Livestock Science 222 (2019) 25–30

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

The response of performance in grower and finisher pigs to diets formulated to different tryptophan to lysine ratios

Liu J.B.[a,b,*], Yan H.L.[a], Cao S.C.[a], Liu J.[a], Li Z.X.[a], Zhang H.F.[a,b]

[a] School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang 621010, PR China
[b] State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China

ARTICLE INFO

Keywords:
Broken-line
Pig
Requirement
Standardized ileal digestible
Tryptophan to lysine ratio

ABSTRACT

Three experiments were conducted to determine the optimal dietary standardized ileal digestible (SID) Trp to Lys ratio (Trp:Lys) for grower-finisher pigs with body weight of 20–50 (Experiment (Exp.) 1), 50–80 (Exp. 2), and 80–110 kg (Exp. 3). A total of 4032 Duroc × Yorkshire × Landrace barrows and gilts were used in the experiments with a randomized complete blocked design. For each experiment, 1344 pigs were randomly assigned into 6 dietary treatments (SID Trp:Lys levels: 0.150, 0.165, 0.180, 0.195, 0.210, and 0.225) with 8 replicate pens per treatment (14 barrows and 14 gilts per pen). Synthetic Trp was used to adjust the dietary SID Trp:Lys, while the Lys content in all 6 diets remained fixed in each experiment. In Exp. 1, the average daily gain (ADG), average daily feed intake (ADFI), gain to feed ratio (G:F), and serum urea N (SUN) were increased quadratically ($P < 0.01$) as the SID Trp:Lys increased from 0.150 to 0.225. The optimal SID Trp:Lys for ADG estimated using broken-line and curvilinear-plateau models were 0.171 and 0.188, respectively. For G:F, the break-points were 0.192 and 0.207 for the linear and quadratic models, respectively. In Exp. 2, a quadratic increase was observed in ADG, ADFI, G:F, and SUN as the SID Trp:Lys increased from 0.150 to 0.225 ($P < 0.05$). The optimal SID Trp:Lys for ADG estimated using the broken-line and curvilinear-plateau models were 0.183 and 0.200, respectively. The break-points for G:F estimated using broken-line and curvilinear-plateau models were 0.195 and 0.213, respectively. In Exp. 3, the ADG, G:F, and SUN increased quadratically with increasing SID Trp:Lys, while ADFI increased linearly ($P < 0.05$). The optimal SID Trp:Lys for ADG estimated using the broken-line and curvilinear-plateau models were 0.184 and 0.201, respectively. Likewise for G:F, the best ratios were 0.174 and 0.198 for the linear and curvilinear models, respectively. In summary, the optimal Trp:Lys for ADG estimated using the broken-line model in pigs weighing 20 to 50, 50 to 80, and 80 to 110 kg were 0.171, 0.183, and 0.184, respectively. The optimal Trp:Lys for G:F estimated using the broken-line model in pigs weighing 20 to 50, 50 to 80, and 80 to 110 kg were 0.192, 0.195, and 0.174, respectively.

1. Introduction

The requirement of pigs for individual amino acid (AA) can be expressed as the AA ratio with respect to Lys. In the latest version of the NRC (2012), the requirement of standardized ileal digestible (SID) Lys were estimated by using the factorial method based on the requirements for maintenance, lean deposition of Lys, and the presumed coefficients of digestibility and bioavailability of Lys. The requirements of other indispensable AA were estimated based on the optimum ratio for supporting the main body functions and estimates of the efficiency of AA utilization (NRC, 2012). This estimation can be questionable for some AA that play key roles in biological reactions other than protein synthesis. Therefore, it is necessary to investigate whether the estimation of AA requirements from the factorial method are consistent with empirical studies.

Tryptophan is an indispensable AA and is a component of serotonin and melatonin (Yao et al., 2011). The optimal SID Trp:Lys recommended by the NRC (2012) in grower pigs ranges from 0.173 at 25 kg of BW to 0.180 at 125 kg of BW. This ratio is consistent with the ideal protein concept, in which the Trp:Lys is 0.18 (Chung and Baker, 1992; Fuller et al., 1989). The values are also generally in agreement with empirical studies (Eder et al., 2003; Sato et al., 1987; Susenbeth and Lucanus, 2005). However, the determined optimal digestible Trp:Lys varied among studies (Naatjes et al., 2014; Susenbeth, 2006; Zhang et al., 2012). These variations indicate that the optimal SID Trp:Lys may be affected by factors such as diet type, experimental animals, and their physiological condition. Tryptophan is also involved in other biological functions such as the immune response.

