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Effect of Dietary Crude Protein and Acid-Binding Capacity on Growth Performance, Serum Cytokine Concentration, and Intestinal Morphology in Enterotoxigenic Escherichia coli F18 Challenged Nursery Pigs

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Summary

A total of 80 weanling pigs (241 × 600 DNA; initially 14.1 ± 1.03 lb) were used in a 14-d study using two groups (40 pigs/group) to evaluate effects of crude protein (CP) and acid-binding capacity at a pH of 4 (ABC-4) on growth performance, fecal dry matter (DM), serum cytokines, haptoglobin concentrations, and intestinal morphology in Enterotoxigenic Escherichia coli F18 (ETEC)-challenged nursery pigs. Within group, pigs were randomly allotted to one of four dietary treatments in a generalized randomized block design. Diets were corn-soybean meal-based without pharmacological levels of Zn or Cu and arranged in a 2 × 2 factorial treatment design. The first factor, ABC-4, compared a low ABC-4 diet formulation (200 and 250 meq/kg in phases 1 and 2, respectively) to a traditional formulation (TF) concept where the ABC-4 level was not specifically considered (≥ 418 and ≥ 382 meq/kg in phases 1 and 2, respectively). The second factor, CP, was tested at 21% (1.35% SID Lys) and 18% (1.15% SID Lys). There were 20 pigs per dietary treatment and diets were fed from d -7 to 0 (phase 1) and d 0 to 14 (phase 2). Pigs were group housed for a 7-d acclimation period, then moved to individual pens and inoculated with ETEC (d 0). Overall (d 0 to 14), pigs fed low ABC-4 diets tended to have increased (P = 0.066) G:F compared with those fed TF diets. No interactions were observed for fecal DM. However, fecal DM increased (P ≤ 0.039) on d 0 and 10 for pigs fed low ABC-4 diets compared with those fed TF diets, with no differences observed on d 3, 7, or 14. An ABC-4 × day interaction was observed (P = 0.014) for serum haptoglobin. Haptoglobin tended to decrease (P = 0.091) on d 14 in pigs fed low ABC-4 diets compared to pigs fed diets using a

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TF strategy, with no differences on d 0 or 7. An ABC-4 × CP × day interaction was observed (P = 0.050) for TNFα where on d 14 (P = 0.003) pigs fed the low ABC-4 diet at 18% CP had increased TNFα concentrations, while pigs fed the TF diet at 21% CP had increased concentrations. Additionally, ABC-4 × day interactions were observed (P ≤ 0.035) for pro-inflammatory (IL-1α, IL-2, IL-6, IL-18) and anti-inflammatory (IL-4 and IL-10) cytokines where on d 7 pigs fed low ABC-4 diets had increased (P ≤ 0.036) concentrations compared to pigs fed TF diets. An ABC-4 × CP interaction was observed (P = 0.007) for duodenal crypt depth, where pigs fed the low ABC-4 diet at 21% CP had greater (P < 0.05) crypt depth compared with pig fed the low ABC-4 diet at 18%, whereas the opposite trend occurred for TF diets. In summary, using a low ABC-4 diet formulation strategy tended to improve feed efficiency, increased fecal DM, and increased cytokine concentrations in ETEC F18-challenged nursery pigs.

Introduction

In the swine industry, Escherichia coli poses many threats to both animal health and food safety for pork consumers. As it relates to the weanling pig, Enterotoxigenic E. coli (ETEC) is a primary cause of post-weaning diarrhea (PWD), which can result in reduced growth performance and increased morbidity and mortality. Historically, ETEC infections have been treated with the use of antibiotics or heavy metals such as zinc oxide. However, due to consumer and regulatory pressures to practice more prudent antibiotic stewardship and environmental concerns, the swine industry needs alternative interventions. Many management and nutritional strategies have been investigated to combat PWD caused by ETEC, including delayed weaning, alterations in crude protein, dietary acidifiers, fiber fractions, and more. However, the efficacy of these practices is quite variable.

Dietary protein serves as an important source of amino acids, but excessive protein intake and an imbalance of amino acids in the diet can lead to a greater risk of diarrhea due to the proliferation of pathogenic bacteria and fermentation in the hindgut. Limbach et al.[5] observed an improvement in nursery pig growth performance as crude protein levels increased from 16 to 22%. However, fecal consistency worsened as crude protein increased. The use of acidifiers has been an area of interest to overcome the post-weaning lag by lowering stomach pH, thereby improving growth performance and fecal consistency. Stas et al.[6] observed a benefit to low ABC-4 diets formulated to 200 and 250 meq/kg in phases 1 and 2, respectively, on nursery pig performance and fecal DM when compared with diets formulated at higher ABC-4 levels. The interactive impact of ABC-4 formulation strategy and CP level has not previously been tested in ETEC challenged pigs. Therefore, the objective of this experiment was to evaluate the effects of ABC-4 formulation strategy, CP levels, and their interactive effects on growth performance, serum cytokine concentration, and intestinal morphology in Enterotoxigenic Escherichia coli F18-challenged nursery pigs.

[5] Limbach, J. R., C. D. Espinosa, E. Perez-Calvo, and H. H. Stein. 2021. Effect of dietary crude protein level on growth performance, blood characteristics, and indicators of intestinal health in weanling pigs. J. Anim. Sci. 99:skab166. doi:10.1093/jas/skab166.

[6] Stas, E. B., M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, and J. T. Gebhardt. 2025. Evaluation of dietary acid-binding capacity level on nursery pig growth performance and fecal dry matter. J. Anim. Sci. 103:skaf039. doi:10.1093/jas/skaf039.

