

2021

The Effect of Increasing Valine, Isoleucine, and Tryptophan:Lysine Ratios on Pigs' Growth Performance and Carcass Characteristics When Fed Diets with Increased Levels of Dietary Leucine:Lysine

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Recommended Citation

Williams, Hadley R.; Tokach, Mike D.; Woodworth, Jason C.; DeRouchey, Joel M.; Goodband, Robert D.; Gebhardt, Jordan T.; Hastad, Chad W.; and Post, Zach B. (2021) "The Effect of Increasing Valine, Isoleucine, and Tryptophan:Lysine Ratios on Pigs' Growth Performance and Carcass Characteristics When Fed Diets with Increased Levels of Dietary Leucine:Lysine," *Kansas Agricultural Experiment Station Research Reports*: Vol. 7: Iss. 11. <https://doi.org/10.4148/2378-5977.8202>

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Funding Source

Appreciation is expressed to New Fashion Pork, Jackson, MN, and New Horizon Farms, Pipestone, MN, for their technical support and expertise in conducting this experiment.

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The Effect of Increasing Valine, Isoleucine, and Tryptophan:Lysine Ratios on Pigs' Growth Performance and Carcass Characteristics When Fed Diets with Increased Levels of Dietary Leucine:Lysine¹

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Summary

A total of 4,076 pigs (initially 86.5 ± 1.24 lb) were used across 2 experiments to evaluate the effect of increasing ratios of Val, Ile, and Trp to Lys on pig growth performance and carcass characteristics in corn-soybean meal-DDGS-based diets containing increased levels of dietary Leu:Lys. In both experiments, the 4 dietary treatments were as follows: 1) high soybean meal and low feed grade amino acids (control); 2) low soybean meal and high feed grade amino acids, with Val:Lys, Ile:Lys, and Trp:Lys at 67, 55, and 18, respectively, (low ratio); 3) same as diet 2 except Val:Lys, Ile:Lys, and Trp:Lys increased to 72, 60, and 21, respectively, (medium ratio); and 4) same as diet 2 except Val:Lys, Ile:Lys, and Trp:Lys increased to 80, 65, and 23, respectively (high ratio). All diets contained 30% DDGS until pigs reached approximately 220 lb, and then 20% DDGS until trial completion. Overall ADG and average ADFI increased (AA ratio; linear, $P < 0.05$) as Val, Ile, and Trp ratios increased from low to high. Pigs fed the control diet exhibited increased ADG when compared to pigs fed low ratio diets, while pigs fed medium and high ratio diets showed intermediate performance. In summary, the soybean meal level can be reduced, and synthetic amino acid levels increased in high DDGS diets as long as ratios of Val, Ile, and Trp to Lys are increased.

Introduction

The use of corn DDGS and other corn co-products allows swine nutritionists to formulate diets with higher concentrations of synthetic amino acids while reducing the level of soybean meal, leading to lower diet costs. This diet formulation strategy results in high levels of Leu that potentially leads to an unbalanced branch chain amino acid (BCAA) relationship. The relationship between BCAA and large neutral amino acids (LNAA) may be less than ideal to support optimal growth performance. Diets with

¹ Appreciation is expressed to New Fashion Pork, Jackson, MN, and New Horizon Farms, Pipestone, MN, for their technical support and expertise in conducting this experiment.

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increased Leu commonly reduce the feed intake and growth performance of pigs; this result could be attributed to imbalanced supply of BCAA that results from increased catabolism of Val and Ile. A study conducted by Morales et al. (2016)⁴ demonstrated that supplementing Ile and Val above the pig's requirement in diets with excess Leu ameliorated the negative effect of excess Leu on absorption and degradation of BCAA. Additions of Ile and Val above the normal requirement may prevent the negative effects of excess Leu.

