Kansas Agricultural Experiment Station Research Reports

Volume 8
Issue 10 *Swine Day*

Article 15

2022

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Recommended Citation

Becker, Larissa L.; Gebhardt, Jordan T.; Tokach, Mike D.; Goodband, Robert D.; DeRouchey, Joel M.; Woodworth, Jason C.; Amachawadi, Raghavendra G.; Kalam, Ramya; Shi, Xiaorong; and Nagaraja, T. G. (2022) "Evaluation of Clay-Based Binders and In-Feed Antimicrobials on Growth Performance and Biological Measurements in Nursery Pigs," *Kansas Agricultural Experiment Station Research Reports*: Vol. 8: Iss. 10. https://doi.org/10.4148/2378-5977.8368

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K-STATE Research and Extension

SWINE DAY 2022

Evaluation of Clay-Based Binders and In-Feed Antimicrobials on Growth Performance and Biological Measurements in Nursery Pigs

Larissa L. Becker, Jordan T. Gebhardt,[1] Mike D. Tokach, Robert D. Goodband, Joel M. DeRouchey, Jason C. Woodworth, Raghavendra G. Amachawadi,[1] Ramya Kalam,[1] Xiaorong Shi,[1] and T.G. Nagaraja[1]

Summary

A total of 360 barrows (DNA 200 × 400; initially 14.2 ± 0.08 lb) were used in a 42-d growth study to evaluate clay-based binders or an in-feed antimicrobial on growth performance and biological measurements including fecal and blood analysis in nursery pigs. Pigs were weaned at approximately 21 d of age and randomly allotted to 1 of 4 dietary treatments in a completely randomized design. There were 5 pigs per pen and 18 replications per treatment. Dietary treatments were corn-soybean meal-based and fed in two phases from d 0 to 9 (phase 1) and 9 to 21 (phase 2) after weaning. Either Protek (0.40% of the diet; Nutriquest, Mason City, IA); Protect-8 Plus (0.10% of the diet; Essential Ag Solutions, Sioux Falls, SD); or Kavault (0.04% of the diet; Avilamycin; Elanco, Greenfield, IN) were added to the control diet to create the experimental treatments. A common phase 3 diet was fed to all pigs from d 21 to 42. Overall (d 0 to 42), pigs fed Kavault had increased ($P < 0.05$) final BW, ADG, and ADFI compared to all other treatments. There was evidence that frequency of fecal scores with softer feces increased over time ($P < 0.001$), with d 21 having the greatest frequency of diarrhea and soft feces. Fecal *Escherichia coli* colony count was lower ($P < 0.001$) on d 21 compared to d 9. For fecal myeloperoxidase (MPO), concentrations were lower ($P < 0.05$) on d 21 compared to d 6 and 9. For fecal DM, pigs fed Kavault had decreased ($P < 0.05$) DM percentage compared to all other treatments. Fecal DM percentage was higher ($P < 0.05$) on d 6 and 9 compared to d 21. No differences ($P > 0.10$) were observed across treatments for fecal scores, fecal *E. coli* colony count, fecal MPO or virulence genes associated with *E. coli*. Similarly, no differences ($P > 0.10$) were observed across treatments for TNF-alpha and IL-6 blood assays. For IL-6, concentrations were greater ($P < 0.05$) on d 9 compared to d 21. In summary, pigs fed Kavault had increased BW, ADG, and ADFI, compared to those fed the 2 clay-based additives or the control diet. There were no treatment effects on fecal score, fecal MPO, or blood measurements. However, we observed a day effect indicating that feces were softer and had less DM on d 21 compared to d 6 and 9. Additionally, fecal *E. coli* colony count and

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MPO had lower concentrations of d 21 compared to d 9. There was a strong negative correlation between fecal DM and score ($P < 0.001$) on d 6, 9, and 21 indicating that as fecal DM increased, the score became closer to 0, representing a firmer fecal sample. Fecal DM on d 6 and fecal DM on d 9 were negatively correlated with ADG from d 0 to 9 meaning that as growth rate increased, fecal DM decreased.

