Evaluating lysine requirements of nursery pigs fed low protein diets with different sources of nonessential amino acids[1]

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ABSTRACT: The Lys requirement of nursery pigs may be dependent on the source of nonessential AA (NEAA) nitrogen or the source of Lys itself. However, little peer-reviewed data examines these phenomena. The objectives of these experiments were to determine if the Lys requirement of pigs is altered when 1) low protein diets are supplemented with different sources of NEAA nitrogen or 2) Lys is supplied as a crystalline source instead of intact protein such as soybean meal (SBM). Two 14-d experiments were conducted using 450 (Exp. 1) and 540 (Exp. 2) pigs (PIC C22/C29 × 337). There were 10 treatments in each experiment, each aligned as a 2 × 5 factorial. In Exp. 1, there were 2 sources of NEAA (L-Gln + L-Gly or L-Gly + L-Ala + L-Pro + L-His) and 5 levels of Lys (1.2, 1.3, 1.4, 1.5, and 1.6%). In Exp. 2, there were 2 sources of proteins providing additional Lys (L-Lys HCl or SBM) and the same 5 levels of Lys. Following weaning at 18 to 22 d of age, pigs were fed a common starter diet for 5 d postweaning followed by a 14-d treatment period. Pigs were weighed and feed disappearance determined on d 0, 7, and 14 of the experiment. Data were analyzed using the MIXED and NLIN procedures of SAS (SAS Inst., Cary, NC). In Exp. 1, increasing CP and Lys resulted in a quadratic increase ($P < 0.05$) in ADG and a linear improvement ($P < 0.05$) in G:F during the 14-d treatment period. Breakpoint regression analyses revealed that optimum ADG was obtained at 1.36% Lys, while optimum G:F was obtained at 1.45% Lys. The source of NEAA did not affect ($P > 0.10$) growth performance during the treatment period. In Exp. 2, both ADG and G:F increased linearly ($P < 0.05$) with increasing Lys. Optimal ADG was obtained at 1.47% Lys, but the breakpoint for optimum G:F was above tested levels. Source of Lys did not affect ($P > 0.10$) ADG, but pigs fed additional Lys from crystalline sources had improved ($P < 0.05$) G:F than those fed additional Lys from intact protein at 1.50% Lys; however, the analyzed Lys values at this level differ. Overall, these data show that the standardized ileal digestibility Lys requirement of pigs is not altered when low protein diets are supplemented with different sources of NEAA nitrogen. Feed efficiency appears to be maximized when additional Lys is supplied by L-Lys HCl instead of SBM, but more research is needed to confirm this phenomenon.

Key words: amino acid, essential, lysine, pig, protein, soybean meal

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INTRODUCTION

Through the use of crystalline AA, the CP concentration of nursery pigs diets can be decreased, which reduces both diet cost and nitrogen excretion. In fact, total nitrogen losses are reduced nearly 8% for each percentage reduction in CP (Kerr and Easter, 1995). Both cost savings and environmental regulations have led to the widespread use of crystalline AA, especially in nursery diets. This use lowers the overall diet CP concentrations because less soybean meal (**SBM**) is used in formulation, raising the question if a minimum CP requirement exists in nursery pigs so that sufficient N is present to generate nonessential AA (**NEAA**). If insufficient CP is available, some AA typically considered to be nonessential in nursery pig diets may, under these conditions, become essential. These conditions,

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Table 1. Formulated diet ingredient composition (as-fed basis; Exp. 1)[1]

	Nonessential AA source									
	L-Gln and L-Gly					L-Gly, L-Ala, L-Pro, and L-His				
	Calculated SID[2] Lys, %									
Ingredient, %	1.20	1.30	1.40	1.50	1.60	1.20	1.30	1.40	1.50	1.60
Corn	35.79	35.66	35.53	35.40	35.27	35.71	35.58	35.45	35.32	35.19
Soybean meal, 46.5% CP	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Whey (spray-dried)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Lactose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Monocalcium phosphate	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Zinc oxide (72%)	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Vitamin premix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix[4]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Ala	–	–	–	–	–	0.60	0.60	0.60	0.60	0.60
L-Gln	1.15	1.15	1.15	1.15	1.15	–	–	–	–	–
L-Gly	1.15	1.15	1.15	1.15	1.15	0.60	0.60	0.60	0.60	0.60
L-His	–	–	–	–	–	0.58	0.58	0.58	0.58	0.58
L-Ile	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-Leu	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-Lys HCl	–	0.13	0.26	0.39	0.52	–	0.13	0.26	0.39	0.52
DL-Met	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
L-Phe	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
L-Pro	–	–	–	–	–	0.60	0.60	0.60	0.60	0.60
L-Thr	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
L-Trp	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-Val	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]Treatments were created by mixing 10 separate premixes with a single basal diet.

[2]SID = standardized ileal digestibility.

[3]Provided (per kilogram of complete diet): 3,063 IU vitamin A, 350 IU vitamin D_3, 25 IU vitamin E, 1.5 mg menadione, 6 mg riboflavin, 28 mg niacin, 14 mg pantothenic acid, and 0.025 mg vitamin B_{12}.

[4]Provided (per kilogram of complete diet): 165 mg $ZnSO_4$, 165 mg $FeSO_4$, 39 mg $MnSO_4$, 17 mg $CuSO_4$, 0.3 mg $Ca(IO_3)_2$, and 0.3 mg Na_2SeO_3.

such as feeding low protein diets, may result in deceptively high Lys requirements or requirements that differ with different sources of either NEAA or Lys.

