Evaluation of Trypsin Inhibitor Unit Level with or without the Addition of Protease and an In-feed Acidifier on Nursery Pig Growth Performance, Fecal Dry Matter, and Nutrient Digestibility

Sierra M. Collier, Skyler D. Ward, Katelyn N. Gaffield, Robert D. Goodband, Mike D. Tokach, Jason C. Woodworth, Joel M. DeRouchey, Hari B. Krishnan,[1] and Jordan T. Gebhardt[2]

Summary

A total of 360 barrows (DNA 200 × 400; initially 12.9 ± 0.59 lb) were used in a 40-d growth trial to evaluate the effects of increasing trypsin inhibitor with or without the addition of protease and benzoic acid on nursery pig growth performance, fecal dry matter, and nutrient digestibility. Pigs were weaned at approximately 21 d of age and randomly allotted to pens. Pens of pigs were blocked by initial BW and allotted to one of eight treatments in a randomized complete block design with five pigs per pen and nine pens per treatment across two barns. The eight treatments were arranged in a 2 × 2 × 2 factorial design with main effects of trypsin inhibitor (1.4 or 2.8 TIU/mg of complete feed), protease (none or 50 mg/kg ProAct 360), and benzoic acid (none or 0.5% VevoVitall). Soy flour (80 TIU/mg) was added at the expense of soybean meal (7 TIU/mg) to create the 1.4 or 2.8 TIU/mg of complete feed levels. Diet formulation was based on analyzed nutrient values of the soy flour and soybean meal, assuming digestibility coefficients for soy flour equivalent to those of soybean meal. Experimental diets were fed in three phases: phase 1 from d 0 to 10, phase 2 from d 10 to 24, and phase 3 from d 24 to 40. Feces were collected from three pigs per pen on d 10 and 40 to determine fecal DM, and d 40 fecal samples were used to determine apparent total tract digestibility (ATTD) of DM and CP. The main effect of TIU negatively impacted ($P \leq 0.014$) growth performance in all phases, while the main effect of acidifier tended ($P \leq 0.089$) to improve ADG and F/G in phase 3. No significant main effects of protease in the phases were observed. Overall, from d 0 to 40, there was a three-way interaction observed ($P = 0.024$) where in low TIU diets, pigs fed diets with either acidifier or protease had numerically lower ADG compared to the low TIU control and the low TIU diet with both acidifier and protease. However, in high TIU diets, pigs fed either acidifier or protease had greater ADG compared to the high TIU control diet

[1] Agricultural Research Service, U.S. Department of Agriculture and Division of Plant Science and Technology, University of Missouri, Columbia, MO 65211.
[2] Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.

without any additive and the high TIU diet with both acidifier and protease. A similar TIU × protease × acidifier interaction was marginally significant for ADFI ($P = 0.054$). Pigs fed diets with 2.8 TIU/mg of complete feed had increased d 10 fecal DM; however, there were no differences on d 40. For the ATTD of DM and CP, there was an interaction ($P = 0.028$) between TIU and acid, where pigs fed the acidifier had improved ATTD compared to pigs fed no acid in a diet containing 1.4 TIU/mg of complete feed; however, pigs fed 2.8 TIU/mg of complete feed had similar ATTD regardless of acid inclusion. For overall main effects, increased TIU worsened ($P < 0.05$) ADG, ADFI, and F/G; protease tended to worsen ($P = 0.067$) F/G; and acidifier had no effects on growth performance. This study suggests that increased dietary TIU negatively affects nursery pig performance, but the inclusion of either a protease or benzoic acid may help mitigate a portion of the effect of high TIU, even as low as 2.8 TIU/mg of complete feed.

Introduction

The nursery phase is a critical period when weaned pigs are first exposed to solid feed that often contains soybean meal (SBM) as a primary protein source. While nutritionally valuable, SBM contains anti-nutritional factors, particularly trypsin inhibitors (TI), which reduce protein and amino acid digestibility, decrease growth performance, and contribute to post-weaning diarrhea. A recent study by Collier et al. (2025)[3] observed that increasing dietary trypsin inhibitor unit (TIU) concentrations above 1.4 TIU/mg of complete feed decreased nursery pig growth performance and nutrient digestibility. These findings highlight the importance of developing effective strategies to mitigate the effects of trypsin inhibitors in nursery diets.

