Estimation of the optimal ratio of standardized ileal digestible tryptophan to lysine for finishing barrows fed low protein diets supplemented with crystalline amino acids

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ABSTRACT: Optimum standardized ileal digestible (SID) tryptophan (Trp) to lysine (Lys) ratio was estimated for 67–96 kg barrows fed low protein diets supplemented with crystalline amino acids (AA). One hundred and fifty Yorkshire × Landrace barrows, with an average initial body weight (BW) of 67.3 ± 3.2 kg, were used in a 28-day feeding trial. All dietary treatments were based on corn, wheat bran, and soybean meal and were formulated to provide 10.5% crude protein and 12.6 MJ/kg metabolizable energy. The dietary SID Lys was set at 0.61% ensuring that Lys was marginally deficient for barrows of this weight range. Graded levels of crystalline L-Trp were added to the basal diet to produce diets providing SID Trp to Lys ratios of 0.131, 0.164, 0.197, 0.230, and 0.262. There were improvements in weight gain (linear and quadratic effect $P < 0.01$) and feed intake (linear effect $P = 0.04$) with increasing dietary SID Trp to Lys ratio. Increased SID Trp to Lys ratio resulted in a decrease in the serum urea nitrogen (SUN) content (linear and quadratic effect $P < 0.01$). The serum concentration of Trp increased with increasing dietary SID Trp to Lys ratio (linear effect $P = 0.03$, quadratic effect $P = 0.08$). Estimates of the optimum SID Trp to Lys ratios were 0.203, 0.197, and 0.214 for weight gain, feed conversion ratio, and SUN, respectively, using a broken-line model while a quadratic model produced optimum SID Trp to Lys ratios of 0.251, 0.224, and 0.249 for the same parameters. The results of this experiment indicate that the SID Trp to Lys ratio for finishing barrows is at least 0.203, which is higher than the SID ratio of Trp to Lys currently recommended by the National Research Council (NRC, 2012).

Keywords: tryptophan requirement; pig; performance; amino acid ratio

Tryptophan (Trp) is generally considered to be the second or third limiting amino acid (AA) in typical corn-soybean meal diets fed to growing and early-finishing pigs, while in late-finishing diets it is the second limiting AA (Lewis, 2001; Cromwell, 2004). For economic and environmental reasons, a reduction in dietary crude protein (CP) and supplementation with crystalline AA is a desirable goal for the pig industry (Kerr et al., 1995). Therefore, it is critical to determine the optimum dietary Trp to Lys ratio to allow greater flexibility in diet formulation and potentially greater utilization of low-protein AA supplemented diets (Kendall et al., 2007). It is anticipated that improved ideal AA profiles prevent the reductions in pig performance often observed when low protein diets are fed (Tuitoek et al., 1997).

Several studies have been conducted to estimate the optimum Trp to lysine (Lys) ratio for nursery

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and growing pigs (Susenbeth, 2006; Quant et al., 2008; Zhang et al., 2012). However, few studies have determined the optimum Trp to Lys ratio for finishing pigs (Kendall et al., 2007), especially on the basis of standardized ileal digestible (SID) AA. Ileal digestibility coefficients for AA can be expressed as apparent, standardized or true basis. However, apparent ileal digestibility values should not be used in practical diet formulation because they do not take into account endogenous AA losses and are not always additive in mixed diets (Stein et al., 2005). Likewise, true ileal digestibility values should not be used in diet formulations unless specific ileal endogenous AA losses are measured and considered in determining the animal's AA requirement (Stein et al., 2007a). Therefore, SID values are recommended for use in diet formulations (Stein et al., 2007b).

The objective of the present study was to estimate the optimum ratio of SID Trp to Lys required to maximize weight gain or optimize the feed conversion ratio (FCR) and serum urea nitrogen (SUN) levels of finishing barrows fed low protein diets supplemented with crystalline AA.

MATERIAL AND METHODS

All experimental procedures and animal care were approved by the China Agricultural University Animal Care and Use Committee (Beijing, P.R. China).