Numerous experiments have evaluated the optimum AA concentration in various growth stages of pigs, but at times these AA requirements may be confounded by the nature of the basal diet. For instance, diets low in CP but high in AA due to crystalline AA addition typically result in poorer performance than conventional diets (Davis et al., 1997; de Rodas et al., 1997; Chung et al., 1999; Bradley et al., 2008). This phenomenon suggests that some aspect of AA nutrition is not well understood and may imply that some AA that are typically thought to be nonessential actually become essential when the CP concentration of the diet is below a certain quantity (Nemechek, 2011). Better understanding of these AA requirements may create an opportunity to decrease feed costs, particularly during times of high protein prices. The authors hypothesized that pigs fed a low protein diet and supplemented with NEAA from L-Gln + L-Gly would have a greater Lys requirement than those supplemented with NEAA from L-Gly + L-Ala + L-Pro + L-His. Therefore, the objective of Exp. 1 was to determine if the Lys requirement for pigs is altered when low protein diets are supplemented with different sources of NEAA nitrogen.

In addition to the source of NEAA nitrogen, the Lys requirement may also be confounded by the source of Lys itself. Theoretically, performance should be similar whether AA are provided by either crystalline sources or intact protein. However, maximum performance is not always achieved by experiments with high crystalline AA concentrations, which often restricts the use of high L-Lys HCl in commercial diets (Kats et al., 1994; Nemechek et al., 2011). Understanding the differences between crystalline and intact protein inclusion may allow producers to maximize profitability by taking advantage of flexibility in feed ingredients. The authors

Table 2. Formulated diet nutrient composition (as-fed basis; Exp. 1)

	Nonessential AA source									
	L-Gln and L-Gly					L-Gly, L-Ala, L-Pro, and L-His				
	Calculated SID[1] Lys, %									
Item	1.20	1.30	1.40	1.50	1.60	1.20	1.30	1.40	1.50	1.60
Total Lys, %	1.35	1.45	1.55	1.65	1.75	1.35	1.45	1.55	1.65	1.75
SID Lys:CP ratio, %	5.3	5.7	6.1	6.4	6.8	5.3	5.7	6.1	6.4	6.8
SID AA, %										
Arg	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31
His	0.52	0.52	0.52	0.52	0.52	1.09	1.09	1.09	1.09	1.09
Ile	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Leu	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60
Lys	1.18	1.28	1.38	1.48	1.58	1.18	1.28	1.38	1.48	1.58
Met	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Pro	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Thr	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04
Trp	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Val	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12
Total sulfur	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
CP, %	25.3	25.4	25.5	25.7	25.8	25.3	25.4	25.5	25.7	25.8
ME, Mcal/kg	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55
SID Lys:ME, g/Mcal	4.7	5.1	5.5	5.8	6.2	4.7	5.1	5.5	5.8	6.2
Ca, %	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
P, %	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Available P, %	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40

[1]SID = standardized ileal digestibility.

hypothesized that the Lys requirement of pigs would be greater when pigs were fed low protein diets with Lys from L-Lys HCl compared with SBM because more Lys is required for the generation of NEAA. The objective of Exp. 2 was to determine if the Lys requirement for pigs is altered when Lys is supplied by crystalline AA instead of intact protein.

MATERIALS AND METHODS

Experimental procedures used in these studies were approved by the Iowa State University Institutional Animal Care and Use Committee (**IACUC** number 8-10-7007-S and number 11-10-7040-S for Exp. 1 and 2, respectively).

Animals and Housing

A total of 740 (Exp. 1) or 605 (Exp. 2) weanling pigs (PIC C22/C29 × 337; Carthage Veterinary Service, Carthage, IL) were purchased and transported to the Iowa State University Swine Nutrition farm (Ames, IA), where they were weighed and tagged with an individual identification number. After a 5-d acclimation period, 540 (Exp. 1; 6.7 ± 1.84 kg BW) or 450 (Exp. 2; 6.6 ± 1.86 kg BW) pigs were used in the experiment. Pens (1.2 by 1.2 m) contained a 4-hole dry self-feeder and 1 nipple waterer to provide ad libitum access to feed and water.

Experimental Design and Diets

Pigs were blocked by initial weight, and pens were randomly assigned to 1 of 10 different experimental diets within block. There were 6 (Exp. 1) or 5 (Exp. 2) pigs per pen and 9 pens per treatment. Piglet sex was equalized within pens within block and across the experiment. A common commercial diet was fed for 5 d during the acclimation period. Experimental diets were fed for 14 d. After the completion of the experiments, pigs were fed a common nursery diet for 7 d and weighed to determine if carryover effects of treatment diets existed. In Exp. 1, treatments were aligned as a 2 × 5 factorial: 2 sources of NEAA (L-Gln + L-Gly or L-Gly + L-Ala + L-Pro + L-His) and 5 levels of Lys (1.2, 1.3, 1.4, 1.5, or 1.6%; Tables 1 and 2). Two different sources of NEAA were selected to reflect different approaches. Glutamine and Gly are more commonly used while the combination of Gly, Ala, Pro, and His represented a more novel and diverse source of NEAA nitrogen. The concentration of CP was attempted to be held constant while Lys level increased, so the resultant Lys:CP ratio changed as a method to determine if a minimum Lys:CP exists. In Exp. 2, treatments were aligned as a 2 × 5 factorial: 2 sources of proteins providing additional Lys (L-Lys HCl; increasing concentrations of L-Lys HCl and constant concentrations of SBM, fish meal, and whey or SBM vs. increasing concentrations of SBM and constant concentrations of L-Lys HCl,

Table 3. Formulated diet ingredient composition (as-fed basis; Exp. 2)[1]