Procedures

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee and Institutional Biosafety Committee. This study was conducted at the K-State Swine Enteric Health Research Center facility in Manhattan, KS. Each pen had metal tri-bar flooring, a stainless-steel self-feeder, and nipple waterer to provide ad libitum access to feed and water. Pigs were placed in solid-sided pens to prevent cross-contamination between adjacent pens.

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 (1,2) fucosyltransferase-1 (FUT1) gene via 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 parities were selected within each group of pigs used in the experiment. A tail sample was collected at litter processing from the selected piglets. Samples were frozen and shipped on dry ice to the ISU VDL for analysis. After identifying susceptible piglets from the initial group, 40 pigs with similar body weights, evenly distributed across litters, were randomly chosen to be used within each group of the experiment (80 pigs total).

Bacterial Strains, Culture Preparations, and Inoculation Methods

The ETEC F18 strain was obtained and cultured at the Kansas State University College of Veterinary Medicine. 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 field clinical case submission. On d 0, all pigs were inoculated with 6 mL of solution containing approximately 109 CFU/mL of ETEC F18. The inoculum was administered via orogastric gavage with a rubber catheter. Briefly, the pig was manually restrained in an upright position with an open mouth and the head elevated compared with the abdomen. The catheter was gently maneuvered into the caudal aspect of the oropharynx. A swallow reflex was stimulated and when visually observed, the tube was slowly introduced into the esophagus. Once the inoculum was dispensed, the catheter was kinked during the removal process to ensure no residual material was deposited in the oropharynx during the removal process.

Animals and diets

A total of 80 weanling pigs (241 × 600 DNA; initially 14.1 ± 1.03 lb) were used in a 14-d study using two groups (40 pigs/group). Pigs were randomly allotted to one of four dietary treatments in a generalized randomized block design and grouped with five to six pigs per pen during the 7-d adaptation period. Diets were corn-soybean meal-based and arranged in a 2 × 2 factorial. The first factor, ABC-4, was tested using a low ABC-4 diet formulation concept set at 200 meq/kg and 250 meq/kg in phases 1 and 2, respectively, compared to a traditional formulation (TF) concept where the ABC-4 level was not specifically considered (≥ 418 and ≥ 382 meq/kg in phases 1 and 2, respectively). The second factor, CP, was tested at 21% (1.35% SID Lys) and 18% (1.15% SID Lys). Treatment diets consisted of 1) low ABC-4 at 21% CP, 2) low ABC-4 at 18% CP, 3) TF at 21% CP, and 4) TF at 18% CP. All diets met or exceeded the nutrient requirement estimates of other amino acids as a ratio to Lys (NRC, 2012)[7] for the weight

[7] NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

ranges of the pigs (Table 1). Treatment diets did not include pharmacological levels of Zn or Cu. Diets were fed in two phases from d −7 to 0 (phase 1) and d 0 to 14 (phase 2). Treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS, and fed in pelleted form for phase 1 and meal form for phase 2. On the day of inoculation (d 0), pigs were moved to individual pens. Pig weights and feed disappearance were measured on d 0, 7, and 14 to determine ADG, ADFI, and G:F.

Fecal Dry Matter and Scoring

On d 0, 3, 7, 10, and 14, fecal samples were collected from each pig to determine fecal dry matter (DM) and fecal score. Using a cotton-tipped applicator to stimulate defecation, fecal samples were collected into plastic bags and frozen at -4°F until fecal dry matter analysis. Fecal scoring of the bags was conducted by two independent scorers blinded to treatments on d 0, 3, 7, 10, and 14 using a numerical scale where 0 = hard, pellet-like feces; 1 = a firm, formed stool; 2 = a soft, moist stool that retains shape; 3 = a soft, unformed stool; and 4 = a watery, liquid stool. Fecal scores ≥ 3 were considered diarrhea. Fecal samples were dried at 131°F in a forced air oven for 48 h, and the ratio of dried-to-wet fecal weight determined the percentage fecal DM. Fecal DM from d 3 and 14 only represent group 2 due to missing samples.

Enumeration of E. coli

Fecal samples (from d 0, 7, and 14 of group 1) were tested using 3M Petrifilm plates (3M Microbiology, St. Paul, MN). One gram of fecal sample was diluted in 10 mL of phosphate buffered saline (PBS; pH 7.2) and the tube was vortexed to make a uniform suspension before serially diluting the fecal suspension to achieve 100, 10-1, 10-2, 10-3, 10-4, 10-5, and 10-6 concentrations. Using a sterile pipette, 1 mL of the homogenized fecal sample at each dilution was placed in the center of the Petrifilm in triplicate. All dilutions were vortexed before plating to ensure equal distribution of fecal inoculum. The top film was then rolled down onto sample gently to prevent pushing sample off film and to reduce air bubbles. The 3M Petrifilm spreader was placed on top film over inoculum. Pressure was gently applied to 3M Petrifilm spreader to distribute sample inoculum over the circular area of 20 cm2 on the bottom film. The plates were incubated for 48 ± 2 h at 98.6°F to detect Escherichia coli as per manufacturer's guidelines. After incubation, the confirmed E. coli colonies – blue colony phenotype along with associated gas bubbles – were counted. The counting ranges were 15 to 150 colonies for E. coli plates. Petrifilm colony counts were expressed as colony-forming units per gram of fecal sample and the bacterial counts were expressed as an average of two separate runs conducted in duplicate with a different fecal sample.