One strategy to improve protein digestibility in weaned pigs is the inclusion of exogenous protease enzymes (Zuo et al., 2015).[4] Additionally, dietary acidifiers, such as benzoic acid, may lower the pH of the gastrointestinal tract, leading to improvements in growth performance and nutrient digestion, while also potentially creating a more favorable environment for optimal exogenous protease activity. However, limited data exist on the combined effects of protease and acidifiers under varying TIU concentrations. Therefore, the objective of this study was to evaluate the effects of dietary protease and benzoic acid in nursery diets containing low and high levels of TIU on pig performance, fecal dry matter, and nutrient digestibility.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This study was conducted at the Kansas State University Segregated Early Weaning Research Facility in Manhattan, KS. The facility has two identical barns that are completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a four-hole, dry self-feeder and a cup waterer for *ad libitum* access to feed and water. Pens (4 × 4 ft) had metal tri-bar floors and allowed approximately 2.7 ft^2/pig.

[3] Collier, S. M., P.T. Soper, K. N. Gaffield, R. D. Goodband, M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, H. B. Krishnan, and J. T. Gebhardt. 2025. Evaluation of increasing trypsin inhibitor on nursery pig growth performance, fecal dry matter, and nutrient digestibility.

[4] Zuo, J., B. Ling, L. Long, T. Li, L. Lahaye, C. Yang, and D. Feng. 2015. Effect of dietary supplementation with protease on growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression of weaned piglets. Anim. Nutr. 1:276–282. doi:10.1016/j.aninu.2015.10.003.

Animals and diets

A total of 360 barrows (DNA 200 × 400; initially 12.9 ± 0.59 lb) were used across two barns in a 40-d growth trial. Pigs were weaned at approximately 21 d of age and assigned to pens blocked by initial body weight (BW). Pens of five pigs were randomly allotted to one of eight dietary treatments in a randomized complete block design with nine replicates per treatment. Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of trypsin inhibitor (1.4 or 2.8 TIU/mg of complete feed), protease (none or 50 mg/kg ProAct 360, DSM Nutritional Products, Parsippany, NJ), and benzoic acid (none or 0.5% VevoVitall, DSM Nutritional Products, Parsippany, NJ). Diets were formulated with a blend of soybean meal (7 TIU/mg) and soy flour (80 TIU/mg) to achieve the target TIU/mg of complete feed while having the same total soy product inclusion. Dietary TIU, protease, and acidifier were formulated to remain consistent among the three phases. Samples of soybean meal and soy flour were collected prior to diet formulation and analyzed for proximate analysis, amino acid profiles, and TIU (Table 1). Diet formulation was based on analyzed nutrient values of the soy flour and soybean meal, assuming digestibility coefficients equivalent to those of soybean meal suggested by the NRC (2012).[5] Pigs were fed treatment diets in meal form for all three nursery phases with phase 1 from d 0 to 10, phase 2 from d 10 to 24, and phase 3 from d 24 to 40 (Table 2). Treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

Pig weights and feed disappearance were measured on d 0, 10, 17, 24, 31, and 40 to determine ADG, ADFI, and feed efficiency (F/G). Feces were collected from three pigs per pen on d 10 and 40 to determine percentage fecal dry matter. Additionally, titanium dioxide was included in phase 3 diets as an indigestible marker to determine apparent total tract digestibility (ATTD) of DM and CP from samples collected on d 40.

Fecal DM and digestibility analysis

At the conclusion of the study, fecal samples from d 10 and 40 were dried at 130°F for 48 h. The loss of weight was used to calculate fecal DM percent. Fecal samples were analyzed separately for each pig, and the average of the three samples from each pen was then used for statistical analysis.

Following fecal DM determination, both ground feed and d 40 fecal samples were dried at 275°F for 2 h to determine percentage DM of the samples used for titanium analysis. The titanium dioxide concentration in both dried feed and fecal samples was determined utilizing procedures outlined by Leone (1973).[6] The ATTD of DM and CP were determined using the index method described by Adeola (2001).[7]

Statistical analysis

Growth data were analyzed as a randomized complete block design for a three-way ANOVA using the lmer function from the lme4 package in R Studio (version 3.5.2, R Core team. Vienna, Austria). Pen served as the experimental unit with the fixed effect of dietary treatment and random effect of block. Three-way interactions, two-way inter-

[5] Nutrient Requirements of Swine: Eleventh Revised Edition. 2012. National Academies Press, Washington, D.C.

