Kansas Agricultural Experiment Station Research Reports

Volume 11
Issue 7 *Swine Day*

Article 9

2025

Evaluation of Increasing Trypsin Inhibitor on Nursery Pig Growth Performance, Fecal Dry Matter, and Nutrient Digestibility

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Recommended Citation

Collier, Sierra M.; Soper, Paige T.; Gaffield, Katelyn N.; Goodband, Robert D.; Tokach, Mike D.; Woodworth, Jason C.; DeRouchey, Joel M.; Krishnan, Hari B.; and Gebhardt, Jordan T. (2025) "Evaluation of Increasing Trypsin Inhibitor on Nursery Pig Growth Performance, Fecal Dry Matter, and Nutrient Digestibility," *Kansas Agricultural Experiment Station Research Reports*: Vol. 11: Iss. 7. https://doi.org/10.4148/2378-5977.8743

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Funding Source

Appreciation is expressed to the United Soybean Board for partial financial support.

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K-STATE Research and Extension

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Evaluation of Increasing Trypsin Inhibitor on Nursery Pig Growth Performance, Fecal Dry Matter, and Nutrient Digestibility

Sierra M. Collier, Paige T. Soper, 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 13.4 ± 0.07 lb) were used in a 42-d growth trial to evaluate the effects of increasing trypsin inhibitor on nursery pig growth performance. At weaning, pigs were randomly assigned to pens with five pigs per pen and 12 pens per treatment. There were six dietary treatments formulated to provide 1.4, 2.1, 2.8, 3.5, 4.2, and 4.9 trypsin inhibitor units (TIU)/mg of complete feed. Soy flour (76 TIU/mg) was added at the expense of soybean meal (5 TIU/mg) to create the treatment TIU levels. Diet formulation was based on analyzed nutrient values of the soy flour and soybean meal but assumed the same digestibility coefficients for each, which matched that of the 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 42. Across all three individual dietary phases and overall (d 0 to 42), increasing TIU decreased (linear, $P < 0.001$) ADG and ADFI and worsened (linear, $P < 0.001$) F/G. Fecal DM on d 10 increased (linear, $P < 0.05$; quadratic, $P = 0.088$) as TIU increased, with the majority of increase in fecal DM tending to be from 3.5 to 4.9 TIU/mg of complete feed. However, there were no differences in fecal DM on d 42. The apparent total tract digestibility (ATTD) of DM decreased then increased (linear, $P = 0.001$; quadratic, $P = 0.003$) as TIU increased in the diet, with the lowest ATTD of DM occurring at 4.2 TIU/mg of complete feed. Furthermore, the ATTD of CP decreased (linear, $P < 0.001$) as the level of TIU/mg of complete feed increased in the diet. In conclusion, these data suggest that increasing dietary TIU above 1.4 TIU/mg of complete feed results in poorer nursery pig growth performance and reduced ATTD of nutrients, although feces were drier on d 10 but not at the end of the study.

Introduction

After weaning, pigs have their first exposure to a solid diet that often contains soybean meal (SBM) as a main source of protein. During this period, pigs commonly experience a transitory period of hypersensitivity to anti-nutritional factors in SBM, resulting in post-weaning diarrhea and decreased growth performance. The primary anti-nu-

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tritional factor, trypsin inhibitor (TI), impairs protein and amino acids digestibility, consequently hindering growth performance. The results of a recent industry survey by Gaffield et al. (2024)[3] revealed a wide variation in TI across SBM sources in the US, with values ranging from 1.67 to 10.57 trypsin inhibitor units (TIU)/mg of seed powder. This considerable variation in soybean meal TI has prompted a need for revisiting the acceptable levels of TIU that can be included in nursery diets without reducing performance. Therefore, the objective of this study was to investigate the effects of increasing TIU in the diet on nursery 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 facility in Manhattan, KS. The facility has two identical barns that are completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder and a cup waterer 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.