	Lys source									
	L-Lys HCl					SBM[2]				
	Calculated SID[3] Lys, %									
Ingredient, %	1.20	1.30	1.40	1.50	1.60	1.20	1.30	1.40	1.50	1.60
Corn	50.98	50.48	49.98	49.48	48.98	62.72	58.76	54.80	50.84	46.89
SBM, 46.5% CP	27.40	27.40	27.40	27.40	27.40	14.65	18.63	22.60	26.58	30.55
Fish meal	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Whey (spray-dried)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
Limestone	0.35	0.34	0.16	0.32	0.31	0.40	0.38	0.36	0.33	0.31
Monocalcium phosphate	0.45	0.45	0.16	0.45	0.45	0.55	0.53	0.50	0.48	0.45
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Zinc oxide (72%)	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Vitamin premix[4]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix[5]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Gln	–	0.10	0.20	0.30	0.40	–	–	–	–	–
L-Gly	–	0.10	0.26	0.30	0.40	–	–	–	–	–
L-Lys HCl	–	0.13	0.45	0.39	0.51	0.41	0.41	0.41	0.41	0.41
DL-Met	0.04	0.10	0.33	0.23	0.29	0.16	0.18	0.21	0.23	0.25
L-Thr	0.03	0.09	0.35	0.22	0.29	0.20	0.21	0.22	0.23	0.24
L-Trp	–	0.01	0.26	0.04	0.06	0.05	0.05	0.04	0.04	0.04
L-Val	–	0.04	0.20	0.12	0.16	0.10	0.10	0.11	0.11	0.11
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]Treatments were created by mixing diets containing 1.20 and 1.60% Lys and blending them to create diets containing 1.30, 1.40, and 1.50% Lys.

[2]SBM = soybean meal.

[3]SID = standardized ileal digestibility.

[4]Provided (per kilogram of complete diet): 3,063 IU vitamin A, 350 IU vitamin D_3, 25 IU vitamin E, 1.5 mg menadione, 6 mg riboflavin, 28 mg niacin, 14 mg pantothenic acid, and 0.025 mg vitamin B_{12}.

[5]Provided (per kilogram of complete diet): 165 mg $ZnSO_4$, 165 mg $FeSO_4$, 39 mg $MnSO_4$, 17 mg $CuSO_4$, 0.3 mg $Ca(IO_3)_2$, and 0.3 mg Na_2SeO_3.

Table 4. Formulated diet nutrient composition (as-fed basis; Exp. 2)

	Lys source									
	L-Lys HCl					SBM[1]				
	Calculated SID[2] Lys, %									
Item	1.20	1.30	1.40	1.50	1.60	1.20	1.30	1.40	1.50	1.60
Total Lys, %	1.32	1.42	1.52	1.62	1.72	1.30	1.41	1.52	1.62	1.73
SID AA, %										
Arg	1.26	1.26	1.26	1.26	1.26	0.89	1.01	1.12	1.24	1.35
His	0.53	0.53	0.53	0.53	0.53	0.41	0.45	0.49	0.52	0.56
Ile	0.83	0.83	0.83	0.82	0.82	0.62	0.69	0.75	0.82	0.88
Leu	1.68	1.68	1.67	1.67	1.66	1.38	1.47	1.56	1.65	1.74
Lys	1.18	1.28	1.38	1.48	1.58	1.19	1.29	1.39	1.48	1.58
Met	0.39	0.48	0.52	0.58	0.64	0.45	0.49	0.53	0.57	0.61
Pro	0.91	0.91	0.91	0.91	0.91	0.69	0.76	0.83	0.89	0.96
Thr	0.77	0.84	0.90	0.97	1.03	0.77	0.83	0.90	0.96	1.02
Trp	0.23	0.25	0.26	0.28	0.29	0.21	0.23	0.25	0.27	0.29
Val	0.92	0.96	1.00	1.03	1.07	0.81	0.88	0.94	1.01	1.07
Total sulfur	0.70	0.76	0.82	0.88	0.94	0.70	0.76	0.82	0.87	0.93
CP, %	22.0	22.2	22.4	22.6	22.8	17.7	19.3	20.8	22.4	23.9
ME, Mcal/kg	3.51	3.51	3.51	3.51	3.51	3.51	3.51	3.51	3.51	3.51
Ca, %	0.71	0.71	0.70	0.70	0.69	0.70	0.70	0.70	0.70	0.70
P, %	0.68	0.68	0.68	0.67	0.67	0.64	0.65	0.67	0.68	0.69
Available P, %	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40

[1]SBM = soybean meal.

[2]SID = standardized ileal digestibility.

Table 5. Analyzed total AA composition of diets (as-fed basis; Exp. 1)[1]