Animals, housing, and experimental diets. A total of 150 Yorkshire × Landrace barrows, with an average initial BW of 67.3 ± 3.2 kg, were used in a 28-day performance trial. This experiment was conducted under commercial conditions at the Beijing Resource Breeding Pig Company (Beijing, P.R. China). Barrows were placed in partially slatted concrete floored pens (3.0 m × 4.0 m) that provided 2.4 m^2 per pig in a naturally ventilated finishing facility. Pens of pigs (five barrows per pen) were allotted to one of five dietary treatments in a randomized complete block design with six pens per treatment. Each pen was equipped with a five-hole conventional dry feeder and two nipple waterers. Feed was given three times a day (7:30, 13:30, and 17:30 h) and water was available *ad libitum*. Barrows were weighed after an overnight fast (feeders were cleaned out) at the beginning and at the end of the experiment to determine weight gain. Feed disappearance was measured weekly to calculate feed intake and FCR.

All dietary treatments were based on corn, wheat bran, and soybean meal and were formulated to provide 10.5% CP and 0.61% SID Lys ensuring that Lys was marginally deficient for pigs of this weight range (National Swine Nutrition Guide – NSNG, 2010). Crystalline L-Trp was added to the basal diet to formulate SID Trp to Lys ratios of 0.131, 0.164, 0.197, 0.230, and 0.262 (Table 1). The SID ratios of the remaining indispensable AAs to SID Lys in the experimental diets were formulated to meet the recommendations of the NSNG (2010). All raw materials were analyzed for total AA content prior to the start of the experiment. SID AA values for the complete diets were estimated by multiplying the analyzed AA levels in corn, soybean meal, and wheat bran by the SID coefficients of the corresponding AA in those feedstuffs using AminoDat software (Version 3.0, 2005) and summing the values.

Table 1. Ingredient composition of the experimental basal diet (g/kg, as-fed)

Ingredients	Basal diet
Corn (7.8% CP)	795.8
Wheat bran (16.8% CP)	135
Soybean meal (43.5% CP)	35
Limestone	10
Dicalcium phosphate	7
Salt	4
L-Lysine hydrochloride (78.8%)	4.4
DL-Methionine (99.0%)	0.9
L-Threonine (98.5%)	1.7
L-Tryptophan (98.5%)[1]	0
L-Isoleucine (98.0%)	0.7
L-Valine (98.0%)	0.5
Vitamin-mineral premix[2]	5

CP = crude protein

[1]L-tryptophan was added at 0, 0.2, 0.4, 0.6, and 0.8 g/kg of the diet to provide dietary standardized ileal digestible tryptophan to lysine ratios of 0.131, 0.164, 0.197, 0.230, and 0.262, respectively

[2]supplied per kg of diet (as-fed basis): vitamin A 7200 IU, vitamin D_3 1400 IU, vitamin E 20 IU, vitamin K_3 0.5 mg, thiamine 1.6 mg, pyridoxine 2 mg, vitamin B_{12} 24.0 μg, riboflavin 4.0 mg, pantothenic acid 12.0 mg, niacin 24.0 mg, folic acid 0.8 mg, choline chloride 400 mg, Fe (as ferrous sulfate) 120 mg, Cu (as copper sulfate) 75 mg, Zn (as zinc sulfate) 100 mg, Mn (as manganese sulfate) 40 mg, I (as calcium iodate) 0.3 mg, Se (as sodium selenite) 0.3 mg; provided by the Beijing Resource Feed Company (Beijing, P.R. China)

Collection and sampling. Representative feed samples were taken weekly and pooled for later analysis. Blood samples were collected from six barrows per treatment (one pig per pen) at the termination of the experiment. The pigs chosen for bleeding were those with a BW nearest to the average of their pen. After an overnight fast (14 h), pigs were bled via jugular vein puncture before the morning feeding using uncoated vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, USA). After collection, all blood samples were quickly transferred to Heraeus Biofuge 22R Centrifuge (Heraeus Holding GmbH, Hanau, Germany) tubes and centrifuged at 1200 *g* for 15 min at room temperature. The serum was carefully removed and subdivided into two plastic vials and stored at –80°C until needed for analysis of serum AA and urea nitrogen (SUN).

