Antibiotic inclusion in the diet did not alter the standardized ileal digestible tryptophan to lysine ratio for growing pigs^{1,2}

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ABSTRACT: Two 21-d experiments were conducted to determine the optimum standardized ileal digestible (SID) Trp:Lys ratio for growing pigs; 1 experiment fed diets supplemented with or without an antibiotic. The primary response variables in both experiments were ADG, ADFI, G:F, and plasma urea N (PUN) concentrations with the optimum SID Trp:Lys ratio detected using broken-line analysis. Experiment 1 evaluated the optimum SID Trp:Lys ratio in growing pigs fed diets supplemented with an antibiotic. This experiment used 120 crossbred pigs that were blocked by sex and initial BW (24.13 ± 2.72 kg) and allotted to 6 SID Trp:Lys ratios in 4 replicates. Dietary treatments were formulated by the addition of crystalline Trp to create 6 SID Trp:Lys ratios (13.08%, 14.06%, 15.04%, 17.00%, 18.95%, and 20.91%) with a constant SID Lys level of 0.655%. As SID Trp:Lys ratios increased, ADG, ADFI, and G:F increased, and PUN concentrations decreased linearly ($P < 0.05$) and quadratically ($P < 0.05$). Linear broken-line analysis yielded optimum SID Trp:Lys ratios of 17.93% ($P < 0.001$) and 16.17% ($P = 0.009$) for ADG and PUN, respectively, resulting in a mean optimum SID Trp:Lys ratio of 17.05%. Experiment 2 evaluated the optimum SID Trp:Lys ratio in growing

pigs fed diets supplemented with or without an antibiotic. It used a total of 324 crossbred pigs (initial BW: 30.81 ± 3.56 kg) that were allotted to 6 SID Trp:Lys ratios in 6 replicates. Dietary treatments were formulated by the addition of crystalline Trp to create 6 SID Trp:Lys ratios (12.52%, 14.86%, 17.20%, 19.54%, 21.88%, and 24.22%) with a constant SID Lys level of 0.67%. As SID Trp:Lys ratios increased, ADG, ADFI, and G:F increased, and PUN concentrations decreased linearly ($P < 0.001$) and quadratically ($P < 0.001$) regardless of antibiotic inclusion. There were no differences by the antibiotic treatment in ADG, ADFI, G:F, or PUN concentrations ($P > 0.49$) and no interactions between antibiotics and Trp:Lys ratios ($P > 0.29$). When the data for all pigs were pooled for the various Trp:Lys ratios, the optimum SID Trp:Lys ratios for ADG and PUN based on linear broken-line analysis were 14.58% ($P < 0.001$) and 14.54% ($P < 0.001$), respectively, resulting in an optimum SID Trp:Lys ratio of 14.56% as the mean of the determined optima for ADG and PUN responses. These results demonstrate that the optimum SID Trp:Lys ratio for 30- to 50-kg growing pigs is not impacted by the dietary inclusion of an antibiotic as long as the diets are formulated on an SID AA basis.

Keywords: antibiotic, lysine, pigs, standard ileal digestible tryptophan:lysine ratio, tryptophan

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INTRODUCTION

The ideal protein concept that expresses AA needs relative to the Lys requirement of the pig (Fuller et al., 1989; Wang and Fuller, 1989; Chung and Baker, 1992) is firmly engrained in the swine industry as the basis for diet formulation. Thus, formulating diets according to the ideal protein concept allows for meeting the need of the pigs without excessive supplementation of AA other than Lys and can be an efficient and economical use of dietary protein while minimizing N excretion to the environment (Wang and Fuller 1989; Boisen, 1993; Canh et al., 1998; Boisen et al., 2000). Many studies have reported the required ratio of all other AA relative to Lys in swine (NRC, 2012), but one of the more disputed ratios is that of Trp relative to Lys (Kerr, 2006; Susenbeth, 2006). Literature data clearly indicate that the optimal Trp:Lys ratios in swine diets vary greatly, with European research reporting that the Trp:Lys ratio is in excess of 20%, whereas most North American research reports that it is less than 17% (Van Cauwenberghe and Relandeau, 2000; Kerr, 2006; Susenbeth, 2006; Salyer et al., 2013). The reason for the disparity of perspective has not yet been identified. To some degree, the issue can be attributed to one of statistical philosophy (Gurnow, 1973; Baker, 1986) and perhaps the type of cereal or the level of dietary CP (Jansman et al., 2000; Quant et al., 2012). None of these potential reasons, however, has fully explained the differing perspectives.

