research indicates there are no additive effects when pharmacological levels of Zn and Cu are used together compared to using only pharmacological levels of Zn (Smith et al., 1997). Whereas Pérez et al. (2011) indicated that pharmacological Zn (3,000 mg/kg diet as ZnO) and pharmacological Cu (100 mg/kg diet as Cu amino acid complex or 250 or 315 mg/kg diet as $CuSO_4$) were additive in promoting the growth of weaned pigs. Other studies have indicated minimal additive improvement when feeding pharmacological levels of Zn and Cu together (Hill et al., 2000; Shelton et al., 2011). To further evaluate the use of pharmacological levels of Cu and Zn together, pharmacological levels of Zn were included within the diet of the current trial. Hill et al. (2000) observed an improvement in G:F during the second week after weaning when pharmacological levels of Zn and Cu were fed together. In the current study, no differences in G:F were observed, but rather the improvement in ADG in phase 2 appears to be the result of the increase in ADFI. Similarly, Zhu et al. (2011) observed increased ADFI with pharmacological levels of Cu.

While numerous studies have characterized pharmacological Cu use within the nursery phase, pharmacological levels of Cu can also be used to increase growth performance in finishing pigs (Braude and Hosking, 1982). However, results are variable with some trials indicating Cu level did not influence overall performance (Carpenter et al., 2019; Bradley et al., 2023). Of the trials that have observed a response from additional pharmacological levels of Cu, many are similar to the current study with increased growth performance in the grower period

(Hastad et al., 2001; Coble et al., 2017; 2018). However, others have observed improved gain in the finishing period as well (Davis et al., 2002; Zhao et al., 2014). Zheng et al. (2018) observed a tendency for $CuSO_4$ to have a greater ADG than pigs fed TBCC, unlike the current trial, where Cu source did not affect ADG when fed at pharmacological levels, which is similar to results of Hastad et al. (2001) and Coble et al. (2017). The gain response observed from Cu is often a function of feed intake (Li et al., 2008). Coble et al. (2017; 2018) observed an increase in ADFI when feeding additional Cu in the diet in the initial few weeks of the growing period. Other research has observed an improvement in G:F when pharmacological levels of Cu were fed (Barber et al., 1955b; Davis et al., 2002; Hernández et al., 2008). In the current trial, the improvement in gain seems to primarily be associated with feed intake, however G:F was slightly improved for pigs fed pharmacological levels of Cu in the growing period.

Some previous research agrees with the observed improvement in final BW and carcass weight with pharmacological levels of Cu due to improvements in growth (Coble 2017). In addition, some have observed increases in loin depth, lean percentage, and dressing percentage (Myres and Bowland, 1973a; Hernandez et al., 2007; Coble et al., 2017). Myres and Bowland (1973b) thought copper may alter adipose tissue fatty acid composition by disrupting the balance between triglyceride lipolysis and free fatty acid esterification and therefore influencing carcass quality. However, these responses have not been consistent, as other studies have reported no effects on carcass characteristics (Omale, 1980; Bowland, 1990; Coble et al., 2018).

In conclusion, these data suggest that in the nursery (Exp. 1), when pharmacological levels of Zn are used, ADG and ADFI can be improved by the addition of pharmacological levels of Cu in the diet from d 10 to 24. The improvement in ADG and ADFI within phase 2 from the addition of pharmacological levels of Cu did not influence overall performance, removals, or mortality.

However, pigs fed pharmacological Cu in phases 2 and 3 had numerically greater ADG compared with those fed other feeding strategies. In grow-finish diets (Exp. 2), these data suggest there were no differences in pig performance due to Cu source when fed at 150 mg/kg. However, feeding pharmacological levels of Cu regardless of source improved growth performance, with the greatest response observed in the early growing period.

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Table 2.1. Composition of experimental diets, Exp. 1 (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3
Ingredient, %			
Corn	40.57	57.63	57.42
Soybean meal, 47.7% CP	17.76	29.92	28.53
Whey powder	25.00	---	---
Dried distillers grains with solubles	5.00	7.50	10.00
Microbial enhanced soybean meal[2]	5.00	---	---
Corn oil	2.50	---	---
Calcium carbonate	0.45	0.75	0.85
Monocalcium phosphate, 21% P	1.10	1.10	0.70
Sodium chloride	0.15	0.45	0.29
Liquid lysine, 55%[3]	---	0.75	0.70
L-Lys-HCl	0.45	---	---
DL-Met	0.26	0.23	0.19
L-Thr	0.18	---	---
Thr[4]	---	0.28	0.24
L-Trp	0.03	0.02	---
L-Val	0.11	0.11	0.08
Vitamin premix[5]	0.25	0.25	0.25
Trace mineral premix[6]	0.15	0.15	0.15
Choline chloride, 60%	0.04	---	---
Phytase[7]	0.06	0.06	0.06
Zinc oxide	0.40	0.26	---
Mycotoxin binder[8]	0.55	0.55	0.55
TBCC, 58%[9]	+/-	+/-	+/-
Total	100	100	100
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.35	1.35	1.30
Ile:Lys	59	56	58
Leu:Lys	120	122	129
Met:Lys	39	39	38
Met and Cys:Lys	60	60	60
Thr:Lys	65	65	65
Trp:Lys	20.3	20.3	20.6
Val:Lys	70	70	70
His:Lys	34	37	39
Net energy, kcal/kg	2,578	2,376	2,394
Crude protein, %	20.7	22.2	22.2
Ca, %	0.72	0.71	0.68
STTD P, %[10]	0.62	0.51	0.44
Ca:P	1.04	1.10	1.20
Zn, mg/kg	3,000	2,000	110

[1] Phase 1 and 2 diets were provided using a feed budget of 1.8 and 5.4 kg/pig, phase 3 was fed for the remainder of the trial.

[2] ME-PRO, Prairie Aquatech, Brookings, SD.

[3] L-Lysine 55% liquid CJ America Bio, Downers Grove, IL.

[4] ThrPro CJ America Bio, Downers Grove, IL.

[5] Provided per kg of feed: 4,134 IU of vitamin A as retinyl acetate; 1,653 IU of vitamin D3 as cholecalciferol; 44 IU vitamin E as dl-α-tocopherol acetate; 3 mg of vitamin K as menadione; 0.03 mg of vitamin B12 as cyanocobalamin; 50 mg of niacin; 26 mg of pantothenic acid as d-calcium pantothenate; 8 mg of riboflavin.

[6] Provided per kg of feed: 17 mg of Cu as copper sulfate; 0.3 mg I as ethylenediamine dihydriodide; 110 mg Fe as ferrous sulfate; 33 mg Mn as manganese oxide; 0.3 mg Se as sodium selenite; 110 mg of Zn as zinc sulfate.

[7] HiPhorius 2,400 (DSM-Firmenich, Maastricht, Netherlands) was added at 1,500 FYT/kg and provided an estimated release of 0.12% STTD P.

[8] Feed Aid NutriQuest, Mason City, IA.

[9] Tribasic copper chloride, (NexTrace TBCC, 58%, SAM Nutrition Bloomington, MN) added at 0.03% of the diet was included at the expense of corn to provide 150 mg/kg of Cu.

[10] Standardized total tract digestible phosphorus.

Table 2.2. Analyzed Cu concentration of experimental diets, Exp. 1 (as-fed basis)[1]

	Cu, mg/kg[2]
Phase 1	
Base level Cu	29
Pharmacological Cu	172
Phase 2	
Base level Cu	27
Pharmacological Cu	171
Phase 3	
Base level Cu	28
Pharmacological Cu	195

[1] Values represent the mean of two samples analyzed in duplicate (Cumberland Valley Analytical Services, Inc., Waynesboro, PA).
[2] Tribasic copper chloride (NexTrace TBCC, 58%, SAM Nutrition, Bloomington, MN) provided an added 150 mg/kg of Cu in treatments labeled as pharmacological Cu.

