Validating a Dietary Approach to Determine Amino Acid:Lysine Ratios for Pigs[1,2]

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Summary

Standardized ileal digestible (SID) amino acid:lysine (AA:Lys) ratio experiments are commonly conducted to estimate the AA requirement of pigs relative to lysine (Lys) and allow for accurate diet formulation. The objective of the studies herein was to validate a dietary approach to determine the optimal SID AA:Lys ratio for pigs using tryptophan (Trp) as a model. Four 21-d experiments were conducted in which pigs (337 × 1050; PIC) were fed corn-soybean meal–based diets with 30% corn dried distillers grains with solubles (DDGS). A total of 1,188, 1,232, 1,204, and 1,183 pigs with initial BW of 28.5 ± 0.4, 50.1 ± 1.3, 127.0 ± 2.5, and 192.5 ± 2.6 lb were used in experiments 1, 2, 3, and 4, respectively. Each experiment had 11 pens per treatment with 24 to 28 pigs per pen. In Experiment 1, each pen housed the same number of barrows and gilts, whereas in Experiments 2 to 4 only gilts were used. Dietary treatments were: (1) High CP, High Lys, and High Trp:Lys ratio (HHH); (2) Low CP, High Lys, and High Trp:Lys ratio (LHH); (3) Low CP, Low Lys, and High Trp:Lys ratio (LLH); and (4) Low CP, Low Lys, and Low Trp:Lys ratio (LLL). The SID Trp concentrations used were 14.5 vs. 20% of Lys, CP was at least 3 percentage units different, and SID Lys levels were 0.01 percentage unit above the estimated requirement at the expected initial BW and 0.10 or 0.05 percentage units below requirement at the expected final BW of the Experiment 1 (nursery) and Experiments 2, 3, and 4 (finishing), respectively. In Experiment 1, decreasing CP (HHH vs. LHH) did not influence ADG but increased ($P < 0.05$) F/G. Decreasing Lys (LHH vs. LLH) and decreasing the SID Trp:Lys ratio (LLH vs. LLL) reduced ($P < 0.05$) ADG and increased ($P < 0.05$) F/G. In Experiment 2, decreasing CP (HHH vs. LHH) did not affect ADG but increased ($P < 0.05$) F/G. Decreasing Lys (LHH vs. LLH) and the SID Trp:Lys ratio (LLH vs. LLL) decreased ($P < 0.05$) ADG and increased ($P < 0.05$) F/G. In Experiment 3, decreasing CP (HHH vs. LHH) or Lys (LHH vs. LLH) did not influence ADG or F/G. Decreasing the SID Trp:Lys ratio (LLH vs. LLL) reduced ($P < 0.05$) ADG and increased ($P < 0.05$) F/G. In Experiment 4, decreasing CP (HHH vs. LHH) did not influence ADG but increased ($P < 0.05$) F/G. Decreasing Lys (LHH vs. LLH) had no effect on performance, but decreasing the SID Trp:Lys ratio (LLH vs. LLL) reduced ($P < 0.05$) ADG and increased ($P < 0.05$) F/G.

In conclusion, low-CP diets formulated 0.10 and 0.05 percentage units below the SID Lys requirement at the end of the experiment's weight range appear to ensure pigs are

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below their Lys requirement when determining the optimal SID Trp:Lys ratio for 29- to 52-lb and 50- to 80-lb pigs, respectively. For pigs heavier than 80 lb, formulating diets at 0.05 percentage units below the SID Lys requirement at the end of the experiment's weight range can limit the ability to provide statistical evidence that pigs are under their lysine requirement.

Key words: amino acid ratio, lysine, tryptophan

Introduction

Low-CP, amino acid (AA)-fortified diets are commonly fed in the swine industry due to the increased availability and decreased cost of feed-grade AA. Pigs fed low-CP diets have similar performance to pigs fed high-CP diets as long as essential AA are supplemented to meet the pigs' requirements. The tryptophan (Trp) requirement can be expressed in different ways; however, the standardized ileal digestible (SID) Trp requirement expressed as a ratio to lysine (Trp:Lys) is considered a practical approach for diet formulation. Lysine is the first limiting AA in most of the cereal grain diets used in swine. Because the Lys requirement when reported as a percentage of the diet decreases as BW increases, if the experimental diet is not limiting in Lys at the end of the experiment's BW range, the ratio of other AA to Lys will be underestimated; therefore, Lys must be the second limiting AA throughout the experiment. The objective of these studies was to validate a dietary approach to determining the optimal SID AA:Lys ratio for pigs using Trp as a model.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The studies were conducted at 2 commercial research barns in southwestern Minnesota. The nursery barn in which Experiment 1 was conducted was totally enclosed, environmentally controlled, and mechanically ventilated. Each pen (3.7 × 2.3 m) was equipped with a 6-hole stainless steel dry self-feeder (SDI Industries, Alexandria, SD) and a pan waterer. The finishing barn was naturally ventilated and double-curtain-sided. Each pen (5.5 × 3.0 m) was equipped with a 4-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a cup waterer. Both barns had completely slatted flooring and deep pits for manure storage. Each facility was equipped with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) that delivered and recorded daily feed additions and diets as specified. Pigs had ad libitum access to feed and water.

