

## Effects of standardized ileal digestible tryptophan: lysine ratio on growth performance of nursery pigs<sup>1,2</sup>

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**ABSTRACT:** Two experiments were conducted to estimate the standardized ileal digestible (SID) Trp:Lys ratio requirement for growth performance of nursery pigs. Experimental diets were formulated to ensure that lysine was the second limiting AA throughout the experiments. In Exp. 1 (6 to 10 kg BW), 255 nursery pigs (PIC 327 × 1050, initially 6.3 ± 0.15 kg, mean ± SD) arranged in pens of 6 or 7 pigs were blocked by pen weight and assigned to experimental diets (7 pens/diet) consisting of SID Trp:Lys ratios of 14.7%, 16.5%, 18.4%, 20.3%, 22.1%, and 24.0% for 14 d with 1.30% SID Lys. In Exp. 2 (11 to 20 kg BW), 1,088 pigs (PIC 337 × 1050, initially 11.2 kg ± 1.35 BW, mean ± SD) arranged in pens of 24 to 27 pigs were blocked by average pig weight and assigned to experimental diets (6 pens/diet) consisting of SID Trp:Lys ratios of 14.5%, 16.5%, 18.0%, 19.5%, 21.0%, 22.5%, and 24.5% for 21 d with 30% dried distillers grains with solubles and 0.97% SID Lys. Each experiment was analyzed using general linear mixed models with heterogeneous residual variances. Competing heteroskedastic models included broken-line linear (BLL), broken-line quadratic (BLQ), and quadratic polynomial (QP). For each response, the best-fitting model was selected using

Bayesian information criterion. In Exp. 1 (6 to 10 kg BW), increasing SID Trp:Lys ratio linearly increased ( $P < 0.05$ ) ADG and G:F. For ADG, the best-fitting model was a QP in which the maximum ADG was estimated at 23.9% (95% confidence interval [CI]: [ $<14.7\%$ ,  $>24.0\%$ ]) SID Trp:Lys ratio. For G:F, the best-fitting model was a BLL in which the maximum G:F was estimated at 20.4% (95% CI: [14.3%, 26.5%]) SID Trp:Lys. In Exp. 2 (11 to 20 kg BW), increasing SID Trp:Lys ratio increased ( $P < 0.05$ ) ADG and G:F in a quadratic manner. For ADG, the best-fitting model was a QP in which the maximum ADG was estimated at 21.2% (95% CI: [20.5%, 21.9%]) SID Trp:Lys. For G:F, BLL and BLQ models had comparable fit and estimated SID Trp:Lys requirements at 16.6% (95% CI: [16.0%, 17.3%]) and 17.1% (95% CI: [16.6%, 17.7%]), respectively. In conclusion, the estimated SID Trp:Lys requirement in Exp. 1 ranged from 20.4% for maximum G:F to 23.9% for maximum ADG, whereas in Exp. 2 it ranged from 16.6% for maximum G:F to 21.2% for maximum ADG. These results suggest that standard NRC (2012) recommendations may underestimate the SID Trp:Lys requirement for nursery pigs from 11 to 20 kg BW.

**Key words:** amino acid ratio, growth, lysine, nursery pig, tryptophan

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### INTRODUCTION

Tryptophan is an important limiting AA in corn and soybean meal-based diets of nursery and finishing pigs (Lewis, 2000). As the availability of feed-grade AA, including Trp, increases, so does their use as replacement for intact protein sources in swine diets. The Trp requirement in swine diets can be

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expressed in different ways. In particular, the standardized ileal digestible (SID) Trp requirement expressed as a ratio to Lys (**Trp:Lys**) is considered a practical approach for diet formulation (Stein et al., 2007); however, the observed SID Trp:Lys ratio requirement varies considerably among studies. For example, NRC (2012) estimates the SID Trp requirement at 16.3% of Lys for nursery pigs, but Guzik et al. (2005) estimated a SID Trp:Lys requirement of greater than 19.5%, and Simongiovanni et al. (2012) concluded that it was 17% to 22% of Lys. The objective of these studies was to estimate the SID Trp:Lys ratio requirement for growth performance in nursery pigs.

## MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Experiment 1 was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, and Exp. 2 was conducted at a commercial research-nursery barn in southwestern Minnesota.

### Experiment 1: 6 to 10 kg BW

A total of 255 nursery pigs (PIC 327 × 1050, with initial and final BW of  $6.3 \pm 0.15$  and  $9.8 \pm 0.46$  kg, respectively, mean ± SD) were used in a 14-d growth trial. Pigs were weaned at 21 d of age and placed in the nursery facility, where they were fed a common diet for 3 d. At d 3 after weaning, pigs were weighed in pens, and pens were randomly assigned to dietary treatments in a randomized complete block design blocked by initial average pen BW. Therefore, d 3 after weaning was d 0 of the trial. Each treatment consisted of 7 pens of 6 to 7 pigs/pen, and each pen comprised barrows and gilts. A 4-hole, dry self-feeder and a nipple waterer were used in each pen (1.2 × 1.5 m) to provide ad libitum access to feed and water. Experimental diets consisted of corn and soybean meal and had 6 ratios of SID Trp:Lys, namely, 14.7%, 16.5%, 18.4%, 20.3%, 22.1%, and 24.0%. Feed-grade L-Trp was added at the expense of corn starch in the basal diet to achieve the desired ratios of SID Trp:Lys (Table 1). Nutrients and SID AA digestibility values used for diet formulation were obtained from NRC (1998). Large batches of the 14.7% and 24.0% SID Trp:Lys diets were manufactured, then blended to achieve the intermediate SID Trp:Lys ratios. The percentages of low- and high-SID Trp:Lys blends to create the treatment diets were 100% and 0%, 80% and 20%, 60% and 40%, 40% and 60%, 20% and 80%, and 0% and 100% for 14.7%, 16.5%, 18.4%, 20.3%, 22.1%, and 24.0% SID Trp:Lys, respectively. Diets were

**Table 1.** Diet composition, Exp. 1 (as-fed basis)<sup>1</sup>

Item	Basal diet
Ingredient, %	
Corn	58.09
Soybean meal (46.5% CP)	25.20
Spray-dried whey	10.00
Soybean oil	1.00
Monocalcium P (21% P)	1.10
Limestone	0.90
Salt	0.35
Zinc oxide	0.25
Trace mineral premix <sup>2</sup>	0.15
Vitamin premix <sup>3</sup>	0.25
L-Lys HCl	0.533
DL-Met	0.220
L-Thr	0.230
L-Ile	0.100
L-Val	0.160
Gln	0.630
Gly	0.630
Phytase <sup>4</sup>	0.085
Corn starch <sup>5</sup>	0.123
L-Trp	—
Total	100
Calculated analysis	
SID AA, <sup>6</sup> %	
Lys	1.30
Ile:Lys	60
Leu:Lys	111
Met:Lys	36
Met and Cys:Lys	58
Thr:Lys	64
Trp:Lys	14.7
Val:Lys	70
Total Lys, %	1.42
ME, kcal/kg	3,341
NE, kcal/kg	2,239
SID lysine:ME, g/Mcal	3.89
SID lysine:NE, g/Mcal	5.27
CP, %	20.4
Ca, %	0.72
P, %	0.64
Available P, %	0.47

<sup>1</sup>Diets were fed from 6.8 to 9.8 kg BW. Nutrient content was calculated on the basis of values from NRC (1998).