	Nonessential AA source									
	L-Gln and L-Gly					L-Gly, L-Ala, L-Pro, and L-His				
	Calculated SID[2] Lys, %									
Item	1.20	1.30	1.40	1.50	1.60	1.20	1.30	1.40	1.50	1.60
Analyzed SID Lys,[3] %	1.14	1.21	1.32	1.40	1.48	1.14	1.21	1.26	1.43	1.46
Essential AA, %										
Arg	1.39	1.43	1.45	1.41	1.47	1.44	1.43	1.40	1.45	1.41
His	0.58	0.56	0.58	0.56	0.57	0.99	0.99	0.95	1.01	1.02
Ile	1.06	1.03	1.07	1.06	1.06	1.05	1.04	1.00	1.06	1.05
Leu	1.88	1.84	1.90	1.87	1.87	1.88	1.85	1.48	1.89	1.84
Lys	1.41	1.49	1.63	1.73	1.82	1.41	1.49	1.55	1.76	1.80
Met	0.73	0.75	0.74	0.75	0.74	0.76	0.73	0.71	0.75	0.74
Phe	1.19	1.16	1.20	1.16	1.17	1.18	1.16	1.12	1.19	1.14
Thr	1.23	1.22	1.21	1.25	1.19	1.20	1.22	1.19	1.24	1.19
Trp	0.35	0.36	0.35	0.36	0.36	0.34	0.37	0.37	0.36	0.37
Val	1.31	1.30	1.32	1.30	1.30	1.31	1.29	1.25	1.35	1.29
Nonessential AA, %										
Ala	1.19	1.15	1.19	1.16	1.16	1.77	1.75	1.71	1.79	1.78
Asp	2.44	2.38	2.46	2.39	2.41	2.42	2.40	2.30	2.46	2.36
Cys	0.33	0.32	0.33	0.32	0.32	0.32	0.32	0.31	0.33	0.32
Gln	5.15	5.05	5.18	5.05	5.10	3.91	3.86	3.70	3.95	3.79
Gly	2.28	2.31	2.34	2.30	2.25	1.68	1.67	1.62	1.71	1.67
Pro	0.00	0.00	0.00	0.00	1.21	1.73	1.75	1.66	1.80	1.78
Ser	1.14	1.10	1.14	1.11	1.12	1.12	1.11	1.07	1.14	1.10
Tyr	0.54	0.52	0.55	0.59	0.53	0.54	0.52	0.49	0.53	0.50

[1]Results are a mean of 1 sample analyzed in duplicate.

[2]SID = standardized ileal digestibility.

[3]Calculated as the sum of SID of ingredients by multiplying the formulated total ingredient inclusion by its published SID value (National Swine Nutrition Guide, 2010). Values included corn, 0.78; soybean meal, 0.89; whey, 0.87; lactose, 0.87; fish meal, 0.94; L-Lys HCl, 0.788; DL-Met, 0.99; L-Thr, 0.99; L-Trp, 0.985; L-Val, 0.965; and L-Ile, L-Pro, L-Leu, L-Gln, L-Gly, L-Ala, L-Pro, and L-His, 1.0.

fish meal, and whey) and 5 levels of Lys (1.2, 1.3, 1.4, 1.5, or 1.6%; Tables 3 and 4). Differing concentrations of crystalline AA other than L-Lys HCl were added to maintain minimum AA:Lys ratios according to the ideal protein ratio concept (NRC, 2012).

All diets were analyzed for essential and NEAA concentrations (Ajinomoto Heartland LLC, Chicago, IL) using HPLC (method 993.12; AOAC, 2000). After conversion to a standardized ileal digestibility (**SID**) basis, total analyzed Lys concentrations were similar to formulated levels in Exp. 1 (Table 5). However, analyzed Lys concentrations in Exp. 2 varied by up to 22% compared with formulated levels (Table 6). Due to the complexity of accurate AA analyses, especially in diets containing milk products, data have been presented according to calculated concentrations. Pigs were weighed on d 0, 14, and 21 of the experiment, and feed disappearance was measured from d 0 to 14 to determine ADG, ADFI, and G:F.

Statistical Analyses

Data were analyzed by the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. The model statement included the fixed effects of source of NEAA and calculated SID Lys:CP (Exp. 1) or source of additional Lys (Exp. 2), the Lys level, and the interaction of the 2. There were no interactions ($P > 0.24$) in either experiment, so the interaction term was removed from the model. Weight block was considered a random effect. Least squared means and pooled SEM were calculated by the LSMEANS and DIFFS options in SAS. Linear, quadratic, cubic, and quartic effects of increasing calculated Lys level were tested and pairwise comparisons were used to contrast differences in protein sources at each Lys level whenever the main effect of Lys was significant. Results were considered significant or trends if their P-values were <0.05 or <0.10, respectively. Various statistical regression models were conducted using the REG procedure of SAS, and the nutrient requirement (i.e., breakpoint regression analysis) was determined using the NLIN procedure of SAS according to Kaps and Lamberson (2009) and Robbins et al. (2006).

Table 6. Analyzed total AA composition of diets (as-fed basis; Exp. 2)[1]

	Lys source									
	L-Lys HCl					SBM[2]				
	Calculated SID[3] Lys, %									
Item	1.20	1.30	1.40	1.50	1.60	1.20	1.30	1.40	1.50	1.60
Analyzed SID Lys,[4] %	1.11	1.20	1.23	1.31	1.25	1.05	1.10	1.16	1.18	1.36
Essential AA, %										
Arg	1.51	1.65	1.65	1.82	1.25	1.46	1.52	1.54	1.48	1.87
His	0.58	0.50	0.68	0.55	0.51	0.64	0.67	0.68	0.70	0.55
Ile	0.77	0.56	1.06	0.95	0.86	1.02	1.14	1.06	1.07	0.98
Leu	1.53	1.65	1.84	1.78	1.74	1.64	1.56	1.66	1.68	1.82
Lys	1.41	1.52	1.54	1.66	1.59	1.36	1.42	1.48	1.50	1.72
Met	0.47	0.53	0.57	0.58	0.54	0.51	0.79	0.64	0.70	0.62
Phe	0.87	0.94	1.27	1.04	0.95	1.12	1.25	1.12	1.29	1.07
Thr	0.93	1.04	1.20	1.12	1.06	1.10	1.41	1.29	1.16	1.15
Trp	0.24	0.29	0.26	0.28	0.20	0.23	0.24	0.23	0.24	0.26
Val	1.07	1.17	1.39	1.27	1.15	1.11	1.53	1.44	1.50	1.32
Nonessential AA, %										
Ala	1.06	1.12	1.41	1.20	1.16	1.38	1.39	1.41	1.42	1.23
Asp	1.74	1.95	2.12	2.17	1.90	2.48	2.60	2.64	2.03	2.24
Cys	0.27	0.29	0.39	0.31	0.32	0.37	0.38	0.39	0.39	0.32
Gln	2.98	3.27	3.49	3.59	3.30	3.18	4.74	3.61	4.78	3.69
Gly	0.93	1.01	1.38	1.10	0.99	1.23	1.75	1.52	1.64	1.13
Pro	1.05	1.13	0.86	1.21	0.64	0.84	0.82	0.89	0.88	1.17
Ser	0.89	0.97	1.28	1.07	0.96	1.22	1.26	1.28	1.31	1.10
Tyr	0.42	0.46	0.63	0.52	0.42	0.60	0.29	0.42	0.31	0.53