Tryptophan is an LNAA that is a precursor for serotonin, which plays a role in appetite regulation. Large neutral amino acids share the same brain transporters as BCAA, and an excess in Leu has been negatively correlated with Trp uptake and brain serotonin levels, which leads to decreased feed intake and growth performance. Recently, a meta-analysis was conducted to predict the influence of BCAA and LNAA on growth performance of pigs (Cemin et al., 2019).⁵ The authors found that the prediction equation suggests increasing Leu:Lys negatively impacts the ADG due to reduction in G:F and ADFI, caused by insufficient levels of other BCAA (Ile and Val) and LNAA relative to Leu. The model predicts the addition of Ile, Val, and Trp in combination or alone has the potential to counteract the negative effects of high dietary Leu. A study conducted by Kerkaert et al. (2020)⁶ to validate this model, showed that supplementing Trp up to 22.2% Trp:Lys ratio did not improve ADFI in the presence of high Leu, suggesting that Trp alone may not be able to overcome an imbalance in BCAA caused by excess Leu. Additionally, the meta-analysis predicted continued improvements in growth performance as the level of Ile, Val, and Trp are increased, but it is unclear if this response will indeed continue or plateau at high levels of supplementation. Therefore, the objective of this study was to evaluate the growth performance and carcass characteristics of pigs fed increasing levels of Val, Ile, and Trp:Lys with diets containing increased levels of Leu:Lys from DDGS.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used for these experiments. This study was conducted at two commercial research facilities in southwestern Minnesota. Barns were either tunnel- or naturally ventilated with completely slatted concrete flooring over deep pits for manure storage. Within both experiments, pens contained a 3-hole, dry feeder (Thorpe Equipment, Inc., Thorpe, WI) and a pan waterer to allow *ad libitum* access to feed and water.

Animals and diets

A total of 4,076 pigs (initially 85.5 ± 1.24 lb) were used in 2 experiments and 4 groups of pigs, with 20 to 27 pigs per pen (similar number across treatments) and 45 pens per treatment. Pens of pigs were blocked by initial BW and randomly assigned to 1 of 4

⁴ Morales, A., N. Acre, M. Cota, L. Buenabad, E. Avelar, J. K. Htoo, and M. Cervantes. 2016. Effect of dietary excess of branched-chain amino acids on performance and serum concentrations of amino acids in growing pigs. *J. Anim. Physiol. Anim. Nutr.* 100:39-45. doi:10.1111/jpn.12327.

⁵ Cemin, H. S., M. D. Tokach, S.S. Dritz, J. C. Woodworth, J. M. DeRouchey, and R.D. Goodband. 2019. Meta-regression analysis to predict the influence of branched-chain and large neutral amino acids on growth performance of pigs. *J. Anim. Sci.* 97:2505-2514. doi:10.1093/jas/skz118.

⁶ Kerkaert, H. R., H. S. Cemin, J. C. Woodworth, J. M. DeRouchey, S.S. Dritz, M. D. Tokach, R. D. Goodband, K. D. Haydon, C. W. Hastad, and Z. B. Post. 2021. Improving performance of finishing pigs with added valine, isoleucine, and tryptophan: validating a meta-analysis model. *J. Anim. Sci.* 99:1-9. doi:10.1093/jas/skab006.

dietary treatments in a randomized complete block design. Experimental diets were fed in four phases with targeted AA ratios similar in all phases. All diets contained 30% DDGS in the first 3 phases and 20% in phase 4. The control diet contained high soybean meal and low feed grade amino acids with ratios to Lys ranging from 92 to 95% for Val, 79 to 83% for Ile, and 23% for Trp. The other 3 diets contained lower soybean meal levels and high feed-grade amino acids with Val:Lys, Ile:Lys, and Trp:Lys increasing for the three treatments (Low, Medium, and High). For the low treatment, Val:Lys, Ile:Lys, and Trp:Lys were 67, 55, and 18, respectively. For the medium ratios, Val:Lys, Ile:Lys, and Trp:Lys were 72, 60, and 21, respectively. For the high ratios, Val:Lys, Ile:Lys, and Trp:Lys were 80, 65, and 23, respectively (Tables 1 and 2). The Leu:Lys levels ranged from 139 to 154% in the different diet phases for the three treatments with higher levels of feed grade L-Lys.

Experimental diets for Exp. 1 were fed from d 0 to 28, 28 to 56, 56 to 84, and 84 to 107 in group 1 and from d 0 to 15, 15 to 43, 43 to 71, and 71 to 106 in group 2, for phases 1 to 4, respectively. For Exp. 2 diets were fed from d 0 to 27, 27 to 54, 54 to 83, and 83 to 118 in group 1, and from d 0 to 13, 13 to 39, 39 to 82, and 82 to 104 in group 2, for phases 1 to 4, respectively.