Haptoglobin and Immune Parameters

On d 0 (before challenge), 7, and 14, a blood sample was collected via jugular venipuncture into a 10-mL vacutainer tube. Samples were centrifuged (3,000 × g for 10 min) and serum was recovered in four separate aliquots. Serum was used for analysis of haptoglobin (Kansas State University Veterinary Diagnostic Laboratory) adapted from the assay of Makimura and Suzuki[8]. 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. An

[8] Makimura, S., and Suzuki, N. 1974. A simple method for quantitative spectrophotometric determination of serum haptoglobin in mice and rats. Proc. 74th Annual Meet. of Vet. Sci., Obihiro, Japan. p. 377-382.

aliquot of serum was sent to Eve Technologies, Calgary, AB, Canada and analyzed using a multiplex immunoassay that quantifies 13 cytokines (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).

Animal Harvesting and Tissue Collection

On d 7, 15 pigs from group 1 and 12 pigs from group 2 were randomly selected to be euthanized via penetrating captive bolt and opened viscerally for necropsy so that there were 20 remaining pigs in each group after any mortalities prior to d 7. Necropsy procedures took place at the KSU Enteric Health Research Center, with carcasses transported to the KSU Veterinary Diagnostic Lab for disposal following sample collection according to standard operating procedures. For necropsy, the duodenum, jejunum, and ileum were identified, removed from the mesentery, and three 2-cm-long s egments from each section were cut and fixed in 10% formalin for histological analysis at the Kansas State University Veterinary Diagnostic Laboratory. Briefly, 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. Simultaneously, visualization of ETEC attachment to epithelial cells was conducted on the tissue samples.

Statistical analysis

Experimental data were analyzed using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute Inc., Cary, NC). Individual pig served as the experimental unit, with dietary treatment as a fixed effect and BW block and group included as random intercepts. Serum cytokine and haptoglobin data were log-transformed for analysis and back-transformed for presentation of least squares means. E. coli enumeration data were analyzed using a log10 transformation. Data points with absolute studentized residuals greater than 4 were removed from data analysis. Fecal scores were summarized using the FREQ procedure and reported as the percentage of observations within each score category by treatment. Because model assumptions were not met, fecal score data are presented descriptively. 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 at P 0.05 and were considered marginally significant at P 0.10.

Results and Discussion

Growth Performance

For period 1 (d 0 to 7), an ABC-4 × CP interaction was observed (P = 0.044) for ADFI (Table 2). When diets were formulated using the low ABC-4 strategy, pigs fed 21% CP had increased ADFI, whereas under the TF strategy, pigs fed 18% CP had increased ADFI. A tendency for an ABC-4 × CP interaction was also observed (P = 0.095) for G:F, where numerically, pigs fed the low ABC-4 diet with 18% CP had decreased G:F, while pigs fed the TF diet with 21% CP had decreased G:F (P > 0.10).

No treatment differences were observed during period 2 (d 7 to 14; Table 2). Overall (d 0 to 14), pigs fed diets formulated using the TF strategy tended to have decreased G:F (P = 0.066) compared with pigs fed diets using the low ABC-4 formulation strategy.

Haptoglobin

No ABC-4 × CP × day interaction was observed for serum haptoglobin (Table 2). An ABC-4 × day interaction was observed (P = 0.014), where pigs fed diets formulated

using the low ABC-4 strategy tended to have lower (P = 0.091) haptoglobin concentrations on d 14 compared with pigs fed diets formulated using the TF strategy. No differences were observed on d 0 or 7. Haptoglobin is an acute phase protein that increases during an inflammatory response, and so a decrease in haptoglobin concentrations may suggest reduced inflammation.

Fecal Dry Matter

No ABC-4 × CP × day, ABC-4 × day, or CP × day interactions were observed for fecal DM (Table 2). After consuming the low ABC-4 diets from d −7 to 0, pigs fed diets formulated using the low ABC-4 strategy had greater fecal DM (P= 0.039) on d 0 than those fed diets formulated using the TF strategy. Pigs fed diets containing 21% CP also tended to have greater (P = 0.052) fecal DM than those fed 18% CP diets. On d 10, pigs fed diets formulated using the low ABC-4 strategy had higher fecal DM (P = 0.010) than those fed diets formulated using the TF strategy.

Serum Cytokine Panel

An ABC-4 × CP × day interaction was observed (P = 0.050) for TNFα (pro-inflammatory cytokine; Table 3). On d 14 (P = 0.003), pigs fed the low ABC-4 diet with 18% CP had greater TNFα concentrations than those fed the 21% CP diet, whereas pigs fed the TF diet with 21% CP had greater concentrations than those fed the 18% CP diet. A similar, but non-significant, tendency was observed on d 7 (P = 0.071). No differences were observed on d 0. Pro-inflammatory cytokines are produced by immune cells in response to infections, and so decreased concentrations may suggest an improvement in immune status.

A tendency for an ABC-4 × CP interaction was observed on d 0 (P = 0.078) for IL-1β (pro-inflammatory cytokine; Table 3), where pigs fed the low ABC-4 diet formulated to 18% CP had greater concentrations, whereas pigs fed the TF diet formulated to 21% CP had greater concentrations of IL-1β.

Acid binding capacity at a pH of 4 (ABC-4) × day interactions were observed (P ≤ 0.035) for pro-inflammatory (IL-1α, IL-2, IL-6, IL-18) and anti-inflammatory (IL-4 and IL-10) cytokines (Table 3). On d 7 (P ≤ 0.036), pigs fed low ABC-4 diets had greater cytokine concentrations than those fed TF diets, with no significant differences on d 0 or 14. Additionally, pigs fed low ABC-4 diets tended to have greater IL-1β (P = 0.070) concentrations on d 7 and IL-6 (P = 0.089) concentrations on d 14 compared with those fed TF diets.