* Corresponding author at: School of Life Science and Engineering, Qinglong Road 59#, Mianyang 621010, China.
E-mail address: liuswust@163.com (J.B. Liu).

https://doi.org/10.1016/j.livsci.2019.01.016
Received 4 June 2018; Received in revised form 24 November 2018; Accepted 23 January 2019

Xu et al. (2015) suggested that dietary Trp supplementation may improve the immune response in grower pigs challenged by porcine respiratory and reproductive syndrome vaccine. Dietary Trp can also enhance the expression of tight junction proteins in the small intestine of grower pigs (Liu et al., 2017b). Thus, it is reasonable to assume that the dietary requirement of Trp is underestimated by using the ratio of Trp:Lys found in the body composition of pigs. Much of the recent research have been focused on the optimal SID Trp:Lys in nursery pigs (Susenbeth, 2006; Gonçalves et al., 2015; Liu et al., 2017a). Yet studies to determine the optimal Trp:Lys in grower-finisher pigs may be more valuable economically, because of greater feed costs in the grower and finisher phases. In addition, estimates of the requirement of Trp:Lys derived from optimally managed research facilities may underestimate the requirement for grower and finisher pigs in commercial conditions because of the relatively greater animal density and environmental challenge found in commercial conditions. The objective of this study was to determine the optimal SID Trp:Lys in grower and finisher pigs of 20–50, 50–80, and 80–110 kg BW in a commercial swine production facility.

2. Materials and methods

2.1. Experimental design and treatments

The experimental protocols for this study were approved by the Animal Care Advisory Committee of Southwest University of Science and Technology (Mianyang, Sichuan Province, China). A total of 4032 Duroc × Yorkshire × Landrace barrows and gilts were used in the 3 experiments. All 3 experiments were randomized complete blocked design, with the average initial BW of a pen considered as the blocking factor. Each study was conducted with 1344 grower-finisher pigs with 6 dietary treatments. The SID Trp:Lys for all 3 experiments was set to 0.150, 0.165, 0.180, 0.195, 0.210, and 0.225, respectively. There were 8 replicate pens for each treatment with 28 pigs (14 barrows and 14 gilts) in each pen. The initial BW of pigs for experiment 1, 2, and 3 were 19.1 ± 1.0, 50.2 ± 1.1, and 79.8 ± 1.1 kg, respectively. The experimental period was 28d for each experiment. The experimental diets were formulated to contain marginally deficient concentrations of SID Lys and relatively sufficient concentrations of all other AA except for Trp. The SID Lys concentration was set to be 90% of the standard concentration used by the production facility based on the internal performance data of the facility. The ratio of SID Met, Thr, Ile, and Val to Lys were set to be at least 105% of the NRC (2012) recommend ratio to Lys. The diet formulation used was adopted from previous studies to ensure Trp is the limiting AA (Guzik et al., 2005). All diets were fed in mash form and the same batch of ingredients was used for all diets within a phase. The diet composition and analyzed nutrient composition of the treatment diet that contained the lowest SID Trp concentration for each experiment are listed in Table 1 and 2, respectively. Crystallized L-Trp was added into the diets at the expense of corn to adjust the SID Trp:Lys. The standardized ileal digestibility of AA for the ingredients used in formulation was as given by the NRC (2012).

2.2. Animals and procedures

The study was conducted in 3 grower-finisher barns with the same design of pens, feeders, and waterers at a large-scale farm of New Hope Group (Mianyang, Sichuan, China). Prior to the target experimental BW for each experiment, all animals were fed with corn-SBM based diets following the standard practice of the production facility. All nutrient and energy concentrations met or exceeded NRC (2012) recommendations. Pigs were housed in concrete floored pens with a single-sided 4-hole feeder and 4-cup waterers. The stocking densities for experiment 1, 2, and 3 were 0.54, 0.71, and 1.00 m^2 per pig, respectively. The relative humidity inside the animal house for all the experiments ranged from 50 to 70%, and the environmental temperature inside the swine confinement building was controlled at 24, 22, and 20 °C in experiment 1, 2, and 3, respectively. All pigs received ad libitum feed and water throughout the experiment. The pigs and feeders were weighed at the beginning and the termination of each

Table 1
Ingredient composition of the basal diet formulated for experiments (Exp.) 1 to 3.[a]

Item	Exp. 1	Exp. 2	Exp. 3
Ingredient (g/kg)			
Corn	670.6	670.0	715.6
Soybean meal	153.5	116.6	73.2
Wheat bran	60.0	80.0	80.0
Corn DDGS[b]	60.0	80.0	80.0
Soybean oil	10.0	10.0	10.0
Dicalcium phosphate	5.9	5.7	5.7
Limestone	6.6	6.6	6.6
Salt	5.0	5.0	5.0
Vitamin and mineral premix[c]	20.0	20.0	20.0
L-Lys•HCl	5.4	3.8	2.4
L-Trp	0.3	0.2	0.00
DL-Met	1.2	0.9	0.6
L-Thr	1.5	1.2	0.9

[a] Body weight ranges for experiment 1, 2, and 3 were 20 to 50, 50 to 80, and 80 to 110 kg, respectively. Diets with increasing standardized Trp to Lys ratios were formulated using crystalline Trp to replace corn.
[b] Dried distillers grains with solubles.
[c] Premix provide the following items per kilogram of complete diet: vitamin A, 5512 IU; vitamin D_3 2200 IU; vitamin E, 64 IU; vitamin K_3 2.2 mg; niacin, 30.3 mg; choline chloride, 551 mg; Mn. 40 mg; Fe, 100 mg; Zn, 100 mg; Cu, 100 mg; and Se, 0.3 mg.