Introduction

Endotoxins are structural components of bacteria and are part of the outer membrane of Gram-negative bacteria such as *E. coli*. Gram negative bacteria are present in the gastrointestinal tract in large amounts and can serve as a major source of systemic endotoxin.

Endotoxins are released when bacteria are lysed due to the use of antibiotics or the body's natural defense mechanisms. Endotoxins result in pro-inflammatory effects in the pig and can have damaging effects on health status and growth performance. Once in circulation, endotoxins activate the immune system and result in cytokine production and the repartitioning of nutrients for immune function rather than for normal function of nutrients to promote growth. However, detoxification using binders and antimicrobials helps to prevent attachment of *E. coli* and may prevent the negative effects of endotoxins.

This study included two objectives. First, to determine relevant measures to test during research evaluating nutritional and management practices to reduce incidence and severity of *E. coli* in preparation for field testing of potential technologies. Second, to determine the influence of diet including bentonite clay additive and an in-feed antimicrobial on detection of serum endotoxin, cytokine concentration, fecal myeloperoxidase concentration, quantification, and characterization of fecal *E. coli*.

Procedures

Animals and diets

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Segregated Early Weaning Research Facility in Manhattan, KS. Each pen contained a 4-hole, dry self-feeder, and nipple waterer for *ad libitum* access to feed and water. A total of 360 barrows (DNA 200 × 400; initially 14.2 ± 0.08 lb) were used in a 42-d growth trial. Pigs were weaned at approximately 21 d of age and randomly allotted to 1 of 4 dietary treatments in a completely randomized design. There were 5 pigs per pen and 18 replications per treatment. Dietary treatments were corn-soybean meal-based and fed in two phases from d 0 to 9 (phase 1) and d 9 to 21 (phase 2) after weaning (Table 1). Either Protek (0.40% of the diet; Nutriquest, Mason City, IA); Protect-8 Plus (0.10% of the diet; Essential Ag Solutions, Sioux Falls, SD); or Kavault (0.04% of the diet; Avilamycin; Elanco, Greenfield, IN) was added to the control corn-soybean meal diet to create experimental treatments. A common phase 3 diet was fed to all pigs from d 21 to 42.

Fecal collection

On d 6, 9, and 21 of the experiment, fecal samples were collected from 3 pigs per pen. Samples were collected into individual sealable plastic bags for each pig. Fecal dry

matter (DM) was analyzed independently on all 3 samples per pen for all 3 collection days. Fecal DM was determined by drying the fecal sample at 131°F (55°C) for 48 h. One pig was identified for fecal myeloperoxidase (MPO), fecal *E. coli* quantification, and blood analysis for all 3 collection days. Fecal MPO (d 6, 9, and 21), *E. coli* quantification (d 9 and 21), and *E. coli* gene typing (d 21) were analyzed on 1 fecal sample per pen. *E. coli* gene typing was analyzed using the procedures by Warner et al.[2]

Fecal scores

On d 6, 9, and 21 of the experiment, fecal scores were assigned to 3 fecal samples collected from 3 pigs per pen. Fecal samples were collected into individual sealable plastic bags and fecal scores were assigned to each bagged sample. Fecal scores were assigned based on a 0 to 2 scale, with 0 indicating firm feces, 1 as soft feces, and 2 as diarrhea.

Enumeration of E. coli in the fecal samples

One fecal sample per pen (from d 9 and 21) was 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 10^{0}, 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 triplicates. The 3M Petrifilm spreader was placed on the top film over the inoculum. Pressure was gently applied to 3M Petrifilm spreader to distribute sample inoculum over the circular area of 20 cm^2 on the bottom film. The plates were incubated for 48 +/- 2 hours at 98.6°F (37°C) to detect *E. 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 unit per gram of fecal sample and the bacterial counts were expressed as an average of three separate runs conducted in triplicate with a different fecal sample.