E. coli Enumeration and Intestinal Histology

No statistical differences were observed for E. coli enumeration (Table 4).

No statistical differences were observed in villus height for the duodenum, jejunum, or ileum (Table 4). However, an ABC-4 × CP interaction was observed for duodenal crypt depth (P = 0.007). Within the 21% CP diets, pigs fed the low ABC-4 formulation strategy had deeper crypts (P = 0.050) than those fed the TF strategy. Conversely, within the 18% diets, pigs fed the TF strategy had deeper crypts (P = 0.046) than those fed the low ABC-4 formulation strategy. Pigs fed diets formulated to 21% CP also tended to have deeper ileal crypts (P = 0.071) than pigs fed 18% CP diets. Furthermore, a tendency for an ABC-4 × CP interaction was observed for the duodenal villus height-to-crypt depth ratio (P = 0.061). Within the TF strategy, pigs fed the 21% CP diet had a greater ratio (P = 0.029) than those fed 18% CP, with no differences within

the ABC-4 strategy. Within the 18% CP diets, pigs fed the low ABC-4 strategy had a greater ratio (P = 0.023) than those fed the TF strategy, with no differences within the 21% CP diets.

Fecal Scoring

Fecal scores were summarized as the percentage of observations within each score category by treatment. Due to lack of model fit of the data, fecal scores are reported using descriptive statistics with a score of 0 indicating hard feces and 4 indicating diarrhea. On d 0, pigs fed 18% CP diets had a numerically greater frequency of diarrhea (Figures 1 and 2). Across all treatments, the frequency of diarrhea increased on d 3 and 7 post-inoculation. On d 7, pigs fed the 18% CP diets had a lower numerical frequency of diarrhea than pigs fed the 21% CP diets. Furthermore, the occurrence of diarrhea persisted to d 10 for pigs fed the TF diets, with the 21% CP TF diet showing diarrhea on d 14.

Survival Probability

Numerically, the 21% CP diets had the lowest probability of survival by the end of the experiment (70 to 80%), whereas the traditional 18% CP diet had the highest at approximately 90% (Figure 3).

In summary, using a low ABC-4 diet formulation strategy tended to improve feed efficiency, increased fecal DM, and increased cytokine concentrations in ETEC F18-challenged nursery pigs.

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Table 1. Diet composition (as-fed basis)[1]

	Phase 1				Phase 2			
ABC-4 formulation approach:	Low		Traditional		Low		Traditional	
CP, %:	21	18	21	18	21	18	21	18
Ingredient, %								
Corn	49.84	58.03	45.60	53.36	58.84	67.08	56.33	64.11
Soybean meal (47% CP)	15.81	8.02	16.29	8.53	17.72	9.93	18.56	10.79
Enzymatically treated SBM[2]	---	---	12.50	12.50	---	---	10.00	10.00
Specialty soy protein concentrate[3]	12.50	12.50	---	---	10.00	10.00	---	---
Lactose	15.00	15.00	---	---	7.50	7.50	---	---
Whey powder	---	---	15.00	15.00	---	---	10.50	10.50
Whey permeate (80% lactose)	---	---	5.25	5.25	---	---	---	---
Soybean oil	2.00	2.00	2.00	2.00	1.00	1.00	1.00	1.00
Calcium carbonate	0.35	0.35	0.57	0.55	0.60	0.59	0.60	0.58
Monocalcium P (21% P)	1.51	1.62	0.85	0.95	1.30	1.40	0.86	0.96
Salt	0.79	0.80	0.47	0.48	0.69	0.70	0.56	0.58
L-Lys-HCl	0.49	0.48	0.42	0.40	0.52	0.51	0.47	0.46
DL-Met	0.26	0.21	0.25	0.20	0.25	0.20	0.24	0.19
L-Thr	0.23	0.21	0.20	0.18	0.24	0.22	0.22	0.19
L-Trp	0.06	0.06	0.03	0.04	0.06	0.06	0.05	0.05
L-Val	0.13	0.10	0.12	0.10	0.14	0.12	0.14	0.11
L-Ile	0.02	0.03	---	0.01	0.01	0.03	---	0.01
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Phytase[4]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Fumaric acid	0.28	0.07	---	---	0.33	0.11	---	---
Formic acid	0.28	0.07	---	---	0.33	0.11	---	---
Total	100	100	100	100	100	100	100	100

Continued

Table 1. Diet composition (as-fed basis)[1]

	Phase 1				Phase 2			
ABC-4 formulation approach:	Low		Traditional		Low		Traditional	
CP, %:	21	18	21	18	21	18	21	18
Calculated analysis								
SID AA, %								
Lys, %	1.35	1.15	1.35	1.15	1.35	1.15	1.35	1.15
Ile:Lys	60	60	60	60	58	58	58	58
Leu:Lys	114	119	115	119	115	120	115	119
Met:Lys	40	39	38	38	40	39	38	38
Met and Cys:Lys	60	60	60	60	60	60	60	60
Thr:Lys	65	65	65	65	65	65	65	65
Trp:Lys	20.2	20.2	20.2	20.2	20.2	20.2	20.2	20.2
Val:Lys	72	72	72	72	72	72	72	72
His:Lys	35	35	35	35	35	35	36	35
NE, kcal/lb	1,172	1,195	1,174	1,193	1,139	1,164	1,143	1,162
SID Lys:NE, g/Mcal	5.23	4.36	5.21	4.37	5.37	4.48	5.36	4.49
CP, %	21.1	18.0	21.1	18.0	21.1	18.0	21.1	18.0
Ca, %	0.61	0.60	0.70	0.68	0.67	0.65	0.67	0.66
STTD P, %	0.42	0.42	0.42	0.42	0.39	0.39	0.39	0.39
Ca:P	0.96	0.97	1.10	1.10	1.10	1.10	1.10	1.10
Calculated ABC-4, meq/kg	200	200	474	431	250	250	436	392

[1] Phase 1 diets were fed from d −7 to 0. Phase 2 diets were fed from d 0 to 14.