Table 2.3. Composition of experimental diets, Exp. 2 (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3	Phase 4
Ingredient, %				
Corn	57.53	62.81	67.87	85.56
Soybean meal, 47.7% CP	19.48	14.36	9.59	12.23
Dried distiller's grains with solubles	20.00	20.00	20.00	---
Calcium carbonate	1.38	1.35	1.28	0.93
Salt	0.35	0.35	0.35	0.35
Monocalcium phosphate, 21% P	0.23	0.20	0.05	0.23
Liquid Lys, 55%[2]	0.66	0.62	0.59	0.44
DL-Met	0.05	0.01	---	0.02
Thr[3]	0.14	0.12	0.10	0.11
L-Trp	0.04	0.04	0.04	0.03
Vitamin/trace mineral premix[4]	0.11	0.11	0.10	0.08
Phytase[5]	0.05	0.05	0.05	0.05
Cu Source[6]	+/-	+/-	+/-	+/-
Total	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lys	1.10	0.95	0.82	0.72
Ile:Lys	60	60	60	60
Leu:Lys	148	159	171	153
Met:Lys	31	30	31	30
Met and Cys:Lys	57	57	60	58
Thr:Lys	64	64	65	65
Trp:Lys	19.1	19.1	19.0	19.3
Val:Lys	69	72	74	70
His:Lys	42	43	45	43
Net energy, kcal/kg	2,394	2,424	2,460	2,552
Crude protein, %	20.2	18.1	16.2	13.2
Ca, %	0.62	0.59	0.52	0.43
STTD P, %[7]	0.39	0.37	0.33	0.27
Ca:P	1.20	1.21	1.20	1.20

[1] Phase one was fed from approximately 23 to 50 kg , phase 2 from 50 to 75 kg, phase 3 from 75 to 100 kg, and phase 4 from 100 kg to marketing at approximately 127 kg.

[2] L-Lys 55% liquid CJ America Bio, Downers Grove, IL.

[3] ThrPro CJ America Bio, Downers Grove, IL.

[4] Provided per kg of premix: 2,645,520 IU of vitamin A as retinyl acetate; 1,047,185 IU of vitamin D3 as cholecalciferol; 27,778 IU vitamin E as dl-α-tocopherol acetate; 2160 mg of vitamin K as menadione; 22 mg of vitamin B12 as cyanocobalamin; 35,494 mg of niacin; 17,637 mg of pantothenic acid as d-calcium pantothenate; 5070 mg of riboflavin; 14,545 mg of Cu as copper sulfate; 318.18 mg I as ethylenediamine dihydriodide; 88,182 mg Fe as ferrous sulfate; 20,909 mg Mn as manganese oxide; 236.36 mg Se as sodium selenite; 88,182 mg of Zn as zinc sulfate.

[5] Quantum Blue 2G (AB Vista, Marlborough, England) was added at 1,016 FTU/kg and provided an estimated release of 0.12% STTD P.

[6] Cu sources were included at 0.026%, 0.028% and 0.06% of the diet for NexTrace TBCC, 58% (SAM Nutrition, Bloomington, MN), Intellibond C, 54% (Selko, Tilburg, Netherlands) and $CuSO_4$ (25.2%, Phibro Animal Health Corp., Teaneck, NJ) at the expense of corn to provide 150 mg/kg of Cu.

[7] Standardized total tract digestible phosphorus.

Table 2.4. Analyzed Cu concentration of experimental diets, Exp. 2 (as-fed basis)[1]

	Phase 1				Phase 2				Phase 3				Phase 4			
	Base level Cu	TBCC Source 1	TBCC Source 2	$CuSO_4$	Base level Cu	TBCC Source 1	TBCC Source 2	$CuSO_4$	Base level Cu	TBCC Source 1	TBCC Source 2	$CuSO_4$	Base level Cu	TBCC Source 1	TBCC Source 2	$CuSO_4$
Cu, mg/kg	62	188	183	159	27	196	172	190	47	196	180	185	46	145	198	184

[1] Values represent the mean of two samples analyzed in duplicate (Cumberland Valley Analytical Services, Inc., Waynesboro, PA). All treatments contained a base level of $CuSO_4$ provided by the trace mineral premix. Inclusions were 16 mg/kg Cu in phases 1 and 2, 13 mg/kg Cu in phase 3, and 11 mg/kg Cu in phase 4 based on a tapered inclusion of the vitamin/trace mineral premix throughout the experiment. In addition to the base level of Cu in all diets, treatments consisted of adding 150 mg/kg of the respective Cu source, TBCC source 1 (NexTrace TBCC, 58%, SAM Nutrition, Bloomington MN), TBCC source 2 (Intellibond C, 54%, Selko, Tilburg, Netherlands), or $CuSO_4$ (25.2%, Phibro Animal Health Corp., Teaneck, NJ) on top of the base Cu level. Treatment diets were formulated in four phases: phase one from approximately 23 to 50 kg , phase 2 from 50 to 75 kg, phase 3 from 75 to 100 kg, and phase 4 from 100 kg to marketing at approximately 127 kg.

Table 2.5. Effects of copper feeding strategy on growth performance of nursery pigs, Exp. 1[1]

	Cu, mg/kg						
Phase 1:	17	17	17	167		*P* =	
Phase 2:	17	17	167	167			Base vs
Phase 3:	17	167	167	167	SEM	Treatment	Pharmacological Cu[2]
BW, kg							
d 0	5.3	5.3	5.3	5.3	0.09	0.998	---
d 10	6.6	6.4	6.4	6.4	0.10	0.034	0.250
d 24	11.8	11.6	11.7	11.9	0.19	0.503	0.327
d 38	18.3	18.2	18.5	18.5	0.22	0.341	0.636
Phase 1							
ADG, g	103	95	94	89	5.1	0.206	0.129
ADFI, g	171	165	167	164	4.4	0.193	0.162
G:F, g/kg	602	568	564	547	26.3	0.501	0.302
Phase 2							
ADG, g	358[b]	364[ab]	370[ab]	376[a]	7.8	0.047	0.012
ADFI, g	476	478	488	495	8.7	0.108	0.021
G:F, g/kg	753	761	759	760	8.4	0.857	0.748
Phase 3							
ADG, g	453	451	465	454	13.8	0.515	0.644
ADFI, g	735	719	741	745	17.6	0.336	0.976
G:F, g/kg	614	627	628	609	9.7	0.141	0.360
Overall							
ADG, g	314	310	317	314	5.8	0.613	---
ADFI, g	474	464	475	478	7.8	0.273	---
G:F, g/kg	662	668	667	657	5.1	0.237	---
Removals, %	20.8	21.0	22.3	21.5	2.29	0.936	---
Mortality in test pen, %	1.6	2.2	2.2	3.1	0.74	0.450	---

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

[1] A total of 2,204 (PIC 1050 × 337; initially 5.3 ± 0.09 kg) were used in a 38-d trial with 29 pigs per pen and 19 replications per treatment. All treatments contained 17 mg/kg of Cu from the trace mineral premix. Treatments consisted of added 150 mg/kg on top of the base Cu level for differing feeding strategies by phase. Treatment diets were fed based on a feed budget of 1.8 kg/pig for phase 1

(approximately d 0 to 10), 5.4 kg/pig for phase 2 (approximately d 10 to 24), and phase 3 (approximately d 24 to 38) was fed for the remainder of the trial.

[2] Compares the means of treatments with no added Cu (17 mg/kg) to treatments containing pharmacological Cu (167 mg/kg) within phase.