Pens of pigs were weighed, and feed disappearance measured approximately every 14 d for both experiments to determine ADG, ADFI, and F/G. The 3 to 5 heaviest pigs from each pen (similar number within each block) were marketed on d 84 (group 1) and d 85 (group 2) in Exp. 1 and d 99 (group 1), and d 82 (group 2) in Exp. 2. The remaining pigs were marketed at the conclusion of the experiments.

At completion of the studies, final pen weights were recorded, and individual pigs were tagged or tattooed with a pen identification number and transported to a commercial abattoir for processing and carcass data collection. Carcass measurements included HCW, backfat depth, loin depth, and percentage lean. Carcass yields were then calculated by the pen average HCW from the plant divided by the pen average final BW on the farm.

To validate and compare the predicted ADG model from Cemin et al.⁵ to the actual ADG that occurred in the study, the equation's intercept term was adjusted until the predicted ADG matched the actual ADG for the pigs fed the control diet. The equation with the adjusted intercept term was then used to predict the ADG of the three remaining treatments. The relationship between the actual and predicted ADG was calculated (actual ADG/predicted ADG) to illustrate the accuracy of the prediction model.

Statistical analysis

For the statistical analysis, data from both experiments were combined. Data were analyzed as a randomized complete block design for one-way ANOVA using the lmer function from the lme4 package in R (version 3.5.1 (2018-07-02), R Foundation for Statistical Computing, Vienna, Austria), with pen considered as the experimental unit, body weight as blocking factor, and treatment as a fixed effect with 45 replicates/treatment. Preplanned linear and quadratic contrasts were used to determine the effects of increasing ratios of Ile, Val, and Trp:Lys for the Low, Medium, and High ratio treat-

ments. Pairwise comparisons using the Tukey-Kramer adjustment were used to evaluate differences in treatment means. Results were considered significant at $P \leq 0.05$.

Results and Discussion

During phase 1, pigs fed the low ratio diets had decreased ($P < 0.05$) ADG compared with all other treatments. Increased AA ratios from low to medium improved ADG, however, further increases of AA from medium to high did not yield additional benefits (AA ratio; quadratic $P = 0.009$). Increased AA ratios from low to high increased ADFI (AA ratio; linear, $P = 0.020$). Pigs fed the control diet had improved F/G compared to the pigs fed the low and high ratio diets, with pigs fed the medium ratio diets having similar F/G to pigs fed the high ratio and control diets. Increased AA ratios from low to medium improved F/G, but then worsened when increasing to high ratios (AA ratio, quadratic, $P = 0.001$).

During phase 2, pigs fed the control diet had increased ADG compared to pigs fed the low ratio diet, with pigs fed the medium and high ratio diets intermediate ($P < 0.05$). Increasing the AA ratio from low ratio to high ratio increased (linear, $P = 0.035$) ADFI.

During phase 3, pigs fed the control diet had increased ($P < 0.05$) ADG and ADFI compared to pigs fed the low ratio diet, with pigs fed the medium and high ratio diets intermediate. Increasing the AA ratio from low to high increased (linear, $P = 0.013$) ADG.

During phase 4, pigs fed the low ratio diet had increased ADG compared to the pigs fed the control diet with the pigs fed the medium ratio and high ratio intermediate ($P < 0.05$). This increase in ADG led to pigs being fed the low and high ratio diet having improved F/G compared to the pigs fed the control diet, with the pigs fed the medium ratio diet intermediate ($P < 0.05$).

Overall, pigs fed the control diet had increased ($P < 0.05$) ADG compared to the pigs fed the low ratio diet, with pigs fed the medium ratio and high ratio being intermediate. Increasing AA ratios from low to high increased (linear, $P < 0.05$) ADG and ADFI. There were no differences in overall F/G between treatments.

For carcass characteristics, pigs fed the control diets had increased ($P < 0.05$) percentage lean and loin depth compared to pigs fed the medium ratio diet, with pigs fed the low and high ratio intermediate. Pigs fed the medium ratio diet had increased backfat depth compared to pigs fed the control diet, with the pigs fed the low and high ratio being intermediate ($P < 0.05$).