Table 2
Chemically analyzed and calculated energy and nutrients composition of the basal diets for experiments (Exp.) 1–3.[a]

Item	Exp. 1	Exp. 2	Exp. 3
Chemical analyses (%)			
CP[b]	15.61	14.33	13.08
Ether extract	3.20	2.99	2.71
Crude fiber	3.78	3.94	3.88
Ca	0.68	0.58	0.50
Total P	0.50	0.44	0.40
Arg	0.89	0.85	0.77
His	0.39	0.35	0.34
Ile	0.57	0.48	0.41
Leu	1.30	0.87	0.63
Lys	0.97	0.80	0.68
Met + Cys	0.60	0.50	0.44
Thr	0.66	0.55	0.51
Trp	0.14	0.12	0.10
Phe	0.64	0.53	0.47
Val	0.70	0.59	0.50
Calculated energy and nutrients[c] (%)			
Net energy (Mcal/kg)[d]	2.45	2.45	2.45
SID Lys[e]	0.90	0.72	0.60
SID Met + Cys	0.53	0.43	0.44
SID Ile	0.49	0.40	0.41
SID Leu	1.20	0.76	0.64
SID Phe	0.56	0.45	0.38
SID Thr	0.57	0.47	0.41
SID Trp	0.14	0.11	0.09
SID Val	0.61	0.50	0.42

[a] BW ranges for experiment 1, 2, and 3 are 20 to 50, 50 to 80, and 80 to 110 kg, respectively. Diets with increasing standardized Trp to Lys ratios were formulated by using crystalline Trp to replace corn.
[b] Crude protein (CP).
[c] Standardized ileal digestibility of amino acid for ingredients were referenced by NRC (2012).
[d] Net energy was calculated by using a previously reported equation (Noblet et al., 1994).
[e] Standardized ileal digestible (SID).

experiment to determine the BW gain and feed leftover to calculate the ADG, ADFI, and G:F. At the end of each experiment, 2 barrows and 2 gilts that were close to the average BW of the pen were selected to collect blood samples from the anterior vena cava. Blood samples were centrifuged (3000 × g) at 4 °C for 10 min and serum was collected and frozen for the analysis of cortisol and serum urea N (SUN).

2.3. Chemical analysis

The feed ingredient and diet samples were analyzed following AOAC (2006) procedures for CP, ether extract, crude fiber, and Ca and P concentration. The AA profile of ingredients and diets were analyzed using HPLC (Hitachi L-8800 AA Analyzer; Hitachi, Tokyo, Japan) after one of 3 hydrolysis procedures (AOAC, 2006). Most of the AA were determined after acid hydrolysis in 6 N HCl at 110 °C for 24 h. For the determination of S-containing AA, the oxidation process was performed before the acid hydrolysis using performic acid. The analysis of Trp was performed after alkaline hydrolysis. The SUN concentration was determined using a biochemical analytical instrument (Byer Diagnostics Manufacturing Ltd., Dublin, Ireland). The plasma cortisol was determined by using an Enzyme-Linked Immuno-Sorbent Assay kit for porcine cortisol determination (Hengyuan Bio-technology, Shanghai, China). The intra-assay coefficient of variation was 10%.

2.4. Statistical analyses

In all experiments, data were analyzed by one-way Analysis of Variance (ANOVA) using PROC GLM of SAS (9.4). Orthogonal polynomial contrasts were applied to test the linear and quadratic effects for the increasing dietary SID Trp:Lys. The individual pen was considered the experimental unit and an alpha concentration of 0.05 was selected. The optimal SID Trp:Lys for ADG or G:F for each BW period was estimated by using PROC NLIN of SAS (9.4). The linear broken-line [1] and curvilinear-plateau regression [2] models are as previously reported (Robbins et al., 2006). Least square means of each SID Trp:Lys were used for the estimation.

$$y = L + U + (R - x),\ \ (R - x) = 0 \text{ if } x > R; \quad (1)$$

$$y = L + U + (R - x)^2,\ \ (R - x) = 0 \text{ if } x > R. \quad (2)$$

3. Results

3.1. Experiment 1

For 20- to 50-kg pigs, the results of growth performance, SUN, and plasma cortisol concentration are presented in Table 3. The ADG, ADFI, G:F, and SUN were increased quadratically ($P < 0.01$) as the SID Trp:Lys increased from 0.150 to 0.225. In contrast, the plasma cortisol concentration decreased linearly ($P < 0.01$) with increasing Trp:Lys. The final BW increased linearly with increasing Trp concentration ($P < 0.01$). The estimations of optimal SID Trp:Lys ratio are shown in Table 4. The optimal SID Trp:Lys with ADG as the response criterion using broken-line and curvilinear-plateau models were 0.171 and 0.188, respectively. For G:F, the break-points were 0.192 and 0.207 for the linear and quadratic models, respectively.