Four 21-d growth experiments were conducted with two groups of pigs. Experiment 1 was conducted with a group of nursery pigs, and Experiments 2, 3, and 4 were conducted with a group of finishing pigs. A total of 1,188, 1,232, 1,204, and 1,183 pigs (337 × 1050; PIC Hendersonville, TN) with initial BW of 28.5 ± 0.4, 50.1 ± 1.3, 127.0 ± 2.5, and 192.5 ± 2.6 lb were used in Experiments 1, 2, 3, and 4, respectively. Each experiment had 11 pens per treatment with 24 to 28 pigs per pen. In Experiment 1, each pen housed the same number of barrows and gilts, whereas only gilts were used in Experiments 2 to 4. Dietary treatments (Tables 1, 2, 3, and 4) were: (1) High CP, High Lys, and High Trp:Lys ratio (HHH); (2) Low CP, High Lys, and High Trp:Lys ratio (LHH); (3) Low CP, Low Lys, and High Trp:Lys ratio (LLH); and (4) Low CP, Low Lys, and Low Trp:Lys ratio (LLL). Corn-soybean meal–based diets with 30%

DDGS were used with different SID Trp:Lys ratios (14.5% vs. 20%), CP (at least 3 percentage units difference), and SID Lys levels (0.01 percentage unit above requirement at the expected initial BW and 0.10 or 0.05 percentage units below requirement at the expected final BW of the nursery and finishing, respectively). Lysine requirements were estimated using the NRC (2012[5]) model for mixed gender pens of pigs for the nursery phase and for gilts only for the finishing phase. Diets were balanced to have the same NE and Ca:standardized total tract digestible (STTD) P ratio.

Five representative samples of corn, soybean meal, and DDGS were collected each week for 5 wk and analyzed in duplicate for total AA and CP by Ajinomoto Heartland Inc. (Chicago, IL), and values were used in diet formulation. Other nutrients and SID AA digestibility coefficient values used for diet formulation were obtained from NRC (2012).

Pens of pigs were weighed and feed disappearance was measured at the beginning and at d 21 of each experiment to determine ADG, ADFI, and F/G. There were 21-d periods between the finishing experiments, in which pigs were fed a common diet that met or exceeded NRC (2012) nutrient requirements and contained 20% SID Trp:Lys. Caloric efficiency was calculated on a pen basis by multiplying total pen feed intake by the dietary energy level (kcal/lb) and dividing by total pen gain.

Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of each experiment and stored at -20°C, then total Trp, other AA, and CP analysis were conducted on composite samples from each dietary treatment by Ajinomoto Heartland, Inc. Diet samples were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, crude fiber, ash, crude fat, Ca, and P.

Data were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) as a randomized complete block design. Pen was the experimental unit for all data analysis. The model included terms for the fixed effects of dietary treatment with the block (initial average pen BW) as a random effect. In addition, for Experiments 3 and 4, dietary treatment from the previous experiment (2 and 3, respectively) was also considered a random effect. Treatment means were separated using pairwise comparisons of means performed using the DIFFS option from the LSMEANS statement of SAS. Results were considered significant at $P \leq 0.05$ and a tendency at $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

The nutrient and total AA analysis of the diets (Tables 5, 6, 7, and 8) were reasonably within the expected variation reported by Cromwell et al. (1999[6]). The analyzed total Trp for the LLL treatment in Experiment 1 was higher than expected, but due to the reduction in growth performance when comparing the LLH vs. LLL treatments observed in that experiment and because the analysis of free L-Trp agrees with formu-

[5] NRC. 2012. Nutrient Requirements of Swine. 11th ed. Natl. Acad. Press, Washington, DC.

[6] Cromwell, G.L., C.C. Calvert, T.R. Cline, J.D. Crenshaw, T.D. Crenshaw, R.A. Easter, R.C. Ewan, C.R. Hamilton, G.M. Hill, A.J. Lewis, D.C. Mahan, E.R. Miller, J.L. Nelssen, J.E. Pettigrew, L.F. Tribble, T.L. Veum, and J.T. Yen. 1999. Variability among sources and laboratories in nutrient analyses of corn and soybean meal. NCR-42 Committee on Swine Nutrition. North Central Regional-42. J. Anim. Sci. 77:3262–3273.

lated values, the researchers believe that this variation could be due to the analytical procedure.

In Experiment 1, decreasing CP (Table 9; HHH vs. LHH) did not influence ($P > 0.05$) ADG and final BW but increased ($P < 0.05$) ADFI and, consequently, F/G and caloric efficiency. Decreasing Lys (LHH vs. LLH) and decreasing the SID Trp:Lys ratio (LLH vs. LLL) reduced ($P < 0.05$) ADG, ADFI, and final BW but increased ($P < 0.05$) F/G and caloric efficiency. In Experiment 2, decreasing CP (HHH vs. LHH) did not influence ADG and final BW but increased ($P < 0.05$) ADFI and consequently increased F/G and caloric efficiency. Decreasing Lys (LHH vs. LLH) reduced ($P < 0.05$) ADG and final BW but increased ($P < 0.05$) F/G and caloric efficiency, with no change in ADFI. Decreasing the SID Trp:Lys ratio (LLH vs. LLL) reduced ($P < 0.05$) ADG, ADFI, and final BW; however, F/G and caloric efficiency were increased ($P < 0.05$). In Experiment 3, decreasing CP (HHH vs. LHH) did not influence ($P > 0.05$) ADG, F/G, caloric efficiency, or final BW but increased ($P < 0.05$) ADFI. Decreasing Lys (LHH vs. LLH) had no effect on pig performance. Decreasing the SID Trp:Lys ratio (LLH vs. LLL) decreased ($P < 0.05$) ADG, ADFI, and final BW but increased ($P < 0.05$) F/G and caloric efficiency. In Experiment 4, decreasing CP (HHH vs. LHH) did not influence ADG, ADFI, or final BW ($P > 0.05$) but increased ($P < 0.05$) F/G and caloric efficiency. Decreasing Lys (LHH vs. LLH) had no effect ($P > 0.05$) on pig performance. Decreasing the SID Trp:Lys ratio (LLH vs. LLL) reduced ($P < 0.05$) ADG and final BW, whereas ADFI was not affected ($P > 0.05$), so F/G and caloric efficiency were increased ($P < 0.05$).