To assess the accuracy of the model developed by Cemin et al.,⁵ we first adjusted the intercept of the predicted control treatment to match the actual ADG. Then when comparing the predicted ADG from the model to the actual ADG, the model slightly under-predicted ADG for the pigs fed the low ratio and medium ratio (by 2 to 5%). The model over-predicted the performance for the pigs fed the high ratio diet (by 3%). Overall, the pigs fed the medium ratio and high ratio diet had similar ADG compared to the pigs fed the control diet. Interestingly, the pigs fed the high ratio diet had similar performance to the pigs fed the control diet, when the model predicts the high ratio to have improved ADG compared to the control.

In conclusion, pigs fed medium ratios for Ile, Val, and Trp relative to Lys of 60, 72, and 18%, respectively, had similar ADG and F/G to pigs fed the control diet with high soybean and low feed grade amino acids. The results from this study indicate that different diet formulation strategies can be utilized to ameliorate the negative effects of high Leu:Lys. Additional research is warranted to understand if Val, Ile, and Trp independently or cumulatively result in the improved performance.

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Table 1. Phase 1 and 2 diet composition (as-fed basis)^{1,2}

Item	Phase 1				Phase 2			
	Control	Low ratio	Medium ratio	High ratio	Control	Low ratio	Medium ratio	High ratio
Ingredients, %								
Corn	44.20	57.35	57.15	56.90	49.95	61.35	61.15	60.90
Soybean meal (46.5% CP)	22.85	8.55	8.60	8.60	17.30	4.95	4.95	4.95
DDGS, > 6 and < 9% oil	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Choice white grease	0.80	1.00	1.08	1.15	0.78	0.95	1.00	1.10
Calcium carbonate	1.10	1.13	1.13	1.13	1.05	1.08	1.08	1.08
Calcium phosphate	0.28	0.44	0.44	0.44	0.15	0.30	0.30	0.30
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys-HCl	0.11	0.55	0.55	0.55	0.14	0.52	0.52	0.52
DL-Met	---	0.08	0.08	0.08	---	0.05	0.05	0.05
L-Thr	---	0.19	0.19	0.19	---	0.16	0.16	0.16
L-Trp	0.02	0.05	0.08	0.10	0.02	0.05	0.08	0.09
L-Val	---	0.01	0.06	0.14	---	---	0.04	0.12
L-Ile	---	---	0.05	0.10	---	---	0.05	0.09
VTM ³	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase ⁴	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated analysis								
Standardized ileal digestible amino acids, %								
Lys	0.98	0.98	0.98	0.98	0.87	0.87	0.87	0.87
Ile:Lys	82	55	60	65	81	55	60	65
Leu:Lys	177	139	139	139	183	147	147	147
Met:Lys	30	32	32	32	32	31	31	31
Met and Cys:Lys	61	57	57	57	64	57	57	57
Thr:Lys	65	65	65	65	65	65	65	65
Trp:Lys	23.0	18.0	21.0	23.0	23.0	18.0	21.0	23.0
Val:Lys	92	67	72	80	92	67	72	80
Lys:NE, g/mcal	3.86	3.86	3.86	3.86	3.41	3.41	3.41	3.41
CP, %	21.8	16.7	16.8	16.9	19.7	15.3	15.3	15.4
NE, kcal/lb	1,152	1,152	1,152	1,152	1,156	1,156	1,156	1,156
Ca, %	0.60	0.60	0.60	0.60	0.54	0.54	0.54	0.54
STTD P, %	0.41	0.41	0.41	0.41	0.37	0.37	0.37	0.37

¹Phase 1 was fed from approximately 90 to 120 lb, and phase 2 was fed from approximately 120 to 160 lb.

²Diets for Exp. 2 differed slightly based on analyzed nutrient values, but the ratios remained constant for both experiments.

³Vitamin and mineral premix provided per kg of complete diet: 90 mg Zn, 37 mg Fe, 11 mg Mn, 15 mg Cu, 0.18 mg I, 0.30 mg of Se, 2,507 IU vitamin A, 318 IU vitamin D, 12 IU vitamin E, 0.11 mg vitamin B₁₂, 11.3 mg of niacin, 7.4 mg pantothenic acid, and 2.0 mg riboflavin.