3.2. Experiment 2

For 50- to 80-kg pigs, the results of growth performance, SUN, and plasma cortisol concentration are shown in Table 5. Quadratic increases were observed in ADG, ADFI, G:F, and SUN ($P < 0.05$) as the SID Trp:Lys increased from 0.150 to 0.225. The plasma cortisol concentration decreased linearly ($P < 0.01$) with increasing Trp:Lys. The results of the non-linear regression analysis are presented in Table 6. The optimal SID Trp:Lys with ADG as the response criterion using broken-line and curvilinear-plateau models were 0.183 and 0.200, respectively. The break-points for G:F were 0.195 and 0.213 using broken-line and curvilinear plateau models, respectively.

3.3. Experiment 3

Table 7 shows the results of growth performance, SUN, and plasma cortisol for finisher pigs weighing 80 to 110 kg. The ADG, G:F, and SUN increased quadratically with increasing SID Trp:Lys ($P < 0.05$), while ADFI and plasma cortisol concentration increased linearly. Table 8 shows the estimations of the optimal SID Trp:Lys. The optimal SID Trp:Lys for the response of ADG using broken-line and curvilinear-plateau models were 0.184 and 0.201, respectively. The optimal SID Trp:Lys for the response of G:F were 0.174, and 0.198 for the linear and quadratic models, respectively.

4. Discussion

The precision-feeding of an animal to its nutritional requirement is of great economic and environmental importance for animal production. Three experiments were conducted in this study to determine the optimal SID Trp:Lys for grower-finisher pigs with BW of 20 to 50, 50 to 80, and 80 to 110 kg raised in a commercial hog production facility. The ratios for optimal ADG and G:F in each BW range were determined by broken-line analysis, as well as the curvilinear-plateau model. This methodology of AA requirement estimation has been well elaborated by Quant et al. (2012). It is widely accepted that the broken-line analysis can be used for the determination of AA requirements for optimal growth performance. To avoid the subjective bias of setting the arbitrary percentage of the maximum performance response, the linear broken-line analysis was chosen in the current study as the primary method of determining the optimal SID Trp:Lys. In addition to the results of broken-line analysis, the results of the asymptote of the quadratic regression are also reported for a reference. It has been suggested that the application of these methods required at least 5 concentrations of nutrient titration ((Liu et al., 2018). The titration of 6 concentrations of SID Trp:Lys in the current study is statistically adequate to generate 2 linear regressions for the broken-line analysis.

Dietary supplementation of Trp is critical for the optimal growth

Table 3
. Growth performance of grower pigs (initial BW = 19.1 ± 1.0 kg) fed with increasing SID Trp to Lys ratios (Experiment 1).[a, b]

Item	SID Trp to Lys ratio 0.150	0.165	0.180	0.195	0.210	0.225	SEM	*P*-value Linear	Quadratic
Final BW[b] (kg)	37.63	37.75	38.82	38.97	39.02	38.94	0.35	< 0.01	0.12
ADG[b] (kg)	0.66	0.68	0.69	0.69	0.70	0.70	0.036	< 0.01	< 0.01
ADFI[b] (kg)	1.38	1.42	1.41	1.41	1.41	1.42	0.047	< 0.01	< 0.01
G:F[b]	0.47	0.48	0.49	0.49	0.49	0.49	0.003	< 0.01	< 0.01
SUN[b] (mg/dL)	13.75	13.39	11.77	11.31	10.60	10.63	0.115	< 0.01	< 0.01
Plasma cortisol (μg/dL)	4.48	3.88	3.53	3.28	3.36	3.12	0.24	< 0.01	0.11

[a] Least square means of 8 replicate pens per treatment with 28 pigs per pen for the 28-d experiment.
[b] Standardized ileal digestible (SID), body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), gain to feed ratio (G:F), serum urea N (SUN).

Table 4
. Estimate of SID Trp to Lys ratios for ADG and G:F determined using broken-line and curvilinear-plateau models for grower pigs with BW of 20 to 50 kg (Experiment 1).[a, b]

Item	Equation	Estimate	R^b	*P*-value
ADG[b]				
Broken-line model	$y = 0.693 - 1.838 \times (0.171 - x)$	0.171	0.965	<0.01
Curvilinear-plateau model	$y = 0.694 - 26.073 \times (0.188 - x)^b$	0.188	0.970	<0.01
G:F[b]				
Broken-line model	$y = 0.492 - 0.424 \times (0.192 - x)$	0.192	0.982	<0.01
Curvilinear-plateau model	$y = 0.492 - 5.672 \times (0.207 - x)^b$	0.207	0.971	<0.01

[a] Broken-line model: $y = l + U \times (R - x)$, where $x = 0$ when $x > R$; Curvilinear-plateau model: $y = l + U \times (R - x)^2$, where $(R - x) = 0$ when $x > R$ (Robbins et al., 2006).
[b] Standardized ileal digestible (SID), average daily gain (ADG), gain to feed ratio (G:F), body weight (BW).