<sup>2</sup>Provided per kilogram of diet: 39.6 mg Mn from manganese oxide, 165 mg Fe from iron sulfate, 1,965 mg Zn from zinc sulfate, 16.5 mg Cu from copper sulfate, 0.30 mg I from calcium iodate, and 0.30 mg Se from sodium selenite.

<sup>3</sup>Provided per kilogram of diet: 11,020 IU vitamin A, 1,378 IU vitamin D<sub>3</sub>, 44 IU vitamin E, 4 mg vitamin K, 8 mg riboflavin, 28 mg pantothenic acid, 50 mg niacin, and 0.039 mg vitamin B<sub>12</sub>.

<sup>4</sup>Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 509 phytase activity (FTU) per kilogram of diet, with a release of 0.10% available P.

<sup>5</sup>Feed-grade L-Trp was added at the expense of corn starch at 0%, 0.024%, 0.049%, 0.074%, 0.098%, and 0.123% of the diet to provide Trp:Lys ratios of 14.7%, 16.5%, 18.4%, 20.3%, 22.1%, and 24.0% to form the experimental treatments.

<sup>6</sup>SID = standardized ileal digestible.

formulated to 1.30% SID Lys on the basis of data of Nemechek et al. (2011). On the basis of the NRC (2012) model, 1.34% SID Lys is the requirement in a diet with 3,341 kcal ME/kg for 10-kg nursery pigs. Thus, experimental diets were 0.04% below the NRC requirement at the end of the 6- to 10-kg BW nursery phase to ensure that lysine was the second limiting AA throughout the experiment. The 14.7% SID Trp:Lys ratio diet was also reported to be deficient in Trp (Nemechek et al., 2011). Diets contained 10% spray-dried whey and no specialty protein sources, such as spray-dried blood meal or select menhaden fish meal. Experimental diets were fed in meal form and were prepared at the O. H. Kruse Feed Technology Innovation Center in Manhattan, KS.

### Experiment 2: 11 to 20 kg BW

A total of 1,088 pigs (PIC 337 × 1050, with initial and final BW of 11.2 kg ± 1.35 and 20.3 ± 2.16 kg, respectively, mean ± SD) were used in a 21-d growth trial. Pigs were weaned at 16 d of age and grouped into pens of 27 animals (14 gilts and 13 barrows). After weaning, pigs were fed a common pelleted diet for 7 d, followed by common meal diets containing 10% and 20% dried distillers grains with solubles (DDGS) from d 7 to 14 and 14 to 28 after weaning, respectively, with 20% SID Trp:Lys ratio. On d 28 after weaning, pigs were weighed in pens, and pens were blocked by initial average pen BW and randomly assigned dietary treatments in a randomized complete block design with 6 pens per treatment. Therefore, d 28 after weaning was d 0 of the trial. The facility was totally enclosed, environmentally controlled, and mechanically ventilated. Pens had completely slatted flooring and deep pits for manure storage. Each pen (3.7 × 2.3 m) was equipped with a 6-hole stainless steel dry self-feeder and a pan waterer for ad libitum access to feed and water. Daily feed additions to each pen were accomplished through a robotic feeding system (FeedPro, Feedlogic Corp., Willmar, MN) capable of providing and measuring feed amounts for individual pens. Five representative samples of corn, soybean meal, and DDGS were collected each week for 5 wk and were analyzed in duplicate for total AA (method 994.12; AOAC International, 2012) and CP (method 990.03; AOAC International, 2012) by Ajinomoto Heartland Inc. (Chicago, IL) before diet formulation. These values along with standardized digestibility coefficients from NRC (2012) for corn, soybean meal, and DDGS were used in diet formulation. Diets were balanced on an NE basis using NRC (2012) values.

Two experimental corn–soybean meal–based diets with 30% DDGS were formulated (Table 2) to be limiting in Lys and to have 14.5% and 24.5% SID Trp:Lys ratios; then the diets were blended using the robotic

**Table 2.** Diet composition, Exp. 2 (as-fed basis)<sup>1</sup>

Item	SID Trp:Lys	
	Low (14.5%)	High (24.5%)
Ingredient, %		
Corn	55.15	55.05
Soybean meal (46% CP)	10.91	10.92
DDGS	30.00	30.00
Beef tallow	0.50	0.50
Dicalcium phosphate (18.5% P)	0.50	0.50
Limestone	1.48	1.48
Salt	0.35	0.35
Trace mineral premix <sup>2</sup>	0.100	0.100
Vitamin premix <sup>3</sup>	0.125	0.125
L-Lys HCL	0.575	0.575
D,L-Met	0.070	0.070
L-Thr	0.140	0.140
L-Trp	—	0.098
L-Ile	0.010	0.010
L-Val	0.060	0.060
Phytase <sup>4</sup>	0.025	0.025
Total	100	100
Calculated analysis		
SID AA, %		
Lys	0.97	0.97
Ile:Lys	55	55
Leu:Lys	153	153
Met:Lys	35	35
Met and Cys:Lys	60	60
Thr:Lys	65	65
Trp:Lys	14.5	24.5
Val:Lys	70	70
His:Lys	38	38
Phe and Tyr:Lys	106	106
Trp:BCAA <sup>5</sup>	3.9	6.6
Trp:LNAA <sup>6</sup>	2.8	4.8
ME, kcal/kg	3,325	3,328
NE, kcal/kg	2,466	2,468
SID lysine:ME, g/Mcal	2.91	2.91
SID lysine:NE, g/Mcal	3.93	3.93
CP, %	18.1	18.2
Ca, %	0.71	0.71
P, %	0.49	0.49
Available P, %	0.40	0.40

<sup>1</sup>Diets were fed from 11.2 to 20.3 kg BW. Corn, dried distillers grains with solubles (DDGS), and soybean meal were analyzed for CP and total AA content, and NRC (2012) standardized ileal digestible (SID) digestibility values were used in the diet formulation.