⁴Quantum Blue 10p (AB Vista, Marlborough, Wiltshire) provided 284 units of phytase FTU/lb of diet with assumed release of 0.10% STTD P.

Table 2. Phase 3 and 4 diet composition (as-fed basis)^{1,2}

Item	Phase 3				Phase 4			
	Control	Low ratio	Medium ratio	High ratio	Control	Low ratio	Medium ratio	High ratio
Ingredients, %								
Corn	55.75	66.05	65.85	65.70	62.90	73.25	73.10	72.95
Soybean meal (46.5% CP)	11.60	0.50	0.55	0.55	14.40	3.20	3.20	3.25
DDGS, > 6 and < 9% oil	30.00	30.00	30.00	30.00	20.00	20.00	20.00	20.00
Choice white grease	0.88	0.98	1.05	1.10	0.80	0.95	1.00	1.03
Calcium carbonate	0.98	1.08	1.08	1.08	0.95	0.98	0.98	0.98
Calcium phosphate	---	---	---	---	---	0.15	0.15	0.15
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys-HCl	0.18	0.52	0.52	0.52	0.08	4.20	4.20	4.20
DL-Met	---	0.03	0.03	0.03	---	0.03	0.03	0.03
L-Thr	---	0.14	0.14	0.14	---	0.15	0.15	0.15
L-Trp	0.03	0.05	0.08	0.09	0.02	0.04	0.06	0.08
L-Val	---	---	0.04	0.10	---	---	0.03	0.09
L-Ile	---	0.02	0.06	0.10	---	0.01	0.04	0.08
VTM ³	0.10	0.10	0.10	0.10	0.31	0.31	0.31	0.31
Phytase ⁴	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated analysis								
Standardized ileal digestible amino acid, %								
Lys	0.76	0.76	0.76	0.76	0.70	0.70	0.70	0.70
Ile:Lys	79	55	60	65	83	55	59	65
Leu:Lys	191	154	154	154	189	148	148	148
Met:Lys	33	30	30	30	33	31	31	31
Met and Cys:Lys	67	58	58	58	67	58	58	58
Thr:Lys	65	65	65	65	67	67	67	67
Trp:Lys	23.0	18.0	21.0	23.0	23.0	18.0	21.0	23.0
Val:Lys	93	67	72	80	95	67	72	80
Lys:NE, g/Mcal	2.96	2.96	2.96	2.96	2.71	2.71	2.71	2.71
CP, %	17.5	13.5	13.6	13.7	16.2	12.2	12.2	12.3
NE, kcal/lb	1,163	1,163	1,163	1,163	1,172	1,172	1,172	1,172
Ca, %	0.47	0.47	0.47	0.47	0.51	0.51	0.51	0.51
STTD P, %	0.33	0.30	0.30	0.30	0.30	0.30	0.30	0.30

¹Phase 3 was fed from approximately 160 to 220 lb, and phase 4 was fed from approximately 220 to 285 lb.

²Diets for Exp. 2 differed slightly based on analyzed nutrient values, but the ratios remained constant for both experiments.

³For phase 3 vitamin and mineral premix provided per kg of complete diet: 90 mg Zn, 37 mg Fe, 11 mg Mn, 15 mg Cu, 0.18 mg I, 0.30 mg of Se, 2507 IU vitamin A, 318 IU vitamin D, 12 IU vitamin E, 0.01 mg vitamin B₁₂, 11.6 mg niacin, 7.4 mg pantothenic acid, and 2.0 mg riboflavin. For phase 4 vitamin and trace mineral premix provided per kg of premix: 160,090 mg Zn, 134,000 mg Fe, 40,000 mg Mn, 13,340 mg Cu, 666 mg I, 24,255 IU vitamin A, 4,410 IU vitamin D, 132,268 IU vitamin E, 13,228 mg vitamin K, 110.2 mg vitamin B₁₂, 99,212 mg niacin, 90,390 mg pantothenic acid, and 17,640 mg riboflavin.

⁴Quantum Blue 10p (AB Vista, Marlborough, Wiltshire) provided 284 units of phytase FTU/lb of diet with assumed release of 0.10% STTD P.