Blood sampling

On d 9 and 21, one average weight barrow from each pen (18 per treatment) was bled for analysis of circulating cytokine and endotoxin concentrations. These were the same pigs used for analysis of fecal MPO, *E. coli* enumeration, and *E. coli* gene typing. Blood was collected in tubes without anticoagulant to obtain serum for TNF-alpha and IL-6 analysis. Blood was allowed to clot before centrifuging for 15 min at 1,500 × *g* to collect serum, and samples were stored at 0°F (-18°C) until analyzed. For TNF-alpha and IL-6, samples were analyzed in triplicate within a single assay. Serum concentrations were determined using ELISA kits per the instructions of the manufacturer (R&D Systems, Minneapolis, MN).

[2] Warner, A. J., A. L. Gerrard, M. D. Tokach, R. G. Amachawadi, A. Labbé, W. Heuser, R. Kalam, S. Xiaorong. T. G. Nagaraja, J. M. DeRouchey, J. C. Woodworth, R. D. Goodband, and J. T. Gebhardt. 2021. Evaluation of a Dried Fermentation Product Administered Through Drinking Water on Nursery Pig Growth Performance, Fecal Consistency, and Antibiotic Injections. Kansas Agricultural Experiment Station Research Reports: Vol. 7: Iss. 11. https://doi.org/10.4148/2378-5977.8189.

Statistical analysis

Growth performance data were analyzed as a completely randomized design for one-way ANOVA. Pen was considered the experimental unit. Treatment was used as the fixed effect and barn was included in the model as a random effect. Fecal dry matter, fecal MPO, and fecal score were analyzed as repeated measures representing multiple observations on each pen over time, and pen was included in the model as a random intercept to account for subsampling attributed to the multiple observations on each day. Treatment, day, and the associated interactions were considered fixed effects, and pen nested within treatment and the cross product of pen, treatment, and day was included as a random intercept. For fecal score analysis, data were analyzed as categorical outcomes using a generalized linear mixed model with a multinomial response distribution and a cumulative logit link function. Data were summarized using the FREQ procedure of SAS and reported as percentage of observations within each fecal score category by treatment and day. For TNF-α and IL-6 assays, microplate was used as a random effect. A $\log_{10}$ transformation was used for fecal *E. coli* colony counts. Tukey adjustment was used for multiple comparisons. All models were fit using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC). Pearson correlation coefficients were determined using the CORR procedure of SAS. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results and Discussion

Growth performance

From d 0 to 9, pigs fed Kavault had increased ($P < 0.05$) BW, ADG, and improved ($P < 0.05$) feed efficiency compared to pigs fed the control or Protect-8 Plus, with pigs fed Proteck intermediate (Table 2). There was no evidence of a difference ($P > 0.05$) in ADFI between treatments from d 0 to 9. From d 9 to 21, pigs fed Kavault had greater ($P < 0.05$) BW, ADG, and ADFI compared to all other treatments. Pigs fed Kavault had improved ($P < 0.05$) F/G compared to pigs fed Proteck, with pigs fed the control diet or Protect-8 Plus intermediate. For the experimental period (d 0 to 21), pigs fed Kavault had increased ($P < 0.05$) BW, ADG, ADFI, and better F/G compared to those fed the two clay additives. There was no benefit beyond the control diet when pigs were fed the two clay additives.

During the common period (d 21 to 42), a marginally significant ($P = 0.064$) difference in ADFI was observed between treatments, and pigs previously fed Kavault had greater ($P < 0.05$) ADFI compared to pigs previously fed Protect-8 Plus, with the control and Proteck treatments intermediate. Pigs previously fed Protect-8 Plus had improved ($P < 0.05$) F/G compared to pigs previously fed Kavault, with pigs fed the control diet or Proteck intermediate. There was no evidence ($P > 0.10$) of a difference in ADG between dietary treatments.