Table 2.6. Effects of copper source on finishing pig growth performance and carcass characteristics, Exp. 2[1]

		Pharmacological Cu				*P* =	
	Base level Cu	TBCC Source 1	TBCC Source 2	$CuSO_4$	SEM	Treatment	Base level vs Pharmacological Cu[2]
BW, kg							
d 0	24.1	24.1	24.1	24.1	0.60	0.998	---
d 56	76.7	77.9	78.2	78.2	1.31	0.001	< 0.001
Final	125.8	127.9	128.0	127.6	1.36	0.063	0.008
Grower[3]							
ADG, g	933	953	960	956	14.8	0.004	< 0.001
ADFI, g	2,105	2,121	2,150	2,143	48.6	0.131	0.059
G:F, g/kg	445	451	448	448	4.0	0.186	0.057
Finisher[4]							
ADG, g	949	957	961	949	27.1	0.686	0.462
ADFI, g	2,960	2,981	3,010	2,987	53.7	0.468	0.203
G:F, g/kg	319	320	319	317	4.1	0.671	0.683
Overall[5]							
ADG, g	938	953	959	951	10.4	0.087	0.018
ADFI, g	2,507	2,526	2,554	2,539	26.3	0.185	0.072
G:F, g/kg	374	378	376	375	3.0	0.410	0.338
Carcass characteristics							
HCW, kg[6]	93.8	95.0	95.2	94.8	0.98	0.171	0.031
Yield, %	74.1	73.8	74.0	74.0	0.22	0.671	0.363
Backfat, mm	16.72	16.56	16.77	16.47	0.320	0.590	0.543
Loin depth, mm	68.15	67.87	67.80	67.90	0.620	0.888	0.447
Lean, %	56.86	56.94	56.78	56.99	0.224	0.622	0.733
Removals and mortality, %							
Removals	4.26	3.33	3.52	4.44	0.887	0.733	0.586
Mortality	0.56	1.30	1.30	1.85	0.580	0.330	0.113

Removals and mortality	4.81	4.63	4.81	6.30	1.045	0.574	0.725

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

[1] A total of 2,160 pigs (PIC 1050 × 337; initially 24.1 ± 0.60 kg) were used in a 110-d trial with 27 pigs per pen and 20 replications per treatment using two research barns. All treatments contained a base level of $CuSO_4$ provided by the trace mineral premix. Inclusions were 16 mg/kg Cu in phases 1 and 2, 13 mg/kg Cu in phase 3, and 11 mg/kg Cu in phase 4 based on a tapered inclusion of the vitamin/trace mineral premix throughout the experiment. In addition to the base level of Cu in all diets, treatments consisted of adding 150 mg/kg of the respective Cu source, TBCC source 1 (NexTrace TBCC, 58%, SAM Nutrition, Bloomington MN), TBCC source 2 (Intellibond C 54%, Selko, Tilburg, Netherlands), or $CuSO_4$ (25.2%, Phibro Animal Health Corp., Teaneck, NJ) on top of the base Cu level. Treatment diets were formulated in four phases: phase one from approximately 23 to 50 kg , phase 2 from 50 to 75 kg, phase 3 from 75 to 100 kg, and phase 4 from 100 kg to marketing at approximately 127 kg.

[2] Compares the control treatment containing a base level of Cu with the mean of treatments fed pharmacological levels of Cu within the period. Contrasts comparing Cu sources were not significant ($P > 0.10$) throughout the entire trial for any response criteria.

[3] Grower period represents d 0 to 56.

[4] Finisher period represents d 56 to 110.

[5] Overall period represents d 0 to 110.

[6] Hot carcass weight (HCW) served as a covariate for carcass data.

Chapter 3 - Effects of a formaldehyde-based product or a blend of phytochemicals and carboxylic acids on growth performance, serum haptoglobin, serum cytokine levels, and intestinal morphology in enterotoxigenic *Escherichia coli* challenged nursery pigs

Abstract

A total of 84 pigs (DNA 241 × 600; initially 6.2 kg) used in a 21-d study to evaluate the effects of a formaldehyde-based product (FORM; Termin-8, Anitox Corp., Lawrenceville, GA) and a combination of phytochemicals and carboxylic acids (PCA; Finio, Anitox Corp., Lawrenceville, GA) on growth performance, serum haptoglobin, serum cytokines and intestinal morphology in enterotoxigenic *Escherichia coli* (ETEC) F18 challenged nursery pigs. Pigs were allotted to one of six treatments at weaning, with 3 or 4 pigs per pen. Diets were fed in two phases (d -7 to 0 and d 0 to 14), consisting of a standard nursery diet for the challenged and non-challenged control treatments, and the control diet with two sources of feed additive at two levels, FORM (0.2% and 0.4%) and PCA (0.1% and 0.2%). On d 0 (7-d post-weaning), 70 pigs were challenged with 6 mL of ETEC (10^9 cfu/mL) via orogastric gavage. Pig weights and feed disappearance were measured on d -7, 0, 7, and 14 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (G:F). From d 0 to 7, ADG was greater ($P < 0.05$) for both control groups compared to pigs fed 0.4% FORM and 0.1% PCA. Non-challenged pigs had greater ADFI ($P < 0.05$) compared to pigs fed 0.1% PCA and 0.2% PCA, and greater G:F compared with pigs fed 0.4% FORM and 0.1% PCA. From d 0 to 14, both control groups had a greater ADG than 0.1% PCA ($P < 0.05$) with no differences observed in

ADFI or G:F. Fecal samples were taken on d 0, 3, 7, 10, and 14 for determination of dry matter (DM). There was a treatment × day interaction ($P < 0.05$), where on d 3, fecal DM was lower ($P < 0.05$) for pigs fed 0.1% PCA compared to the non-challenge control and 0.2% FORM, while there were no differences among treatments on the other sampling days. Serum was collected on d 0, 7, and 14 for serum haptoglobin and cytokine analysis. There were no differences observed for serum haptoglobin analysis. On d 7, IFN-γ was greater in pigs fed 0.2% PCA than in the non-challenge control pigs and pigs fed 0.2% FORM ($P < 0.05$). On d 14, pigs fed 0.1% PCA and 0.4% FORM had lower ($P < 0.05$) serum TNF-α than pigs fed 0.2% FORM. There were no differences observed for intestinal morphology measurements between treatments. In summary, the FORM and PCA feed additives did not improve growth performance, fecal DM, intestinal morphology, or serum haptoglobin, with only marginal changes in serum cytokine concentrations.

Introduction

Weaned pigs experience both physiological and immunological changes, leading to reduced feed intake and intestinal inflammation that contribute to an imbalance in the gut microbiota (Pluske, 2016). Disruption of the gut barrier allows bacteria and toxins to cross the epithelium, leading to inflammatory responses and post-weaning diarrhea (PWD). One of the causes of PWD is enterotoxigenic *Escherichia coli* (ETEC), where infections are characterized by reduced growth performance, impaired gut health, decreased intestinal function, diarrhea, and increased mortality (Duan et al., 2012; Pluske, 2016; Pan et al., 2017). One factor that illustrates the impact an ETEC challenge can have on gut health and function is modulation of intestinal morphology, such as a decrease in villus height and an increase in crypt depth (McLamb et al., 2013; Duarte and Kim, 2022; Duarte et al., 2023). Due to the significant economic impact caused

by ETEC challenges, there has been a variety of research into management and nutritional strategies to combat PWD caused by ETEC. These strategies include delayed weaning, reduced crude protein, dietary acidifiers, fiber fractions, and others, although the results of these practices are quite variable. Furthermore, a variety of feed additives are commercially available that are suggested to provide beneficial effects on animal health.

Previous research has found that chemical feed mitigants such as formaldehyde-based products have efficacy against *Salmonella* species when added in feed (Wales et al., 2010). Previous research has also shown formaldehyde-based products and combinations of phytochemicals and carboxylic acids can reduce the viral concentration in feed (Harrison et al., 2024). However, to our knowledge, there is little published data on the effects of these additives on nursery pigs within an ETEC challenge model. Therefore, the objective of this experiment was to evaluate the effect of a formaldehyde-based product and a blend of phytochemicals and carboxylic acids on the growth performance, serum haptoglobin, serum cytokine concentration, and intestinal morphology in ETEC F18 challenged nursery pigs.

Materials And Methods

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC #5015) and Institutional Biosafety Committee (IBC-1768). The experiment was conducted under biosafety level 2 (BSL-2) operating conditions at the K-State Swine Enteric Health Research Center facility in Manhattan, KS. Each pen had metal tri-bar flooring and a stainless-steel, 3-hole, dry, self-feeder and nipple waterer to provide *ad libitum* access to feed and water. Pens were solid-sided to prevent cross contamination between adjacent pens during the challenge period.