Chemical analyses. Diets were analyzed for CP, calcium, and phosphorus according to AOAC (2003) procedures. Amino acids except methionine, cystine, and Trp were determined by ion-exchange chromatography using a Hitachi L-8800 Amino Acid Analyzer (Hitachi, Tokyo, Japan) after acid hydrolysis with HCl 6N (refluxed at 110°C for 24 h). Dietary methionine and cystine were measured after oxidation with performic acid and subsequent hydrolysis with HCl while Trp was determined after alkaline hydrolysis at 120°C for 16 h (AOAC, 2003) using reverse-phase HPLC instrument equipped with Waters 2690 separation module (Waters, Milford, USA).

Serum AA concentrations were determined by ion-exchange chromatography under physiological fluid analysis conditions (S-433D Amino Acid Analyzer; Sykam GmbH, Eresing, Germany). Frozen serum samples were first thawed at 4°C and deproteinized using 120 mg of salicylsulfonic acid per ml of serum. After sitting in an ice bath for 20 min, the reaction system was adjusted for pH by adding a lithium hydroxide solution (2 mol/l) and then centrifuged at 11 000 *g* (Beckman Optima

Table 2. Chemical analysis and calculated nutritional content of the experimental diets (g/kg, as-fed)[1]

	SID tryptophan to lysine ratio				
	0.131	0.164	0.197	0.230	0.262
Chemically analyzed values					
Crude protein	102.5	102.6	110.2	103.5	104.6
Calcium	4.9	5.5	6.0	5.9	5.6
Total phosphorus	4.6	4.9	4.7	4.8	4.7
Isoleucine	3.9	4.0	3.9	4.3	3.9
Lysine	6.8	6.9	6.9	7.1	6.7
Methionine and cystine	5.1	5.0	5.2	5.0	5.2
Threonine	5.0	5.1	5.3	5.2	5.1
Tryptophan	1.1	1.3	1.5	1.7	1.9
Valine	6.3	6.1	6.5	6.7	6.2
Calculated values					
Metabolizable energy (MJ/kg)[2]	12.6	12.6	12.6	12.6	12.6
SID lysine[3]	6.1	6.1	6.1	6.1	6.1
SID tryptophan[3]	0.8	1.0	1.2	1.4	1.6

SID = standardized ileal digestible

[1]analyzed values for crude protein and amino acid are shown and are based on a composite sample obtained weekly; all diets were formulated to contain 6.1 g/kg SID lysine, 4.2 g/kg SID methionine plus cystine, 4.4 g/kg SID threonine, 4.8 g/kg valine, and 0.34 g/kg isoleucine

[2]based on data from the China Feed Bank (CAAS, 2009)

[3]SID amino acid values for the complete diets were estimated by multiplying the analyzed amino acid levels in corn, soybean meal, and wheat bran by the SID coefficients of the corresponding amino acids in those feedstuffs and summing (AminoDat, Version 3.0, 2005)

L80-XP; Beckman Coulter Inc., Fullerton, USA) for 30 min. The supernatant was collected and then passed through a filter (0.1 μm). The concentration of SUN was determined with a blood urea nitrogen colour test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analyses. Data were analyzed as a randomized complete block design using the GLM procedure of SAS (Statistical Analysis System, Version 6.12, 1998) with a pen as the experimental unit. The LSMEANS procedure of SAS was used to calculate the means. Regression analyses were performed to determine linear and quadratic relationships. Alpha levels of $P \leq 0.05$ and $P < 0.10$ were the criterion for statistical significance and tendencies. Estimates of requirements for optimum performance and SUN were determined by subjecting the data to least squares, broken-line methodology (Robbins et al., 2006):

$$y = L + U \times (R - x), (R - x) = 0 \text{ when } x > R$$

where:
L = plateau
U = slope
R = breakpoint

using the NLIN procedure of SAS, along with the maximum of the quadratically fitted line.