For the overall period (d 0 to 42), pigs fed Kavault from d 0 to 21 had increased ($P < 0.05$) final BW, ADG, and ADFI compared to all other treatments. We observed no statistical differences ($P > 0.10$) in F/G.

Fecal analysis

For analysis of fecal scores, no treatment × day interaction or treatment differences were observed ($P > 0.10$; Figure 1). Pigs had a higher ($P < 0.001$) frequency of softer

feces on d 21 compared to d 6 and d 9. Fecal *E. coli* colony count was lower ($P < 0.001$) on d 21 compared to d 9 (Table 3). No differences ($P > 0.10$) were observed across treatments for fecal *E. coli* colony count or virulence genes associated with *E. coli* (Table 4). Fecal colony counts indicate the quantity of *E. coli* being shed in the feces. The goal of this analysis was to determine if there could be any differences in fecal coliform counts with different diet strategies.

Myeloperoxidase is an enzyme that is present in neutrophils. With inflammation in the small intestine, neutrophils will migrate to those tissues to assist with the inflammatory process. Neutrophils will then release MPO which can be measured in the feces with greater concentration when gut inflammation is present. For fecal MPO, no treatment × day or treatment differences were observed ($P > 0.10$; Table 3). However, we observed lower ($P < 0.05$) concentrations of fecal MPO on d 9 compared to d 6, and lower concentrations on d 21 compared to both d 6 and 9.

For fecal DM, no treatment × day interaction was observed ($P > 0.10$). Pigs fed Kavault had decreased ($P < 0.05$) fecal DM percentage compared to all other treatments. Fecal DM percentage was higher ($P < 0.05$) on d 6 and 9 compared to d 21.

There was no correlation between fecal *E. coli* count and fecal DM, fecal score, fecal MPO, or ADG throughout the study ($P > 0.10$; Table 5). There was a strong negative correlation between DM and score ($P < 0.001$) on d 6, 9, and 21 indicating that as fecal DM increased, the score became closer to 0, representing a firmer fecal sample. There was no correlation between MPO and score on d 6 and 9 ($P > 0.10$). However, a marginally significant ($P = 0.068$) positive correlation was observed on d 21 between MPO and score. Dry matter and MPO were negatively correlated on d 6 and 9 ($P \leq 0.02$), but no correlation was observed on d 21 ($P > 0.10$). Additionally, no correlation between ADG and score were observed on d 9 or 21 ($P > 0.10$). Fecal DM on d 6 and 9 were negatively correlated with ADG from d 0 to 9, meaning that as growth rate increased, fecal DM decreased. Fecal MPO on d 9 was positively correlated with ADG from d 0 to 9, and fecal MPO on d 21 was positively correlated with ADG from d 0 to 21, meaning that pigs with greater gain generally tended to have greater fecal MPO. Fecal DM on d 6 was negatively correlated with ADG from d 0 to 21, however, fecal DM on d 9 was positively correlated with ADG from d 0 to 21.

Blood analysis

No treatment × day or treatment differences were observed for TNF-α and IL-6 blood assays ($P > 0.10$). For IL-6, concentrations were higher ($P = 0.015$) on d 9 compared to d 21 (Table 3). No evidence of a change in TNF-α concentration over time was observed ($P > 0.10$).

In summary, pigs fed Kavault had increased BW, ADG, and ADFI, but lower fecal DM compared with pigs fed the control diet or those fed the two clay-based additives. However, there were no treatment effects on fecal score, fecal MPO, or blood measurements. We observed a day effect indicating that feces were softer and had less DM on d 21 compared to d 6. Additionally, fecal *E. coli* colony count and fecal MPO had lower concentrations on d 21 compared to d 9. There was a strong negative correlation between fecal DM and score ($P < 0.001$) on d 6, 9, and 21 indicating that as fecal DM increased, the score became closer to 0, representing a firmer fecal sample. Fecal DM on

d 6 and fecal DM on d 9 were negatively correlated with ADG from d 0 to 9 meaning that as growth rate increased, fecal DM decreased.