Table 3. Effects of branched-chain amino acid ratios on growth performance of pigs¹

Item	Control	Low ratio	Medium ratio	High ratio	SEM	<i>P</i> = ²	
						AA Linear	AA Quadratic
Initial BW, lb	86.6	86.5	86.6	86.5	1.24	0.911	0.614
Final BW, lb	291.5	288.1	289.3	290.3	2.39	0.107	0.857
Phase 1							
ADG, lb	1.83 ^a	1.70 ^b	1.79 ^a	1.79 ^a	0.035	< 0.001	0.009
ADFI, lb	3.95	3.92	3.95	4.02	0.072	0.020	0.588
F/G	2.16 ^c	2.32 ^a	2.21 ^{bc}	2.25 ^b	0.022	0.005	0.001
Phase 2							
ADG, lb	2.12 ^a	2.06 ^b	2.09 ^{ab}	2.09 ^{ab}	0.029	0.161	0.386
ADFI, lb	5.38	5.25	5.32	5.37	0.089	0.035	0.695
F/G	2.53	2.54	2.55	2.57	0.022	0.267	0.642
Phase 3							
ADG, lb	2.01 ^a	1.95 ^b	1.97 ^{ab}	1.99 ^{ab}	0.018	0.013	0.870
ADFI, lb	6.28 ^a	6.12 ^b	6.17 ^{ab}	6.24 ^{ab}	0.053	0.057	0.956
F/G	3.13	3.15	3.14	3.13	0.028	0.656	0.931
Phase 4							
ADG, lb	1.85 ^b	1.95 ^a	1.92 ^{ab}	1.92 ^{ab}	0.056	0.258	0.660
ADFI, lb	6.43	6.39	6.42	6.42	0.117	0.731	0.766
F/G	3.58 ^a	3.33 ^b	3.42 ^{ab}	3.41 ^b	0.067	0.216	0.327
Overall							
ADG, lb	1.95 ^a	1.92 ^b	1.94 ^{ab}	1.95 ^{ab}	0.028	0.011	0.564
ADFI, lb	5.55	5.45	5.49	5.54	0.066	0.032	0.959
F/G	2.85	2.85	2.84	2.85	0.023	0.868	0.700
Carcass characteristics							
HCW, lb	214.5	213.0	213.9	214.8	2.09	0.272	0.953
Carcass yield, %	73.4	73.7	73.6	73.7	0.319	0.968	0.784
Lean, %	57.0 ^a	56.6 ^{ab}	56.3 ^b	56.5 ^{ab}	0.254	0.866	0.081
Back fat depth, in.	0.55 ^b	0.57 ^{ab}	0.59 ^a	0.58 ^{ab}	0.101	0.614	0.213
Loin depth, in.	2.70 ^a	2.64 ^{ab}	2.63 ^b	2.67 ^{ab}	0.019	0.166	0.147
Predicted vs. actual ADG ^{3,4}							
Actual overall ADG, lb	1.95	1.91	1.94	1.95	---	---	---
Predicted overall ADG, lb	1.95	1.81	1.91	2.01	---	---	---
Actual vs. predicted, %	100	105	102	97	---	---	---

^{a-c}Means within a row with different superscript differ (*P* < 0.05).

¹A total of 4,076 pigs were used in a study split between 4 groups at 2 commercial facilities in southwestern Minnesota. Within the study, 20 to 27 pigs were placed per pen and there were 45 replicates per treatment.

²Linear and quadratic contrasts were evaluated based on total leucine, isoleucine, valine, and tryptophan per diet by phase.

³Prediction equation used was derived by Cemin 2019 (Cemin, H.S., M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, and R. D. Goodband. 2019. Meta-regression analysis to predict the influence of branched-chain and large neutral amino acids on growth performance of pigs. *J. Anim. Sci.* 97:2505-2514. doi:10.1093/jas/skz11.). The intercept term was adjusted until the predicted ADG matched the actual ADG of the control treatment. The adjusted intercept term was then used to predict the ADG of the remaining treatments.

⁴Actual and predicted ADG is the overall weighted ADG for each phase. Weighted ADG = (phase 1 ADG × phase 1 days on feed) + (phase 2 ADG × phase 2 days on feed) + (Phase 3 ADG × phases 3 days on feed) + (phase 4 ADG × phase 4 days on feed) / total days on feed.