[2] HP 300; Hamlet Protein; Findlay, OH.

[3] AX3 Digest; Protekta; Newport Beach, CA.

[4] Ronozyme HiPhos 2700 (DSM-Firmenich; Parsippany, NJ) provided at an estimated release of 0.14% STTD P with 919 FYT/lb.

Table 2. Effects of acid-binding capacity at pH 4 (ABC-4) and crude protein (CP) on growth performance, haptoglobin levels, and fecal dry matter (DM) of F18 *E. coli* challenged nursery pigs[1,2]

ABC-4 formulation approach:	Low		Traditional			*P* =		
CP, %:	21	18	21	18	SEM	ABC-4 × CP	ABC-4	CP
BW, lb								
d 0	14.1	14.1	14.1	14.2	1.03	0.840	0.975	0.852
d 7	14.2	13.7	12.9	13.8	1.30	0.161	0.240	0.714
d 14	19.6	18.4	17.0	18.9	2.21	0.242	0.450	0.809
Period 1 (d 0 to 7)								
ADG, lb	-0.13	-0.21	-0.24	-0.13	0.074	0.179	0.847	0.843
ADFI, lb[3]	0.30	0.22	0.25	0.29	0.082	0.044	0.650	0.474
G:F	-0.65	-1.16	-1.49	-0.70	0.389	0.095	0.617	0.716
Period 2 (d 7 to 14)								
ADG, lb	0.65	0.70	0.58	0.76	0.166	0.658	0.950	0.402
ADFI, lb	0.77	0.82	0.67	0.82	0.142	0.670	0.652	0.426
G:F	0.96	0.91	0.76	0.91	0.111	0.363	0.355	0.613
Overall (d 0 to 14)								
ADG, lb	0.33	0.30	0.22	0.31	0.109	0.523	0.607	0.777
ADFI, lb	0.53	0.52	0.45	0.55	0.088	0.472	0.719	0.502
G:F	0.71	0.50	-0.02	0.41	0.222	0.146	0.066	0.597
Haptoglobin levels, mg/dL[4]								
d 0	57.6	51.4	43.6	38.1	12.07	0.961	0.146	0.527
d 7	97.7	72.0	122.9	130.5	33.48	0.468	0.105	0.627
d 14	59.4	62.9	98.4	95.9	28.39	0.875	0.091	0.952
Fecal DM, %[5]								
d 0	19.4	18.3	18.0	12.0	1.87	0.186	0.039	0.052
d 3[6]	10.8	10.1	10.9	11.3	3.25	0.861	0.840	0.959
d 7	14.7	18.4	14.8	18.4	2.77	0.991	1.000	0.160
d 10	28.7	32.5	21.8	22.6	3.42	0.631	0.010	0.460
d 14[6]	27.9	26.1	26.0	24.6	2.84	0.948	0.552	0.593

[1] A total of 80 pigs were used in two 14-d growth studies with 1 pig per pen and 20 replicates per treatment.

[2] Pigs were euthanized on d 7 so that 20 pigs remained for each group.

[3] CP effect in low ABC-4 (P = 0.054). ABC-4 effect in low CP (P = 0.081).

[4] ABC-4 × day interaction (P = 0.014). All other interactions (P > 0.10).

[5] No ABC-4 × CP × day, ABC-4 × day, or CP × day interactions (P > 0.10).

[6] Data shown is only from group 2.

Table 3. Effects of acid-binding capacity at pH 4 (ABC-4) and crude protein (CP) on serum cytokine concentrations (fluorescence intensity values) of F18 *E. coli* challenged nursery pigs[1,2]

ABC-4 formulation approach:	Low		Traditional			*P* =		
CP, %:	21	18	21	18	SEM	ABC-4 × CP	ABC-4	CP
GM-CSF								
d 0	18.8	19.3	21.0	18.7	2.86	0.464	0.687	0.658
d 7	18.1	19.2	19.0	18.0	2.75	0.570	0.939	0.983
d 14	19.1	18.0	19.2	16.5	3.06	0.702	0.758	0.411
IFNγ								
d 0	56.4	70.6	63.9	57.6	12.86	0.276	0.793	0.686
d 7	51.7	57.4	57.2	49.9	10.78	0.442	0.900	0.918
d 14	39.5	53.1	47.1	39.8	10.56	0.178	0.744	0.714
IL-1α[3]								
d 0	54.9	94.0	72.2	66.8	27.09	0.281	0.906	0.420
d 7	99.7	185.7	82.1	63.9	56.12	0.144	0.036	0.532
d 14	67.1	125.9	78.5	60.1	41.97	0.182	0.386	0.590
IL-1β								
d 0	80.0	136.6	108.0	82.1	31.73	0.078	0.646	0.568
d 7	138.8	200.8	123.3	94.4	49.30	0.186	0.070	0.831
d 14	94.7	158.2	104.2	99.3	43.52	0.309	0.502	0.400
IL-1RA								
d 0	288.2	363.6	290.2	296.3	67.07	0.426	0.456	0.341
d 7	590.7	740.0	740.1	477.2	237.44	0.241	0.705	0.705
d 14	196.1	320.8	281.5	256.4	79.05	0.188	0.755	0.368
IL-2[3]								
d 0	46.6	74.8	56.6	58.2	23.13	0.461	0.926	0.404
d 7	85.8	147.9	58.9	49.3	48.14	0.251	0.021	0.560
d 14	69.3	99.6	62.8	45.3	35.96	0.335	0.217	0.958
IL-4[3]								
d 0	53.0	87.4	69.1	56.1	23.99	0.200	0.745	0.596
d 7	93.2	178.8	76.2	65.0	51.41	0.158	0.036	0.390
d 14	66.4	125.4	79.7	65.4	39.68	0.194	0.465	0.494
IL-6[3]								
d 0	63.6	90.2	74.4	58.1	24.29	0.247	0.580	0.843
d 7	100.5	158.8	77.8	60.3	44.63	0.183	0.023	0.703
d 14	85.4	120.7	64.9	57.2	37.07	0.430	0.089	0.713