Table 5
. Growth performance of finisher pigs (initial BW = 50.2 ± 1.1 kg) fed with increasing standardized ileal digestible Trp to Lys ratios. (Experiment 2).[a]

	SID[b] Trp to Lys ratio						SEM	*P*-value	
	0.150	0.165	0.180	0.195	0.210	0.225		Linear	Quadratic
Final BW[b] (kg)	73.01	73.35	74.97	75.38	75.65	75.42	0.440	<0.01	0.05
ADG[b] (kg)	0.83	0.86	0.89	0.90	0.90	0.89	0.048	<0.01	<0.01
ADFI[b] (kg)	2.34	2.38	2.42	2.38	2.39	2.39	0.014	0.03	<0.01
G:F[b]	0.36	0.36	0.37	0.38	0.38	0.37	0.003	<0.01	0.04
SUN[b] (mg/dL)	10.09	9.83	9.26	8.90	9.03	8.84	0.117	<0.01	<0.01
Plasma cortisol (μg/dL)	6.77	5.86	6.21	5.41	5.38	4.71	0.33	<0.01	0.92

[a] Least square means of 8 replicate pens per treatment with 28 pigs per pen for the 28-d experiment.
[b] Standardized ileal digestible (SID), body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), gain to feed ratio (G:F), serum urea N (SUN).

Table 6
Estimate of standardized ileal digestible Trp to Lys ratio for ADG and G:F by using broken-line and curvilinear-plateau models for finisher pigs with BW of 50–80 kg (Experiment 2).[a, b]

	Equation	Estimate	R^b	*P*-value
ADG[b]				
Broken-line model	$y = 0.896 - 1.898 \times (0.183 - x)$	0.183	0.989	<0.01
Curvilinear-plateau model	$y = 0.896 - 26.422 \times (0.200 - x)^b$	0.200	0.990	<0.01
G:F[b]				
Broken-line model	$y = 0.375 - 0.408 \times (0.195 - x)$	0.195	0.975	<0.01
Curvilinear-plateau model	$y = 0.375 - 4.856 \times (0.213 - x)^b$	0.213	0.932	0.02

[a] Broken-line model: $y = l + U \times (R - x)$, where $x = 0$ when $x > R$; Curvilinear-plateau model: $y = l + U \times (R - x)^2$, where $(R - x) = 0$ when $x > R$ (Robbins et al., 2006).
[b] Average daily gain (ADG), gain to feed ratio (G:F), body weight (BW).

Table 7
Growth performance of finisher pigs (initial BW = 79.8 ± 1.1 kg) fed with increasing SID Trp to Lys ratios (Experiment 3).[a, b]

	SID Trp to Lys ratio						SEM	*P*-value	
	0.150	0.165	0.180	0.195	0.210	0.225		Linear	Quadratic
Final BW[b] (kg)	101.9	102.4	104.1	104.5	104.7	104.8	0.40	<0.01	0.03
ADG[b] (kg)	0.79	0.83	0.86	0.87	0.86	0.87	0.049	<0.01	<0.01
ADFI[b] (kg)	2.81	2.85	2.94	2.89	2.92	2.98	0.142	<0.01	0.29
G:F[b]	0.28	0.29	0.29	0.30	0.30	0.29	0.002	<0.01	<0.01
SUN[b] (mg/dL)	8.88	8.35	7.75	7.47	7.22	7.14	0.115	<0.01	<0.01
Plasma cortisol (μg/dL)	9.38	9.27	8.58	8.85	9.19	8.41	0.27	0.04	0.75

[a] Least square means of 8 replicate pens per treatment with 28 pigs per pen for the 28-d experiment.
[b] Standardized ileal digestible (SID), body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), gain to feed ratio (G:F), serum urea N (SUN).

performance of pigs because Trp is one of the limiting AA in a typical corn-SBM based diet. The physiological function of Trp is complicated in animals (Yao et al., 2011). In addition to protein synthesis, the physiological functions of Trp also include biosynthesis of serotonin and melatonin, which are important molecules that affect animal activity and behavior (Henry et al., 1992). The stress induced by the grouping and mixing of pigs can be ameliorated by dietary supplementation of Trp acting to reduce plasma cortisol and noradrenaline concentrations (Koopmans et al., 2005; Shen et al., 2012). Therefore, in stressful commercial conditions, the optimal SID Trp:Lys may be greater than the ratio derived from body composition analysis. It is also reported that supplementation of L-Trp in diets can reduce catabolism of dietary AA and reduce fat deposition (Ruan et al., 2014). Tryptophan can also enhance antioxidant activity and intestinal development (Li et al., 2016). Furthermore, Liu et al. (2017b) reported dietary Trp supplementation can improve tight junction protein production in grower pigs and therefore can be beneficial to intestinal health. For these reasons, additional dietary supplementation of Trp can be beneficial in practical swine production.