[6] Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. AOAC. 56(3):535.

[7] Adeola, O. 2001. Digestion and balance techniques in pigs. pp. 903. Swine Nutrition, 2nd ed. A. J. Lewis and L. L. Southern ed. CRC Press, Washington, DC.

actions, and main effects of TIU, protease, and benzoic acid were evaluated. Results were considered significant with $P < 0.05$ and marginally significant at $0.05 < P \le 0.10$.

Results and Discussion

For phase 1 (d 0 to 10), there was a tendency ($P \le 0.072$) for an interaction between TIU and acid, where pigs fed the acidifier had numerically decreased d 10 BW, ADG, and ADFI compared to pigs fed no acid in a diet containing 2.8 TIU/mg of complete feed. However, pigs fed 1.4 TIU mg/complete feed performed similarly, regardless of acid inclusion. There was a main effect of TIU, where pigs consuming 2.8 TIU/mg of complete feed had poorer ($P \le 0.012$) d 10 BW, ADG, ADFI, and F/G compared to pigs fed 1.4 TIU/mg. However, there were no differences ($P > 0.10$) due to the main effects of protease or acid inclusion.

For phase 2 (d 10 to 24), there was an interaction ($P \le 0.041$) between TIU, acid, and protease, where pigs fed the combination of both the acidifier and protease had numerically greater d 24 BW and ADG than when the additives were included individually in diets containing 1.4 TIU/mg of complete feed. Inversely, in diets containing 2.8 TIU/mg complete feed, pigs fed both the acidifier and protease had the poorest performance compared to the other diets with the same TIU. Although the means did not separate, there was an F/G interaction ($P = 0.035$) between TIU, acid, and protease, where pigs fed the acidifier, protease, or a combination of both in a diet containing 1.4 TIU/mg of complete feed had numerically poorer F/G compared to pigs fed no additives. Conversely, pigs fed no additives in the diet containing 2.8 TIU/mg had numerically poorer F/G compared to pigs fed the acidifier or protease either individually or in combination. There was a main effect of TIU, where pigs fed diets with 2.8 TIU/mg of complete feed had decreased ($P \le 0.014$) d 24 BW, ADG, and ADFI compared to pigs fed 1.4 TIU/mg. However, there were no differences ($P > 0.10$) due to the main effects of protease or acid inclusion.

For phase 3 (d 24 to 40), there was a three-way interaction ($P \le 0.020$) observed for the d 40 BW and ADFI and a tendency ($P = 0.055$) for ADG between TIU, acid, and protease. Pigs fed either no additive, or the combination of both the acidifier and protease had greater performance than when the additives were included individually in diets containing 1.4 TIU/mg of complete feed. In diets containing 2.8 TIU/mg complete feed, pigs fed no feed additive or the diet with both acidifier and protease had numerically lower performance than pigs fed either the acid or protease individually. There was a main effect of TIU, with pigs fed diets with 2.8 TIU/mg of complete feed having poorer ($P \le 0.010$) d 40 BW, ADG, ADFI, and F/G compared to pigs fed 1.4 TIU/mg. There tended ($P \le 0.089$) to be a main effect of acidifier, where pigs fed diets with benzoic acid had improved ADG and F/G. There was no main effect difference ($P > 0.10$) due to the inclusion of protease.

Overall (d 0 to 40), there was an interaction ($P = 0.024$) for ADG and a tendency ($P = 0.054$) for an interaction for ADFI between TIU, acid, and protease, where pigs fed either the acidifier or protease had poorer performance than when the additives were included together in diets containing 1.4 TIU/mg of complete feed; however, the opposite was observed in diets containing 2.8 TIU/mg of complete feed. Overall, the main effect of TIU showed pigs fed diets with 2.8 TIU/mg of complete feed had poorer ($P \le 0.005$) ADG, ADFI, and F/G compared to pigs fed 1.4 TIU/mg. There tended ($P = 0.067$) to be a main effect of protease, where pigs fed diets with protease had poorer F/G. There were no overall main effect differences ($P > 0.10$) due to added acidifier.