[1]Results are a mean of 1 sample analyzed in duplicate.

[2]SBM = soybean meal.

[3]SID = standardized ileal digestibility.

[4]Calculated as the sum of SID of ingredients by multiplying the formulated total ingredient inclusion by its published SID value (National Swine Nutrition Guide, 2010). Values included corn, 0.78; soybean meal, 0.89; whey, 0.87; lactose, 0.87; fish meal, 0.94; L-Lys HCl, 0.788; DL-Met, 0.99; L-Thr, 0.99; L-Trp, 0.985; L-Val, 0.965; and L-Ile, L-Pro, L-Leu, L-Gln, L-Gly, L-Ala, L-Pro, and L-His, 1.0.

Table 7. Effects of calculated Lys level or nonessential AA (NEAA) N source on pig growth performance (Exp. 1)[1]

	NEAA source														
	L-Gln and L-Gly					L-Gly, L-Ala, L-Pro, and L-His									
	Calculated SID[2] Lys, %											*P*-value			
Item	1.2	1.3	1.4	1.5	1.6	1.2	1.3	1.4	1.5	1.6	SEM	Lys	NEAA source	Lys × NEAA	Lys:CP
Pens	9	9	9	9	9	9	9	9	9	9					
Weight, kg															
d 0	6.65	6.64	6.66	6.65	6.64	6.65	6.67	6.67	6.66	6.66	0.292	1.000	0.756	1.000	1.000
d 14	11.27	11.83	12.27	12.20	12.17	11.13	11.90	12.09	12.32	12.38	0.452	<0.0001	0.906	0.902	0.0003
d 21	14.32	15.20	15.56	15.62	15.47	14.22	15.09	15.17	15.32	15.55	0.528	0.002	0.429	0.956	0.011
ADG, g															
d 0 to 14	330	362	401	375	395	320	374	387	385	409	17.1	<0.0001	0.815	0.833	0.0002
d 14 to 21	436	481	467	489	472	441	456	440	428	454	23.0	0.712	0.076	0.684	0.417
d 0 to 21	365	402	423	413	421	360	401	405	399	424	15.7	0.001	0.447	0.942	0.013
ADFI, g															
d 0 to 14	451	476	499	469	464	449	486	477	485	477	19.3	0.205	0.789	0.774	0.346
G:F															
d 0 to 14	0.73	0.76	0.80	0.80	0.85	0.71	0.77	0.81	0.80	0.86	0.018	<0.0001	0.898	0.941	<0.0001

[1]A total of 540 pigs (6 pigs/pen and 9 pens/treatment) were used to determine if NEAA N source affected Lys requirement in pigs from 7 to 16 kg. Dietary treatments were fed from 0 to 14 d, and pigs were weighed on d 21 of the experiment to determine if carryover effects of dietary treatments existed.

[2]SID = standardized ileal digestibility.

Table 8. Pairwise comparisons and contrasts of nonessential AA (NEAA) N sources on pig growth performance according to calculated Lys level (Exp. 1)[1]

	Pairwise comparisons[1]								
	Calculated SID[2] Lys, %					Contrasts			
Item	1.2	1.3	1.4	1.5	1.6	Linear	Quadratic	Cubic	Quartic
Weight, kg									
d 14	0.681	0.834	0.589	0.702	0.530	<0.0001	0.018	0.591	0.969
d 21	0.818	0.821	0.404	0.520	0.861	0.0003	0.044	0.420	0.806
ADG, g									
d 0 to 14	0.650	0.601	0.523	0.633	0.542	<0.0001	0.042	0.128	0.275
d 0 to 21	0.812	0.978	0.367	0.511	0.894	0.0002	0.091	0.123	0.666
G:F									
d 0 to 14	0.508	0.662	0.793	0.978	0.778	<0.0001	0.647	0.092	0.108

[1]Values are the pairwise comparisons between NEAA supplied by L-Gln + L-Gly or L-Gly + L-Ala + L-Pro + L-His at varying Lys levels.

[2]SID = standardized ileal digestibility.