Animals and diets

A total of 360 barrows (DNA 200 × 400; initially 13.4 ± 0.07 lb) were used across two barns in a 42-d growth trial. Pigs were weaned at approximately 21 d of age and assigned to pens blocked by initial body weight (BW). Treatments were assigned in a generalized randomized block design. Pigs were blocked into light (initially 11.2 ± 0.05 lb), intermediate (initially 13.3 ± 0.05 lb), and heavy groups (initially 15.6 ± 0.05 lb). Within each block, there were four pens of five pigs per treatment (two pens per weight group in each barn) for a total of 12 replications per treatment (six per barn). Pens of pigs were randomly allotted to one of six dietary treatments. Diets were corn-soybean meal-based and consisted of increasing TIU/mg of complete feed (1.4, 2.1, 2.8, 3.5, 4.2, 4.9). Diets were formulated with a blend of soybean meal (5 TIU/mg) and soy flour (76 TIU/mg) to achieve increasing values of TIU while having the same total soy product inclusion. Dietary TIU values were identical 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).[4] Diets were then formulated using these analyzed values for the soy products and using the soybean meal digestibility coefficients for both soy sources.

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 42 (Tables 2, 3, and 4). 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 42 to determine ADG, ADFI, and feed efficiency (F/G). Feces were collected from three pigs

[3] Gaffield, K. N., R. D. Goodband, J. M. DeRouchey, M. D. Tokach, J. C. Woodworth, G. Denny, P. Smolen, C. Slipher, H. B. Krishnan, and J. T. Gebhardt. 2024. An industry survey of the composition and variability of soybean gums and soapstocks across US soybean processing plants. J. Anim. Sci. 102:skae378. doi:10.1093/jas/skae378.

[4] Kim, S., and H. B. Krishnan. 2023. A fast and cost-effective procedure for reliable measurement of trypsin inhibitor activity in soy and soy products. Methods Enzymol. 680:195-213. doi:10.1016/bs.mie.2022.08.016.

per pen on d 10 and 42 to determine the percentage of fecal dry matter. Fecal samples were analyzed separately for each pig, and the average of the three samples from each pen was then used for statistical analysis. 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 42.

Digestibility analysis

At the conclusion of the study, fecal samples were dried at 130°F for 48 h. The loss of weight was used to calculate fecal DM percentage. Following fecal DM determination, both ground feed and fecal samples were dried at 275°F for 2 h to determine the percentage DM of the samples used for titanium analysis. Titanium dioxide concentration in both dried feed and fecal samples was determined utilizing procedures outlined by Leone (1973).[5] The ATTD of DM and CP were determined using the index method described by Adeola (2001).[6]

Statistical analysis

Data were analyzed as a generalized randomized block design for one-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, dietary treatment and weight block were included as fixed effects, and barn was included as a random effect. Linear and quadratic contrasts were used to test for increasing levels of TIU. Contrast statements were used to test the main effects of treatment, day, and the interaction between treatment and day for fecal DM. Results were considered significant with $P < 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

Analysis of final diets for TIU/mg of complete feed was generally higher compared to diet formulation; however, the expected overall increasing TIU trend was observed for the six dietary treatments (Tables 2 to 4).

In all three dietary phases and overall (d 0 to 42), increasing TIU decreased (linear $P < 0.001$) BW, ADG, ADFI, and worsened (linear, $P < 0.001$) F/G. From d 0 to 10, ADG was decreased by 20% and F/G worsened by 33% for pigs fed 4.9 TIU/mg of complete feed compared with those fed 1.4 TIU/mg. On d 42, pigs fed 4.9 TIU/mg of complete feed were 12 lb lighter than those fed 1.4 TIU/mg. The decrease in ADG was partially driven by a reduction in ADFI (linear, $P < 0.001$) as TIU increased during each phase and overall.

There was a tendency for an interaction (linear, $P = 0.094$) between treatment and day for fecal DM. On d 10, fecal DM increased (linear, $P < 0.05$; quadratic, $P = 0.088$) as TIU/mg of complete feed increased, with most of the increase in fecal DM tending to be from 3.5 to 4.9 TIU/mg. However, there were no differences in fecal DM on d 42. Additionally, there was a main effect of day ($P < 0.05$) with fecal DM being lower on d 10 compared to d 42.