Continued

Table 3. Effects of acid-binding capacity at pH 4 (ABC-4) and crude protein (CP) on serum cytokine concentrations (fluorescence intensity values) of F18 *E. coli* challenged nursery pigs[1,2]

ABC-4 formulation approach:	Low		Traditional			*P* =		
CP, %:	21	18	21	18	SEM	ABC-4 × CP	ABC-4	CP
IL-8								
d 0	283.4	484.1	483.5	601.8	191.76	0.539	0.146	0.145
d 7	372.4	375.9	284.9	364.1	128.51	0.676	0.596	0.652
d 14	222.8	217.3	221.5	280.2	112.08	0.720	0.732	0.772
IL-10[3]								
d 0	61.8	88.5	69.4	58.9	25.46	0.341	0.595	0.722
d 7	110.8	154.3	74.6	58.8	46.19	0.319	0.019	0.869
d 14	71.5	106.3	69.9	55.1	34.59	0.317	0.286	0.802
IL-12								
d 0	957.8	1,039.0	926.2	916.7	202.23	0.646	0.426	0.721
d 7	1,168.3	1,036.5	1,007.0	1,038.1	211.14	0.283	0.293	0.522
d 14	971.7	1,017.2	871.3	1,002.9	183.82	0.504	0.388	0.195
IL-18[3]								
d 0	91.5	140.9	117.7	90.7	31.31	0.117	0.667	0.696
d 7	142.4	209.1	120.5	88.2	48.71	0.130	0.026	0.875
d 14	104.5	170.6	115.6	94.5	43.82	0.181	0.342	0.575
TNFα[4]								
d 0	42.2	51.5	46.6	39.8	8.16	0.163	0.537	0.867
d 7	35.1	43.3	46.4	35.3	7.56	0.071	0.775	0.806
d 14	25.0	47.3	37.8	29.7	8.12	0.003	0.853	0.175

[1] A total of 80 pigs (initially 14.1 ± 1.03 lb) were used in two 14-d growth studies with 1 pig per pen and 20 replicates per treatment. No ABC-4 × CP × day, ABC-4 × day, or CP × day interactions unless otherwise indicated.

[2] Pigs were euthanized on d 7 so that 20 pigs remained for each group.

[3] ABC-4 × day interaction ($P < 0.05$).

[4] ABC-4 × CP × day interaction ($P = 0.050$). CP × day tendency ($P = 0.097$).

Table 4. Effects of acid-binding capacity at pH 4 (ABC-4) and crude protein (CP) on intestinal histology of F18 *E. coli* challenged nursery pigs[1]

ABC-4 formulation approach:	Low		Traditional			*P* =		
CP, %:	21	18	21	18	SEM	ABC-4 CP	ABC-4	CP
E. coli enumeration, log_{10} cfu/g[2]								
d 0	6.16	6.33	6.22	6.51	0.433	0.880	0.766	0.584
d 7	6.78	6.14	6.10	6.80	0.463	0.152	0.988	0.954
d 14	4.60	4.44	4.55	4.72	0.846	0.805	0.869	0.996
Villi height, µm								
Duodenum	413	365	370	317	40.0	0.941	0.207	0.164
Jejunum	401	386	310	362	42.1	0.433	0.177	0.660
Ileum	299	290	263	254	28.8	0.980	0.197	0.737
Crypt depth, µm								
Duodenum[3]	399	330	335	393	23.7	0.007	0.994	0.787
Jejunum	308	281	295	288	16.6	0.541	0.863	0.301
Ileum	266	204	232	228	18.8	0.108	0.785	0.071
Villi height:crypt depth								
Duodenum[4]	1.09	1.14	1.14	0.86	0.087	0.061	0.172	0.196
Jejunum	1.35	1.43	1.10	1.30	0.138	0.662	0.173	0.298
Ileum	1.20	1.49	1.24	1.14	0.136	0.142	0.237	0.464

[1] A total of 40 pigs were used in a 14-d growth study with 1 pig per pen and 10 replicates per treatment.

[2] No ABC-4 × CP × day, ABC-4 × day, or CP × day interactions (P > 0.10).

[3] CP effect in low ABC-4 (P = 0.029). CP effect in traditional ABC-4 (P = 0.074). ABC-4 effect in high CP (P = 0.050). ABC-4 effect in low CP (P = 0.046).

[4] CP effect in traditional ABC-4 (P = 0.029). ABC-4 effect in low CP (P = 0.023).