For fecal DM on d 10, there were no interactive effects between TIU, protease, and acid. There was a main effect of TIU ($P = 0.030$), with pigs fed diets with 2.8 TIU/mg of complete feed having increased d 10 fecal DM. However, there were no differences in fecal DM on d 40. Additionally, there was a main effect of day ($P < 0.001$) with fecal DM being lower on d 10 compared to d 40.

For the ATTD of DM, there was an interaction ($P = 0.028$) between TIU and acid, where pigs fed the acidifier had improved DM digestibility compared to pigs fed no acid in diets containing 1.4 TIU/mg of complete feed; however, there was no response to acidifier in pigs fed 2.8 TIU/mg of complete feed. For the ATTD of CP, there was an interaction ($P = 0.001$) between TIU and acid, where pigs fed the acidifier had decreased CP digestibility compared to pigs fed no acid in a diet containing 2.8 TIU/mg of complete feed. Inversely, pigs fed the acidifier had improved CP digestibility compared to pigs fed no acid in a diet containing 1.4 TIU/mg of complete feed. For the ATTD of DM and CP, there were no differences ($P > 0.10$) due to the main effects of TIU, protease, or acidifier.

In summary, pigs fed diets containing 1.4 TIU/mg of complete feed, with or without the combination of dietary acidifier and protease, performed similarly with greater performance than when acidifier and protease were included individually. Pigs fed diets containing 2.8 TIU/mg of complete feed with acid and protease added individually performed similarly with greater performance than pigs fed diets without or with the combination of the acidifier and protease. Pigs fed diets containing 2.8 TIU/mg of complete feed had poorer growth performance compared to pigs fed diets containing 1.4 TIU/mg throughout the entire study. This data suggest that minimizing dietary TIU exposure in nursery diets is critical for growth performance. Strategic use of feed additives like protease or benzoic acid, when added individually, may offset a portion of the negative impacts of high TIU diets.

Acknowledgment
Appreciation is expressed to DSM Nutritional Products, Parsippany, NJ for financial support of this study.

Table 1. Chemical analysis of soybean meal and soy flour[1]

Item	Soybean meal	Soy flour
Trypsin inhibitor units, TIU/mg	7.12	80.26
Proximate analysis, %		
Moisture	10.30	6.92
Crude protein	45.29	50.15
Crude fat	3.10	0.58
Crude fiber	3.01	3.35
Ash	5.86	6.31
Total amino acid concentration, %		
Cys	0.67	0.77
His	1.21	1.34
Ile	2.20	2.42
Leu	3.51	3.89
Lys	2.95	3.32
Met	0.63	0.68
Thr	1.73	1.97
Trp	0.56	0.61
Val	2.35	2.55

[1] Values represent the mean of samples analyzed in duplicate.

Table 2. Diet composition (as-fed basis)[1]

	Phase 1		Phase 2		Phase 3	
Item TIU:	**1.4**	**2.8**	**1.4**	**2.8**	**1.4**	**2.8**
Ingredients, %						
Corn	51.26	51.30	58.45	58.45	69.54	69.51
Soybean meal	17.57	15.53	24.50	22.51	25.83	23.86
Soy flour	0.55	2.55	0.05	2.05	0.00	2.00
Whey powder	12.50	12.50	5.00	5.00	---	---
Whey permeate, 80% lactose	7.50	7.50	5.00	5.00	---	---
Spray-dried blood plasma	2.50	2.50	---	---	---	---
Fish meal	5.00	5.00	3.25	3.25	---	---
Calcium carbonate	0.53	0.53	0.63	0.63	0.80	0.80
Monocalcium P (21% P)	0.45	0.45	0.75	0.75	0.95	0.95
Salt	0.40	0.40	0.50	0.50	0.60	0.60
L-Lys-HCl	0.34	0.34	0.46	0.46	0.55	0.55
DL-Met	0.19	0.19	0.22	0.22	0.23	0.23
L-Thr	0.18	0.18	0.23	0.23	0.26	0.26
L-Trp	0.05	0.05	0.07	0.07	0.08	0.08
L-Val	0.11	0.11	0.15	0.15	0.16	0.16
L-Ile	0.03	0.03	0.04	0.04	0.04	0.04
Zinc oxide	0.40	0.40	0.25	0.25	---	---
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
Phytase[2]	0.06	0.06	0.06	0.06	0.06	0.06
Protease[3]	+/-	+/-	+/-	+/-	+/-	+/-
Benzoic acid[4]	+/-	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100	100
Calculated analysis						
SID AA, %						
Lys	1.35	1.35	1.35	1.35	1.30	1.30
Ile:Lys	56	56	57	57	56	56
Met and Cys:Lys	58	58	58	58	58	58
Thr:Lys	64	64	64	64	64	64
Trp:Lys	19.2	19.2	19.1	19.1	19.1	19.1
Val:Lys	70	70	70	70	70	70
His:Lys	34	34	34	34	34	34
NE, kcal/lb	1,130	1,111	1,115	1,096	1,102	1,083
STTD P, %	0.57	0.56	0.53	0.52	0.45	0.45
Analyzed composition						
TIU/mg of complete feed	1.20	2.94	1.07	2.53	1.47	2.52