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

Ingredient, %	Phase 1[2]	Phase 2[3]	Phase 3[4]
Corn[5]	44.50	49.81	64.24
Soybean meal, dehulled	17.41	23.55	31.78
Bovine blood plasma	2.00	---	---
DDGS	5.00	7.50	---
Fish meal	2.50	---	---
Whey powder	10.00	---	---
Whey permeate	10.00	10.00	---
Choice white grease	1.00	1.00	---
Calcium carbonate	0.45	0.45	0.90
Monocalcium P	0.80	1.00	1.00
Salt	0.30	0.50	0.60
L-Lys-HCl	0.40	0.55	0.50
DL-Met	0.18	0.22	0.21
L-Thr	0.17	0.22	0.21
L-Trp	0.02	0.04	0.04
L-Val	0.08	0.12	0.13
Vitamin and trace mineral premixes[6]	0.40	0.40	0.40
Zinc oxide	0.40	0.25	---
Microbial-enhanced soy protein[7]	4.00	4.00	---
Proteck[8]	+/-	+/-	---
Protect-8 Plus[9]	+/-	+/-	---
Kavault[10]	+/-	+/-	---
Total	100	100	100

continued

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

Ingredient, %	Phase 1[2]	Phase 2[3]	Phase 3[4]
Calculated analysis			
SID AA, %			
Lys	1.35	1.35	1.35
Ile:Lys	57	57	56
Leu:Lys	120	118	115
Met:Lys	35	37	36
Met and Cys:Lys	58	58	58
Thr:Lys	64	63	63
Trp:Lys	19.2	19.3	19.4
Val:Lys	70	70	70
Total Lys, %	1.51	1.51	1.50
ME, kcal/lb	1,543	1,530	1,487
NE, kcal/lb	1,153	1,135	1,097
SID Lys:ME, g/Mcal	3.97	4.00	4.12
CP, %	21.5	21.5	21.3
Ca, %	0.66	0.54	0.71
STTD P, %	0.58	0.51	0.47

[2]Phase 1 diets were fed from d 0 to 9 (14 to 18 lb).
[3]Phase 2 diets were fed from d 9 to 21 (18 to 29 lb).
[4]A common phase 3 diet was fed to all pigs from d 21 to 42 (29 to 60 lb).
[5]Additives were included at the expense of corn during phase 1 and 2.
[6]Ronozyme HiPhos (DSM, Parsippany, NJ) included at 1,250 FTU/kg provided an estimated release of 0.13% STTD P.
[7]Me-Pro, Prairie AquaTech, Brookings, SD.
[8]Proteck (Nutriquest, Mason City, IA) 0.40% of the diet.
[9]Protect-8 Plus (Essential Ag Solutions, Sioux Falls, SD) 0.10% of the diet.
[10]Kavault (Elanco, Greenfield, IN) 0.04% of the diet.

Table 2. Evaluation of clay-based binders or an in-feed antimicrobial on nursery pig growth performance[1]