RESULTS

Pig performance. The greatest weight gains (1.05 kg/day) and highest feed intakes (2.98 kg/day) were obtained by barrows fed the 0.262 SID Trp to Lys ratio diet. However, dietary SID Trp to Lys ratios of 0.197 and 0.230 produced the best FCR (2.72) (Table 3). There were improvements in final body weight (linear effect $P = 0.09$), weight gain (linear and quadratic effect $P < 0.01$), and feed intake (linear effect $P = 0.04$) as the SID Trp to Lys ratio increased.

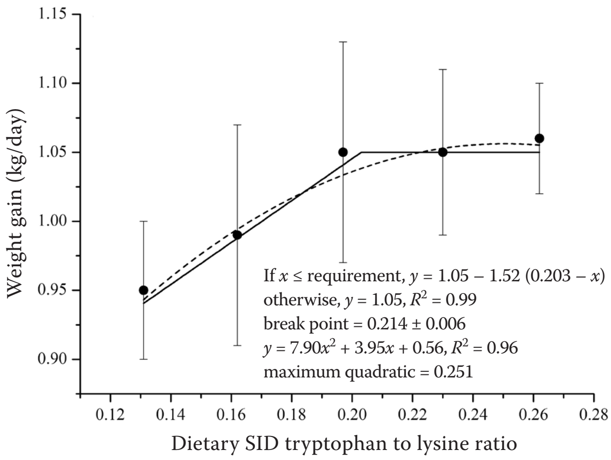

Figure 1. Fitted broken line analysis (—) and quadratic model (---) plot of weight gain plotted against standardized ileal digestible tryptophan to lysine ratios

Serum indispensable amino acids and urea nitrogen. Effects of SID Trp to Lys ratio on serum indispensable AA and SUN are shown in Table 4. Increased ratios of SID Trp to Lys resulted in a decrease in SUN (linear and quadratic effect $P < 0.01$) which was minimized at the SID Trp to Lys ratio of 0.230 (6.8 mg/dl). Serum Trp increased (linear effect $P = 0.03$ and quadratic effect $P = 0.08$) with increasing SID Trp to Lys ratio. There were no significant differences in the concentrations of other serum indispensable AA ($P \geq 0.10$).

Broken-line and quadratic analyses. Based on the linear broken-line and quadratic analyses, the optimum SID Trp to Lys ratios to maximize weight gain were 0.203 and 0.251 (Figure 1) while the

Table 3. Effect of SID Trp to lysine ratio on the performance of 67–96 kg barrows[1]

	Dietary SID Trp to lysine ratio					SEM	*P*-value	
	0.131	0.164	0.197	0.230	0.262		linear	quadratic
Initial body weight (kg)	67.3	66.8	67.2	67.6	67.3	1.54	0.87	0.98
Final body weight (kg)	93.9	94.6	96.7	97.0	96.9	1.63	0.09	0.20
Weight gain (kg/day)	0.95	0.99	1.05	1.05	1.06	0.03	< 0.01	< 0.01
Feed intake (kg/day)	2.74	2.90	2.85	2.85	2.98	0.08	0.04	0.13
Feed conversion ratio	2.90	2.93	2.72	2.72	2.81	0.08	0.15	0.15
Daily SID Trp intake (g/day)	2.20	2.84	3.41	3.98	4.75	0.10	< 0.01	< 0.01

SID = standardized ileal digestible, Trp = tryptophan

[1]represents the mean of six pens per treatment with five barrows per pen in an experiment lasting for 28 days