<sup>2</sup>Provided per kilogram of diet: 33 mg Mn from manganese oxide, 110 mg Fe from iron sulfate, 110 mg Zn from zinc oxide, 16.5 mg Cu from copper sulfate, 0.33 mg I from ethylenediamin dihydroiodide, and 0.30 mg Se from sodium selenite.

<sup>3</sup>Provided per kilogram of diet: 5,290 IU vitamin A, 827 IU vitamin D<sub>3</sub>, 26.4 IU vitamin E, 2.64 mg vitamin K, 16.5 mg pantothenic acid, 30 mg niacin, 4.6 mg riboflavin, and 0.02 mg vitamin B<sub>12</sub>.

<sup>4</sup>OptiPhos 2000 (Enzyvia LLC, Sheridan, IN) provided 1,251 phytase activity (FTU) per kilogram of diet with a release of 0.13% available P.

<sup>5</sup>Amount of Trp in the diet as a ratio to branched-chain AA (BCAA; Ile, Leu, and Val) on an SID basis.

<sup>6</sup>Amount of Trp in the diet as a ratio to large neutral AA (LNAA; Ile, Leu, Val, Phe, and Tyr) on an SID basis.

feeding system to achieve intermediate SID Trp:Lys ratios, thereby defining dietary treatments. The percentages of low- and high-SID Trp:Lys ratios blended to create the treatment diets were 100% and 0%, 80% and 20%, 65% and 35%, 50% and 50%, 35% and 65%, 20% and 80%, and 0% and 100% for 14.5%, 16.5%, 18.0%, 19.5%, 21.0%, 22.5%, and 24.5% SID Trp:Lys, respectively. The SID Trp:Lys ratio was increased by adding feed-grade L-Trp to the control diet at the expense of corn. The NRC (2012) model was used to estimate the SID Lys requirement of pigs fed diets with 2,466 kcal NE/kg at the expected BW at the end of the experiment (22.7 kg). The SID Lys requirement (i.e., 1.07%) was reduced by 0.10% point for diet formulation to ensure that lysine was the second limiting AA throughout the experiment. Diets were fed in meal form and were manufactured at the New Horizon Farms Feed Mill (Pipestone, MN). A preliminary experiment was conducted before Exp. 2 in the same facility and with pigs of the same BW to validate that diets were indeed limiting in Lys. For that preliminary experiment, a total of 1,188 pigs (PIC 337 × 1050, initially 12.9 kg ± 0.66 BW, mean ± SD) were used in a 21-d growth trial with 27 pigs per pen and 11 pens per treatment. Pigs were fed either a high-Lys diet (SID Lys levels were 0.01% above the estimated NRC [2012] requirement at the expected initial BW) or a low-Lys diet (0.97%, which is 0.10% below the estimated NRC [2012] requirement at the expected final BW). In the preliminary study, pigs fed low-Lys diets had lower ( $P < 0.05$ ) ADG, ADFI, and G:F compared with pigs fed high-Lys diets, thus validating the below-requirement SID Lys level of diets used in Exp. 2.

### **Diet Sampling and Analysis**

In Exp. 1 and 2, samples of the diets were submitted to Ward Laboratories Inc. (Kearney, NE) for analysis of DM (method 935.29; AOAC International, 2012), CF (method 978.10 [AOAC International, 2012] for preparation and Ankom 2000 Fiber Analyzer, Ankom Technology, Fairport, NY), ash (method 942.05; AOAC International, 2012), crude fat (method 920.39a [AOAC International 2012] for preparation and ANKOM XT20 Fat Analyzer, Ankom Technology), Ca, and P (method 968.08b [AOAC International, 2012] for preparation using ICAP 6500, ThermoElectron Corp., Waltham, MA). In Exp. 1, CP was analyzed by Ward Laboratories Inc. (method 990.03; AOAC International, 2012); in Exp. 2, CP was analyzed by Ajinomoto Heartland Inc. (method 990.03; AOAC International, 2012).

In Exp. 1, diet samples were collected from feeders at the beginning of the trial and on d 7 and 14. At

the end of the trial, samples of the diets were combined within dietary treatment, and a composite sample from each treatment was analyzed in duplicate for total AA content by Ajinomoto Heartland Inc.

In Exp. 2, diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d before the end of the trial and stored at  $-20^{\circ}\text{C}$ ; then CP and total AA analyses were conducted in duplicate on composite samples by Ajinomoto Heartland Inc.

### **Data Collection**

Pig BW and feed disappearance were measured on d 0 and 14 in Exp. 1 and on d 0 and 21 in Exp. 2 to calculate ADG, ADFI, G:F, grams of SID Trp intake per day, and grams of SID Trp intake per kilogram of gain. Total grams of SID Trp intake per day were calculated on the basis of formulated values by multiplying ADFI by SID Lys level by SID Trp:Lys. The total grams of SID Trp intake were divided by total BW gain to calculate the grams of SID Trp intake per kilogram of gain.

### **Statistical Analysis**

Responses of interest (ADG, ADFI, G:F, BW, grams of SID Trp intake per day, and grams of SID Trp intake per kilogram of gain) measured at the pen level were analyzed using general linear and nonlinear mixed models to accommodate the randomized complete block design of the study. The linear predictor included the fixed effect of dietary treatment presented as a factor and initial average pen BW as a random blocking factor. Pen was the experimental unit. Models were expanded to account for heterogeneous residual variances, as needed. Residual assumptions were checked using standard diagnostics on Studentized residuals and were found to be reasonably met. Linear and quadratic polynomial contrasts were built to evaluate the functional form of the dose response to increasing the dietary SID Trp:Lys ratio on ADG, ADFI, G:F, BW, grams of SID Trp intake per day, and grams of SID Trp intake per kilogram of gain. Polynomial contrast coefficients were adjusted for unequally spaced treatments using the IML procedure of SAS (version 9.3; SAS Inst. Inc., Cary, NC). Degrees of freedom were estimated using the Kenward-Rogers approach. Statistical models were fitted using the GLIMMIX procedure of SAS. Results were considered significant at  $P \leq 0.05$  and marginally significant at  $0.05 < P \leq 0.10$ .