Previous studies have reported that the absolute Trp or essential amino acid (EAA) requirements of the pigs were higher when pigs were fed diets without an antibiotic compared with antibiotic-fortified diets (Becker et al., 1955; Bikker and Dirkzwager, 2003; Bikker et al., 2007). However, evaluation of the dietary Trp:Lys ratio in pigs supplemented with and without an antibiotic is basically nonexistent. Therefore, the objectives of the experiments reported here were to determine the optimal standardized ileal digestible (SID) Trp:Lys ratio for 30- to 50-kg growing pigs and to determine if the optimum SID Trp:Lys ratio differs depending on whether the diets contain an antibiotic.

MATERIALS AND METHODS

Each experiment was conducted with growing pigs under protocols approved by the University of Kentucky Institutional Animal Care and Use Committee. For each experiment, pigs were brought into the growing-finishing facility at approximately 18 to 20 kg BW and were placed on a common corn-soybean meal diet adequate in all nutrients for a minimum period of 1 wk prior to allotment for all experiments.

Experiment 1

Experiment 1 used 120 crossbred pigs with an initial BW of 24.13 ± 2.72 kg allotted to 6 SID Trp:Lys ratios treatments. Pigs were housed in 24 pens (2.73×4.55 m), with concrete slats extending the length of each pen. Each pen was equipped with a single-sided, 2-hole, stainless-steel feeder and 2 nipple waterers. The pigs were randomly allotted on the basis of sex and initial BW in a randomized incomplete block design to 6 diets with 4 replicates of 5 pigs/pen. Two replicates contained 3 barrows and 2 gilts, whereas the remaining 2 replicates contained 2 barrows and 3 gilts for a total of 120 pigs (60 barrows and 60 gilts).

The basal diet was formulated to contain 0.655% SID Lys, whereas the other EAA (except for Trp) were formulated on an ideal ratio basis using an SID Lys level of 0.803% (0.905% total Lys), which was the determined Lys requirement for the pigs at the University of Kentucky (Quant et al., 2012). Dietary treatments consisted of 6 increasing SID Trp:Lys ratios (13.08%, 14.06%, 15.04%, 17.00%, 18.95%, and 20.91%; Table 1), using crystalline L-Trp at the expense of cornstarch to create the high Trp:Lys ratio basal diet, with the intermediate Trp:Lys ratio diets (14.06%, 15.04%, 17.00%, and 18.95%) derived from a blending of the lowest ratio and highest ratio diets in appropriate proportions. All raw materials and the final complete diets were analyzed for CP and total AA content prior to the experiment. The total AA analyses and the SID AA coefficients for all raw materials were used in final diet formulation using SID coefficients (AminoDat 3.0; Evonik-Degussa GmbH, Hanau, Germany). Vitamins and minerals were formulated to meet or exceed NRC (1998) requirement estimates. Ethoxyquin (Santoquin; Novus International Inc., St. Louis, MO) and tylosin phosphate (Tylan-40, Elanco Inc., Greenfield, IN) were also added to the experimental diets. During the 21-d experiment, pigs were provided ad libitum access to feed and water.