Low-CP, AA-fortified diets did not influence ADG or final BW in any experiment compared with pigs fed the high-CP diets with increased soybean meal. Pigs fed low-CP, AA-fortified diets in Experiments 1, 2, and 3 had increased ADFI and consequently increased NE intake compared with those fed high-CP diets. In addition, F/G was increased in Experiments 1, 2, and 4 in pigs fed low-CP diets, which suggests that the NE used for corn was overestimated or that NE values used for soybean meal and added fat sources were underestimated.

The SID Lys concentrations used in diet formulation were 92, 95, 94, and 93% of SID Lys requirement estimates suggested by NRC (2012) at the end of the BW range for Experiments 1, 2, 3, and 4, respectively. Using diets with 92 and 95% of the estimated SID Lys requirement at the end of the BW range for 29- to 52-lb and 50- to 80-lb pigs was sufficient to statistically reduce growth performance (LHH vs. LLH); however, for 127- to 162-lb and 193- to 237-lb pigs, using diets with 94 and 93% of SID Lys requirement at the end of the BW range resulted in only a numerical increase in F/G and reduction in ADG and final BW between the LHH and LLH diets. This result suggests that one should formulate SID Lys to be less than 93% of the NRC (2012) requirement estimate of the final BW of the experiment when determining AA:Lys in pigs heavier than 80 lb.

In all experiments, pigs fed diets with 14.5% SID Trp:Lys had decreased performance compared with pigs fed diets with 20% SID Trp:Lys, which indicates that Trp was definitely the first limiting AA in the LLL diet. Also, in Trp:Lys ratio studies, a Trp ratio of 14.5% of Lys may be a good starting point for observing a response to increasing Trp.

We recommend the following procedures as critical to successful characterization of AA:Lys ratios:

- Analyze dietary ingredients before formulation and use analyzed AA concentrations in the diet formulation.
- Ensure that diets are formulated to be below the SID Lys requirement for the entire feeding period using the NRC (2012) for the specific weight range, energy level, and gender. Based on the results of these studies, formulating diets with 0.05 percentage units below the SID Lys requirement estimate at the final BW of the experiment is enough for pigs under 80 lb, but for pigs heavier than that, it only numerically increased F/G and reduced ADG and final BW.
- Conduct experiments with a short length (e.g., 3 to 4 wk) to ensure Lys is below the requirement for the entire period.
- To ensure that diets were indeed formulated below the SID Lys requirement, conduct a preliminary experiment or include a dietary treatment in which Lys is above pigs' requirement.
- Ensure that all other AA are high enough to decrease the probability that another essential AA is deficient (e.g., SID ratios of 65% and 68% for Thr in early and late finishing phases, respectively; 70% Valine:Lys; 55% Isoleucine:Lys; 60% Methionine & Cysteine:Lys; 100% Leucine:Lys; 32% Histidine:Lys).
- Analyze diets after formulation to determine actual Lys and other AA levels that were fed.
- In conclusion, low-CP diets formulated 0.10 and 0.05 percentage units below the SID Lys requirement at the end of the experiment's weight range appear to ensure pigs are below their Lys requirement when determining the optimal SID Trp:Lys ratio for 29- to 52-lb and 50- to 80-lb pigs, respectively. For pigs heavier than 80 lb, formulating diets at 0.05 percentage units below the SID Lys requirement at the end of the experiment's weight range can limit the ability to provide statistical evidence that pigs are under their Lys requirement.

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

Item	HHH[2]	LHH	LLH	LLL
Ingredient, %				
Corn	31.48	41.59	55.10	55.16
Soybean meal (46% CP)	32.79	23.09	10.91	10.91
DDGS	30.00	30.00	30.00	30.00
Corn oil	3.00	1.80	0.50	0.50
Calcium phosphate (dicalcium)	0.15	0.30	0.50	0.50
Limestone	1.50	1.49	1.48	1.48
Salt	0.35	0.35	0.35	0.35
Trace mineral premix[3]	0.100	0.100	0.100	0.100
Vitamin premix[4]	0.125	0.125	0.125	0.125
L-lysine-HCL	0.340	0.625	0.575	0.575
DL-methionine	0.075	0.160	0.070	0.070
L-threonine	0.065	0.190	0.140	0.140
L-tryptophan	---	0.053	0.054	---
L-valine	---	0.105	0.060	0.060
L-isoleucine	---	---	0.010	0.010
Phytase[5]	0.025	0.025	0.025	0.025
Total	100	100	100	100

continued

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

Item	HHH[2]	LHH	LLH	LLL
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lysine	1.29	1.29	0.97	0.97
Isoleucine:lysine	67	55	55	55
Leucine:lysine	152	135	153	153
Methionine:lysine	34	37	35	35
Met & Cys:lysine	60	60	60	60
Threonine:lysine	65	65	65	65
Tryptophan:lysine	20.0	20.0	20.0	14.5
Valine:lysine	73	70	70	70
Histidine:lysine	43	37	38	38
Total lysine, %	1.51	1.48	1.13	1.13
ME, kcal/lb	1,563	1,541	1,512	1,511
NE, kcal/lb	1,121	1,121	1,122	1,121
SID lysine:ME, g/Mcal	3.74	3.80	2.91	2.91
SID lysine:NE, g/Mcal	5.22	5.22	3.92	3.92
CP, %	26.1	22.9	18.2	18.1
Ca, %	0.71	0.71	0.71	0.71
P, %	0.52	0.51	0.49	0.49
Available P, %	0.37	0.38	0.40	0.40
STTD P[6] with phytase, %	0.40	0.40	0.40	0.40
Ca:P	1.36	1.39	1.44	1.44
Ca:P (STTD P with phytase)	1.79	1.79	1.79	1.79

[1] Diets were fed from 28.5 to 51.9 lb BW. Corn, dried distillers grains with solubles (DDGS) and soybean meal were analyzed for total amino acid content, and NRC (2012) SID digestibility values were used in the diet formulation.