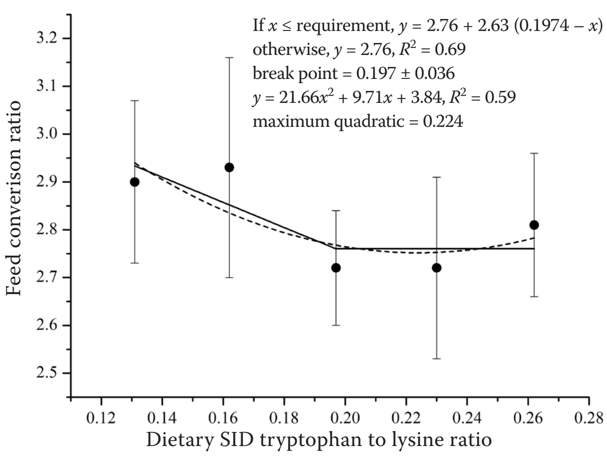

Figure 2. Fitted broken line analysis (—) and quadratic model (---) plot of feed conversion ratio plotted against standardized ileal digestible tryptophan to lysine ratios

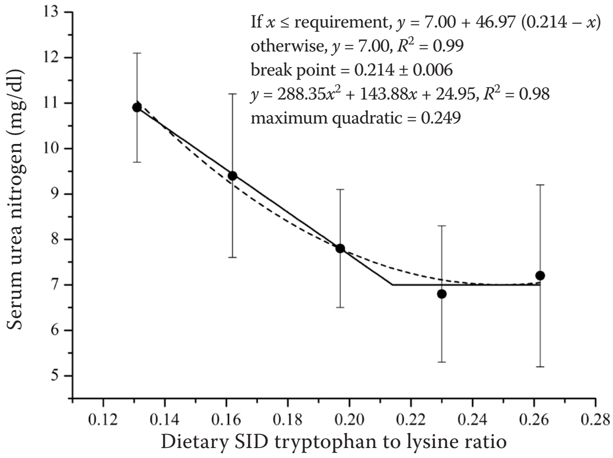

Figure 3. Fitted broken line analysis (—) and quadratic model (---) plot of serum urea nitrogen plotted against standardized ileal digestible tryptophan to lysine ratios

optimum SID Trp to Lys ratios to optimize FCR were 0.197 and 0.224 (Figure 2), and 0.214 and 0.249 to minimize SUN, respectively (Figure 3).

DISCUSSION

The analyzed CP and AA contents of the diets are shown in Table 2. Some variation in chemical content between the diets is apparent. However, it should be pointed out that all the diets were formulated using the same batch of ingredients and should have contained the same level of each ingredient with the exception of Trp. Therefore, the variations observed are likely the normal variation related to sampling and chemical analysis rather than reflecting actual differences in the chemical content of the diets. In addition, since all indispensable AAs (except for Lys and Trp) were formulated to provide at least 110% of the SID requirements relative to Lys as recommended

Table 4. Effects of SID tryptophan to lysine ratio on serum urea nitrogen and indispensable amino acid levels in 67–96 kg barrows[1]

	Dietary SID tryptophan to lysine ratio					SEM	*P*-value	
	0.131	0.164	0.197	0.230	0.262		linear	quadratic
Serum urea nitrogen (mg/dl)	10.9	9.4	7.8	6.8	7.2	0.80	< 0.01	< 0.01
Serum indispensable amino acids (nmol/ml)								
Arginine	262	306	318	292	271	37.7	0.97	0.45
Histidine	111	124	132	125	118	15.7	0.80	0.59
Isoleucine	109	132	138	140	109	15.1	0.93	0.10
Leucine	350	363	346	365	332	38.6	0.73	0.87
Lysine	342	312	341	386	319	67.3	0.93	0.97
Methionine	66	70	67	74	64	8.8	0.97	0.85
Phenylalanine	110	130	127	120	129	19.5	0.66	0.85
Threonine	183	205	176	186	169	21.5	0.43	0.65
Tryptophan	45	58	58	73	72	9.7	0.03	0.08
Valine	315	369	342	387	313	38.6	0.97	0.40

SID = standardized ileal digestible

[1]blood samples were collected from six pigs per treatment (one pig per pen) after an overnight fast (14 h) at the termination of the experiment

by the NSNG (2010), these differences would not be expected to affect pig performance.