Experiment 2

A total of 324 crossbred pigs were used in 3 blocks to determine the optimal SID Trp:Lys ratio for growing pigs fed diets with and without added antibiotic. In each block, the pigs were randomly divided into 12 groups and fed 2 levels of antibiotic and 6 SID Trp:Lys ratios in a 2×6 factorial design. Block 1 used 120 crossbred pigs with an initial BW of 28.56 ± 2.07 kg; pigs were randomly assigned to 12 groups (24 pens, 1.83×3.05 m) by sex and BW, each of which was replicated 2 times with 5 pigs per pen, with each pen containing 2 barrows and 3 gilts. Block 2 used 108 crossbred pigs with an initial BW of 30.34 ± 3.10 kg; pigs were randomly assigned to 12 groups (24 pens, $1.83 \times$

Table 1. Composition of the basal diets (% , as-fed basis)

Item	Exp. 1		Exp. 2	
	Low Trp:Lys	High Trp:Lys	Low Trp:Lys	High Trp:Lys
Ingredient				
Corn, ground	74.725	74.725	79.605	79.605
Field peas, ground	10.00	10.00	5.00	5.00
Soybean meal, 48% CP	3.00	3.00	5.00	5.00
Corn gluten meal, 60% CP	2.50	2.50	5.00	5.00
Grease	3.00	3.00	1.00	1.00
Cornstarch ¹	2.00	1.95	0.50	0.41
L-Lysine	0.28	0.28	0.37	0.37
L-Threonine	0.10	0.10	0.15	0.15
L-Isoleucine	0.00	0.00	0.03	0.03
L-Tryptophan	0.00	0.05	0.00	0.09
DL-Methionine	0.05	0.05	0.05	0.05
Dicalcium phosphate	1.35	1.35	1.25	1.25
Limestone	0.75	0.75	0.85	0.85
Clay ²	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Trace-mineral premix ³	0.075	0.075	0.075	0.075
Vitamin premix ⁴	0.10	0.10	0.10	0.10
Santoquin ⁵	0.02	0.02	0.02	0.02
Tylan-40 ⁶	0.05	0.05		
Total	100.00	100.00	100.00	100.00
Calculated energy and nutrient profile ⁷				
ME, kcal/kg	3,249	3,247	3,198	3,195
CP, ⁸ %	12.00 (12.32)	12.05 (12.38)	12.81 (12.91)	12.90 (13.07)
Total Lys, ⁸ %	0.7408 (0.715)	0.7408 (0.715)	0.7552 (0.778)	0.7552 (0.778)
Total Trp, ⁸ %	0.1049 (0.10)	0.1544 (0.15)	0.1068 (0.1102)	0.1959 (0.1872)
Total Trp:Lys, ⁸ %	0.1416 (0.1399)	0.2084 (0.2098)	0.1414 (0.1374)	0.2594 (0.2406)
SID Lys, %	0.6554	0.6554	0.6735	0.6735
SID Trp, %	0.0867	0.1362	0.0868	0.1759
SID Trp:Lys, ⁹ %	0.1322 (0.1308)	0.2078 (0.2091)	0.1288 (0.1252)	0.2611 (0.2422)
Ca, %	0.61	0.61	0.63	0.63
P, %	0.53	0.53	0.53	0.53

¹L-Tryptophan was added at the expense of cornstarch to the high standardized ileal digestibility (SID) Trp:Lys ratio diet.

²AB-20, Prince Agriproducts, Quincy, IL.

³Provided the following per kilogram of diet: Zn, 131 mg as ZnO; Fe, 131 mg as FeSO₄·H₂O; Mn, 45 mg as MnO; Cu, 13 mg as CuSO₄·H₂O; I, 1.5 mg as CaI₂O₆; Co, 0.23 mg as CoCO₃; Se, 0.28 mg as Na₂SeO₃.

⁴Provided the following per kilogram of diet: vitamin A, 6,600 IU; vitamin D₃, 880 IU; vitamin E, 44 IU; vitamin K (as menadione sodium bisulfite complex), 6.4 mg; thiamin, 4.0 mg; riboflavin, 8.8 mg; pyridoxine, 4.4 mg; vitamin B₁₂, 33 µg; folic acid, 1.3 mg; niacin, 44 mg; pantothenic acid, 22 mg; d-biotin, 0.22 mg.

⁵The Santoquin product (Novus International Inc., St. Louis, MO) supplied 130 mg of ethoxyquin per kilogram of basal diet.