Additional models adapted from Robbins et al. (2006) and Pesti et al. (2009) were fitted to ADG and G:F to further estimate SID Trp:Lys requirements using an inverse prediction strategy. Specifically, competing statistical models fitted to the data included a broken-line linear

ascending (**BLL**) model, a broken-line quadratic ascending (**BLQ**) model, and a quadratic polynomial (**QP**) model. As in Robbins et al. (2006), competing models described growth performance as a function of SID Trp:Lys levels as follows: For the BLL ascending model,

$$y_{ij} = L_{BLL} + U_l(R_{BLL} - X_i) + b_j + e_{ij} \quad \text{for } X_i < R_{BLL},$$

$$y_{ij} = L_{BLL} + b_j + e_{ij} \quad \text{for } X_i \geq R_{BLL}.$$

For the BLQ ascending model,

$$y_{ij} = L_{BLQ} + U_q(R_{BLQ} - X_i)^2 + b_j + e_{ij} \quad \text{for } X_i < R_{BLQ},$$

$$y_{ij} = L_{BLQ} + b_j + e_{ij} \quad \text{for } X_i \geq R_{BLQ}.$$

For the QP model,

$$y_{ij} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + b_j + e_{ij}.$$

Here  $y_{ij}$  is the response associated with the pen in block  $j$  assigned to dietary treatment  $i$ ,  $X_i$  is the SID Trp:Lys level of the  $i$ th dietary treatment, and  $L_{BLL}$  and  $L_{BLQ}$  indicate the unknown maximum growth response to dietary treatments (i.e., plateau) under the BLL and BLQ models, respectively.  $R_{BLL}$  and  $R_{BLQ}$  are the unknown minimum levels of SID Trp:Lys required to reach the plateau under the BLL and BLQ models, respectively. In the last equation,  $\beta_0$  is the intercept;  $U_l$ ,  $U_q$ ,  $\beta_1$ , and  $\beta_2$  are the corresponding unknown rates of change of the response as a function of  $X_i$ ;  $b_j$  is the random blocking effect of initial average pen BW associated with  $j$ th block, where  $b_j \sim N(0, \sigma_b^2)$ ; and  $e_{ij}$  is a random error associated with the pen in the  $j$ th block that received the  $i$ th treatment, where  $e_{ij} \sim N(0, \sigma_{e_i}^2)$ . Note that  $b_j$  and  $e_{ij}$  are assumed to be independent of each other.

Broken-line regression models were fitted using the NLMIXED procedures of SAS. The optimization technique used was the dual quasi-Newton algorithm, as specified by default in the NLMIXED procedure. Competing statistical models were compared using maximum likelihood-based fit criteria, specifically the Schwarz Bayesian information criterion (**BIC**; Milliken and Johnson, 2009). Results reported here correspond to inference yielded by the best-fitting models.

For the best-fitting models, the estimated requirement of SID Trp:Lys to reach plateau performance (i.e.,  $R_{BLL}$  and  $R_{BLQ}$  in the broken-line models) or to reach maximum performance (i.e., in the QP) of ADG and G:F are reported with a 95% confidence interval

(**CI**). In the quadratic polynomial model, the level of the SID Trp:Lys ratio that maximized ADG and G:F was estimated by equating the first derivative of the regression equation to zero, then solving for the SID Trp:Lys ratio (Pesti et al., 2009). The corresponding 95% CI were computed using the inverse regression approach proposed by Lavagnini and Magno (2006).

## RESULTS AND DISCUSSION

The analyzed total AA, DM, CP, CF, Ca, P, fat, and ash contents of diets for Exp. 1 and 2 (Tables 3 and 4, respectively) were consistent with calculated values based on variation reported by Cromwell et al. (1999).

In Exp. 1 (6 to 10 kg BW), increasing the SID Trp:Lys ratio linearly increased (Table 5) ADG ( $P = 0.022$ ), G:F ( $P = 0.012$ ), grams of SID Trp intake per day ( $P = 0.001$ ), and grams of SID Trp intake per kilogram of gain ( $P = 0.001$ ). Increasing the SID Trp:Lys ratio also induced marginal linear increases in ADFI ( $P = 0.057$ ) and final BW ( $P = 0.052$ ).

For ADG in the 6- to 10-kg BW pigs (Exp. 1), the best-fitting model was a QP (BIC: 215.3) compared with BLL and BLQ models (BIC: 217.4 and 217.8, respectively). The estimated regression equation for the best-fitting QP model (Fig. 1) was

$$\text{ADG} = 42.7 + 1,819.7(\text{Trp:Lys}) - 3,810.1(\text{Trp:Lys})^2$$

whereby the SID Trp:Lys explanatory variable is expressed as a proportion (i.e., 0.180) rather than a percentage (i.e., 18.0%) for numerical stability of computations. Based on the best-fitting QP model, the maximum ADG was estimated at a 23.9% (95% CI: [ $<14.7\%$ ,  $>24.0\%$ ]) SID Trp:Lys ratio. Note the substantial width of this CI, thereby indicating considerable uncertainty for inference on SID Trp:Lys requirements that maximized ADG. This uncertainty is probably related to the large amount of unaccounted variability in ADG relative to an apparently minor effect of SID Trp:Lys ratios on ADG in Exp. 1, which is further supported by the nonsignificant  $P$ -value of the quadratic regression coefficient for ADG ( $P = 0.484$ ) during this phase.

Also for Exp. 1 (6 to 10 kg BW), the best-fitting model for G:F was a BLL (BIC: 253.6) compared with QP and BLQ (BIC: 255.0 and 255.0, respectively). The best-fitting estimated regression equations for the best-fitting BLL model (Fig. 2) were

$$\text{G:F} = 0.733 - 0.6034 \times (0.204 - \text{Trp:Lys}) \quad \text{if SID Trp:Lys} < 20.4\%$$