In AA ratio dose-response trials, Lys must be the second limiting amino acid after Trp in order to ensure that the derived Trp to Lys ratio is not underestimated by an excess level of Lys (Boisen, 2003). The four following recommended estimates of the requirements of pigs for SID AA are most widely utilized in pig diet formulations: the NSNG (2010) estimates (the SID Lys requirement of 81–102 kg barrows to be 0.72% for high lean gain lines and 0.62% for medium lean gain lines), Evonik Industries (Rademacher et al., 2009) estimates (the SID Lys requirement of 70–105 kg pigs to be 0.71%), the NRC (2012) estimates (the Lys requirement of 75–105 kg pigs to be 0.73% SID Lys), the British Society of Animal Science (BSAS, 2003) estimates (the SID Lys requirement of 60–90 kg pigs to be 0.51% for slow growing pigs, 0.71% for intermediate growing pigs, and 0.89% for fast growing pigs). Thus in the present study, 0.61% of SID Lys was chosen, which is by about 15% less than previously recommended values for high lean gain pigs.

The concept of an ideal protein and proposals for ideal AA profiles for pigs were first introduced by the Agricultural Research Council in the early 1980's (ARC, 1981), where a total Trp to Lys ratio of 0.150 was recommended based on whole carcass AA concentration. The ideal protein was later revisited and further improved upon by several researchers, in which estimates of the ratio of total Trp to Lys ranged from 0.170 to 0.190 for growing/finishing pigs (Fuller et al., 1989; Wang and Fuller, 1989; Chung and Baker, 1992; Boisen et al., 2000). Based on these estimates of ideal protein, BSAS (2003) recommend a SID Trp to Lys ratio of 0.190 and Evonik Industries (Rademacher et al., 2009) recommend a SID Trp to Lys ratio of 0.185 for finishing pigs, while lower SID Trp to Lys ratios were recommended by the NSNG (2010) (0.153–0.160) and NRC (2012) (0.176–0.180).

To date, there has been only one empirical study which determined the optimum SID Trp to Lys ratio for finishing pigs (Kendall et al., 2007). This study estimates the optimum SID Trp to Lys ratio to be at least 0.145 (broken-line analysis), but not greater than 0.220 (quadratic model) for 90–125 kg of BW pigs. There are two studies dealing with the SID Trp requirement of finishing pigs. Guzik et al. (2005) reported a SID Trp requirement of 0.096% by broken-line regression analysis for pigs weighing 69.4 kg, whereas Eder et al. (2003) estimated a SID Trp requirement between 0.084 and 0.122% for 50–80 kg pigs and a requirement in excess of 0.171% for 80–115 kg pigs (95% of the maximum response), respectively. In the present study, diets were formulated to be limiting in Lys. Therefore, the potential to compare these studies is limited. However, at least a SID Trp to Lys ratio of 0.200 was required when the performance was used as the primary response variable with a corresponding SID Trp content of 0.122%. Overall, our estimates are higher than most of current recommendations and previous studies.

There are three factors that may account for the higher SID Trp to Lys ratio obtained in the current study. Firstly, crystalline AAs may be more susceptible to reactions with other compounds in the feed than protein-bound AAs. In particular, free Trp is known to be sensitive to destruction from free radicals produced from the oxidation of unsaturated fatty acids during storage (Boisen, 2003). Secondly, Trp is involved in several biological functions such as appetite regulation, stress, and immune responses (Le Floc'h et al., 2012). Our experiment was conducted in a commercial finishing environment in which a lower sanitary environment might have increased the requirement for Trp (Le Floc'h et al., 2006), compared with a more controlled environment. Thirdly, Quant (2008) found that supplying other indispensable AA (other than Trp and Lys) at higher levels to meet their absolute requirement in reduced CP diets yielded a higher optimum Trp to Lys ratio of 0.171 while a lower SID Trp to Lys ratio of 0.156 was observed when the AA were balanced to meet their ideal ratio based on the same suboptimal SID Lys level of 0.66% in the diets. In the present study, the basal diet was formulated to contain 0.61% SID Lys (about 15% lower than NSNG's recommendation), while the other indispensable AAs (except for Trp) were formulated to provide at least 110% of the SID requirements relative to Lys for all other AA as recommended by the NSNG (2010).