[2] HHH: high CP, high SID Lys, and high SID Trp:Lys; LHH: low CP, high SID Lys, and high SID Trp:Lys; LLH: low CP, low SID Lys, and high Trp:Lys; LLL: low CP, low SID Lys, and low SID Trp:Lys.

[3] Provided per pound of diet: 33 ppm Mn from manganese oxide, 110 ppm Fe from iron sulfate, 110 ppm Zn from zinc oxide, 16.5 ppm Cu from copper sulfate, 0.33 ppm I from ethylenediamin dihydroiodide, and 0.30 ppm Se from sodium selenite.

[4] Provided per pound of diet: 4,000 IU vitamin A, 625 IU vitamin D_3, 20 IU vitamin E, 2.0 mg vitamin K, 12.5 mg pantothenic acid, 22.5 mg niacin, 3.5 mg riboflavin, and 15 μg vitamin B_{12}.

[5] OptiPhos 2000 (Enzyvia LLC, Sheridan, IN) provided 568 phytase units (FTU) per pound of diet.

[6] Standardized total tract digestible phosphorus.

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

Item	HHH[2]	LHH	LLH	LLL
Ingredient, %				
Corn	39.59	49.60	57.14	57.20
Soybean meal (46% CP)	25.32	16.10	9.56	9.55
DDGS	30.00	30.00	30.00	30.00
Choice white grease	2.70	1.35	0.50	0.50
Limestone	1.49	1.45	1.43	1.43
Salt	0.35	0.35	0.35	0.35
Trace mineral premix[3]	0.100	0.100	0.100	0.100
Vitamin premix[4]	0.075	0.075	0.075	0.075
L-lysine-HCL	0.305	0.575	0.550	0.550
DL-methionine	0.020	0.100	0.050	0.050
L-threonine	0.035	0.150	0.125	0.125
L-tryptophan	---	0.050	0.051	---
L-valine	---	0.070	0.050	0.050
Phytase[5]	0.025	0.025	0.025	0.025
Total	100	100	100	100

continued

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

Item	HHH[2]	LHH	LLH	LLL
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lysine	1.09	1.09	0.92	0.92
Isoleucine:lysine	68	55	55	55
Leucine:lysine	165	147	159	159
Methionine:lysine	32	36	34	34
Met & Cys:lysine	60	60	60	60
Threonine:lysine	65	65	65	65
Tryptophan:lysine	20.0	20.0	20.0	14.5
Valine:lysine	76	70	70	70
Histidine:lysine	45	38	39	39
Total lysine, %	1.29	1.26	1.08	1.08
ME, kcal/lb	1,557	1,536	1,520	1,520
NE, kcal/lb	1,131	1,131	1,131	1,131
SID lysine:ME, g/Mcal	3.18	3.22	2.75	2.75
SID lysine:NE, g/Mcal	4.37	4.37	3.69	3.69
CP, %	23.2	20.2	17.6	17.6
Ca, %	0.65	0.61	0.58	0.58
P, %	0.46	0.42	0.40	0.40
Available P, %	0.33	0.32	0.31	0.31
STTD P[6] with phytase, %	0.36	0.33	0.32	0.32
Ca:P	1.40	1.44	1.46	1.46
Ca:P (STTD P with phytase)	1.82	1.82	1.82	1.82

[1] Diets were fed from 50.1 to 80.3 lb BW. Corn, dried distillers grains with solubles (DDGS) and soybean meal were analyzed for total amino acid content and NRC (2012) SID digestibility values were used in the diet formulation.

[2] HHH: high CP, high SID Lys, and high SID Trp:Lys; LHH: low CP, high SID Lys, and high SID Trp:Lys; LLH: low CP, low SID Lys, and high Trp:Lys; LLL: low CP, low SID Lys, and low SID Trp:Lys.

[3] Provided per pound of diet: 33 ppm Mn from manganese oxide, 110 ppm Fe from iron sulfate, 110 ppm Zn from zinc oxide, 16.5 ppm Cu from copper sulfate, 0.33 ppm I from ethylenediamin dihydroiodide, and 0.30 ppm Se from sodium selenite.

[4] Provided per pound of diet: 2,400 IU vitamin A, 375 IU vitamin D_3, 12 IU vitamin E, 1.2 mg vitamin K, 7.5 mg pantothenic acid, 13.5 mg niacin, 2.1 mg riboflavin, and 9 μg vitamin B_{12}.

[5] OptiPhos 2000 (Enzyvia LLC, Sheridan, IN) provided 568 phytase units (FTU) per pound of diet.

[6] Standardized total tract digestible phosphorus.