$$\text{G:F} = 0.733 \quad \text{if SID Trp:Lys} \geq 20.4\%$$

**Table 3.** Chemical analysis of the diets, Exp. 1 (as-fed basis)<sup>1</sup>

Item	Standardized ileal digestible Trp:Lys <sup>2</sup>					
	14.7%	16.5%	18.4%	20.3%	22.1%	24.0%
Proximate analysis, %						
DM	91.07 (89.43)	91.06 (89.43)	91.23 (89.43)	91.12 (89.43)	91.17 (89.43)	91.21 (89.43)
CP	20.2 (20.4)	20.5 (20.4)	20.3 (20.4)	20.4 (20.4)	20.6 (20.5)	21.0 (20.5)
CF	2.2 (2.3)	2.2 (2.3)	2.3 (2.3)	2.3 (2.3)	2.4 (2.3)	2.3 (2.3)
Ca	0.87 (0.72)	0.85 (0.72)	0.76 (0.72)	0.84 (0.72)	0.86 (0.72)	0.87 (0.72)
P	0.69 (0.64)	0.63 (0.64)	0.67 (0.64)	0.70 (0.64)	0.73 (0.64)	0.77 (0.64)
Fat	3.3 (3.7)	3.2 (3.7)	3.4 (3.7)	3.1 (3.7)	3.2 (3.7)	3.2 (3.7)
Ash	6.04 (5.54)	5.93 (5.54)	5.71 (5.54)	5.81 (5.54)	6.10 (5.54)	6.11 (5.54)
AA, %						
Lys	1.43 (1.42)	1.43 (1.42)	1.42 (1.42)	1.38 (1.42)	1.37 (1.42)	1.42 (1.42)
Ile	0.90 (0.87)	0.95 (0.87)	0.93 (0.87)	0.94 (0.87)	0.91 (0.87)	0.93 (0.87)
Leu	1.61 (1.61)	1.63 (1.61)	1.60 (1.61)	1.60 (1.61)	1.53 (1.61)	1.60 (1.61)
Met	0.50 (0.50)	0.46 (0.50)	0.49 (0.50)	0.47 (0.50)	0.49 (0.50)	0.49 (0.50)
Met and Cys	0.82 (0.82)	0.78 (0.82)	0.81 (0.82)	0.78 (0.82)	0.80 (0.82)	0.81 (0.82)
Thr	0.95 (0.93)	0.97 (0.93)	0.95 (0.93)	0.94 (0.93)	0.94 (0.93)	0.95 (0.93)
Trp	0.22 (0.22)	0.23 (0.24)	0.24 (0.26)	0.27 (0.29)	0.30 (0.31)	0.30 (0.34)
Val	1.07 (1.02)	1.05 (1.02)	1.04 (1.02)	1.05 (1.02)	1.03 (1.02)	1.05 (1.02)
His	0.47 (0.48)	0.46 (0.48)	0.46 (0.48)	0.45 (0.48)	0.44 (0.48)	0.46 (0.48)
Phe	0.92 (0.86)	0.92 (0.86)	0.91 (0.86)	0.90 (0.86)	0.88 (0.86)	0.89 (0.86)

<sup>1</sup>Diet samples were collected from feeder at the beginning of the trial and on d 7 and 14. At the end of the trial, samples of each diet were combined, and a composite sample was analyzed for total AA analysis by Ajinomoto Heartland Inc. (Chicago, IL). Samples of the diets were also submitted to Ward Laboratories Inc. (Kearney, NE) for analysis of DM, CP, CF, Ca, P, ash, and crude fat.

<sup>2</sup>Values in parentheses indicate those calculated and are based on values from NRC (1998).

**Table 4.** Chemical analysis of the diets, Exp. 2 (as-fed basis)<sup>1</sup>

Item	Standardized ileal digestible Trp:Lys <sup>2</sup>						
	14.5%	16.5%	18.0%	19.5%	21.0%	22.5%	24.5%
Proximate analysis, %							
DM	90.48 (88.26)	90.06 (88.27)	90.21 (88.27)	90.25 (88.27)	90.35 (88.27)	89.91 (88.27)	89.78 (88.28)
CP	19.0 (18.1)	19.4 (18.2)	18.8 (18.2)	18.7 (18.2)	18.9 (18.2)	19.1 (18.2)	18.2 (18.2)
CF	3.8 (4.2)	3.8 (4.2)	4 (4.2)	3.9 (4.2)	3.5 (4.2)	3.8 (4.2)	4.0 (4.2)
Ca	0.88 (0.71)	0.93 (0.71)	0.97 (0.71)	1.11 (0.71)	1.04 (0.71)	1.10 (0.71)	1.25 (0.71)
P	0.52 (0.49)	0.52 (0.49)	0.55 (0.49)	0.54 (0.49)	0.52 (0.49)	0.53 (0.49)	0.54 (0.49)
Fat	4.8 (5.2)	4.7 (5.2)	4.9 (5.2)	4.9 (5.2)	4.7 (5.2)	4.7 (5.2)	4.7 (5.2)
Ash	4.89 (4.73)	4.75 (4.73)	4.82 (4.73)	5.39 (4.72)	5.35 (4.72)	5.18 (4.72)	5.57 (4.72)
AA, %							
Lys	1.19 (1.13)	1.18 (1.13)	1.22 (1.13)	1.22 (1.13)	1.17 (1.13)	1.16 (1.13)	1.19 (1.13)
Ile	0.73 (0.65)	0.75 (0.65)	0.75 (0.65)	0.75 (0.65)	0.74 (0.65)	0.76 (0.65)	0.77 (0.65)
Leu	1.82 (1.74)	1.86 (1.74)	1.86 (1.74)	1.87 (1.74)	1.85 (1.74)	1.90 (1.74)	1.89 (1.74)
Met	0.40 (0.39)	0.39 (0.39)	0.40 (0.39)	0.40 (0.39)	0.40 (0.39)	0.39 (0.39)	0.40 (0.39)
Met and Cys	0.70 (0.70)	0.71 (0.70)	0.72 (0.70)	0.71 (0.70)	0.72 (0.70)	0.72 (0.70)	0.73 (0.70)
Thr	0.82 (0.78)	0.81 (0.78)	0.83 (0.78)	0.81 (0.78)	0.83 (0.78)	0.80 (0.77)	0.81 (0.77)
Trp	0.19 (0.17)	0.19 (0.19)	0.19 (0.21)	0.20 (0.22)	0.23 (0.24)	0.23 (0.25)	0.24 (0.27)
Val	0.93 (0.83)	0.96 (0.83)	0.96 (0.83)	0.96 (0.83)	0.95 (0.83)	0.96 (0.83)	0.96 (0.83)
His	0.47 (0.44)	0.48 (0.44)	0.48 (0.44)	0.48 (0.44)	0.47 (0.44)	0.49 (0.44)	0.49 (0.44)
Phe	0.88 (0.81)	0.91 (0.81)	0.90 (0.81)	0.91 (0.81)	0.90 (0.81)	0.93 (0.81)	0.93 (0.81)

<sup>1</sup>Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d before the end of the trial and were stored at -20°C; then CP and AA analysis was conducted on composite samples by Ajinomoto Heartland Inc. (Chicago, IL). Samples of the diets were also submitted to Ward Laboratories Inc. (Kearney, NE) for analysis of DM, CF, Ca, P, ash, and crude fat.