Estimates of requirement have been shown to be affected by the statistical methodology used in the study with derived estimates from broken-line models almost always resulting in requirement estimates lower than those estimated from quadratic models (Kendall et al., 2007), which agrees with the present experiment. Quadratic models can effectively estimate increases and decreases, and the shape of the curve represents the response to graded concentrations of a nutrient up to the point when it becomes "safe" or "meets requirements" (Pesti et al., 2009). Their advantage over linear models consists in that they more accurately describe the physiological course (Robbins et al., 2006). However, these models may not be appropriate if the response criterion does not further respond to a higher level of nutrient, and their estimates are influenced by additional input levels below or above the requirement (Pesti et al., 2009). In addition, the quadratic estimation of the amount of AA to reach 100% of the maximum commonly overestimates the requirement (Baker, 1986). In the present study, the quadratic estimates were all near the highest level and therefore may be somewhat suspect. Therefore, additional research should be conducted using higher SID Trp to Lys ratios to confirm the results of the present study.

Linear broken line models fit the ascending and plateau portions of the curve well and the break-point of two lines is selected as the requirement. However, this requirement corresponds to a theoretical average pig without considering the variation within the population (Baker, 1986). Moreover, it assumes that the dose response of a nutrient is linear until the requirement is met and above which no significant change in response can be expected. As such, the broken-line model may underestimate the requirement (Robbins et al., 2006). To facilitate comparison with sources in the literature and decrease the population variation, both linear broken line and quadratic models were chosen in the present study.

The performance response was supported by the shape of the SUN response curve. In the present study, the optimum SID Trp to Lys ratio obtained from SUN was similar to that estimated for weight gain. Urea is the main end product originating from the catabolism of AA (D'Mello, 2003). The use of blood urea nitrogen as a rapid response parameter for testing the protein quality of a diet has been investigated (Pedersen and Boisen, 2001). Our results agree with the findings of Guzik et al. (2005) and Quant (2008), indicating that SUN could be used to determine AA requirements for pigs. However, the estimates derived by using weight gain and FCR as the response criteria are more accepted in practical production for economic reasons.

There were some differences in evaluating the optimum SID Trp to Lys ratio between serum AAs and performance in the present study. Although most of the serum indispensable AAs were minimized in pigs fed the 0.262 SID Trp to Lys ratio diet when the dietary Trp increased, there were no significant improvements in either weight gain or FCR at SID Trp to Lys ratios greater than 0.197. In a typical AA dose-titration study, Loughmiller et al. (1998) pointed out that plasma concentrations of other non-limiting indispensable AA will be minimized once the requirement of the limiting AA was met because protein synthesis is maximal. Moreover, the present results demonstrating a linear response for serum Trp are in agreement with other studies (Loughmiller et al., 1998; Kendall et al., 2007).

Changes in the plasma concentrations of a test AA have been used to determine the optimum AA needs of pigs. Wiltafsky et al. (2009) found that increasing dietary supply of an AA from deficit to oversupply had little effect on plasma concentrations until the requirement was met, but linearly increased plasma concentration of the test AA when the diet contained adequate or excess amounts. However, no sharp increase was found when dietary AA exceeded the pig's requirement in the present study. The reason for the observed difference might be derived from the length of the fast following which blood samples were collected. In the present study, pigs were bled after an overnight fast (14 h) while a fasting period of approximately 2.5 h was used by Wiltafsky et al. (2009).

CONCLUSION

On the basis of this study, maximized weight gain using estimates provided by the linear broken-line and quadratic model were 0.203 and 0.251, respectively, while 0.197 and 0.224 optimized FCR and 0.214 and 0.249 minimized SUN. Our data suggest that the SID Trp to Lys ratio is at least 0.203 for barrows fed a low protein corn, wheat bran, and soybean meal based diet in which the corresponding SID Trp content of the diet was 0.122%. This estimate is greater than the current NRC recommendation (NRC, 2012).

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