Item	Control	Proteck[2]	Protect-8 Plus[3]	Kavault[4]	SEM	*P* =
BW, lb						
d 0	14.2	14.2	14.2	14.2	0.08	0.965
d 9	17.3^{b}	17.6^{a,b}	17.3^{b}	17.9^{a}	0.21	0.011
d 21	28.6^{b}	28.7^{b}	28.4^{b}	30.8^{a}	0.38	< 0.001
d 42	59.2^{b}	59.1^{b}	58.7^{b}	61.5^{a}	0.87	0.002
d 0 to 9 (phase 1)						
ADG, lb	0.34^{b}	0.38^{a,b}	0.34^{b}	0.41^{a}	0.018	0.003
ADFI, lb	0.40	0.41	0.39	0.43	0.021	0.158
F/G	1.19^{a}	1.10^{a,b}	1.17^{a}	1.06^{b}	0.022	0.003
d 9 to 21 (phase 2)						
ADG, lb	0.95^{b}	0.92^{b}	0.93^{b}	1.07^{a}	0.042	< 0.001
ADFI, lb	1.13^{b}	1.11^{b}	1.10^{b}	1.26^{a}	0.046	< 0.001
F/G	1.19^{a,b}	1.22^{a}	1.19^{a,b}	1.18^{b}	0.007	0.077
d 0 to 21 (experimental period)						
ADG, lb	0.69^{b}	0.69^{b}	0.68^{b}	0.79^{a}	0.021	< 0.001
ADFI, lb	0.81^{b}	0.81^{b}	0.80^{b}	0.90^{a}	0.022	< 0.001
F/G	1.19^{a}	1.19^{a}	1.18^{a}	1.15^{b}	0.007	0.003
d 21 to 42 (common period)						
ADG, lb	1.45	1.45	1.44	1.46	0.025	0.931
ADFI, lb	2.16^{a,b}	2.15^{a,b}	2.11^{b}	2.21^{a}	0.042	0.064
F/G	1.49^{a,b}	1.49^{a,b}	1.46^{b}	1.52^{a}	0.005	0.002
d 0 to 42						
ADG, lb	1.07^{b}	1.06^{b}	1.06^{b}	1.12^{a}	0.022	0.005
ADFI, lb	1.49^{a,b}	1.48^{b}	1.45^{b}	1.56^{a}	0.031	0.003
F/G	1.39	1.39	1.37	1.39	0.004	0.233

[1]A total of 360 barrows (initially 14.2 ± 0.08 lb) were used with 5 pigs per pen and 18 replications per treatment. Treatment diets were fed in phases 1 and 2. A common phase 3 diet was fed to all pigs from d 21 to 42.
[2]Nutriquest, Mason City, IA.
[3]Essential Ag Solutions, Sioux Falls, SD.
[4]Elanco, Greenfield, IN.

Table 3. Main effects of clay-based binders or an in-feed antimicrobial on nursery pig fecal characteristics and serum cytokine concentrations[1]

	Treatment						Day				
Item	Control	Proteck[2]	Protect-8 Plus[3]	Kavault[4]	SEM	*P* =	6	9	21	SEM	*P* =
Fecal sample											
E. coli colony count, $\log_{10}$	3.20	2.49	3.01	3.45	0.453	0.485	---[6]	4.03[a]	2.04[b]	0.320	< 0.001
MPO, nmol/min/mL[5]	0.036	0.041	0.038	0.042	0.0032	0.447	0.059[a]	0.045[b]	0.014[c]	0.0031	< 0.001
DM, %	27.39[a]	26.29[a]	26.81[a]	25.04[b]	0.498	< 0.001	26.64[a]	27.63[a]	24.88[b]	0.439	< 0.001
Serum											
TNF-α	173.5	174.3	170.7	163.4	12.29	0.836	---	173.4	167.6	12.20	0.530
IL-6	6.6	7.0	6.2	6.8	1.65	0.983	---	9.6[a]	3.7[b]	1.45	0.015

[1]A total of 360 barrows (initially 14.2 ± 0.08 lb) were used with 5 pigs per pen and 18 replications per treatment. Fecal samples were collected individually from 3 pigs per pen (216 pigs total) on d 6, 9, and 21.

[2]Nutriquest, Mason City, IA.

[3]Essential Ag Solutions, Sioux Falls, SD.

[4]Elanco, Greenfield, IN.

[5]MPO = fecal myeloperoxidase.

[6]Fecal samples were not analyzed for *E. coli* enumeration and serum was not collected on d 6.