The ATTD of DM decreased then increased (linear, $P = 0.001$; quadratic, $P = 0.003$) as TIU/mg of complete feed increased in the diet, with the lowest ATTD of DM occur-

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

[6] 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.

ring at 4.2 TIU/mg. Furthermore, the ATTD of CP decreased (linear, $P < 0.001$) as the level of TIU/mg of complete feed increased in the diet.

In summary, increasing dietary TIU worsened growth performance throughout the entire nursery period and lowered nutrient digestibility. Based on the results in this study, including soybean meal with greater than 6 TIU/mg at a rate similar to that used in this study would result in dietary TIU greater than 1.4 TIU/mg of complete feed, which will lead to a reduction in nursery growth performance and nutrient digestibility. Therefore, the soybean meal TIU concentration should be used to dictate the amount of soybean meal included in nursery pig diets to maintain performance and nutrient digestibility.

Acknowledgement

Appreciation is expressed to the United Soybean Board for partial financial support.

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

Item	SBM	Soy flour
Trypsin inhibitor units, TIU/mg	5.41	75.87
Proximate analysis, %		
Moisture	8.08	6.96
Crude protein	48.45	50.18
Crude fat	3.00	0.62
Crude fiber	3.09	3.97
Ash	6.05	6.02
Total amino acid concentration, %		
Cys	0.71	0.74
His	1.30	1.33
Ile	2.28	2.33
Leu	3.69	3.79
Lys	3.21	3.27
Met	0.73	0.68
Thr	1.92	1.95
Trp	0.58	0.59
Val	2.38	2.40

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

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

	Trypsin inhibitor units (TIU), per mg of complete feed					
Item	**1.4**	**2.1**	**2.8**	**3.5**	**4.2**	**4.9**
Ingredients, %						
Corn	51.26	51.28	51.30	51.28	51.27	51.29
Soybean meal	17.57	16.55	15.53	14.55	13.56	12.54
Soy flour	0.55	1.55	2.55	3.55	4.55	5.55
Whey powder	12.50	12.50	12.50	12.50	12.50	12.50
Whey permeate, 80% lactose	7.50	7.50	7.50	7.50	7.50	7.50
Spray-dried blood plasma	2.50	2.50	2.50	2.50	2.50	2.50
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00
Calcium carbonate	0.53	0.53	0.53	0.53	0.53	0.53
Monocalcium P (21% P)	0.45	0.45	0.45	0.45	0.45	0.45
Salt	0.40	0.40	0.40	0.40	0.40	0.40
L-Lys-HCl	0.34	0.34	0.34	0.34	0.34	0.34
DL-Met	0.19	0.19	0.19	0.19	0.19	0.19
L-Thr	0.18	0.18	0.18	0.18	0.18	0.18
L-Trp	0.05	0.05	0.05	0.05	0.05	0.05
L-Val	0.11	0.11	0.11	0.11	0.11	0.11
L-Ile	0.03	0.03	0.03	0.03	0.03	0.03
Zinc oxide	0.40	0.40	0.40	0.40	0.40	0.40
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
Total	100	100	100	100	100	100
						continued
Calculated analysis						
SID AA, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	56	56	56	56	56	56
Leu:Lys	112	112	112	112	112	113
Met:Lys	37	37	37	36	36	36
Met and Cys:Lys	58	58	58	58	58	58
Thr;Lys	64	64	64	64	64	64
Trp:Lys	19.2	19.2	19.2	19.2	19.2	19.2
Val:Lys	70	70	70	70	70	70
His:Lys	34	34	34	34	34	34
NE, kcal/lb	1,137	1,127	1,118	1,108	1,099	1,090
SID Lys:NE, g/Mcal	5.39	5.43	5.48	5.52	5.57	5.62
STTD P, %	0.57	0.57	0.57	0.57	0.57	0.57

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

	Trypsin inhibitor units (TIU), per mg of complete feed					
Item	**1.4**	**2.1**	**2.8**	**3.5**	**4.2**	**4.9**
Analyzed composition						
TIU/mg of complete feed	1.86	2.84	3.48	4.52	5.29	4.59
CP, %	18.8	18.1	17.1	20.0	18.7	19.3
DM, %	89.0	89.1	89.1	89.0	89.1	89.2

[1] Diets were fed from d 0 to 10.
[2] HiPhorius 2400 included at 1,500 FTU/kg feed provided an estimated release of 0.13% STTD P.