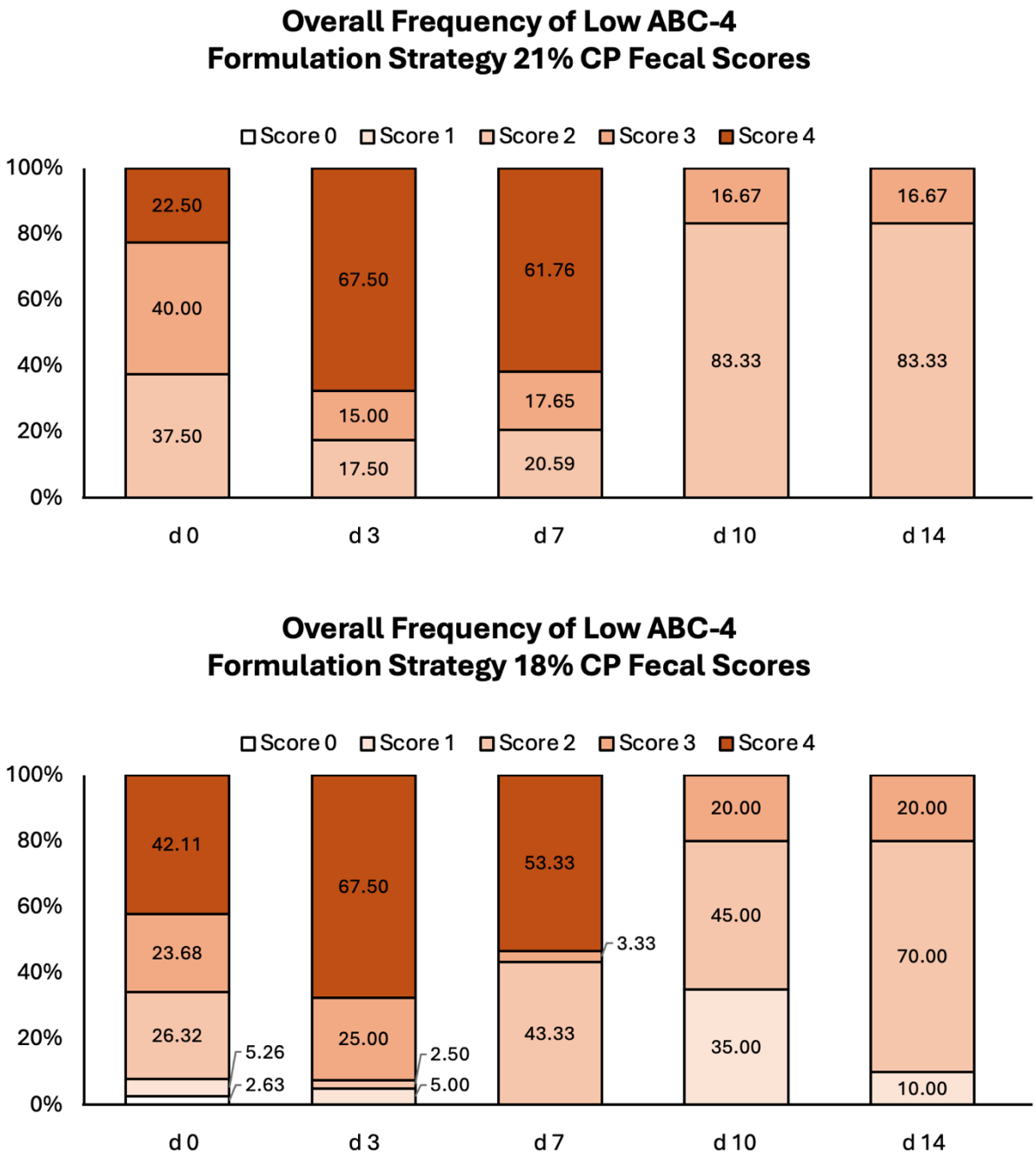


Figure 1. Fecal scores are presented on a 4-point scale: 0 = hard, pellet-like lumps; 1 = firm, formed feces; 2 = normal feces; 3 = mild looseness; and 4 = diarrhea scored by two observers