Animal Selection Criteria

Selected piglets came from sows not previously vaccinated for *E. coli* F18. Piglets were confirmed for genetic susceptibility to the F18 strain of ETEC by sequencing the α(1,2) fucosyltransferase-1 (FUT1) gene via polymerase chain reaction (PCR) at the Iowa State University Veterinary Diagnostic Laboratory (VDL). To identify susceptible piglets, an initial group of 88 piglets with similar body weights and an equal distribution of litters were selected. A tissue sample (tail) was collected at litter processing at approximately d 2 from the selected piglets. Samples were frozen and shipped on dry ice to the Iowa State University VDL for analysis. After identifying fully susceptible piglets (genotype GG) from the initial group, 42 pigs with similar body weights, evenly distributed across litters, were randomly chosen. This procedure was repeated for the second group to total 84 pigs used in this experiment.

Animals and Diets

The selected 84 weanling pigs (DNA 241 × 600; initially 6.2 kg) from two groups (42 pigs/group) were used in a 21-d study. Pigs were weaned and randomly allotted to one of six treatments in a generalized randomized block design and grouped with 4 pigs per pen on d -7, prior to ETEC inoculation on d 0. Diets consisted of a standard nursery diet for the challenged and non-challenged control treatments, and a control diet with two sources of feed additives added at two levels, a formaldehyde-based feed additive (FORM; Termin-8, Anitox Corp., Lawrenceville, GA) included at 0.2% and 0.4% of the diet and a blend of phytochemicals and carboxylic acids (PCA; Finio, Anitox Corp., Lawrenceville, GA) included at 0.1% and 0.2% of the diet (Table 1). Diets were fed in 2 phases, phase 1 from d -7 to d 0, and phase 2 from d 0 to d 14. A single batch of phase 1 and phase 2 diets were manufactured for each group of pigs. Using a common batch of feed, experimental diets were then mixed utilizing a pilot-scale mixer with

the respective additives applied. All feed being fed was within the 21-d label claim for the FORM. Following the first group of pigs, the same procedures were used to manufacture the phase 1 and phase 2 diets used for the second group of pigs. Treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS and fed in meal form. On the day of inoculation (considered d 0 of the study), pigs were moved to individual pens in a BSL-2 facility (KSU Swine Enteric Health Research Center). Pig weights and feed disappearance were measured on d -7 (weaning), 0, 7, and 14 to determine ADG, ADFI, and G:F.

Bacterial Strains and Culture Preparations

Propagation of inoculum occurred at the Kansas State University College of Veterinary Medicine (Manhattan, KS) and targeted an inoculum titer of 10^9 CFU/mL. The isolate used was an F18 fimbrial type, with genes for heat-labile enterotoxin (LT), heat-stable toxin A (Sta), heat-stable toxin B (STb), and shiga toxin type 2e (Stx2e) as determined via PCR and was derived from a clinical case submission to the Iowa State University VDL.

Inoculation Methods

On d 0, 70 pigs over both groups were inoculated with 6 mL of solution containing 10^9 CFU/mL of ETEC F18. Inoculum was administered via orogastric gavage using a 10 Fr feeding tube and each pig received 6 mL of the respective inoculum concentration followed by 6 mL of sterile phosphate buffered saline. The 14 non-challenged pigs received 6 mL of sterile phosphate buffered saline utilizing the same method to simulate inoculation.

Haptoglobin and Immune Parameters

A blood sample was collected via jugular venipuncture into a 10 mL vacutainer tube on d 0 (before challenge), 7, and 14. Samples were placed on ice until transported to the laboratory,

where they were centrifuged (2,000 × *g* for 10 min) and serum was recovered. Serum samples (d 0 before challenge, 7, and 14) were submitted to the Kansas State University VDL (Manhattan, KS) to measure haptoglobin concentration. The method used was adapted from the assay of Makimura and Suzuki (1982). Haptoglobin concentration was calculated with a standard curve prepared by incubating known amounts of hemoglobin with a serum sample containing a haptoglobin concentration greater than 150 mg/dL. Serum samples were also sent to Eve Technologies (Calgary, AB, Canada) where cytokines were analyzed using a multiplex immunoassay that quantifies the following 13 cytokines, granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-1α, IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and TNF-α, from which data was reported using fluorescence intensity.

Fecal Consistency

Fecal samples were collected on d 0, 3, 7, 10, and 14, for the measurement of fecal dry matter. Samples were collected into plastic bags using a sterile, cotton-tipped applicator to stimulate defecation and frozen at -20°C until matter analysis. Fecal samples were dried at 55°C in a forced air oven for 48 h, and the ratio of dried-to-wet fecal weight determined the percentage fecal DM.

Animal Harvesting and Tissue Collection

On d 7, pigs were euthanized for sampling via captive bolt, such that approximately half of the pigs remained for d 7 to 14 (n = 45). Pigs were then opened viscerally for necropsy. During necropsy, the duodenum, jejunum, and ileum were identified, removed from the mesentery, and three 2-cm-long segments from each section were cut and fixed in 10% formalin, one additional 4-cm long segment was cut from the jejunum approximately 30 cm distally from the first cut. Samples were sent to the Kansas State University VDL for histology analysis, where

tissue samples were stained with hematoxylin and eosin and evaluated for villus height, crypt depth, and the villus height to crypt depth ratio using a microscope at 10× magnification. On d 14, the remaining pigs were euthanized and necropsied following the same procedure for d 7. Evaluation of intestinal morphology measurements was performed by a veterinary anatomic pathologist blinded to treatment.

Data Analysis

Experimental data were analyzed using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute Inc., Cary, NC) with pig serving as the experimental unit, dietary treatment as a fixed effect, and weight block and group as random intercepts. Individual data points falling outside of 4 studentized residuals were removed from analysis. Cytokine panel was reported using fluorescence intensity and were analyzed using a $\log_{10}$ transformation. When treatment was a significant source of variation, differences were determined by pairwise comparison using the Tukey-Kramer multiplicity adjustment to control for Type I Error. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results

Growth Performance

On the day of inoculation (d 0), there was no evidence of a difference in body weight between dietary treatments (Table 2). On d 7, pigs fed 0.1% PCA were lighter ($P < 0.05$) compared to all pigs fed the control and FORM diets, with 0.2% PCA being intermediate. From d 0 to 7, average daily gain for pigs fed the control diet, both challenged and non-challenged pigs, was greater ($P < 0.05$) than pigs fed 0.4% FORM and 0.1% PCA, with those treatments losing weight during the peak infection time period. Non-challenged pigs had greater ADFI ($P <$

0.05) when compared to pigs fed 0.1% PCA and 0.2% PCA, with all other treatments being intermediate. Non-challenge control pigs also had greater G:F ($P < 0.05$) than pigs fed 0.4% FORM and 0.1% PCA, with other treatments being intermediate. From d 7 to 14, there were no significant differences observed in ADG, ADFI, or G:F. From d 0 to 14, both the challenged and non-challenged pigs fed the control diet had greater ($P < 0.05$) ADG than pigs fed 0.1% PCA, with other treatments being intermediate. No differences were observed for ADFI or G:F.

Fecal Dry Matter

There was a treatment × day ($P = 0.011$) interaction for fecal DM, where on d 3, fecal DM was lower ($P < 0.05$) for pigs fed 0.1% PCA compared to the non-challenge control and 0.2% FORM. There was no evidence of a difference between treatments for other sampling days. Fecal DM was lower ($P < 0.05$) on d 3 compared to all other days (d 0, 3, 7, 10 and 14 being 23.1, 12.8, 19.2, 22.3, and 20.6% DM, respectively).

Serum Haptoglobin and Serum Cytokine Panel

There was no evidence of a treatment × day interaction for serum haptoglobin concentrations. A significant day effect was observed ($P < 0.001$; Table 3) for serum haptoglobin. Serum haptoglobin was greater ($P < 0.05$) on d 7 compared to d 0 (181.7 vs. 65.2 mg/mL, respectively), and d 14 serum haptoglobin was lower ($P < 0.05$) than d 7 (110.9 vs. 181.7 mg/mL, respectively).