<sup>2</sup>Values in parentheses indicate those calculated and are based on values from NRC (2012), with the exception of CP and total AA content from corn, soybean meal, and dried distillers grains with solubles, which were analyzed before diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).

**Table 5.** Least squares mean estimates ( $\pm$ SEM) for growth performance of 6- to 10-kg nursery pigs fed dietary treatments consisting of standardized ileal digestible (SID) Trp:Lys ratios ranging from 14.7% to 24.0% (Exp. 1)<sup>1</sup>

Item	SID Trp:Lys						Probability P	
	14.7%	16.5%	18.4%	20.3%	22.1%	24.0%	Linear	Quadratic
ADG, g	226 $\pm$ 14.1	244 $\pm$ 8.5	244 $\pm$ 8.5	266 $\pm$ 14.1	258 $\pm$ 14.1	260 $\pm$ 8.5	<0.022	<0.484
ADFI, g	325 $\pm$ 11.6	342 $\pm$ 11.6	342 $\pm$ 11.6	349 $\pm$ 11.6	341 $\pm$ 11.6	363 $\pm$ 11.6	<0.057	<0.939
G:F	0.718 $\pm$ 0.011	0.697 $\pm$ 0.011	0.694 $\pm$ 0.011	0.750 $\pm$ 0.011	0.751 $\pm$ 0.011	0.716 $\pm$ 0.011	<0.012	<0.500
BW, kg								
d 0	6.3 $\pm$ 0.06	6.3 $\pm$ 0.06	6.2 $\pm$ 0.06	6.3 $\pm$ 0.06	6.2 $\pm$ 0.06	6.3 $\pm$ 0.06	<0.753	<0.870
d 14	9.4 $\pm$ 0.19	9.7 $\pm$ 0.19	9.7 $\pm$ 0.09	10.0 $\pm$ 0.19	9.9 $\pm$ 0.19	9.9 $\pm$ 0.19	<0.052	<0.294
SID Trp intake, g/d	0.621 $\pm$ 0.029	0.736 $\pm$ 0.029	0.818 $\pm$ 0.029	0.917 $\pm$ 0.029	0.978 $\pm$ 0.029	1.132 $\pm$ 0.029	<0.001	<0.687
SID Trp g/kg gain	2.8 $\pm$ 0.11	3.0 $\pm$ 0.06	3.4 $\pm$ 0.06	3.5 $\pm$ 0.11	3.8 $\pm$ 0.11	4.4 $\pm$ 0.11	<0.001	<0.075

<sup>1</sup>A total of 255 nursery pigs (PIC 327  $\times$  1050, initially 6.3 kg and 3 d postweaning) were used in a 14-d trial with 6 to 7 pigs per pen and 7 pens per treatment. Diets were formulated to 1.30% SID Lys based on data of Nemechek et al. (2011). The SID Trp intake and gram per kilogram gain were evaluated using diet calculated values for Trp.

whereby the SID Trp:Lys explanatory variable is expressed as a proportion (i.e., 0.180) rather than a percentage (i.e., 18.0%) for numerical stability. Based on the best-fitting BLL model, the estimated minimum SID Trp:Lys requirement to achieve maximum G:F was 20.4% (95% CI: [14.3%, 26.5%]).

To the author's knowledge, this is the first attempt to try to quantify uncertainty around the SID Trp:Lys ratio requirement. Therefore, after this first experiment, a subsequent study was conducted on a larger scale in a commercial facility with a 7-point titration and slightly wider treatment ranges to reduce the uncertainty around the estimates and evaluate the response to increasing SID Trp:Lys ratios.

In Exp. 2 (11 to 20 kg BW), increasing SID Trp:Lys ratio quadratically increased (Table 6) ADG ( $P = 0.001$ ), ADFI ( $P = 0.006$ ), G:F ( $P = 0.002$ ), final BW ( $P = 0.001$ ), grams of SID Trp intake per day ( $P = 0.026$ ), and grams of SID Trp intake per kilogram of gain ( $P = 0.006$ ).

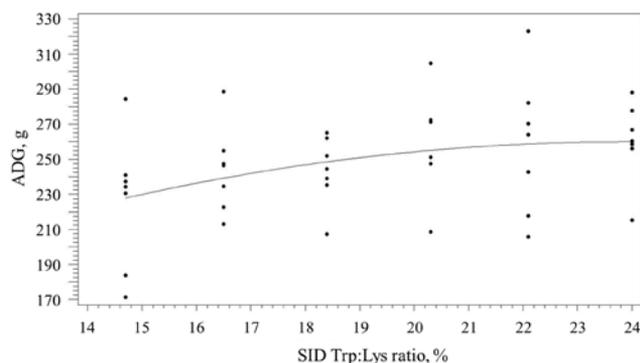
For ADG in the 11- to 20-kg BW pigs (Exp. 2), the best-fitting model was a QP (BIC: 198.1) compared with BLL and BLQ models (BIC: 204.8 and 204.8, re-

spectively). The estimated regression equation for the best-fitting QP model (Fig. 3) was

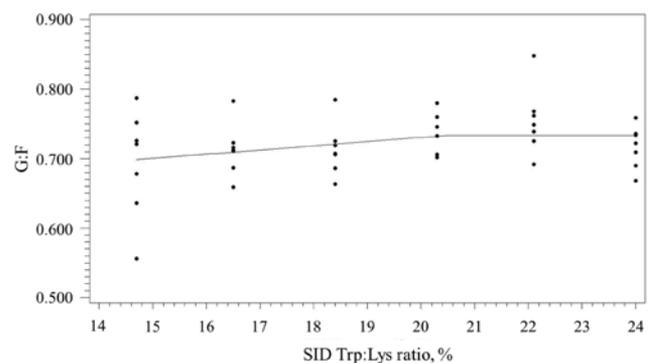
$$\text{ADG} = -317 + 7,259 \times (\text{Trp:Lys}) - 17,110 \times (\text{Trp:Lys})^2$$

On the basis of the best-fitting QP model, the maximum ADG was estimated at 21.2% (95% CI: 20.5% to 21.9%) SID Trp:Lys. Note the reduced uncertainty (i.e., narrower CI) around the estimated Trp:Lys ratio requirement for maximum ADG compared with that in Exp 1.