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

	Trypsin inhibitor units (TIU), per mg of complete feed					
Item	**1.4**	**2.1**	**2.8**	**3.5**	**4.2**	**4.9**
Ingredients, %						
Corn	58.45	58.47	58.45	58.44	58.46	58.48
Soybean meal	24.50	23.48	22.51	21.52	20.50	19.48
Soy flour	0.05	1.05	2.05	3.05	4.05	5.05
Whey powder	5.00	5.00	5.00	5.00	5.00	5.00
Whey permeate, 80% lactose	5.00	5.00	5.00	5.00	5.00	5.00
Fish meal	3.25	3.25	3.25	3.25	3.25	3.25
Calcium carbonate	0.63	0.63	0.63	0.63	0.63	0.63
Monocalcium P (21% P)	0.75	0.75	0.75	0.75	0.75	0.75
Salt	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys-HCl	0.46	0.46	0.46	0.46	0.46	0.46
DL-Met	0.22	0.22	0.22	0.22	0.22	0.22
L-Thr	0.23	0.23	0.23	0.23	0.23	0.23
L-Trp	0.07	0.07	0.07	0.07	0.07	0.07
L-Val	0.15	0.15	0.15	0.15	0.15	0.15
L-Ile	0.04	0.04	0.04	0.04	0.04	0.04
Zinc oxide	0.25	0.25	0.25	0.25	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
Total	100	100	100	100	100	100

continued

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

	Trypsin inhibitor units (TIU), per mg of complete feed					
Item	**1.4**	**2.1**	**2.8**	**3.5**	**4.2**	**4.9**
Calculated analysis						
SID AA, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	57	57	57	57	57	57
Leu:Lys	109	109	109	109	109	109
Met:Lys	39	39	39	39	39	39
Met and Cys:Lys	58	58	58	58	58	58
Thr;Lys	64	64	64	64	64	64
Trp:Lys	19.1	19.1	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	1,122	1,113	1,103	1,094	1,084	1,075
SID Lys:NE, g/Mcal	5.46	5.50	5.55	5.60	5.65	5.70
STTD P, %	0.53	0.53	0.53	0.53	0.53	0.53
Analyzed composition						
TIU/mg of complete feed	1.99	2.65	3.77	4.40	4.63	4.90
CP, %	19.1	19.1	19.0	18.9	18.8	19.2
DM, %	88.2	87.9	88.1	87.8	87.7	87.7

[1] Diets were fed from d 10 to 24.
[2] HiPhorius 2400 included at 1,500 FTU/kg feed provided an estimated release of 0.13% STTD P.

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

	Trypsin inhibitor units (TIU), per mg of complete feed					
Item	**1.4**	**2.1**	**2.8**	**3.5**	**4.2**	**4.9**
Ingredients, %						
Corn	69.54	69.53	69.51	69.53	69.54	69.54
Soybean meal	25.83	24.84	23.86	22.84	21.84	20.83
Soy flour	0.00	1.00	2.00	3.00	4.00	5.00
Calcium carbonate	0.80	0.80	0.80	0.80	0.80	0.80
Monocalcium P (21% P)	0.95	0.95	0.95	0.95	0.95	0.95
Salt	0.60	0.60	0.60	0.60	0.60	0.60
L-Lys-HCl	0.55	0.55	0.55	0.55	0.55	0.55
DL-Met	0.23	0.23	0.23	0.23	0.23	0.23
L-Thr	0.26	0.26	0.26	0.26	0.26	0.26
L-Trp	0.08	0.08	0.08	0.08	0.08	0.08
L-Val	0.16	0.16	0.16	0.16	0.16	0.16
L-Ile	0.04	0.04	0.04	0.04	0.04	0.04
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
Titanium dioxide[3]	0.50	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100	100
Calculated analysis						
SID AA, %						
Lys	1.30	1.30	1.30	1.30	1.30	1.30
Ile:Lys	56	56	56	56	56	56
Leu:Lys	109	109	109	109	109	110
Met:Lys	38	38	38	38	38	38
Met and Cys:Lys	58	58	58	58	58	58
Thr;Lys	64	64	64	64	64	64
Trp:Lys	19.1	19.1	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	1,109	1,100	1,090	1,081	1,071	1,062
SID Lys:NE, g/Mcal	5.32	5.36	5.41	5.46	5.50	5.55
STTD P, %	0.45	0.45	0.45	0.45	0.45	0.45
Analyzed composition						
TIU/mg of complete feed	1.74	3.07	4.01	4.70	5.21	5.19
CP, %	18.2	18.4	18.0	18.2	18.7	18.3
DM, %	86.9	86.9	87.0	87.2	87.0	87.0