RESULTS AND DISCUSSION

Experiment 1

Increasing concentrations of CP and Lys resulted in heavier weights on both d 14 ($P < 0.05$) and 21 ($P < 0.05$) due to improved ADG during the treatment period ($P < 0.05$; Table 7). Although there was no effect ($P > 0.10$) of Lys during the 7-d carryover period, ADG was still improved ($P < 0.05$) over the entire 21 d. This is in contrast to the findings of Nemechek (2011), who reported no overall effect of Lys level because pigs fed low Lys during the treatment period had high compensatory gain during the carryover period. It has been established that both the extent and duration of AA restriction affects compensatory gain (Prince et al., 1983; Kamalakar et al., 2009), so perhaps the pigs fed the lower Lys treatments in our experiment were not restricted to a degree resulting in compensatory gain. Feed intake was not affected ($P > 0.10$); however, G:F was improved ($P < 0.05$) with increasing Lys levels from d 0 to 14. Expectedly, similar effects were seen with increasing Lys:CP ratio. The highest tested Lys:CP ratio was 6.8%, which is substantially lower than ratios thought to decrease growth performance due to the generation of NEAA from essential AA (Mahan and Shields, 1998). In addition, source of NEAA did not significantly affect ($P > 0.05$) any performance variables. This suggests that even the low protein diets with only L-Gln + L-Gly supplementation had sufficient N to allow for the generation of Ala, Pro, and His for optimum growth. Perhaps these results would be different if the dietary CP concentrations were lowered even further, but these formulations represent realistic low protein diets.

Pairwise comparisons using formulated Lys concentrations between source of NEAA N at different Lys:CP ratios revealed no differences in any measured variables ($P > 0.10$; Table 8), which reinforces our conclusion that Gly, Ala, Pro, and His were not restricted to the level at which the supplied NEAA nitrogen could be transaminated to meet AA requirements. Weights at d 14 and 21 as well as ADG from d 0 to 14 increased quadratically ($P < 0.05$) with increasing CP. Similarly, ADG from d 0 to 21 and G:F increased in a linear ($P < 0.05$) manner.

Linear and curvilinear regression demonstrated that a variety of models fit the presented data and were used to predict responses to CP. The R^2, adjusted R^2, or root means square error (**RMSE**) are thought to be the best determinants of model fit (Kaps and Lamberson, 2009; Robbins et al., 2006). All models fit the data similarly; however, the selection of a specific model can dramatically affect the identification of the nutrient requirement. One- or 2-slope breakpoint models are most commonly used to set nutritional requirements, but they assume that the response is linear before the breakpoint. Alternately, exponential and quadratic models account for curvilinear responses but may overestimate requirements (Robbins et al., 2006; Nemechek, 2011). For this reason, we have analyzed and reported data according to all possible models (Table 9). However, we have chosen to only discuss the results of the 1-slope breakpoint model in an attempt to be concise and be easily compared with other literature. The ADG breakpoint of all treatments according to this model was 1.36% Lys. The breakpoint of treatments with NEAA supplied by L-Gln + L-Gly was greater (1.40 vs. 1.33%) than when supplied by L-Gly + L-Ala + L-Pro + L-His. The G:F breakpoint of all treatments according to the 1-slope breakpoint model was 1.45%. The breakpoint of treatments with NEAA supplied by L-Gln + L-Gly was again greater (1.48 vs. 1.43%) than when supplied by L-Gly + L-Ala + L-Pro + L-His. Therefore, the SID Lys requirement of pigs in our experiment can be set at 1.36% for ADG and 1.45% for G:F.

In summary, data from Exp. 1 suggest that ADG and G:F were improved with increasing Lys levels up to 1.36 or 1.45% for ADG and G:F, respectively. There was no difference in NEAA source, but nursery pig performance

Table 9. Effects of calculated Lys level or nonessential AA N source on model determination and Lys breakpoint (Exp. 1)

	Variable									
	ADG					G:F				
	Model									
Item	Treatment *P*-value	R^2	Adjusted R^2	RMSE[1]	Breakpoint	Treatment *P*-value	R^2	Adjusted R^2	RMSE	Breakpoint
All treatments										
Linear	0.002	0.72	0.68	16.47	n/a[2]	<0.0001	0.91	0.90	0.015	n/a
Exponential	0.002	0.72	0.69	16.27	1.40	<0.0001	0.91	0.90	0.015	1.53
Quadratic breakpoint	0.002	0.83	0.78	13.71	1.44	<0.0001	0.91	0.90	0.019	2.25
1-slope breakpoint	0.0004	0.89	0.86	10.80	1.36	0.0002	0.91	0.88	0.016	1.45
2-slope breakpoint	0.004	0.90	0.87	10.69	1.34	0.0001	0.92	0.90	0.019	1.33
L-Gln + L-Gly only										
Linear	0.112	0.63	0.51	19.97	n/a	0.013	0.95	0.93	0.012	n/a
Exponential	0.104	0.64	0.52	19.76	1.40	0.024	0.89	0.86	0.021	1.51
Quadratic breakpoint	0.176	0.82	0.64	17.13	1.44	0.049	0.95	0.93	0.012	11.05
1-slope breakpoint	0.187	0.81	0.61	17.95	1.40	0.047	0.95	0.90	0.014	1.48
2-slope breakpoint	0.112	0.89	0.78	13.28	1.40	0.047	0.95	0.90	0.014	1.40
L-Gly + L-Ala + L-Pro + L-His only										
Linear	0.041	0.81	0.74	16.87	n/a	0.023	0.89	0.86	0.021	n/a
Exponential	0.044	0.81	0.75	16.59	1.40	0.014	0.85	0.93	0.012	1.55
Quadratic breakpoint	0.126	0.87	0.74	16.89	1.43	0.023	0.89	0.86	0.021	1.86
1-slope breakpoint	0.103	0.90	0.81	14.49	1.33	0.096	0.90	0.80	0.024	1.43
2-slope breakpoint	0.032	0.97	0.95	7.58	1.30	0.074	0.93	0.87	0.020	1.31

[1]RMSE = root means square error.

[2]n/a = not applicable.