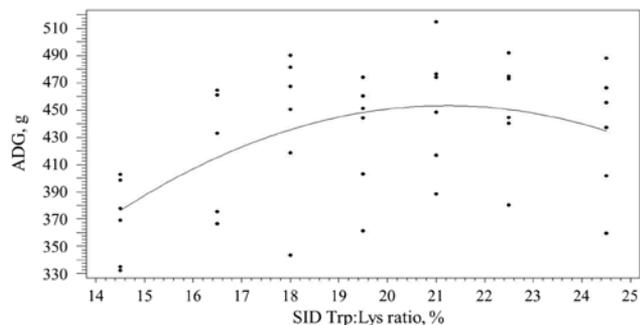
For G:F in the 11- to 20-kg BW pigs (Exp. 2), BLL and BLQ models had comparable fits (BIC: 346.1 and 346.1, respectively), whereas QP showed a less adequate fit (BIC: 355.2). The comparable fit of these models may be explained by the scarcity of information on G:F for the range of SID Trp:Lys ratios for which the functional relationship is estimated; that is, G:F observations were available for only 2 SID Trp:Lys ratios before the estimated plateau in G:F was detected. Note that the same number of unknown fixed-effect parameters occur in the BLL model (i.e.,  $L_{BLL}$ ,  $U_b$ , and  $R_{BLL}$ ) and the BLQ model (i.e.,  $L_{BLQ}$ ,  $U_q$ , and  $R_{BLQ}$ ), so the



**Figure 1.** Fitted quadratic polynomial regression model on ADG as a function of increasing standardized ileal digestible (SID) Trp:Lys in 6- to 10-kg pigs (Exp. 1). The maximum ADG was estimated at 23.9% (95% confidence interval: [ $<14.5\%$ ,  $>24.5\%$ ]) SID Trp:Lys.



**Figure 2.** Fitted broken-line linear regression model on G:F as a function of increasing standardized ileal digestible (SID) Trp:Lys in 6- to 10-kg pigs (Exp. 1). The maximum G:F was estimated at 20.4% (95% confidence interval: [14.3%, 26.5%]).



**Figure 3.** Fitted quadratic polynomial regression model on ADG as a function of increasing standardized ileal digestible (SID) Trp:Lys in 11- to 20-kg pigs (Exp. 2). The maximum ADG was estimated at 21.2% (95% confidence interval: [20.5%, 21.9%]) SID Trp:Lys.

principle of parsimony favoring simpler models would, in this case, not contribute to model selection. Taken together, these issues can help explain, at least partially, the impaired discrimination in differential fit between BLL and BLQ models. On the basis of the best-fitting BLL and BLQ models, alternative estimated regression equations (Fig. 4) were

$$G:F = 0.5844 - 1.95(0.166 - \text{Trp:Lys}) \\ \text{if SID Trp:Lys} < 16.6\%$$

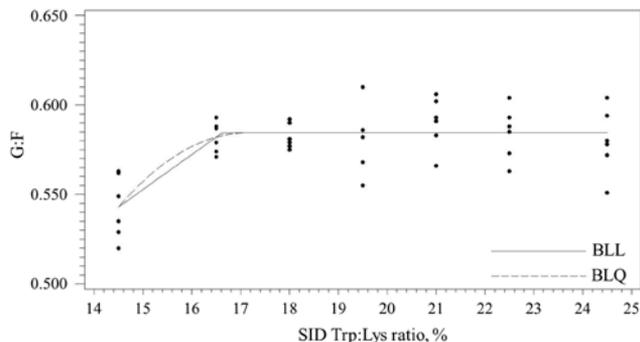
$$G:F = 0.5844 \text{ if SID Trp:Lys} \geq 16.6\%$$

for the BLL model and

$$G:F = 0.5844 - 59.80(0.171 - \text{Trp:Lys})^2 \\ \text{if SID Trp:Lys} < 17.1\%$$

$$G:F = 0.5844 \text{ if SID Trp:Lys} \geq 17.1\%$$

for the BLQ model.



**Figure 4.** Fitted broken-line linear (BLL) and broken-line quadratic (BLQ) regression models on G:F as a function of increasing standardized ileal digestible (SID) Trp:Lys in 11- to 20-kg pigs (Exp. 2). The maximum G:F was estimated at 16.6% (95% confidence interval [CI]: [16.0%, 17.3%]) and 17.1% (95% CI: [16.6%, 17.7%]) SID Trp:Lys in the BLL and BLQ models, respectively.

The estimated SID Trp:Lys requirements for G:F were 16.6% (95% CI: 16.0 to 17.3) and 17.1% (95% CI: 16.6 to 17.7) based on the BLL and BLQ models, respectively. Again, similar to the estimated SID Trp:Lys requirements for maximum ADG, the CI for SID Trp:Lys requirements for maximum G:F was narrower than that in Exp. 1.

Broken-line models and QP models can be used (through inverse prediction) to estimate the minimum level of requirement that maximizes average growth performance in the intended swine population. From a conceptual standpoint, both types of models may address the question of interest, but finer differences between the fit of the models become apparent when describing the relationship between growth performance and SID Trp:Lys. This illustrates the importance of objectively selecting a model based on its fit to the data (Littell et al., 2006). When developing requirement curves, the coefficient of determination  $R^2$  traditionally has been used as the primary indicator of model fit to select among competing models (Pesti et al., 2009).

**Table 6.** Least squares mean estimates ( $\pm$ SEM) for growth performance of 11- to 20-kg nursery pigs fed dietary treatments of standardized ileal digestible (SID) Trp:Lys ratio ranging from 14.5% to 24.5% (Exp. 2)<sup>1</sup>