[1] Phase 1 diets were from d 0 to 10, phase 2 from d 10 to 24, and phase 3 from d 24 to 40.
[2] HiPhorius 2400 added at 1,500 FTU/kg feed provided an estimated release of 0.13% STTD P.
[3] Protease (ProAct 360, DSM Nutritional Products, Parsippany, NJ) at 50 mg/kg was included at the expense of corn.
[4] Benzoic acid (VevoVitall, DSM Nutritional Products, Parsippany, NJ) at 0.50% of the diet was included at the expense of corn.

Table 3. Interactive effects of trypsin inhibitor unit (TIU) level in diets with or without the addition of protease and an in-feed acidifier on nursery pig performance[1]

	TIU/mg of complete feed									P =[2]	
	1.4				2.8						
Protease:[3]	No		Yes		No		Yes			TIU × protease × acid	TIU × acid
Benzoic acid:[4]	No	Yes	No	Yes	No	Yes	No	Yes	SEM		
Body weight, lb											
d 0	12.9	12.9	12.9	12.9	12.9	12.9	13.0	13.0	0.59	0.972	0.834
d 10	17.3	17.1	16.8	17.2	16.7	16.1	16.8	16.4	0.72	0.345	0.065
d 24	32.0[a]	30.8[ab]	31.0[ab]	31.9[a]	30.1[ab]	30.0[ab]	30.3[ab]	29.6[b]	1.09	0.041	0.663
d 40	52.9[a]	51.8[ab]	51.1[ab]	53.0[a]	49.1[b]	50.0[ab]	50.2[ab]	49.2[b]	1.53	0.020	0.658
Phase 1 (d 0 to 10)											
ADG, lb	0.44	0.42	0.39	0.43	0.37	0.32	0.39	0.35	0.019	0.333	0.056
ADFI, lb	0.51	0.48	0.47	0.52	0.48	0.43	0.49	0.45	0.021	0.169	0.072
F/G	1.17	1.19	1.24	1.21	1.29	1.36	1.26	1.32	0.029	0.734	0.226
Phase 2 (d 10 to 24)											
ADG, lb	1.05[a]	0.98[ab]	1.01[ab]	1.04[ab]	0.97[ab]	0.99[ab]	0.96[ab]	0.94[b]	0.029	0.018	0.469
ADFI, lb	1.38	1.32	1.37	1.40	1.33	1.30	1.31	1.28	0.043	0.355	0.812
F/G	1.32	1.35	1.36	1.35	1.39	1.32	1.36	1.36	0.014	0.035	0.092
Phase 3 (d 24 to 40)											
ADG, lb	1.31[a]	1.32[a]	1.26[ab]	1.32[a]	1.19[b]	1.25[ab]	1.24[ab]	1.23[ab]	0.035	0.055	0.759
ADFI, lb	2.02[a]	1.99[ab]	1.96[ab]	2.04[a]	1.87[b]	1.94[ab]	1.98[ab]	1.92[ab]	0.056	0.011	0.736
F/G	1.54	1.51	1.55	1.54	1.57	1.55	1.60	1.57	0.016	0.538	0.991
Overall (d 0 to 40)											
ADG, lb	1.00[a]	0.97[ab]	0.95[ab]	1.00[a]	0.90[b]	0.93[ab]	0.93[ab]	0.91[b]	0.025	0.024	0.647
ADFI, lb	1.42	1.38	1.38	1.43	1.33	1.34	1.37	1.33	0.040	0.054	0.543
F/G	1.42	1.42	1.45	1.43	1.47	1.45	1.47	1.47	0.010	0.431	0.646
Fecal DM, %[5]											
d 10	26.6	26.8	27.5	26.8	28.0	27.7	28.6	28.5	0.82	0.653	0.818
d 40	28.4	28.1	29.6	28.6	29.6	28.1	29.7	29.8	0.91	0.129	0.691
ATTD of DM[6]	84.3	85.5	82.9	84.8	85.9	84.1	85.1	85.4	0.73	0.456	0.028
ATTD of CP[6]	78.4	81.1	77.3	80.3	82.4	78.6	80.3	79.6	1.05	0.347	0.001