Table 3. Diet composition, Experiment 3 (as-fed basis)[1]

Item	HHH[2]	LHH	LLH	LLL
Ingredient, %				
Corn	46.84	57.00	60.82	60.87
Soybean meal (46% CP)	18.95	9.52	6.23	6.23
DDGS	30.00	30.00	30.00	30.00
Corn oil	2.10	0.85	0.50	0.50
Limestone	1.28	1.26	1.25	1.25
Salt	0.35	0.35	0.35	0.35
Trace mineral premix[3]	0.100	0.100	0.100	0.100
Vitamin premix[4]	0.075	0.075	0.075	0.075
L-lysine-HCL	0.275	0.552	0.495	0.495
DL-methionine	---	0.050	0.005	0.005
L-threonine	0.005	0.125	0.090	0.090
L-tryptophan	---	0.052	0.045	---
L-valine	---	0.045	0.010	0.010
Phytase[5]	0.025	0.025	0.025	0.025
Total	100	100	100	100

continued

Table 3. Diet composition, Experiment 3 (as-fed basis)[1]

Item	HHH[2]	LHH	LLH	LLL
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lysine	0.92	0.92	0.80	0.80
Isoleucine:lysine	70	55	56	56
Leucine:lysine	181	159	174	174
Methionine:lysine	33	34	32	32
Met & Cys:lysine	63	60	60	60
Threonine:lysine	65	65	65	65
Tryptophan:lysine	20.0	20.0	20.0	14.5
Valine:lysine	80	70	70	70
Histidine:lysine	48	39	41	41
Total lysine, %	1.10	1.08	0.95	0.95
ME, kcal/lb	1,554	1,532	1,524	1,524
NE, kcal/lb	1,142	1,142	1,142	1,142
SID lysine:ME, g/Mcal	2.69	2.72	2.38	2.38
SID lysine:NE, g/Mcal	3.65	3.65	3.18	3.18
CP, %	20.7	17.6	16.3	16.2
Ca, %	0.55	0.52	0.51	0.51
P, %	0.44	0.40	0.38	0.38
Available P, %	0.32	0.31	0.30	0.30
STTD P[6] with phytase, %	0.34	0.32	0.31	0.31
Ca:P	1.27	1.31	1.32	1.32
Ca:P (STTD P with phytase)	1.63	1.63	1.63	1.63

[1] Diets were fed from 127.0 to 162.1 lb BW. Corn, dried distillers grains with solubles (DDGS), and soybean meal were analyzed for total amino acid content, and NRC (2012) SID digestibility values were used in the diet formulation.

[2] HHH: high CP, high SID Lys, and high SID Trp:Lys; LHH: low CP, high SID Lys, and high SID Trp:Lys; LLH: low CP, low SID Lys, and high Trp:Lys; LLL: low CP, low SID Lys, and low SID Trp:Lys.

[3] Provided per pound of diet: 33 ppm Mn from manganese oxide, 110 ppm Fe from iron sulfate, 110 ppm Zn from zinc oxide, 16.5 ppm Cu from copper sulfate, 0.33 ppm I from ethylenediamin dihydroiodide, and 0.30 ppm Se from sodium selenite.

[4] Provided per pound of diet: 2,400 IU vitamin A, 375 IU vitamin D_3, 12 IU vitamin E, 1.2 mg vitamin K, 7.5 mg pantothenic acid, 13.5 mg niacin, 2.1 mg riboflavin, and 9 μg vitamin B_{12}.

[5] OptiPhos 2000 (Enzyvia LLC, Sheridan, IN) provided 568 phytase units (FTU) per pound of diet.

[6] Standardized total tract digestible phosphorus.

Table 4. Diet composition, Experiment 4 (as-fed basis)[1]

Item	HHH[2]	LHH	LLH	LLL
Ingredient, %				
Corn	51.86	61.19	65.19	65.23
Soybean meal (46% CP)	14.19	5.65	2.21	2.21
DDGS	30.00	30.00	30.00	30.00
Choice white grease	2.10	0.90	0.50	0.50
Limestone	1.12	1.11	1.10	1.10
Salt	0.35	0.35	0.35	0.35
Trace mineral premix[3]	0.050	0.050	0.050	0.050
Vitamin premix[4]	0.050	0.050	0.050	0.050
L-lysine-HCL	0.250	0.500	0.423	0.423
DL-methionine	---	0.005	---	---
L-threonine	0.005	0.115	0.065	0.065
L-tryptophan	---	0.046	0.036	---
L-valine	---	0.015	---	---
Phytase[5]	0.025	0.025	0.025	0.025
Total	100	100	100	100

continued

Table 4. Diet composition, Experiment 4 (as-fed basis)[1]

Item	HHH[2]	LHH	LLH	LLL
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lysine	0.79	0.79	0.65	0.65
Isoleucine:lysine	73	56	60	60
Leucine:lysine	198	174	201	201
Methionine:lysine	36	32	36	36
Met & Cys:lysine	68	60	67	67
Threonine:lysine	68	68	68	68
Tryptophan:lysine	20.0	20.0	20.0	14.5
Valine:lysine	84	70	75	75
Histidine:lysine	50	41	45	45
Total lysine, %	0.96	0.94	0.79	0.79
ME, kcal/lb	1,555	1,536	1,528	1,528
NE, kcal/lb	1,154	1,154	1,154	1,154
SID lysine:ME, g/Mcal	2.30	2.33	1.93	1.93
SID lysine:NE, g/Mcal	3.11	3.11	2.55	2.56
CP, %	18.9	16.0	14.6	14.6
Ca, %	0.48	0.45	0.44	0.44
P, %	0.42	0.38	0.37	0.37
Available P, %	0.31	0.30	0.30	0.30
STTD P[6] with phytase, %	0.33	0.31	0.30	0.30
Ca:P	1.16	1.20	1.20	1.20
Ca:P (STTD P with phytase)	1.47	1.47	1.47	1.47

[1] Diets were fed from 192.5 to 237.4 lb BW. Corn, dried distillers grains with solubles (DDGS), and soybean meal were analyzed for total amino acid content, and NRC (2012) SID digestibility values were used in the diet formulation.