In the current study, which is conducted under commercial hog

Table 8
Estimate of standardized ileal digestible Trp to Lys ratio for ADG and G:F determined using broken-line and curvilinear-plateau model for finisher pigs with BW of 80 to 110 kg (Experiment 3).[a, b]

	Equation	Estimate	R^b	P-value
ADG[b]				
Broken-line model	$y = 0.869 - 2.402 \times (0.184 - x)$	0.184	0.978	< 0.01
Curvilinear-plateau model	$y = 0.869 - 32.486 \times (0.201 - x)^b$	0.201	0.977	< 0.01
G:F[b]				
Broken-line model	$y = 0.295 - 0.657 \times (0.174 - x)$	0.174	0.883	0.04
Curvilinear-plateau model	$y = 0.296 - 7.163 \times (0.198 - x)^b$	0.198	0.921	0.02

[a] Broken-line model: $y = l + U \times (R - x)$, where $x = 0$ when $x > R$; Curvilinear-plateau model: $y = l + U \times (R - x)^2$, where $(R - x) = 0$ when $x > R$ (Robbins et al., 2006).

[b] Average daily gain (ADG), gain to feed ratio (G:F), body weight (BW).

production conditions, the determined optimal Trp:Lys for grower and finisher pigs was numerically greater than NRC (2012) recommendations (from 0.17 to 0.18 in BW period comparable with current study), but similar to some studies with similar scale of experimental animals (Naatjes et al., 2014; Salyer et al., 2013). These results agree with our assumptions stated above. In addition, the increasing concentration of dietary SID Trp:Lys linearly decreased plasma cortisol concentration in all 3 BW ranges, which may indicate reduced stress with the addition of Trp. This result is similar to previous studies, in which lower cortisol concentration was observed in grower pigs fed diets with greater SID Trp:Lys (Koopmans et al., 2006; Shen et al., 2012). However, Guzik et al. (2006) reported that no reduction of plasma cortisol was observed in finisher pigs given additional dietary Trp which does not agree with the current study. This discrepancy might be because of the Trp:Lys exceeding the requirement of the pigs in that study, where the true digestible Trp:Lys was formulated to be 0.21 in the control diet.

For the past decade, the growth potential of pigs has been improved by modern breeding and genetic technologies. With the improvement of growth performance, the requirement of SID Lys and other AA for the grower pigs has increased as well (NRC, 2012). The utilization of Trp in the animal body can be interfered by other large neutral AA (LNAA; valine, leucine, isoleucine, tyrosine, and phenylalanine), because of competition for transporters to cross the blood-brain barrier (Markus et al., 2000). In addition, some feed ingredients in modern swine diets, such as distillers' grains, have relatively greater concentration of LNAA. Therefore, it is possible that the requirement of Trp is increased compared with a decade ago. This might also contribute to the relatively greater optimal SID Trp:Lys observed in the current and several recent studies compared with previous publications (Susenbeth, 2006; Salyer et al., 2013; Zhang et al., 2012).

Among the empirical studies, there was considerable variation in the determined optimal digestible Trp:Lys. Boisen et al. (2000) reviewed previous studies and summarized that optimal Trp:Lys ratio for grower pigs ranged from 0.17 to 0.19. Susenbeth (2006) concluded that 0.17 is the optimal Trp:Lys for pigs, which is an average value from a review of the literature and includes a safety margin to account for variation in reported requirements. But it should be noted that the range of optimal Trp:Lys from the review was approximately between 0.15 and 0.21. A study conducted by Quant et al. (2012) reported the optimal SID Trp:Lys in grower pigs fed with corn-based or barley-based diets to be 0.158 and 0.156, respectively. The results in the current study are greater than these values. This disagreement could be a result of different genetic backgrounds, or differences in the concentrations of Trp in the experiments. In the study of Quant et al. (2012), a quadratic effect of the increasing SID Trp:Lys in G:F was not observed. This phenomenon may suggest that the SID Trp:Lys were not at the optimal range required to exhibit the plateau of the response. As a result, the determined coefficients of the broken-line analysis and asymptote of the quadratic regression might be affected.