Item	SID Trp:Lys							Probability, <i>P</i>	
	14.5%	16.5%	18.0%	19.5%	21.0%	22.5%	24.5%	Linear	Quadratic
d 0 to 21									
ADG, g	369 $\pm$ 20.2	428 $\pm$ 20.2	442 $\pm$ 20.2	432 $\pm$ 20.2	453 $\pm$ 17.6	451 $\pm$ 17.6	435 $\pm$ 17.6	<0.001	<0.001
ADFI, g	682 $\pm$ 35.2	735 $\pm$ 31.1	759 $\pm$ 35.2	749 $\pm$ 35.2	768 $\pm$ 31.1	773 $\pm$ 31.1	750 $\pm$ 31.1	<0.001	<0.006
G:F	0.543 $\pm$ 0.008	0.582 $\pm$ 0.005	0.582 $\pm$ 0.005	0.578 $\pm$ 0.008	0.590 $\pm$ 0.005	0.584 $\pm$ 0.005	0.580 $\pm$ 0.008	<0.002	<0.002
BW, kg									
d 0	11.3 $\pm$ 0.55	11.3 $\pm$ 0.55	11.2 $\pm$ 0.55	11.2 $\pm$ 0.55	11.2 $\pm$ 0.55	11.3 $\pm$ 0.55	11.2 $\pm$ 0.55	<0.844	<0.952
d 21	19.0 $\pm$ 0.87	20.2 $\pm$ 0.87	20.7 $\pm$ 0.87	20.3 $\pm$ 0.94	20.8 $\pm$ 0.87	20.7 $\pm$ 0.87	20.4 $\pm$ 0.87	<0.001	<0.001
SID Trp intake, g/d	0.959 $\pm$ 0.069	1.176 $\pm$ 0.063	1.325 $\pm$ 0.069	1.417 $\pm$ 0.069	1.564 $\pm$ 0.063	1.686 $\pm$ 0.063	1.783 $\pm$ 0.063	<0.001	<0.026
SID Trp, g/kg gain	2.6 $\pm$ 0.04	2.8 $\pm$ 0.02	3.0 $\pm$ 0.02	3.3 $\pm$ 0.04	3.5 $\pm$ 0.04	3.7 $\pm$ 0.04	4.1 $\pm$ 0.04	<0.001	<0.006

<sup>1</sup>A total of 1,088 pigs (PIC 337  $\times$  1050, initially 11.2 kg BW and 28 d postweaning) were used in a 21-d growth trial with 24 to 27 pigs per pen and 6 pens per treatment. The NRC (2012) model was used to determine the Lys requirement of mixed-gender pens of pigs at the end of the BW range (22.7 kg), and that value was reduced by 0.10%. The SID Trp intake and gram per kilogram gain were evaluated using diet calculated values for Trp.

However, within a mixed-model framework as in this study, the calculation and interpretation of  $R^2$  is fraught with ambiguities. First,  $R^2$  is not uniquely defined when multiple sources of random variability are present in the data (Schabenberger and Pierce, 2002). Such multiple definitions of  $R^2$  in the mixed-model framework impair the arguably intuitive interpretation of  $R^2$  as a proportion of variability explained by X that has been so appealing in the animal sciences. Furthermore, attempts to calculate  $R^2$  in the mixed-model framework fail to take into account model complexity in the presence of random effects (Kvalseth, 1985). In turn, other information criteria that take into consideration the design structure of the experiment are available for model selection in the mixed-model framework. These include, but are not limited to, the maximum likelihood-based Akaike information criterion and Schwarz's BIC (Milliken and Johnson, 2009). Schwarz's BIC is often used in mixed models, and its calculation is slightly more conservative than the AIC and tends to favor more parsimonious models (Schwarz, 1978); for this reason BIC was the primary indicator of model fit when assessing the competing dose response models. A 2-point difference in BIC is usually considered to be indicative of improved fit of the model with lower BIC (Raftery, 1996). The results indicate that for ADG in both early and late nursery pigs, the QP model was the single best fitting model. In contrast, for G:F the BLL was the best-fitting model in early nursery pigs. In late nursery pigs, the BLL and BLQ models had comparable BIC, indicating comparable fits to the data, so the regression equations for both models were provided. Although the CI for SID Trp:Lys ratio requirements overlap for the BLL and BLQ models, the functional forms assumed by the models induced a point estimate for BLQ that was numerically slightly higher than that of the BLL model.

In addition to considering biologic performance, nutritionists should also take into account economic considerations during diet formulation. It is generally economically unfeasible to formulate diets that meet requirements to achieve 100% of the maximized average performance, so arbitrary target levels of average performance (i.e., 95%) are commonly used in the industry (Pesti et al., 2009). Therefore, the current study also provides fitted prediction equations that can be used to estimate ADG and G:F on the basis of different SID Trp:Lys requirements. The SID Trp:Lys ratios needed to achieve different target average ADG and G:F levels for nursery pigs are demonstrated in Table 7. An approximately 18% SID Trp:Lys ratio would be needed to achieve 95% of the maximum ADG and 98% of the maximum G:F in Exp. 1, whereas the same ratio would achieve 96% of the maximum ADG and 100% of the maximum G:F in Exp. 2. In early and late nursery pigs, 99% of the

**Table 7.** Standardized ileal digestible (SID) Trp:Lys ratio at different target performance levels of nursery pigs<sup>1</sup>

Item	Percentage of maximum performance					
	95%	96%	97%	98%	99%	100%
Exp. 1 (6- to 10-kg BW pigs)						
ADG						
QP	18.1	18.7	19.4	20.2	21.3	23.9
G:F						
BLL	14.3	15.5	16.7	18.0	19.2	20.4
Exp. 2 (11- to 20-kg BW pigs)						
ADG						
QP	17.6	18.0	18.4	18.9	19.6	21.2
G:F						
BLL	15.1	15.4	15.7	16.0	16.3	16.6
BLQ	14.9	15.1	15.4	15.7	16.1	17.1

<sup>1</sup>Derived from equations presented in the text. BLL = broken-line linear; BLQ = broken-line quadratic; QP = quadratic polynomial.

maximum ADG was reached at 21.3% and 19.6% SID Trp:Lys, respectively. These relative differences in performance can be translated into economic terms on the basis of localized economic conditions while taking into account the underlying biologic performance.

In general, these findings add to the body of literature in which low levels of SID Trp in the diets of pigs reduce ADFI and, consequently, reduce ADG and G:F (Simongiovanni et al., 2012). The absorbed Trp is converted into 5-hydroxytryptophan, which is a metabolite that can cross the blood-brain barrier and work as a precursor for serotonin after being decarboxylated (Le Floc'h et al., 2011). As a result, the inclusion of different levels of Trp in pig diets has been shown to manipulate serotonin-mediated feed intake (Batterham et al., 1994; Simongiovanni et al., 2012). Although the results on SID Trp:Lys requirements for the 6- to 10-kg BW phase (Exp. 1) were inconclusive, the study for the 11- to 20-kg BW phase (Exp. 2) indicates the SID Trp:Lys requirements for maximum ADG were greater than those for maximum G:F, which is consistent with other studies (Ma et al., 2010; Petersen, 2011). In turn, the NRC (2012) recommended SID Trp:Lys ratio is 16.3% for nursery pigs, whereas the National Swine Nutrition Guide (2010) recommended SID Trp:Lys at 16.8% for nursery pigs. It is worth noticing that neither of these 2 standards distinguish between requirements for ADG and requirements for G:F; in fact, these standards correspond closely with estimates of SID Trp:Lys requirements for maximum G:F, but they may underestimate requirements for maximum ADG.

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