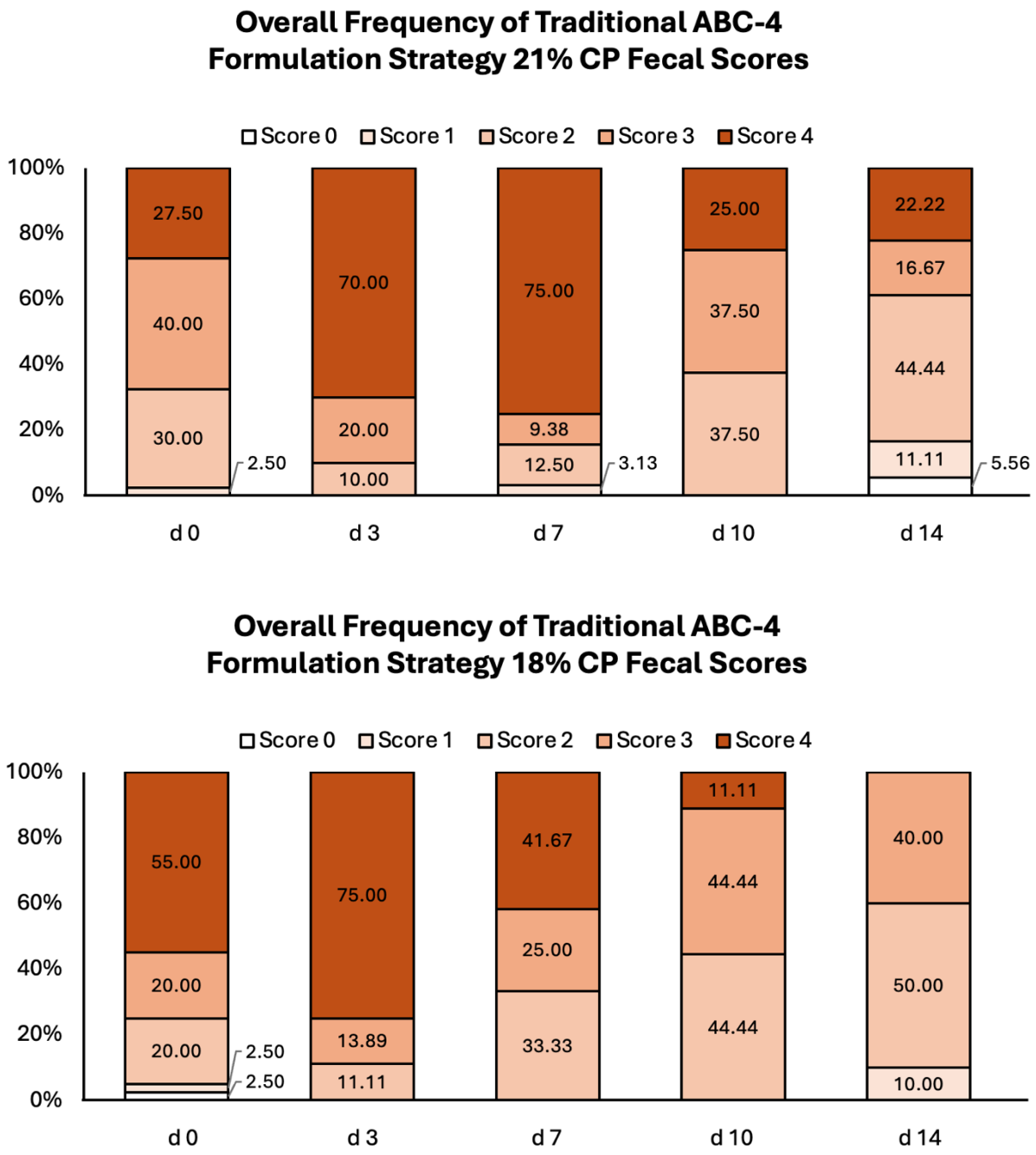


Figure 2. Fecal scores are presented on a 4-point scale: 0 = hard, pellet-like lumps; 1 = firm, formed feces; 2 = normal feces; 3 = mild looseness; and 4 = diarrhea scored by two observers

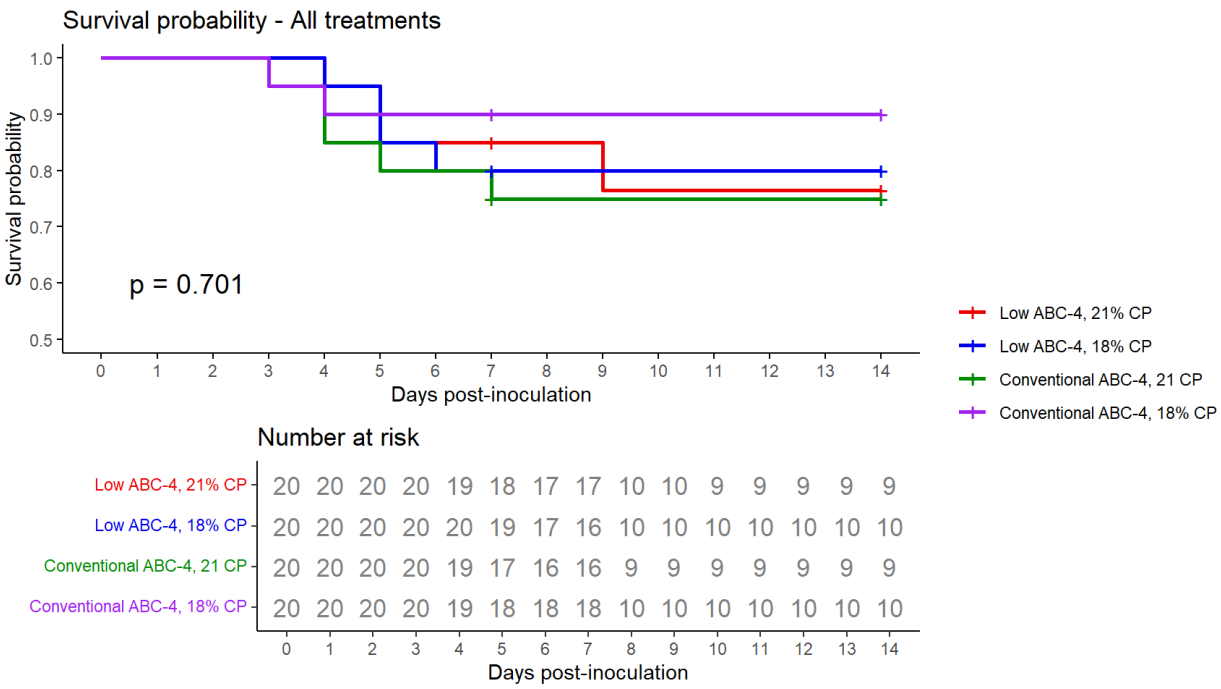


Figure 3. Survival probability for all treatments across the duration of the trial.