Kendall et al. (2007) determined the true ileal digestible (TID) Trp:Lys for finisher pigs from 90 to 125 kg of BW to be at least 0.145 but no greater than 0.17. That result is lower than the values determined in the current study. Although it should be noted that there might be inherent difference between TID and SID Trp:Lys, the total Trp:Lys at the corresponding concentration in that study was also lower than the present study. Guzik et al. (2005) determined the requirement of TID Trp:Lys to be 0.21, which is similar to the current study. The differences among the studies could be a result of the genetic background of the experimental animals or the diet composition. There was no protein source other than corn and synthetic AA in the study from Kendall et al. (2007), while the current study and Guzik et al. (2005) provided a small amount of protein source other than corn in the diets. It has been argued that the crude protein concentration in the diets that contains crystalline AA should be maintained at a certain concentration to provide adequate N, dispensable AA, and small peptide to optimize the growth performance. There are also studies that have indicated a greater SID Trp:Lys requirement for finisher pigs. Eder et al. (2003) determined the requirement of SID Trp for 80 kg pigs to be 1.22 g/kg of diet, and the SID Trp:Lys was calculated to be 0.217. However, the SID of AA cited in the study of Eder et al. (2003) was from the 1990s. Therefore, there might be difference in the SID of AA in feed ingredients used by Eder et al. (2003) and the current study.

Theoretically, the optimal SID Trp:Lys in finisher pigs is slightly greater compared with grower pigs. The ratio recommended by the NRC (2012) was increased from 0.17 to 0.18 for pigs ranged from 25 to 125 kg of BW. This estimation was based on the body composition of pigs at different BW ranges. Salyer et al. (2013) reported that the optimal SID Trp:Lys for 36- to 72-kg pigs is 0.165 and is 0.195 for 72- to 120-kg pigs. The present data indicate a similar trend. But it is worth noting that the break-point of the broken-line analysis for G:F decreased in the finisher phase of the current study. This might be caused by the linear increase of feed intake observed with increasing Trp concentrations in the current study, which negatively impacted G:F and therefore affected the non-linear regression analysis.

5. Conclusion

The optimal SID Trp:Lys for 20- to 50-kg grower pigs are estimated to be 0.171 and 0.192 for ADG and G:F, respectively. For 50- to 80-kg pigs, the ratios are determined to be 0.183 and 0.195 for ADG and G:F, respectively. For 80- to 110-kg pigs, the ratios are estimated to be 0.184 and 0.174 for ADG and G:F, respectively.

Conflict of interest statement

The authors declare no competing interests.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2016YFD0500505, China), the Foundation of Sichuan Science and Technology Program (2018JY0225, Sichuan, China) and Education Department of Sichuan Province of China (17ZA0413, Sichuan, China).

References

AOAC, 2006. Official Methods of Analyses, 17th ed. Assoc. Off. Anal. Chem, Arlington, VA.

Boisen, S., Hvelplund, T., Weisbjerg, M.R., 2000. Ideal amino acid profiles as a basis for feed protein evaluation. Livest. Prod. Sci. 64, 239–251.

Chung, T.K., Baker, D.H., 1992. Ideal amino acid pattern for 10-kilogram pigs. J. Anim. Sci. 70, 3102–3111.

Eder, K., Nonn, H., Kluge, H., Peganova, S., 2003. Tryptophan requirement of growing pigs at various body weights. J. Anim. Physiol. Anim. Nutr. 87, 336–346.

Fuller, M.F., McWilliam, R., Wang, T.C., Giles, L.R., 1989. The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. Br. J. Nutr. 62, 255–267.

Gonçalves, M.A., Nitikanchana, S., Tokach, M.D., Dritz, S.S., Bello, N.M., Goodband, R.D., Touchette, K.J., Usry, J.L., DeRouchey, J.M., Woodworth, J.C., 2015. Effects of standardized ileal digestible tryptophan: lysine ratio on growth performance of nursery pigs. J. Anim. Sci. 93, 3909–3918.

Guzik, A.C., Shelton, J.L., Southern, L.L., Kerr, B.J., Bidner, T.D., 2005. The tryptophan requirement of growing and finisher barrows. J. Anim. Sci. 83, 1303–1311.

Guzik, A.C., Matthews, J.O., Kerr, B.J., Bidner, T.D., Southern, L.L., 2006. Dietary tryptophan effects on plasma and salivary cortisol and meat quality in pigs. J. Anim. Sci. 84, 2251–2259.

Henry, Y., Sève, B., Colléaux, Y., Ganier, P., Saligaut, C., Jégo, P., 1992. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs, in relation to plasma free amino acids and hypothalamic serotonin. J. Anim. Sci. 70, 1873–1887.

Kendall, D.C., Gaines, A.M., Kerr, B.J., Allee, G.L., 2007. True ileal digestible tryptophan to lysine ratios in ninety-to one hundred twenty-five-kilogram barrows 1. J. Anim. Sci. 85, 3004–3012.

Koopmans, S.J., Guzik, A.C., Van Der Meulen, J., Dekker, R., Kogut, J., Kerr, B.J., Southern, L.L., 2006. Effects of supplemental l-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling piglets. J. Anim. Sci. 84, 963–971.

Koopmans, S.J., Ruis, M., Dekker, R., van Diepen, H., Korte, M., Mroz, Z., 2005. Surplus dietary tryptophan reduces plasma cortisol and noradrenaline concentrations and enhances recovery after social stress in pigs. Physiol. Behav. 85, 469–478.