⁶The Tylan-40 premix (Elanco Animal Health, Indianapolis, IN) supplied 88 g tylosin per kilogram of diet.

⁷Values for ME, Ca, and P were obtained from the feedstuff values listed in NRC (1998). The primary feedstuffs were analyzed for AA; SID values for feedstuffs were those of Evonik-Degussa (Hanau, Germany).

⁸Analyzed values for CP and AA, as well as SID values and ratios, are presented in parentheses and are expressed as a percentage as fed; Trp level is based on a regression of analyzed values divided by the mean analyzed Lys for all 6 diets in Exp. 1 and all 12 diets in Exp. 2, respectively.

⁹The total Trp:Lys ratios based on the analyzed values were different from that of the ratio based on calculated AA values. Thus, the computed ratio in parentheses is the original calculated ratio reduced by the same percentage as the analyzed total ratio is reduced from the calculated total ratio (e.g., $0.1322 \times [0.1399/0.1416] = 0.1308$).

3.05 m) by sex and BW, each of which was replicated 2 times, with 1 replicate containing 1 barrow and 3 gilts/pen and the other replicate containing 2 barrows and 3 gilts/pen. Block 3 used 96 crossbred pigs with an initial BW of 34.27 ± 2.28 kg; pigs were randomly assigned to 12 groups (24 pens, 1.83×3.05 m) by sex and BW, each of which was replicated 2 times, with 1

replicate containing 2 barrows and 2 gilts/pen and the other replicate containing 3 barrows and 1 gilt/pen.

The diet composition was similar to that of Exp. 1 except for the use of an antibiotic. The total AA analysis and the SID AA content of all raw feedstuffs were used for final diet formulation as described in Exp. 1. The basal diets were formulated to a suboptimal Lys

Table 2. Growth performance of growing pigs fed increasing SID Trp:Lys ratios (Exp. 1)¹

Parameter	SID Trp:Lys						SEM	P-value	
	13.08%	14.06%	15.04%	17.00%	18.95%	20.91%		Linear	Quadratic
ADG, kg/d	0.43	0.48	0.56	0.71	0.77	0.79	0.025	<0.001	0.004
ADFI, kg/d	1.14	1.25	1.32	1.58	1.64	1.72	0.049	<0.001	0.044
G:F	0.37	0.38	0.42	0.45	0.47	0.46	0.012	<0.001	0.008
PUN, mg/dL	10.62	9.51	8.83	7.93	8.08	8.63	0.556	0.011	0.015

¹Represents the least squares means of 4 pens per treatment with 5 pigs/pen for the 21-d experiment. SID = standardized ileal digestible. PUN = plasma urea nitrogen.

level of 0.674% SID Lys, which was 84.65% of the determined Lys requirement for the pigs at the University of Kentucky, whereas the other EAA (except for Trp) were formulated on an ideal ratio basis using an SID Lys level of 0.803% (0.905% total Lys). The composition of basal diets and their main contents are shown in Table 1. Dietary treatments consisted of 6 increasing SID Trp:Lys ratios (12.52%, 14.86%, 17.20%, 19.54%, 21.88%, and 24.22%) with or without antibiotic supplementation. Vitamins and minerals in all diets met or exceeded requirement estimates of NRC (1998). The experiment utilized the same diet mixing procedure as in Exp. 1. The antibiotic chosen to be used was a broad-spectrum antibiotic (Tylan-40, tylosin phosphate; Elanco Inc., Greenfield, IN) that was previously used in an experiment evaluating Trp:Lys ratios in our facilities (Quant et al., 2012). Antibiotic and crystalline L-Trp were added to the diets at the expense of cornstarch. All samples (>100 g per sample) of the basal diets and the final diets were analyzed as in Exp. 1.

Pigs were preselected, moved into the growing-finishing facility, and fed a common starter diet with or without 0.05% broad-spectrum antibiotic (Tylan-40; Elanco Inc.) for 10 d before the AA ratio studies began. During each 21-d study, pigs were provided ad libitum access to feed and water. Each pen was equipped with a single-sided, 2-hole steel feeder and 2 nipple waterers.