[a,b] Means with different superscripts differ, $P \leq 0.05$.

[1] A total of 360 barrows (DNA 200 × 400, initially 12.9 ± 0.59 lb) were used with five pigs per pen and nine replicates per treatment.

[2] No significant TIU × protease or acid × protease interactions observed.

[3] Protease (ProAct 360, DSM Nutritional Products, Parsippany, NJ) at 50 mg/kg was included at the expense of corn.

[4] Benzoic acid (VevoVitall, DSM Nutritional Products, Parsippany, NJ) at 0.50% of the diet was included at the expense of corn.

[5] Feces from three pigs per pen were weighed and dried to measure fecal DM. TIU × protease × acid × day, $P = 0.429$; TIU × protease × acid, $P = 0.659$; TIU × protease, $P = 0.893$; TIU × acid, $P = 0.708$; protease × acid, $P = 0.935$; TIU, $P = 0.030$; protease, $P = 0.356$; acid, $P = 0.119$; day, $P < 0.001$.

[6] Both ground feed and fecal samples were dried at 275°F for 2 h to determine DM percentage of the samples used for titanium determination. Titanium dioxide concentration in both dried feed and fecal samples was analyzed in duplicate. ATTD = apparent total tract digestibility.

Table 4. Main effect of trypsin inhibitor of complete diets on nursery pig performance[1]

	Trypsin inhibitor unit (TIU)[2]			
Item	1.4	2.8	SEM	*P* =
Body weight, lb				
d 0	12.9	12.9	0.59	0.507
d 10	17.1	16.5	0.71	0.001
d 24	31.4	30.0	1.07	< 0.001
d 40	52.2	49.6	1.49	< 0.001
Phase 1 (d 0 to 10)				
ADG, lb	0.42	0.36	0.016	< 0.001
ADFI, lb	0.50	0.46	0.019	0.012
F/G	1.20	1.31	0.021	0.001
Phase 2 (d 10 to 24)				
ADG, lb	1.02	0.96	0.027	0.001
ADFI, lb	1.37	1.31	0.039	0.014
F/G	1.34	1.36	0.010	0.321
Phase 3 (d 24 to 42)				
ADG, lb	1.30	1.23	0.033	< 0.001
ADFI, lb	2.00	1.93	0.054	0.004
F/G	1.54	1.57	0.013	0.010
Overall (d 0 to 42)				
ADG, lb	0.98	0.92	0.023	< 0.001
ADFI, lb	1.40	1.34	0.037	0.005
F/G	1.43	1.47	0.008	< 0.001
Fecal DM, %[3]				
d 10	26.9	28.2	0.38	0.018
d 40	28.8	29.3	0.32	0.269
ATTD of DM[4]	84.4	85.1	0.40	0.131
ATTD of CP[4]	79.3	80.2	0.53	0.202

[1] A total of 360 barrows (DNA 200 × 400, initially 12.9 ± 0.59 lb) were used with five pigs per pen and 36 replicates per treatment.
[2] Soy flour and soybean meal were blended to create the TIU/mg complete feed for dietary treatments.
[3] Feces from three pigs per pen were weighed and dried to measure fecal DM.
[4] Both ground feed and fecal samples were dried at 275°F for 2 h to determine percentage DM of the samples used for titanium determination. Titanium dioxide concentration in both dried feed and fecal samples was analyzed in duplicate. ATTD = apparent total tract digestibility.