[2] HHH: high CP, high SID Lys, and high SID Trp:Lys; LHH: low CP, high SID Lys, and high SID Trp:Lys; LLH: low CP, low SID Lys, and high Trp:Lys; LLL: low CP, low SID Lys, and low SID Trp:Lys.

[3] Provided per pound of diet: 16.5 ppm Mn from manganese oxide, 55 ppm Fe from iron sulfate, 55 ppm Zn from zinc oxide, 8.3 ppm Cu from copper sulfate, 0.17 ppm I from ethylenediamin dihydroiodide, and 0.15 ppm Se from sodium selenite.

[4] Provided per pound of diet: 1,600 IU vitamin A, 250 IU vitamin D_3, 8 IU vitamin E, 0.80 mg vitamin K, 5.0 mg pantothenic acid, 9.0 mg niacin, 1.4 mg riboflavin, and 6 µg vitamin B_{12}.

[5] OptiPhos 2000 (Enzyvia LLC, Sheridan, IN) provided 568 phytase units (FTU) per pound of diet.

[6] Standardized total tract digestible phosphorus.

Table 5. Chemical analysis of the diets, Experiment 1 (as-fed basis)[1,2]

Item	HHH	LHH	LLH	LLL
Proximate analysis, %				
DM	91.28 (82.06)	91.11 (82.26)	90.79 (83.23)	90.8 (83.28)
CP	27.6 (26.1)	23.9 (22.9)	20.2 (18.2)	20.1 (18.1)
Crude fiber	4.3 (4.6)	3.9 (4.4)	3.6 (4.2)	3.4 (4.2)
Ca	0.87 (0.71)	1.05 (0.71)	0.74 (0.71)	0.88 (0.71)
P	0.52 (0.52)	0.51 (0.51)	0.51 (0.49)	0.50 (0.49)
Fat	7.1 (7.2)	6.0 (6.2)	4.7 (5.2)	4.6 (5.2)
Ash	5.57 (3.68)	5.32 (3.20)	4.28 (2.61)	4.71 (2.61)
Total amino acids, %				
Lysine	1.48 (1.51)	1.52 (1.48)	1.21 (1.13)	1.14 (1.13)
Isoleucine	1.07 (1.01)	0.96 (0.85)	0.76 (0.65)	0.76 (0.65)
Leucine	2.38 (2.27)	2.21 (2.03)	1.94 (1.74)	1.95 (1.74)
Methionine	0.51 (0.50)	0.57 (0.54)	0.41 (0.39)	0.42 (0.39)
Methionine & Cysteine	0.95 (0.91)	0.97 (0.90)	0.76 (0.70)	0.76 (0.70)
Threonine	1.03 (1.03)	1.08 (1.01)	0.86 (0.78)	0.87 (0.78)
Tryptophan	0.29 (0.30)	0.29 (0.30)	0.22 (0.23)	0.27 (0.17)
Valine	1.23 (1.14)	1.18 (1.08)	0.97 (0.83)	0.98 (0.83)
Histidine	0.67 (0.65)	0.60 (0.56)	0.49 (0.44)	0.49 (0.44)
Phenylalanine	1.29 (1.22)	1.18 (1.04)	0.93 (0.81)	0.94 (0.81)
Free lysine	0.26 (0.34)	0.45 (0.63)	0.49 (0.58)	0.44 (0.58)
Free threonine	0.09 (0.07)	0.22 (0.19)	0.18 (0.14)	0.20 (0.14)
Free tryptophan	0.02 (---)	0.04 (0.05)	0.04 (0.05)	0.01 (---)

[1] Values in parentheses indicate those used in diet formulation and are from NRC (2012), with the exception of CP and total amino acid content from corn, soybean meal, and dried distillers grains with solubles, which were analyzed prior to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).

[2] Diet samples were collected from feeders, stored at -20°C, and submitted to Ward Laboratories, Inc. (Kearney, NE) for proximate analysis, with the exception of CP and total amino acids, which were analyzed by Ajinomoto Heartland, Inc. (Chicago, IL).

Table 6. Chemical analysis of the diets, Experiment 2 (as-fed basis)[1,2]