Data and Sample Collection

In both Exp. 1 and 2, pig and feeder weights were obtained at the start and conclusion of the experiment as well as weekly for determination of growth performance and feed intake. On d 21, blood samples were collected via jugular venipuncture using a 10-mL syringe and then transferred into a 10-mL tube coated with sodium heparin (Vacutainer System, Becton Dickinson, Rutherford, NJ). On the morning of scheduled blood collection, pigs were aroused at 0700 h to stimulate feed consumption, with blood collection commencing at 0845 h. Blood samples were immediately placed on ice for a minimum of 2 h prior to centrifugation (20 min at 2,500 ×g, 4°C). After centrifugation, plasma was harvested in 1-mL aliquots and stored at -20°C.

Laboratory Analyses

The N content of the diets was determined using a thermocombustion method (AOAC, 1998). Dietary AA concentrations were determined by ion exchange chromatography with postcolumn derivatization with ninhydrin. Amino acids were oxidized with performic acid, which was neutralized with sodium metabisulfite (Llames and Fontaine, 1994; European Commission, 1998). Amino acids were liberated from the protein by hydrolysis with 6 N HCl for 24 h at 110°C and quantified with the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction, 280 nm; emission, 356 nm) after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110°C (European Commission, 2000). Chemical analyses were conducted in duplicate. Blood samples were analyzed for plasma urea N (PUN) with a colorimetric procedure (Kit B755-10, Point Scientific Inc., Lincoln Park, MI) with a Varian Cary 50 spectrophotometer (Varian Analytical Instruments, Walnut Creek, CA).

Statistical Analysis

Prior to statistical analysis, data for both Exp. 1 and Exp. 2 were evaluated to identify any statistical outliers within individual pens. First, the pens displaying intrapen CV values higher than 25% for ADG were identified for further examination. Then individual pig performance within the selected pens was evaluated to identify the pig most divergent from its pen mates. This performance was then compared with littermates on other treatments in the experiment to determine whether the observed abnormalities were simply a genetic response. The suspected outliers were also compared to the performance of other pigs in other pens on that same dietary treatment. In the majority of evaluations, the pig was not removed from the data set; in a few instances it was decided that removal of the selected pigs from the experiment was appropriate on the basis of criteria used by Van de Ligt (1999). When a pig was removed, the feed intake of the remaining pigs within those pens was estimated using the model developed by Lindemann and Kim (2007).

Figure 1. Linear and quadratic broken-line analysis of the ADG response to increasing standardized ileal digestible (SID) Trp:Lys ratios fed to growing pigs (Exp. 1). The optimum SID Trp:Lys (breakpoint of the 2 linear lines) was 17.93% ($P < 0.001$). The asymptote of the quadratic curve was 21.14% ($P < 0.001$).

Data were then analyzed using the generalized linear model procedure (Proc GLM of SAS; SAS Inst. Inc., Cary, NC) with the pen of pigs considered the experimental unit. The statistical model included the Trp:Lys ratio, antibiotic, block, replicate within block, ratio $\times$ antibiotic, ratio $\times$ block, antibiotic $\times$ block, and ratio $\times$ antibiotic $\times$ block. Appropriate coefficients for determination of the polynomial contrasts for linear and quadratic responses across the dietary treatments were computed using the interactive matrix language procedure (Proc IML). The results were also subjected to linear broken-line regression analysis (Robbins et al., 2006), where the identified breakpoint represents the estimated optimum Trp:Lys ratio. Although it was not the primary approach used for determination of the optimum Trp:Lys ratio, the results were also subjected to quadratic broken-line analysis to facilitate comparison with sources in the literature that utilized curvilinear or exponential statistical models to determine their requirements.

Figure 3. Linear and quadratic broken-line analysis of the ADFI response to increasing standardized ileal digestible (SID) Trp:Lys ratios in growing pigs (Exp. 1). The optimum SID Trp:Lys ratio (breakpoint of the 2 straight lines) was 19.40% ($P < 0.001$). The asymptote of the quadratic curve was 22.13% ($P < 0.001$).