[1] Diets were fed from d 24 to 42.
[2] HiPhorius 2400 included at 1,500 FTU/kg feed provided an estimated release of 0.13% STTD P.
[3] Utilized as an indigestible marker for apparent total tract digestibility calculations.

Table 5. Effect of increasing trypsin inhibitor on nursery pig performance[1]

	Trypsin inhibitor units (TIU), per mg of complete feed[2]							*P* =	
Item	1.4	2.1	2.8	3.5	4.2	4.9	SEM	Linear	Quadratic
BW, lb									
d 0	13.3	13.4	13.4	13.4	13.4	13.3	0.07	0.926	0.620
d 10	18.8	18.2	18.0	17.8	17.5	16.8	0.25	< 0.001	0.681
d 24	33.1	31.8	30.9	29.6	28.8	27.1	0.43	< 0.001	0.622
d 42	61.2	59.7	57.3	54.3	51.2	49.0	0.77	< 0.001	0.401
Phase 1 (d 0 to 10)									
ADG, lb	0.54	0.48	0.46	0.44	0.41	0.35	0.021	< 0.001	0.773
ADFI, lb	0.69	0.65	0.65	0.64	0.64	0.57	0.026	< 0.001	0.394
F/G	1.28	1.37	1.42	1.46	1.57	1.65	0.039	< 0.001	0.616
Phase 2 (d 10 to 24)									
ADG, lb	1.02	0.96	0.93	0.85	0.81	0.73	0.023	< 0.001	0.728
ADFI, lb	1.40	1.34	1.31	1.23	1.18	1.11	0.033	< 0.001	0.712
F/G	1.37	1.39	1.42	1.45	1.46	1.52	0.029	< 0.001	0.526
Phase 3 (d 24 to 42)									
ADG, lb	1.56	1.53	1.46	1.37	1.24	1.22	0.025	< 0.001	0.383
ADFI, lb	2.50	2.44	2.41	2.29	2.13	2.08	0.044	< 0.001	0.144
F/G	1.60	1.60	1.65	1.68	1.72	1.71	0.024	< 0.001	0.618
Overall (d 0 to 42)									
ADG, lb	1.14	1.08	1.04	0.98	0.90	0.85	0.018	< 0.001	0.488
ADFI, lb	1.70	1.63	1.63	1.54	1.46	1.40	0.025	< 0.001	0.236
F/G	1.50	1.51	1.56	1.59	1.62	1.64	0.015	< 0.001	0.993
Fecal DM, %[3]									
d 10	25.87	25.42	25.44	25.83	26.80	27.59	0.637	0.015	0.088
d 42	27.46	26.87	26.15	27.65	26.36	27.54	0.637	0.943	0.261
ATTD of DM[4]	85.65[a]	84.27[ab]	84.69[ab]	83.26[b]	80.74[c]	84.88[ab]	0.647	0.001	0.003
ATTD of CP[4]	79.87	77.19	77.55	75.22	75.22	75.55	0.952	< 0.001	0.105

[1]A total of 360 barrows (DNA 200 × 400, initially 13.4 ± 0.07 lb) were used with five pigs per pen and 12 replicates per treatment.

[2] Soy flour and soybean meal were blended to create the TIU/mg of complete feed for dietary treatments.

[3] Feces from three pigs per pen were weighed and dried to measure fecal DM. Linear treatment × day interaction, $P = 0.094$; Treatment, $P = 0.120$; Day, $P = 0.023$.

[4] Both ground feed and fecal samples were dried in a 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.