Item	HHH	LHH	LLH	LLL
Proximate analysis, %				
DM	91.16 (86.94)	91.10 (88.04)	90.79 (88.67)	90.97 (88.66)
CP	24.0 (23.2)	20.7 (20.2)	20.5 (17.6)	18.9 (17.6)
Crude fiber	4.2 (4.4)	3.6 (4.3)	3.2 (4.2)	3.6 (4.2)
Ca	0.78 (0.65)	0.84 (0.61)	0.74 (0.58)	0.64 (0.58)
P	0.44 (0.46)	0.40 (0.42)	0.39 (0.40)	0.40 (0.40)
Fat	6.0 (7.1)	5.2 (6.0)	4.7 (5.3)	4.9 (5.3)
Ash	4.87 (4.92)	4.71 (4.44)	4.53 (4.10)	4.15 (4.10)
Total amino acids, %				
Lysine	1.30 (1.29)	1.23 (1.26)	1.16 (1.08)	1.08 (1.08)
Isoleucine	0.93 (0.89)	0.81 (0.73)	0.77 (0.62)	0.72 (0.62)
Leucine	2.19 (2.09)	2.04 (1.86)	1.96 (1.71)	1.91 (1.71)
Methionine	0.42 (0.41)	0.48 (0.45)	0.39 (0.37)	0.40 (0.37)
Methionine & Cysteine	0.82 (0.79)	0.83 (0.78)	0.73 (0.67)	0.75 (0.67)
Threonine	0.91 (0.89)	0.89 (0.86)	0.81 (0.74)	0.78 (0.74)
Tryptophan	0.26 (0.26)	0.24 (0.25)	0.23 (0.22)	0.17 (0.17)
Valine	1.08 (1.01)	1.02 (0.92)	0.94 (0.80)	0.92 (0.80)
Histidine	0.60 (0.58)	0.53 (0.49)	0.50 (0.43)	0.47 (0.43)
Phenylalanine	1.15 (1.08)	1.01 (0.91)	0.96 (0.79)	0.90 (0.79)
Free lysine	0.23 (0.31)	0.37 (0.58)	0.37 (0.55)	0.38 (0.55)
Free threonine	0.06 (0.04)	0.15 (0.15)	0.12 (0.13)	0.13 (0.13)
Free tryptophan	0.01 (---)	0.03 (0.05)	0.02 (0.05)	0.01 (---)

[1] Values in parentheses indicate those used in diet formulation and are from NRC (2012), with the exception of CP and total amino acid content from corn, soybean meal, and DDGS, which were analyzed prior to diet formulation by Ajinomoto Heartland, Inc. (Chicago, IL).
[2] Diet samples were collected from feeders, stored at -20°C, and submitted to Ward Laboratories, Inc. (Kearney, NE) for proximate analysis, with the exception of CP and total amino acids, which were analyzed by Ajinomoto Heartland, Inc. (Chicago, IL).

Table 7. Chemical analysis of the diets, Experiment 3 (as-fed basis)[1,2]

Item	HHH	LHH	LLH	LLL
Proximate analysis, %				
DM	91.44 (87.33)	90.86 (88.34)	90.35 (88.57)	90.77 (88.57)
CP	21.5 (20.7)	19.1 (17.6)	17.2 (16.3)	17.6 (16.2)
Crude fiber	3.9 (4.3)	3.1 (4.2)	3.1 (4.1)	3.2 (4.1)
Ca	0.77 (0.55)	0.64 (0.52)	0.62 (0.51)	0.56 (0.51)
P	0.49 (0.44)	0.42 (0.40)	0.4 (0.38)	0.42 (0.38)
Fat	7.2 (6.6)	5.4 (5.6)	4.9 (5.4)	5.1 (5.4)
Ash	4.54 (4.41)	3.74 (3.93)	3.57 (3.77)	3.40 (3.77)
Total amino acids, %				
Lysine	1.23 (1.10)	1.37 (1.08)	1.03 (0.95)	1.11 (0.95)
Isoleucine	0.86 (0.78)	0.71 (0.62)	0.65 (0.56)	0.65 (0.56)
Leucine	2.10 (1.94)	1.95 (1.71)	1.85 (1.63)	1.85 (1.63)
Methionine	0.39 (0.36)	0.40 (0.37)	0.33 (0.31)	0.34 (0.31)
Methionine & Cysteine	0.77 (0.71)	0.74 (0.67)	0.65 (0.59)	0.66 (0.59)
Threonine	0.84 (0.76)	0.80 (0.74)	0.71 (0.66)	0.74 (0.66)
Tryptophan	0.21 (0.22)	0.20 (0.22)	0.18 (0.19)	0.16 (0.15)
Valine	1.02 (0.90)	0.89 (0.79)	0.80 (0.70)	0.81 (0.70)
Histidine	0.55 (0.52)	0.49 (0.43)	0.45 (0.40)	0.46 (0.40)
Phenylalanine	1.06 (0.96)	0.91 (0.79)	0.84 (0.73)	0.85 (0.73)
Free lysine	0.31 (0.28)	0.68 (0.55)	0.43 (0.50)	0.47 (0.50)
Free threonine	0.05 (0.01)	0.12 (0.13)	0.10 (0.09)	0.13 (0.09)
Free tryptophan	0.02 (---)	0.04 (0.05)	0.03 (0.05)	0.01 (---)

[1] Values in parentheses indicate those used in diet formulation and are from NRC (2012), with the exception of CP and total amino acid content from corn, soybean meal, and DDGS, which were analyzed prior to diet formulation by Ajinomoto Heartland, Inc. (Chicago, IL).
[2] Diet samples were collected from feeders, stored at -20°C, and submitted to Ward Laboratories, Inc. (Kearney, NE) for proximate analysis, with the exception of CP and total amino acids, which were analyzed by Ajinomoto Heartland Inc.

Table 8. Chemical analysis of the diets, Experiment 4 (as-fed basis)[1,2]