Li, X., Jiang, M., Ruan, Z., Mi, S., Wu, X., Yao, K., Xiong, X., Zhou, Y., Yin, Y., 2016. Tryptophan increases intestinal permeability and decreases intestinal tight junction protein expression in weanling piglets. J. Anim. Sci. 94, 87–90.

Liu, H., Hu, C.A., Bai, M., Liu, G., Tossou, M., Xu, K., Li, F., Liao, P., Kong, X., Wu, X., 2017a. Short-term supplementation of isocaloric meals with l-tryptophan affects pig growth. Amino Acids 49, 2009–2014.

Liu, W., Mi, S., Ruan, Z., Li, J., Shu, X., Yao, K., Jiang, M., Deng, Z., 2017b. Dietary tryptophan enhanced the expression of tight junction protein ZO-1 in intestine. J. Food Sci. 82, 562–567.

Liu, J.B., Xue, P.C., Cao, S.C., Liu, J., Chen, L., Zhang, H.F., 2018. Effects of dietary phosphorus concentration and body weight on postileal phosphorus digestion in pigs. Anim. Feed Sci. Technol. 242, 86–94.

Markus, C.R., Olivier, B., Panhuysen, G.E., Van der Gugten, J., Alles, M.S., Tuiten, A., Westenberg, H.G., Fekkes, D., Koppeschaar, H.F., de Haan, E.E., 2000. The bovine protein α-lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. Am. J. Clin. Nutr. 71, 1536–1544.

Naatjes, M., Htoo, J., Walter, K., Tölle, K., Susenbeth, A., 2014. Effect of dietary tryptophan to lysine ratio on growth of young pigs fed wheat–barley or corn based diets. Livest. Sci. 163, 102–109.

Noblet, J., Fortune, H., Shi, X., Dubois, S., 1994. Prediction of net energy value of feeds for growing pigs. J. Anim. Sci. 72, 344–354.

National Research Council, 2012. Nutrient Requirements for Swine, 11th rev. ed. Natl. Acad. Press, Washington, DC.

Quant, A.D., Lindemann, M.D., Kerr, B.J., Payne, R.L., Cromwell, G.L., 2012. Standardized ileal digestible tryptophan-to-lysine ratios in growing pigs fed corn-based and non-corn-based diets. J. Anim. Sci. 90, 1270–1279.

Robbins, K.R., Saxton, A.M., Southern, L.L., 2006. Estimation of nutrient requirements using broken-line regression analysis1. J. Anim. Sci. 84, E155–E165.

Ruan, Z., Yang, Y., Wen, Y., Zhou, Y., Fu, X., Ding, S., Liu, G., Yao, K., Wu, X., Deng, Z., 2014. Metabolomic analysis of amino acid and fat metabolism in rats with l-tryptophan supplementation. Amino Acids 46, 2681–2691.

Salyer, J., Tokach, M., DeRouchey, J., Dritz, S., Goodband, R., Nelssen, J., 2013. Effects of standardized ileal digestible tryptophan: lysine in diets containing 30% dried distillers grains with solubles on finisher pig performance and carcass traits. J. Anim. Sci. 91, 3244–3252.

Sato, H., Kobayashi, T., Jones, R.W., Easter, R.A., 1987. Tryptophan availability of some feedstuffs determined by pig growth assay. J. Anim. Sci. 64, 191–200.

Shen, Y.B., Voilqué, G., Odle, J., Kim, S.W., 2012. Dietary L-tryptophan supplementation with reduced large neutral amino acids enhances feed efficiency and decreases stress hormone secretion in nursery pigs under social-mixing stress. J. Nutr. 142, 1540–1546.

Susenbeth, A., 2006. Optimum tryptophan: lysine ratio in diets for growing pigs: analysis of literature data. Livest. Sci. 101, 32–45.

Susenbeth, A., Lucanus, U., 2005. The effect of tryptophan supplementation of diets of restricted- and unrestricted-fed young pigs. J. Anim. Physiol. Anim. Nutr. 89, 331–336.

Xu, S., Zhao, Y., Shen, J., Lin, Y., Fang, Z., Che, L., Wu, D., 2015. Threonine and tryptophan supplementation enhance porcine respiratory and reproductive syndrome (PRRS) vaccine-induced immune responses of growing pigs. Anim. Sci. J. 86, 294–304.

Yao, K., Fang, J., Yin, Y., Feng, Z.M., Tang, Z.R., Wu, G., 2011. Tryptophan metabolism in animals: important roles in nutrition and health. Front. Biosci. 3, 286–297.

Zhang, G., Song, Q., Xie, C., Chu, L., Thacker, P., Htoo, J., Qiao, S., 2012. Estimation of the ideal standardized ileal digestible tryptophan to lysine ratio for growing pigs fed low crude protein diets supplemented with crystalline amino acids. Livest. Sci. 149, 260–266.