Table 10. Effects of calculated Lys level or source of additional Lys on pig growth performance (Exp. 2)[1]

	Lys source													
	L-Lys HCl					SBM[2]								
	Calculated SID[2] Lys, %											*P*-value		
Item	1.2	1.3	1.4	1.5	1.6	1.2	1.3	1.4	1.5	1.6	SEM	Lys level	Lys source	Level × source
Pens	9	9	9	9	9	9	9	9	9	9				
Weight, kg														
d 0	6.61	6.60	6.62	6.57	6.60	6.55	6.63	6.64	6.66	6.56	0.292	0.978	0.896	0.940
d 14	10.99	11.44	11.77	11.90	11.73	10.97	11.31	11.50	11.93	11.87	0.495	0.0001	0.702	0.892
d 21	14.61	15.00	15.24	15.02	15.05	14.23	14.53	14.86	15.38	15.49	0.602	0.068	0.671	0.464
ADG, g														
d 0 to 14	312	338	368	381	367	316	334	347	365	379	16.9	<0.0001	0.476	0.542
d 14 to 21	518	523	495	446	474	466	461	479	493	484	28.2	0.907	0.401	0.247
d 0 to 21	381	399	410	403	403	366	376	391	408	414	17.3	0.048	0.322	0.589
ADFI, g														
d 0 to 14	421	425	452	450	432	440	429	443	460	439	19.6	0.290	0.522	0.910
G:F														
d 0 to 14	0.74	0.80	0.81	0.85	0.85	0.72	0.78	0.78	0.79	0.86	0.015	<0.0001	0.011	0.242

[1]A total of 450 pigs (5 pigs/pen and 9 pens/treatment) were used to determine if Lys supplied by L-Lys HCl or SBM affected Lys requirement in pigs from 7 to 16 kg. Dietary treatments were fed from 0 to 14 d, and pigs were weighed on d 21 of the experiment to determine if carryover effects of dietary treatments existed.

[2]SBM = soybean meal.

[3]SID = standardized ileal digestibility.

Table 11. Pairwise comparisons and contrasts of additional Lys source on pig growth performance according to calculated Lys level (Exp. 2)

Calculated SID[2] Lys, %	Pairwise comparisons[1]					Contrasts			
	1.2	1.3	1.4	1.5	1.6	Linear	Quadratic	Cubic	Quartic
Weight, kg									
d 14	0.945	0.662	0.367	0.924	0.644	<0.0001	0.071	0.567	0.620
ADG, g									
d 0 to 14	0.810	0.802	0.183	0.310	0.420	<0.0001	0.082	0.530	0.928
G:F									
d 0 to 14	0.178	0.424	0.171	0.014	0.643	<0.0001	0.309	0.069	0.418

[1]Values are the pairwise comparisons between additional Lys supplied by L-Lys HCl or by intact soybean meal.

[2]SID = standardized ileal digestibility.

was maximized at a higher CP and Lys level when AA were supplied by L-Gln + L-Gly compared with L-Gly + L-Ala + L-Pro + L-His.

Experiment 2

Increasing levels of Lys resulted in heavier weights on d 14 ($P < 0.05$) due to improved ($P < 0.05$) ADG during the treatment period (Table 10). Although there was no effect ($P > 0.10$) during the 7-d carryover period, ADG was still improved ($P < 0.05$) over the entire 21 d. Feed intake was not affected ($P > 0.10$); however, G:F was improved ($P < 0.05$) with increasing Lys levels. The increased ADG and G:F but not ADFI response to increasing Lys level is in agreement with data from other researchers (Gaines et al., 2003; Kendall et al., 2008). The source of additional Lys did not affect ($P > 0.10$) pig weights, ADG, or ADFI but did affect ($P < 0.05$) G:F.

Pairwise comparisons between source of additional Lys at different formulated Lys levels revealed ($P > 0.10$) no differences in weight at d 14 or ADG from d 0 to 14 (Table 11). However, G:F was improved ($P < 0.05$) at 1.5% Lys when the additional Lys was supplied by L-Lys HCl compared with SBM, which was the level closest to the breakpoint. The source of this difference can be debated. The analyzed Lys concentration of the diet where Lys was supplied by L-Lys HCl was 1.66% compared to 1.50% in diets where Lys was supplied by SBM. The result could simply be the result of incorrect mixing and thus a common Lys quantity response. As stated previously, however, the inclusion of whey in these diets complicates AA analysis and these results may not be reflective of actual SID Lys concentrations. Alternate causes for the G:F improvement may be attributed to the antinutritional factors associated with SBM, which are known to cause gut hypersensitivity in nursery pigs (Li et al., 1990, 1991). Indeed, the fermentation of SBM has been shown to improve G:F, which may suggest that the trypsin inhibitors and oligosaccharides responsible for decreased pig performance in traditional SBM may also inhibit AA digestibility (Min et al., 2004; Kim et al., 2007; Cho et al., 2007; Jones et al., 2010). Our data suggests that supplementing diets with crystalline AA improves G:F compared with traditional SBM, at least at levels closest to the nutrient requirement. However, this effect should be reevaluated in diets where analyzed values are more closely related. As it stands, it is difficult to determine the true effect of Lys source because we are unable to refine the origin of the observed effect to only 1 variable.