A treatment × day interaction was observed for IL-1α ($P = 0.024$), but no treatment effect was detected at any sampling day. There were tendencies for treatment × day interactions for IL-2, IL-6, IL-10, and TNF-α ($P = 0.054$, 0.069, 0.052, and 0.081, respectively). No treatment × day interactions were observed for GM-CSF, IFN-γ, IL-1β, IL-1RA, IL-4, IL-8, IL-12, or IL-18.

A treatment effect was observed for IL-1RA ($P = 0.045$), with non-challenged control pigs having lower serum IL-1RA than pigs fed 0.1% PCA and other treatments intermediate. A treatment effect was also observed for TNF-α ($P = 0.009$), where pigs fed 0.2% PCA had a lower ($P < 0.05$) serum TNF-α than pigs fed 0.1% PCA, with all other treatments being intermediate. On d 7, IFN-γ was greater in pigs fed 0.2% PCA than in the non-challenge control pigs and pigs fed 0.2 FORM ($P < 0.05$). On d 0 and d 7, pigs fed 0.1% PCA had lower ($P < 0.05$) serum TNF-α than pigs fed 0.2% PCA, with all other treatments being intermediate. However, on d 14, pigs fed 0.1% PCA and 0.4% FORM had a lower ($P < 0.05$) serum TNF-α than pigs fed 0.2% FORM, with all other treatments being intermediate. A slight tendency for a treatment effect was observed for GM-CSF but the means did not separate ($P = 0.094$). No treatment effects were observed for IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, or IL-18 ($P \geq 0.119$).

Day effects were observed for GM-CSF, IL-1RA, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, and TNF-α ($P \leq 0.021$). For GM-CSF, serum concentrations decreased from d 0 to d 7 ($P < 0.05$) and from d 7 to d 14 ($P < 0.05$; 3.07, 2.80, and 2.59 $\log_{10}$ fluorescence intensity, respectively). For IFN-γ, concentrations were greater on d 7 than d 14, with d 0 intermediate (4.60, 4.66, and 4.37 $\log_{10}$ fluorescence intensity, d 0, 7, 14, respectively; $P = 0.023$). For IL-1β, concentrations were greater on d 0 than d 7 or d 14 (4.47, 4.26, and 4.01 $\log_{10}$ fluorescence intensity; $P = 0.003$). For IL-1RA, concentrations were lower on d 14 than d 0 or d 7 (5.60, 5.72, and 4.90 $\log_{10}$ fluorescence intensity, d 0, 7, 14, respectively; $P < 0.001$). For IL-2, concentrations were lower on d 14 than d 0, with d 7 being intermediate (3.85, 3.67, and 3.4237 $\log_{10}$ fluorescence intensity, d 0, 7, 14, respectively; $P = 0.007$). For IL-4, concentrations were lower on d 14 compared to d 0, with d 7 intermediate (4.05, 3.90, and 3.70 $\log_{10}$ fluorescence intensity, d 0, 7, 14, respectively; $P = 0.021$). For IL-6, concentrations were lower on d 14 than d

0 or d 7 (4.22, 3.97, and 3.63 $\log_{10}$ fluorescence intensity, d 0, 7, 14, respectively; $P < 0.001$). For IL-10, IL-12, IL-18 concentrations were lower on d 7 and d 14 than d 0 ($\log_{10}$ fluorescence intensity on d 0, 7, 14, for IL-10: 4.09, 3.81, and 3.62; $P < 0.001$, IL-12: 6.95, 6.47, and 6.42; $P < 0.001$, IL-18: 4.60, 4.21, and 4.21; $P < 0.001$). For TNF-α, concentrations were lower on d 14 than d 0 or d 7 (4.46, 4.40, and 4.12 $\log_{10}$ fluorescence intensity, d 0, 7, 14; $P = 0.009$). No day effect was observed for IL-8.

Intestinal Morphology

Villus height, crypt depth, and the villus height to crypt depth did not significantly differ between treatments (Table 4). Although a tendency ($P = 0.057$) was observed for jejunum crypt depth as well as a slight tendency ($P = 0.095$) was observed for duodenum villi height, with the non-challenge control having the numerically greatest villi height.

Survival Probability

The survival probability curve (Figure 1) indicates that all treatments have a similar probability of survival ($P = 0.318$). However, pigs not inoculated with ETEC F18 fed the same diet as the control pigs had the best survival probability.

Discussion

Among ETEC infections, the primary adhesive fimbriae types associated with PWD are F4 and F18 (Fairbrother et al., 2005; Luise et al., 2019). The age-dependent expression of these receptors explains why F4 ETEC infections are most prevalent during the neonatal and immediate post-weaning periods, whereas ETEC F18 infections typically occur later in the post-weaning period (Luise et al., 2019). The primary enteroxins of ETEC F18 include heat-labile toxin (LTI and LTII), heat-stable toxins (Sta and STb), enteroaggregative heat-stable toxin 1 (EAST1), and Shiga toxins (Stx1, Stx2 and subtypes; Francis et al., 2002; Yang et al., 2021).

Infections can be accompanied by reduced growth performance, impaired gut health and intestinal function, diarrhea, and increased mortality (Duan et al., 2012; Pluske, 2016; Pan et al., 2017). A high variation in diarrhea severity and incidence has been observed in some research (Luise et al. 2019). This variation in pig response to ETEC challenge models may be due to differences in age, immune competence, natural exposure, and alpha(1,2)-fructosyltransferase gene (FUT1) genetic susceptibility (Jensen et al., 2012; McLamb et al., 2013). Therefore, within this study, variation within these factors was minimized as much as possible by selecting pigs of the same age and genetic susceptibility across equal sow parties within the Kansas State University Swine Unit sow herd.

Although not statistically significant, ETEC F18 numerically reduced growth performance, similar to previous research (Kim et al., 2019; Becker et al., 2020). In pigs challenged with a similar dose of 10^9 CFU, Li et al. (2019) observed a negative impact on ADG, ADFI, and body weight at 7 days post inoculation. Previous research in ETEC challenged pigs by Duarte et al. (2020) observed negative effects later in a 20 d trial rather than observing negative effects early on post-challenge. The inverse was observed for the current trial, where in the second half of the challenge period, pigs challenged with ETEC F18 appeared to experience some compensatory gain, as all treatments except for pigs fed 0.1% PCA had a slightly greater ADG than the non-challenged control pigs.

Enterotoxigenic *Escherichia coli* infections can impose significant economic losses in swine production due to increased mortality and morbidity. Feed mitigants such as FORM and blends of PCA used in the present study can be used to reduce microbial contamination and viral load in feed (Harrison et al., 2024; Palowski et al., 2025). Previous research has shown formaldehyde can reduce bacterial load in complete feeds for poultry and swine (Carrique-Mas

et al., 2007; Sbardella et al., 2015; Williams et al., 2018). When added to feed, it is effective against *Salmonella* species and *Enterobacteriaceae* (Wales et al., 2010; Williams et al., 2018; Ricke et al., 2019).

The growth performance impact from the addition of formaldehyde-based feed additives has been researched more extensively in poultry than in swine. Some researchers have found that the inclusion of FORM in poultry diets did not show adverse growth effects (Ricke et al., 2019), whereas other research has noted a reduction in growth performance when added at 0.5% in the diet or greater (Babar et al., 2001; Khan et al., 2006). To our knowledge, there has not been published research evaluating the effects of FORM on ETEC F18 challenged nursery pigs. In previous research in non-challenged pigs, the addition of 0.3% FORM applied to complete feed in nursery diets tended to have lower feed intake than that of pigs fed a diet without the addition of FORM (DeRouchey et al., 2004), suggesting a reduction in palatability when FORM is included in the diet. In more recent research, a reduction in growth performance was observed by Williams et al. (2018) and Sica et al. (2016) in non-challenged nursery pigs when a formaldehyde-based product was included at 0.32% and 0.3% of the diet. The addition of FORM to nursery pig diets negatively impacted ADG, ADFI and G:F (Sica et al., 2016). This is similar to the reduction in growth performance observed in the current study in pigs fed either feed mitigant after ETEC inoculation.