Table 4. Effect of clay-based binders or an in-feed antimicrobials on detection of virulence genes with fecal *E. coli*[1]

Item	Control	Proteck[2]	Protect-8 Plus[3]	Kavault[4]	*P* =
Virulence gene					
ehxA	0/720	0/720	0/720	10/720	1.000
estA	6/720	10/720	0/720	0/720	1.000
estB	6/720	10/720	0/720	0/720	1.000
astA	10/720	13/720	14/720	1/720	0.976
hlyA	80/720	44/720	84/720	51/720	0.113
eae	29/720	40/720	23/720	62/720	0.777
stx1	0/720	0/720	0/720	1/720	1.000
O8	1/720	0/720	0/720	0/720	1.000
O9	0/720	0/720	1/720	0/720	1.000
O26	0/720	0/720	0/720	1/720	1.000
O111	1/720	0/720	0/720	0/720	1.000
O180	1/720	1/720	0/720	1/720	1.000
O182	1/720	1/720	0/720	0/720	1.000
K88	1/720	0/720	0/720	0/720	1.000

[1] A total of 360 barrows (initially 14.2 ± 0.08 lb) were used with 5 pigs per pen and 18 replications per treatment. Detection of virulence genes associated with *E. coli* were analyzed on 1 pig per pen (18 pigs per treatment). Values represent the count of detection of *E. coli* virulence genes of 720 *E. coli* isolates (10 isolates per sample). *BfpA, stx2,* and *elt* were non-detectable for all samples.

[2]Nutriquest, Mason City, IA.

[3]Essential Ag Solutions, Sioux Falls, SD.

[4]Elanco, Greenfield, IN.

Table 5. Correlations between fecal characteristics on d 6, 9, and 21 of the experiment[1,2]

	Day 6			Day 9				Day 21			
	DM	Score	MPO[3]	DM	Score	MPO	*E. coli* count	DM	Score	MPO	*E. coli* count
Day 6											
Score	r = -0.61										
	P = 0.001										
MPO	r = -0.40	r = 0.09									
	P = 0.001	*P* = 0.461									
Day 9											
DM	r = 0.32	r = -0.19	r = 0.05								
	P < 0.001	*P* = 0.006	*P* = 0.664								
Score	r = -0.13	r = 0.12	r = -0.18	r = -0.67							
	P = 0.053	*P* = 0.072	*P* = 0.122	*P* < 0.001							
MPO	r = -0.004	r = 0.11	r = 0.16	r = -0.29	r = 0.18						
	P = 0.971	*P* = 0.366	*P* = 0.176	*P* = 0.016	*P* = 0.130						
E. coli count	r = 0.13	r = -0.11	r = 0.001	r = 0.04	r = -0.06	r = 0.03					
	P = 0.280	*P* = 0.347	*P* = 0.999	*P* = 0.747	*P* = 0.591	*P* = 0.801					
Day 21											
DM	r = 0.13	r = -0.11	r = 0.08	r = 0.28	r = -0.22	r = 0.23	r = 0.01				
	P = 0.058	*P* = 0.101	*P* = 0.534	*P* < 0.001	*P* = 0.001	*P* = 0.056	*P* = 0.922				
Score	r = -0.11	r = 0.18	r = -0.10	r = -0.13	r = 0.07	r = -0.03	r = -0.03	r = -0.51			
	P = 0.111	*P* = 0.007	*P* = 0.397	*P* = 0.070	*P* = 0.301	*P* = 0.830	*P* = 0.804	*P* < 0.001			
MPO	r = -0.021	r = 0.067	r = 0.272	r = -0.27	r = 0.307	r = 0.29	r = 0.06	r = -0.10	r = 0.22		
	P = 0.860	*P* = 0.578	*P* = 0.021	*P* = 0.022	*P* = 0.01	*P* = 0.015	*P* = 0.600	*P* = 0.400	*P* = 0.068		
E. coli count	r = 0.075	r = -0.05	r = 0.009	r = 0.121	r = -0.10	r = -0.06	r = 0.09	r = -0.12	r = -0.08	r = -0.05	
	P = 0.533	*P* = 0.696	*P* = 0.942	*P* = 0.315	*P* = 0.416	*P* = 0.595	*P* = 0.433	*P* = 0.318	*P* = 0.495	*P* = 0.663	
d 0 to 9 ADG	r = -0.22	r = -0.09	r = 0.18	r = -0.19	r = -0.01	r = 0.43	r = 0.15	r = -0.01	r = 0.05	r = 0.27	r = -0.01
	P = 0.001	*P* = 0.193	*P* = 0.140	*P* = 0.006	*P* = 0.947	*P* = 0.001	*P* = 0.202	*P* = 0.897	*P* = 0.422	*P* = 0.022	*P* = 0.926
d 0 to 21 ADG[2]	r = -0.16	r = 0.07	r = 0.13	r = 0.23	r = 0.08	r = 0.28	r = -0.10	r = -0.05	r = 0.07	r = 0.28	r = -0.14
	P = 0.017	*P* = 0.300	*P* = 0.290	*P* = 0.001	*P* = 0.264	*P* = 0.017	*P* = 0.396	*P* = 0.447	*P* = 0.276	*P* = 0.019	*P* = 0.245