All measured variables increased linearly ($P < 0.05$) with increasing Lys levels. Breakpoint regression of all treatments according to ADG was 1.47% Lys (Table 12). The breakpoint of treatments with additional Lys supplied by L-Lys HCl was lower (1.42 vs. 1.59%) than when supplied by SBM. When analyzed for G:F, the breakpoint was above tested levels. There was a breakpoint for the diets containing added Lys from L-Lys HCl (1.49%), but the breakpoint for diets with added Lys from SBM was again above tested levels, suggesting that the Lys requirement for optimal G:F in pigs fed added Lys from crystalline sources is higher compared with intact protein. However, additional research is required using higher Lys concentrations to determine the nutrient requirement and confirm these findings.

Overall, the results from Exp. 2 suggest that ADG was improved with increasing Lys levels up to 1.47% for ADG. No requirement could be defined based on G:F. While the source of Lys did not affect ADG, supplying Lys from L-Lys HCl compared with SBM resulted in improved G:F, particularly at the 1.5% Lys level.

The SID Lys requirements for 6.6- to 12.4-kg pigs established by Exp. 1 or Exp. 2 are substantially higher than those from the NRC (2012), which reports the Lys requirement to be 19 g/kg BW gain or approximately 1.10% for 5- to 10-kg pigs. However, the NRC (2012) is clear to point out its data to make this calculation is limited to 2 datasets, which do not use modern genotypes (Lewis et al., 1980; Martinez and Knabe, 1990). Numerous other experiments have suggested that the NRC (2012) underestimates the SID Lys requirements for modern pig genotypes and that the requirement is actually

Table 12. Effects of calculated Lys level or source of additional Lys on model determination and Lys breakpoint (Exp. 2)

	Variable									
	ADG					G:F				
	Model									
Item	Treatment *P*-value	R^2	Adjusted R^2	RMSE[1]	Breakpoint	Treatment *P*-value	R^2	Adjusted R^2	RMSE	Breakpoint
All treatments										
Linear	0.0001	0.85	0.83	10.24	n/a[2]	0.001	0.80	0.78	0.016	n/a
Exponential	<0.0001	0.91	0.90	8.09	4.19	0.0004	0.81	0.79	0.022	2.11
Quadratic breakpoint	0.0001	0.85	0.83	10.24	1.62	0.001	0.80	0.78	0.016	2.05
1-slope breakpoint	0.0001	0.92	0.90	7.90	1.47	0.001	0.80	0.78	0.022	1.61
2-slope breakpoint	0.0001	0.92	0.90	7.90	1.47	0.004	0.80	0.75	0.024	1.30
L-Lys HCl										
Linear	0.061	0.75	0.67	16.02	n/a	0.023	0.89	0.85	0.007	n/a
Exponential	0.018	0.89	0.86	10.53	4.11	0.008	0.94	0.92	0.013	4.14
Quadratic breakpoint	0.023	0.98	0.96	5.67	1.52	0.021	0.89	0.85	0.007	1.61
1-slope breakpoint	0.023	0.98	0.96	5.83	1.42	0.051	0.95	0.90	0.008	1.49
2-slope breakpoint	0.022	0.98	0.96	5.67	1.50	0.051	0.95	0.90	0.015	1.47
SBM[3]										
Linear	<0.0001	0.998	0.997	1.40	n/a	0.032	0.85	0.80	0.008	n/a
Exponential	0.010	0.95	0.94	6.30	0.28	0.028	0.82	0.76	0.024	0.07
Quadratic breakpoint	<0.0001	0.998	0.997	1.40	5.06	0.029	0.85	0.80	0.022	5.05
1-slope breakpoint	0.002	0.998	0.996	1.58	1.59	0.031	0.85	0.80	0.008	1.71
2-slope breakpoint	<0.0001	0.998	0.997	1.40	1.61	0.031	0.85	0.80	0.022	1.52

[1]RMSE = root means square error.
[2]n/a = not applicable.
[3]SBM = soybean meal.

1.30 to 1.45% (Lenehan et al., 2003; Gaines et al., 2003; Hill et al., 2007; Dean et al., 2007; Kendall et al., 2008). Of these experiments, Nemechek (2011) offers the most current and robust establishment of the Lys requirement of nursery pigs. Our Lys requirement levels are slightly higher than those suggested by Nemechek (2011), whose analyses revealed breakpoints at 1.30 and 1.39% for ADG and G:F, respectively. The breakpoint analyses in our experiments revealed that SID Lys concentration explained more variation in growth performance than in the Nemechek (2011) trials. While those experiments did not report adjusted R^2 or RMSE, the R^2 for breakpoint analysis in the Nemechek (2011) trials were 0.42 and 0.88 for ADG and G:F, respectively, compared with 0.89 (Exp. 1) or 0.92 (Exp. 2) for ADG and 0.91 (Exp.1) for G:F in our experiments. This is expected because our 2 experiments were conducted in a single facility and with a single pig genotype. Meanwhile, the Nemechek (2011) breakpoint analysis was conducted with 4 different pig sources and environments, inherently introducing more variability. Still, our data largely agrees Nemechek (2011) and other researchers (Hill et al., 2007; Dean et al., 2007; Kendall et al., 2008) in that the SID Lys requirement ranges between 1.3 and 1.5%, which is substantially higher than that suggested by the NRC (2012).

In conclusion, we have shown that the SID Lys requirement of pigs is not substantially altered when low protein diets are supplemented with different sources of NEAA nitrogen. However, G:F in nursery pigs appears to be maximized when additional Lys is supplied by L-Lys HCl instead of intact protein, and the SID Lys requirement of pigs fed additional Lys from L-Lys HCl appears to be lower than when the additional Lys is supplied from SBM. However, more research is needed to confirm these findings when analyzed and formulated Lys values are more consistent. Finally, the SID Lys requirement for optimum growth performance is dependent on pig environment and genotype but appears to range from 1.3 to 1.5%.

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