Formaldehyde products are designed primarily to reduce microbial contamination in feed, the chemical interactions that reduce microbial load may also influence nutrient utilization and could partially explain the reduced growth performance observed early in the challenge period of this study. Formaldehyde treatment of feed ingredients has been shown to alter protein availability through the formation of cross-links between proteins and amino acids, which may

influence nutrient digestibility under certain conditions (Spears et al., 1980). The potential impact of formaldehyde on the gut microbiome may also contribute to the negative effect observed on growth (Jian et al., 2025). A reduction in desirable bacteria and an increase in possibly harmful bacteria have been demonstrated with the addition of a formaldehyde-based product (Williams et al., 2018). In addition, previous work in poultry has shown that ingestion of formaldehyde-containing compounds can alter physiological responses and hematological parameters and may influence intestinal microbial populations (Babar et al., 2001; Khan et al., 2006). Although these studies were conducted in poultry, they suggest that formaldehyde-based feed treatments may influence gut microbial ecology and host physiology, which could contribute to the reduction in growth performance observed early in the present study.

Gosling et al. (2021) found that PCA at 2.5% appeared to eliminate avian pathogenic *Escherichia coli* from poultry diets. Organic carboxylic acids and phytochemicals have also been explored as alternatives to antibiotic growth promoters in swine diets because of their ability to inhibit pathogenic bacteria, stabilize gut microflora, and promote intestinal health (Nhara et al., 2024). In contrast to formaldehyde-based products, phytochemical and carboxylic acid blends may influence gut microbial populations through different mechanisms. Organic carboxylic acids can reduce gastrointestinal pH and disrupt bacterial metabolism, while plant-derived phytochemicals may alter bacterial membrane integrity and inhibit pathogen colonization (Nhara et al., 2024). Together, these mechanisms may influence microbial populations within the gastrointestinal tract and subsequently impact gut health and nutrient utilization. These organic carboxylic acid compounds may also stimulate digestive enzyme secretion and improve nutrient digestibility, which has been associated with improved gut health and performance in some studies (Nhara et al., 2024). Ahmed et al. (2014) challenged pigs with 5 mL of culture fluid

containing 2.3 × 10^8 cfu/mL of *E. coli* KCTC 2571 and 5.9 × 10^8 cfu/mL of *S. typhimurium, then* fed pigs 0.5% citric acid or 0.4% of an acidifier blend of 17.2% formic acid, 4.1% propionic acid, 10.2% lactic acid, 9.5% phosphoric acid, 34.0% SiO_2. At the conclusion of the trial, ADFI was lower for pigs fed these feed additives than pigs fed a basal diet. Gain was greatest for pigs fed a basal diet and 0.5% citric acid compared to the 0.4% acidifier blend. These results are similar to the numerical reduction in ADFI and ADG for PCA when compared to the control pigs in the current study.

To our knowledge, the impact of FORM and PCA on fecal dry matter is uncharacterized in nursery pigs within current literature. However, with the exception of d 3, the addition of the feed additives did not impact fecal DM. Previous research suggests that blends of PCA may reduce diarrheal incidence by altering gut pH and suppressing pathogenic bacteria such as *E. coli*, while promoting beneficial microbial populations within the gastrointestinal tract of non-challenged pigs (Nhara et al., 2024). However, this was not observed within the current study, fecal DM was more consistent with previous research in ETEC challenge models, where pigs challenged with ETEC F18 had reduced fecal DM post-inoculation (Xu et al., 2022; Duarte et al., 2023; Jang et al., 2023). Coddens et al. (2017) observed fecal matter was loosest 4 days post inoculation, similar to that of the current trial, where pigs had the greatest occurrence of diarrhea on d 3. Feces became firmer for ETEC-challenged pigs after the first week post inoculation, similar to that observed by Kim et al. (2019).

There are measurements that can give an indicator of the intestinal inflammatory responses, including increased circulating white blood cells, elevated levels of pro-inflammatory cytokines and haptoglobin concentration. Haptoglobin is an acute-phase protein that increases during an inflammatory response (Quaye, 2008). Pro-inflammatory, IL-1α, IL-2, IL-6, IL-18,

and TNF-α, initiate immune responses contributing to immune cell activation and recruitment. Therefore, elevated levels may be an indication of infections, and decreased concentrations may suggest improved immune status. Anti-inflammatory cytokines, IL-4 and IL-10, help control immune responses by suppressing excessive inflammation and preventing tissue damage (Dinarello, 2000). Previous work by Liu et al. (2013) demonstrated that infection with ETEC F18 elicits both systemic and intestinal inflammatory responses, including increased circulating white blood cells, elevated serum TNF-α and haptoglobin concentrations, and greater ileal infiltration of macrophages and neutrophils. Similarly, haptoglobin was greatest on d 7 post-inoculation, in addition, IFN-γ was greatest on d 7, indicating inflammation from the ETEC challenge. Serum TNF-α and IFN-γ responses indicated lower inflammation in pigs fed the lower concentration of PCA. However, pigs on this treatment had the greatest reduction in growth performance. Boeckman et al. (2022) observed an increase in IL-6 and IL-8 in ETEC-challenged pigs. In the current study, IL-8 concentration was unaffected, whereas the IL-6 concentration decreased over time and was not significantly impacted by treatment. Along with IL-6, the GM-CSF, IL-1β, IL-1RA, IL-2, IL-4, IL-10, IL-12, IL-18, and TNF-α decreased over time**.** This pattern may reflect the majority of the inflammatory response was associated with weaning itself rather than solely the ETEC challenge. Weaning is associated with a prolonged and transient response in gene expression of pro- and anti-inflammatory cytokines and IgA-producing cells in the intestine (Lalles et al., 2004; Groot et al., 2021). Groot et al. (2021) indicated an intestinal inflammatory response for at least 15 d post weaning, with many cytokines upregulated at 30 d post weaning.

Infection from ETEC F18 also compromises the gut barrier function, integrity, and recovery, often leading to reduced villus height and increased crypt depth, to compensate for the

damage (Jensen et al., 1998; Duarte et al., 2020). Due et al. (2025) observed a significant response in intestinal morphology where non-challenged pigs had a greater villus height and villus height: crypt depth ratio in the jejunum than ETEC challenged pigs after d 5, while no significant differences were observed in the ileum, and no samples were collected from the duodenum for morphology measurements. In an experiment done by Gao et al. (2013), crypt depth was reduced in pigs challenged with *E. coli,* similar to the numerical reduction in crypt depth observed in the current study. The limited morphological response may be partially explained by the timing of sample collection, as many samples were obtained at 7 or 14 d post-inoculation, when intestinal recovery may have already begun.

In conclusion, although previous research has demonstrated antimicrobial properties of FORM and PCA in feed, their impact on the performance and health of ETEC-challenged nursery pigs has not been well characterized. In this study, the inclusion of these feed mitigants did not alleviate the physiological or performance responses associated with the ETEC challenge. Growth performance responses during the early post-inoculation period appeared consistent with previously reported impacts of ETEC infection and potential adverse effects of these feed mitigants on the gut microbial environment. Likewise, fecal DM and inflammatory indicators followed patterns consistent with ETEC-induced intestinal disturbance and the post-weaning inflammatory response. In summary, these results suggest that when dietary treatments were provided to pigs immediately post-weaning and challenged with ETEC F18 7 d later, FORM and PCA did not improve overall growth performance, fecal DM, or serum haptoglobin, with only marginal changes in serum cytokine concentrations.