Table 5. Main effect of protease on nursery pig performance[1]

	Protease[2]			
Item	**No**	**Yes**	**SEM**	***P* =**
Body weight, lb				
d 0	12.9	12.9	0.59	0.552
d 10	16.8	16.8	0.71	0.978
d 24	30.7	30.7	1.07	0.997
d 40	50.9	50.9	1.49	0.904
Phase 1 (d 0 to 10)				
ADG, lb	0.39	0.39	0.016	0.884
ADFI, lb	0.48	0.48	0.019	0.546
F/G	1.25	1.26	0.021	0.779
Phase 2 (d 10 to 24)				
ADG, lb	0.99	0.99	0.027	0.804
ADFI, lb	1.33	1.34	0.039	0.659
F/G	1.34	1.36	0.010	0.255
Phase 3 (d 24 to 42)				
ADG, lb	1.27	1.26	0.033	0.824
ADFI, lb	1.95	1.97	0.054	0.420
F/G	1.54	1.57	0.013	0.123
Overall (d 0 to 42)				
ADG, lb	0.95	0.95	0.023	0.752
ADFI, lb	1.37	1.38	0.037	0.588
F/G	1.44	1.46	0.008	0.067
Fecal DM, %[3]				
d 10	27.7	27.4	0.38	0.667
d 40	29.3	28.8	0.32	0.254
ATTD of DM[4]	85.0	84.5	0.40	0.380
ATTD of CP[4]	80.1	79.4	0.53	0.296

[1] A total of 360 barrows (DNA 200 × 400, initially 12.9 ± 0.59 lb) were used with five pigs per pen and 36 replicates per treatment.
[2] Protease (ProAct 360, DSM Nutritional Products, Parsippany, NJ) at 50 mg/kg was included at the expense of corn.
[3] Feces from three pigs per pen were weighed and dried to measure fecal DM.
[4] Both ground feed and fecal samples were dried at 275°F for 2 h to determine percentage DM of the samples used for titanium determination. Titanium dioxide concentration in both dried feed and fecal samples was analyzed in duplicate. ATTD = apparent total tract digestibility.

Table 6. Main effect of benzoic acid on nursery pig performance[1]

	Benzoic acid[2]			
Item	**No**	**Yes**	**SEM**	***P* =**
Body weight, lb				
d 0	12.9	12.9	0.59	0.806
d 10	16.9	16.7	0.71	0.257
d 24	30.8	30.5	1.07	0.374
d 40	50.8	51.0	1.49	0.718
Phase 1 (d 0 to 10)				
ADG, lb	0.40	0.38	0.016	0.230
ADFI, lb	0.49	0.47	0.019	0.219
F/G	1.24	1.27	0.021	0.303
Phase 2 (d 10 to 24)				
ADG, lb	0.99	0.99	0.027	0.626
ADFI, lb	1.35	1.32	0.039	0.309
F/G	1.36	1.34	0.010	0.293
Phase 3 (d 24 to 42)				
ADG, lb	1.25	1.28	0.033	0.081
ADFI, lb	1.96	1.97	0.054	0.479
F/G	1.57	1.54	0.013	0.089
Overall (d 0 to 42)				
ADG, lb	0.95	0.95	0.023	0.725
ADFI, lb	1.37	1.37	0.037	0.746
F/G	1.45	1.44	0.008	0.174
Fecal DM, %[3]				
d 10	27.3	27.8	0.38	0.312
d 40	28.7	29.4	0.32	0.110
ATTD of DM[4]	84.6	85.0	0.40	0.433
ATTD of CP[4]	79.6	79.9	0.53	0.690

[1] A total of 360 barrows (DNA 200 × 400, initially 12.9 ± 0.59 lb) were used with five pigs per pen and 36 replicates per treatment.
[2] Benzoic acid (VevoVitall, DSM Nutritional Products, Parsippany, NJ) at 0.50% of the diet was included at the expense of corn.
[3] Feces from three pigs per pen were weighed and dried to measure fecal DM.
[4] Both ground feed and fecal samples were dried at 275°F for 2 h to determine percentage DM of the samples used for titanium determination. Titanium dioxide concentration in both dried feed and fecal samples was analyzed in duplicate. ATTD = apparent total tract digestibility.