[1]A total of 360 barrows (initially 14.2 ± 0.08 lb) were used with 5 pigs per pen and 18 replications per treatment. Fecal samples were collected individually from 3 pigs per pen (216 pigs total) on d 6, 9, and 21.

[2]Correlation between d 0 to 9 ADG and d 0 to 21 ADG: r = 0.72, *P* < 0.001.

[3]MPO = fecal myeloperoxidase.

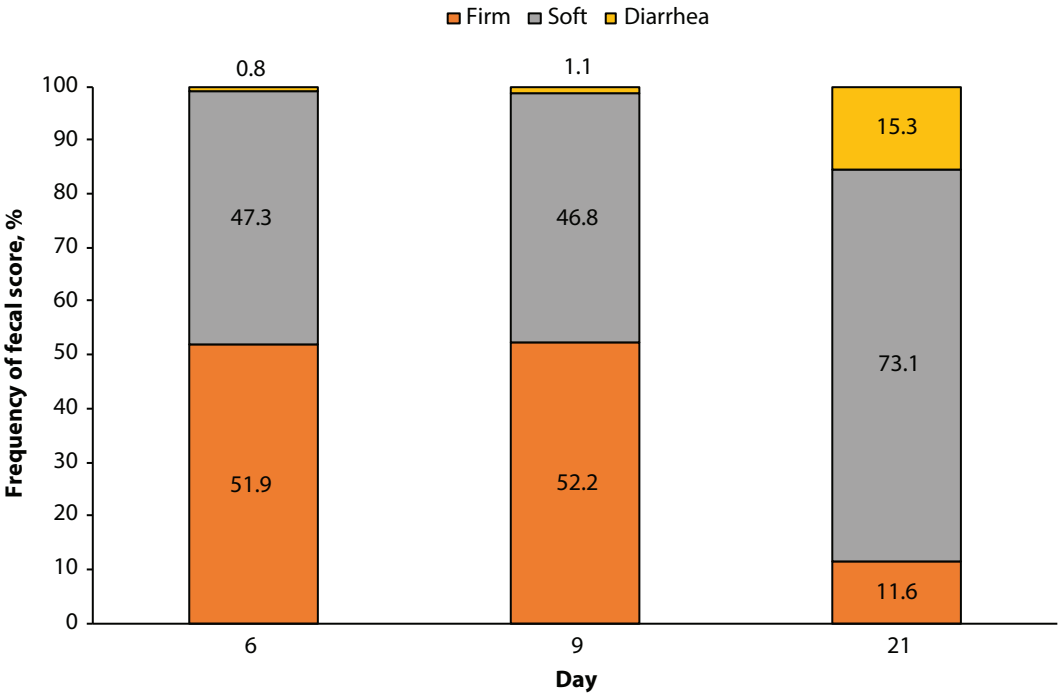

Figure 1. Fecal score frequency by day. Treatment × day, $P = 0.311$; Treatment, $P = 0.326$; Day, $P < 0.001$.