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Table 3.1. Composition of experimental diets (as-fed basis)[1]

Ingredient, %	Phase 1	Phase 2
Corn	42.95	55.13
Soybean meal, 47.7% CP	21.53	24.07
Whey powder	15.00	12.50
Whey permeate, 80% lactose	9.00	---
Soy protein concentrate[2]	6.25	3.75
Soy oil	2.00	1.00
Calcium carbonate	0.40	0.60
Monocalcium phosphate, 21% P	1.05	0.90
Sodium chloride	0.47	0.56
L-Lys-HCl	0.36	0.44
DL-Met	0.22	0.22
L-Thr	0.19	0.21
L-Trp	0.03	0.05
L-Val	0.10	0.12
Vitamin premix[3]	0.25	0.25
Trace mineral premix[4]	0.15	0.15
Phytase[5]	0.06	0.06
FORM/PCA[6]	+/-	+/-
Total	100	100
Calculated analysis		
Standardized ileal digestible amino acids, %		
Lys	1.35	1.35
Ile:Lys	60	58
Leu:Lys	115	115
Met:Lys	37	37
Met and Cys:Lys	58	58
Thr:Lys	65	65
Trp:Lys	20.3	20.1
Val:Lys	70	70
His:Lys	35	35
NE, kcal/kg	2,593	2,293
CP, %	20.7	20.8
Ca, %	0.68	0.69
STTD P, %[7]	0.58	0.52
Ca:P	1.00	1.10

[1] Phase 1 diets were provided from d -7 to d 0, phase 2 diets were provided from d 0 to d 14.

[2] PurePro Soy, Bunge, Chesterfield, MO

[3] Provided per kg of feed: 4,134 IU of vitamin A as retinyl acetate; 1,653 IU of vitamin D_3 as cholecalciferol; 44 IU vitamin E as dl-α-tocopherol acetate; 3 mg of vitamin K as menadione; 0.03 mg of vitamin B_{12} as cyanocobalamin; 50 mg of niacin; 26 mg of pantothenic acid as d-calcium pantothenate; 8 mg of riboflavin.

[4] Provided per kg of feed: 17 mg of Cu as copper sulfate; 0.3 mg I as ethylenediamine dihydriodide; 110 mg Fe as ferrous sulfate; 33 mg Mn as manganese oxide; 0.3 mg Se as sodium selenite; 110 mg of Zn as zinc sulfate.

[5] HiPhorius 2,400 (DSM-Firmenich, Maastricht, Netherlands) was added at 1,500 FYT/kg and provided an estimated release of 0.12% STTD P.

[6] Additives were included at the expense of corn. Dry Termin-8 (FORM; Anitox Corp., Lawrenceville, GA) was added at 0.2% and 0.4% of the diet. Liquid Finio (PCA; Anitox Corp., Lawrenceville, GA) was added at 0.1% and 0.2% of the diet.

[7] Standardized total tract digestible phosphorus.

Table 3.2. Effects of formaldehyde-based product (FORM) or a blend of phytochemicals and carboxylic acids (PCA) on growth performance and fecal dry matter (DM) in Enterotoxigenic *Escherichia coli* F18 challenged nursery pigs[1]

			FORM[2]		PCA[3]			
Item	Non-challenge control	Control	0.2%	0.4%	0.1%	0.2%	SEM	*P* =
BW, kg								
d -7	6.23	6.23	6.21	6.26	6.23	6.23	---	---
d 0	6.98	6.78	6.41	6.58	6.64	6.72	1.048	0.390
d 7	9.42^{a}	9.25^{ab}	7.55^{ab}	7.24^{ab}	6.26^{c}	7.65^{abc}	1.064	< 0.001
d 14	13.14	13.25	11.26	11.13	9.93	11.91	1.665	0.074
d -7 to 0								
n =	14	14	14	14	14	14	---	---
ADG, g	107	79	29	46	60	70	---	---
ADFI, g	128	97	100	98	100	109	---	---
G:F, g/kg	834	806	287	609	586	641	---	---
d 0 to 7							---	---
n =	14	14	14	14	14	14	---	---
ADG, g	349^{a}	208^{ab}	58^{bc}	-57^{c}	-85^{c}	49^{bc}	96.3	< 0.001
ADFI, g	439^{a}	330^{ab}	278^{ab}	336^{ab}	237^{b}	257^{b}	77.8	0.019
G:F, g/kg	771^{a}	187^{ab}	98^{ab}	-455^{b}	-949^{b}	-225^{ab}	447.4	0.002
d 7 to 14								
n =	8	8	8	7	8	6	---	---
ADG, g	533	540	557	576	403	587	109.1	0.648
ADFI, g	623	665	545	596	388	610	101.7	0.143
G:F, g/kg	904	819	1070	995	1134	1023	94.0	0.139
d 0 to 14								
n =	8	8	8	7	8	6	---	---
ADG, g	442^{a}	445^{a}	341^{ab}	320^{ab}	197^{b}	368^{ab}	80.2	0.041

ADFI, g	521	512	438	481	333	452	88.0	0.264
G:F, g/kg	836	857	720	665	778	801	84.8	0.280
Fecal DM, %[4,5]								
d 0	22.4	22.0	25.2	24.2	23.3	21.6	2.02	0.779
d 3	22.4[a]	13.7[bc]	15.4[ab]	9.4[bc]	5.7[c]	10.1[bc]	2.24	< 0.001
d 7	16.6	18.8	21.9	19.7	21.6	16.6	3.33	0.639
d 10	19.5	19.9	24.8	22.1	26.1	21.1	1.95	0.091
d 14	18.8	20.1	22.7	18.5	21.5	22.0	2.21	0.560

[a,b,c] Means in the same row that do not have a common superscript differ ($P < 0.05$).

[1] A total of 84 pigs (DNA 600 × 241) were used in a 21-d nursery trial with four pigs per pen for phase 1, from d -7 to 0 and one pig per pen for phase 2, from d 0 to 14. A total of 70 pigs were inoculated with ETEC F18 on d 0 with 14 non-inoculated pigs serving as the negative control.

[2] Termin-8 (dry; Anitox Corp., Lawrenceville, GA) was added at 0.2% and 0.4% of the diet.

[3] Finio (liquid; Anitox Corp., Lawrenceville, GA) was added at 0.1% and 0.2% of the diet.

[4] Day, $P < 0.05$.

[5] Treatment × day, $P < 0.05$.

Table 3.3. Effects of formaldehyde-based product (FORM) or a blend of phytochemicals and carboxylic acids (PCA) on serum haptoglobin concentration and cytokine fluorescence intensity in Enterotoxigenic *Escherichia coli* F18 challenged nursery pigs[1]

			FORM[2]		PCA[3]			
Item	Non-challenge control	Control	0.2%	0.4%	0.1%	0.2%	SEM	*P* =
Serum haptoglobin[4], mg/mL								
d 0	59.5	71.6	53.3	72.9	80.9	52.8	12.61	0.517
d 7	146.0	113.1	192.0	210.1	249.0	180.1	41.08	0.159
d 14	108.0	103.9	135.2	115.7	111.3	91.7	29.21	0.919
Serum cytokines, fluorescence intensity, log 10								
GM-CSF[4,5]								
d 0	2.67	3.42	2.92	3.07	2.66	3.68	0.337	0.134
d 7	2.62	2.99	2.62	2.78	2.60	3.17	0.224	0.120
d 14	2.55	2.62	2.60	2.63	2.46	2.70	0.161	0.544
IFN-γ[4]								
d 0	4.29	4.89	4.40	4.63	4.23	5.14	0.313	0.150
d 7	4.30[b]	4.79[ab]	4.28[b]	4.79[ab]	4.37[ab]	5.44[a]	0.321	0.030
d 14	4.13	4.39	4.51	4.27	4.37	4.58	0.314	0.839
IL-1α[6,7]								
d 0	3.85	4.73	3.71	4.03	4.03	4.42	0.691	0.238
d 7	3.98	4.24	3.77	3.78	4.24	4.23	0.705	0.808
d 14	4.03	3.72	4.20	3.30	3.99	3.89	0.689	0.385
IL-1β[4]								
d 0	4.20	4.97	4.27	4.25	4.53	4.58	0.536	0.337
d 7	4.30	4.23	4.15	4.10	4.39	4.38	0.551	0.971
d 14	4.13	3.84	4.21	3.61	4.25	4.05	0.559	0.612