Item	HHH	LHH	LLH	LLL
Proximate analysis, %				
DM	90.43 (87.31)	89.92 (88.21)	89.79 (88.46)	89.83 (88.46)
CP	20.9 (18.9)	17.3 (16.0)	15.7 (14.6)	15.9 (14.6)
Crude fiber	3.7 (4.3)	3.8 (4.1)	3.3 (4.1)	3.8 (4.1)
Ca	0.86 (0.48)	0.65 (0.45)	0.66 (0.44)	0.52 (0.44)
P	0.42 (0.42)	0.40 (0.38)	0.39 (0.37)	0.38 (0.37)
Crude fat	6.4 (6.6)	5.7 (5.7)	5.0 (5.5)	5.4 (5.5)
Ash	4.70 (3.96)	3.87 (3.54)	3.45 (3.36)	3.48 (3.36)
Amino acids, %				
Lysine	1.00 (0.96)	0.93 (0.94)	0.84 (0.79)	0.81 (0.79)
Isoleucine	0.81 (0.70)	0.65 (0.55)	0.58 (0.49)	0.56 (0.49)
Leucine	2.05 (1.82)	1.81 (1.61)	1.75 (1.53)	1.75 (1.53)
Methionine	0.37 (0.34)	0.33 (0.30)	0.31 (0.28)	0.31 (0.28)
Methionine & Cysteine	0.74 (0.66)	0.65 (0.59)	0.61 (0.55)	0.60 (0.55)
Threonine	0.77 (0.69)	0.72 (0.67)	0.64 (0.57)	0.64 (0.57)
Tryptophan	0.21 (0.19)	0.18 (0.19)	0.16 (0.16)	0.13 (0.12)
Valine	0.96 (0.82)	0.81 (0.70)	0.71 (0.62)	0.71 (0.62)
Histidine	0.52 (0.48)	0.43 (0.39)	0.41 (0.36)	0.41 (0.36)
Phenylalanine	1.00 (0.87)	0.82 (0.72)	0.76 (0.65)	0.77 (0.65)
Free lysine	0.17 (0.25)	0.32 (0.50)	0.30 (0.42)	0.31 (0.42)
Free threonine	0.04 (0.01)	0.12 (0.12)	0.08 (0.07)	0.08 (0.07)
Free tryptophan	0.01 (---)	0.02 (0.05)	0.02 (0.04)	0.01 (---)

[1] Values in parentheses indicate those used in diet formulation and are from NRC (2012), with the exception of CP and total amino acid content from corn, soybean meal, and DDGS, which were analyzed prior to diet formulation by Ajinomoto Heartland, Inc. (Chicago, IL).
[2] Diet samples were collected from feeders, stored at -20°C, and submitted to Ward Laboratories, Inc. (Kearney, NE) for proximate analysis, with the exception of CP and total amino acids, which were analyzed by Ajinomoto Heartland, Inc.

Table 9. Effects of different standardized ileal digestible (SID) tryptophan:lysine ratios, CP, and SID lysine levels on pig performance[1]

Item	HHH[2]	LHH	LLH	LLL	SEM	Probability, $P <$
Exp. 1						
d 0 BW, lb	28.5	28.5	28.5	28.5	0.37	0.976
ADG, lb	1.23[a]	1.26[a]	1.04[b]	0.89[c]	0.02	0.001
ADFI, lb	1.69[b]	1.82[a]	1.73[b]	1.59[c]	0.03	0.001
F/G	1.38[a]	1.45[b]	1.67[c]	1.78[d]	0.03	0.001
NE caloric efficiency[3]	1,547[a]	1,620[b]	1,873[c]	1,996[d]	31.1	0.001
d 21 BW, lb	54.4[a]	55.3[a]	50.5[b]	47.3[c]	0.61	0.001
Exp. 2						
d 0 BW, lb	50.2	50.1	50.1	50.1	1.3	0.988
ADG, lb	1.57[a]	1.60[a]	1.44[c]	1.08[b]	0.03	0.001
ADFI, lb	2.55[a]	2.75[b]	2.78[b]	2.23[c]	0.08	0.001
F/G	1.62[a]	1.71[b]	1.93[d]	2.06[c]	0.03	0.001
NE caloric efficiency	1,838[a]	1,940[b]	2,178[c]	2,326[d]	34.9	0.001
d 21 BW, lb	83.2[a]	83.8[a]	80.9[c]	73.1[b]	1.8	0.001
Exp. 3						
d 0 BW, lb	126.8	126.9	127.1	127.1	2.5	0.967
ADG, lb	1.67[b]	1.72[b]	1.66[b]	1.46[a]	0.04	0.001
ADFI, lb	4.03[a]	4.24[b]	4.26[b]	4.02[a]	0.06	0.001
F/G	2.43[a]	2.48[a]	2.57[a]	2.77[b]	0.10	0.007
NE caloric efficiency	2,777[a]	2,831[a]	2,934[a]	3,167[b]	77.5	0.007
d 21 BW, lb	163.6[b]	164.1[b]	162.6[b]	158.2[a]	1.2	0.001
Exp. 4						
d 0 BW, lb	192.5	192.5	192.5	192.6	2.6	0.999
ADG, lb	2.28[c]	2.17[bc]	2.13[b]	1.91[a]	0.04	0.001
ADFI, lb	5.90	5.87	5.90	5.64	0.10	0.163
F/G	2.59[a]	2.70[b]	2.78[b]	2.95[c]	0.03	0.001
NE caloric efficiency	2992[a]	3121[b]	3210[b]	3410[c]	42.9	0.001
d 21 BW, lb	240.5[c]	238.6[bc]	237.6[b]	233.0[a]	2.6	0.001

[1]A total of 1,188, 1,232, 1,204, and 1,183 pigs (PIC 337 × 1050) were used for Experiments 1, 2, 3 and 4, respectively, in 21-d growth trials. Each treatment had 11 replications with 24 to 28 pigs per pen.

[2] Dietary treatments were HHH (High CP, High Lys, and High Trp), LHH (Low CP, High Lys, and High Trp), LLH (Low CP, Low Lys, and High Trp), LLL (Low CP, Low Lys, and Low Trp).

[3] Caloric efficiency is expressed as kcal/lb of gain.

[a,b,c,d] Means in same row with different superscripts differ ($P < 0.05$).