IL-1RA[5,7]								
d 0	5.18	6.02	5.59	5.51	5.77	5.51	0.352	0.056
d 7	5.50	4.96	5.62	5.48	6.76	6.00	0.572	0.123
d 14	4.58	4.59	5.40	4.66	5.37	4.79	0.441	0.138
IL-2[4,8]								
d 0	3.52	4.54	3.41	3.84	3.74	4.07	0.619	0.218
d 7	3.65	3.89	3.47	3.44	3.73	3.85	0.622	0.901
d 14	3.43	3.39	3.75	3.11	3.63	3.21	0.584	0.508
IL-4[4]								
d 0	3.80	4.55	3.69	4.01	3.97	4.25	0.562	0.408
d 7	3.74	4.08	3.81	3.68	4.01	4.07	0.563	0.885
d 14	3.59	3.63	4.06	3.26	3.81	3.82	0.545	0.367
IL-6[4,8]								
d 0	3.78	4.76	3.79	4.19	4.44	4.38	0.653	0.160
d 7	3.96	4.08	3.65	3.83	4.24	4.07	0.698	0.906
d 14	3.52	3.48	3.87	3.27	3.91	3.77	0.657	0.581
IL-8								
d 0	5.68	6.33	5.73	5.85	5.83	6.37	0.410	0.352
d 7	6.02	5.43	6.05	5.58	5.37	6.02	0.538	0.664
d 14	6.02	5.32	6.02	5.86	6.10	6.23	0.539	0.668
IL-10[4,8]								
d 0	3.83	4.70	3.69	4.00	4.08	4.23	0.633	0.271
d 7	3.77	4.06	3.59	3.77	3.82	3.88	0.649	0.950
d 14	3.57	3.48	3.93	3.35	3.84	3.54	0.612	0.571
IL-12[4]								
d 0	6.92	7.06	7.01	6.83	7.04	6.88	0.139	0.649
d 7	6.66	6.04	6.65	6.48	6.27	6.70	0.259	0.211
d 14	6.52	6.08	6.66	6.32	6.30	6.64	0.234	0.260
IL-18[4]								

d 0	4.33	5.07	4.40	4.55	4.62	4.64	0.597	0.366
d 7	4.34	4.31	4.10	4.06	4.18	4.28	0.612	0.964
d 14	4.14	4.23	4.40	3.89	4.48	4.12	0.604	0.602
TNF-α[4,5,8]								
d 0	3.98[ab]	4.98[ab]	4.48[ab]	4.24[ab]	3.92[b]	5.19[a]	0.302	0.015
d 7	4.14[ab]	4.79[ab]	4.44[ab]	4.16[ab]	3.77[b]	5.09[a]	0.302	0.029
d 14	3.93[ab]	4.22[ab]	4.69[a]	3.60[b]	3.82[b]	4.45[ab]	0.221	0.004

[a,b] Means in the same row that do not have a common superscript differ ($P < 0.05$).

[1] A total of 84 pigs (DNA 600 × 241) were used in a 21-d nursery trial with four pigs per pen for phase 1, from d -7 to 0 and one pig per pen for phase 2, from d 0 to 14. A total of 70 pigs were inoculated with ETEC F18 on d 0 with 14 non inoculated pigs serving as the negative control. On d 0, n = 84; d 7, n = 60; d 14, n = 44. All treatment × day, treatment, and day effects $P > 0.10$ unless otherwise indicated.

[2] Termin-8 (dry; Anitox Corp., Lawrenceville, GA) was added at 0.2% and 0.4% of the diet.

[3] Finio (liquid; Anitox Corp., Lawrenceville, GA) was added at 0.1% and 0.2% of the diet.

[4] Day, $P < 0.05$.

[5] Treatment, $P < 0.10$.

[6] Treatment × day, $P < 0.05$.

[7] Day, $P < 0.10$.

[8] Treatment × day, $P < 0.10$.

0

Table 3.4. Effect of formaldehyde-based product (FORM) or a blend of phytochemicals and carboxylic acids (PCA) on intestinal morphology in Enterotoxigenic *Escherichia coli* F18 challenged nursery pigs[1]

			FORM[2]		PCA[3]			
Item	Non-challenge control	Control	0.2%	0.4%	0.1%	0.2%	SEM	*P* =
Villi height, µm								
Duodenum	495	424	363	327	296	384	69.1	0.095
Jejunum	474	410	360	295	303	375	70.6	0.187
Ileum	347	311	279	225	190	277	50.2	0.140
Crypt depth, µm								
Duodenum	433	370	355	306	281	336	69.6	0.213
Jejunum	342	298	277	217	210	247	42.5	0.057
Ileum	227	194	199	166	147	185	35.4	0.498
Villi height:crypt depth								
Duodenum	1.16	1.00	0.90	0.76	0.76	1.03	0.165	0.167
Jejunum	1.41	1.18	1.12	0.97	1.02	1.30	0.241	0.383
Ileum	1.44	1.31	1.20	0.87	0.82	1.31	0.204	0.101

[1] A total of 84 pigs (DNA 600 × 241) were used in a 21-d nursery trial with four pigs per pen for phase 1, from d -7 to 0 and one pig per pen for phase 2, from d 0 to 14. A total of 70 pigs were inoculated with ETEC F18 on d 0 with 14 non-inoculated pigs serving as the negative control. Intestinal morphology samples were taken from all pigs within the trial (n = 84).

[2] Termin-8 (dry; Anitox Corp., Lawrenceville, GA) was added at 0.2% and 0.4% of the diet.

[3] Finio (liquid; Anitox Corp., Lawrenceville, GA) was added at 0.1% and 0.2% of the diet.

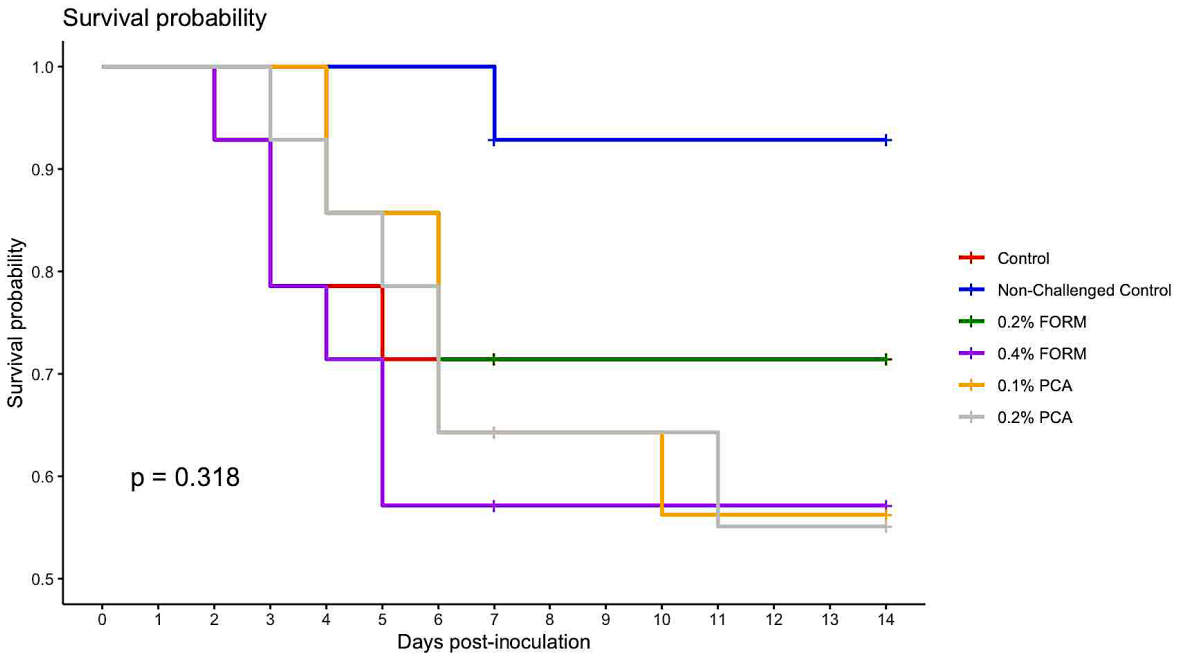

Figure 3.1. Effect of formaldehyde-based product (FORM) or a blend of phytochemicals and carboxylic acids (PCA) on the survival probability curve of Enterotoxigenic *Escherichia coli* F18 challenged nursery pigs. The survival probability curve indicates that all treatments have a similar probability of survival ($P = 0.318$).