Figure 2. Linear and quadratic broken-line analysis of the plasma urea nitrogen (PUN) response to increasing standardized ileal digestible (SID) Trp:Lys ratios fed to growing pigs (Exp. 1). The optimum SID Trp:Lys (breakpoint of the 2 straight lines) was 16.17% for PUN ($P = 0.009$). The asymptote of the quadratic curve was 16.95% ($P < 0.004$).

RESULTS

Results for Exp. 1 are shown in Table 2. As SID Trp:Lys ratios increased, ADG increased linearly ($P < 0.001$) and quadratically ($P = 0.004$). Linear broken-line analysis yielded an optimum SID Trp:Lys ratio of 17.93% ($P < 0.001$) for ADG (Fig. 1). Plasma urea N concentrations decreased linearly ($P = 0.011$) and quadratically ($P = 0.015$) as SID Trp:Lys ratios increased (Table 2), whereas linear broken-line analysis revealed a breakpoint of 16.17% ($P = 0.009$; Fig. 2). Average daily feed intake and G:F increased linearly ($P < 0.001$) and quadratically ($P < 0.05$; Table 2). Linear broken-line analysis yielded an optimum SID Trp:Lys ratio of 19.40% for ADFI ($P < 0.001$; Fig. 3) and 16.71% for G:F ($P < 0.001$; Fig. 4). The mean of the determined optima for ADG and PUN responses from linear broken-line analysis yielded an optimum SID Trp:Lys ratio of 17.05%.

Results for Exp. 2 are shown in Table 3. One pig from block 1 (the lowest Trp:Lys ratio diet without antibiotic) was removed because of health-related issues

Figure 4. Linear and quadratic broken-line analysis of the G:F response to increasing standardized ileal digestible (SID) Trp:Lys ratios in growing pigs (Exp. 1). The optimum SID Trp:Lys ratio (breakpoint of the 2 straight lines) was 16.71% ($P < 0.001$). The asymptote of the quadratic curve was 19.35% ($P < 0.001$).

Table 3. Growth performance of growing pigs fed increasing SID Trp:Lys ratios in the diets with and without antibiotics (Exp. 2)¹

Item	ADG, kg		ADFI, kg		G:F		PUN, mg/dL	
	-Anti	+Anti	-Anti	+Anti	-Anti	+Anti	-Anti	+Anti
SID Trp:Lys ratio, %								
12.52	0.538	0.567	1.456	1.517	0.364	0.373	8.852	8.331
14.86	0.798	0.805	1.854	1.896	0.429	0.424	6.965	7.054
17.20	0.836	0.860	1.889	1.929	0.442	0.446	7.359	7.476
19.54	0.863	0.821	1.972	1.916	0.438	0.429	7.093	6.991
21.88	0.876	0.834	1.999	1.910	0.439	0.436	7.001	7.119
24.22	0.801	0.806	1.816	1.876	0.440	0.430	7.441	7.245
SEM	0.020		0.044		0.006		0.279	
<i>P</i> -value								
Antibiotics	0.779		0.711		0.494		0.610	
Trp:Lys linear	<0.001		<0.001		<0.001		<0.001	
Quadratic	<0.001		<0.001		<0.001		<0.001	
Anti × Trp:Lys	0.295		0.383		0.622		0.843	

¹Represents the least squares means of 6 pens per treatment with 4 or 5 pigs/pen for the 21-d experiment. SID = standardized ileal digestible. PUN = plasma urea nitrogen. Anti = antibiotic.

Figure 5. Linear and quadratic broken-line analysis of the ADG response to increasing standardized ileal digestible (SID) Trp:Lys ratios in growing pigs (Exp. 2). The optimum SID Trp:Lys ratio (breakpoint of the 2 straight lines) was 14.58% ($P < 0.001$). The asymptote of the quadratic curve was 16.15% ($P < 0.001$).

Figure 7. Linear and quadratic broken-line analysis of the ADFI response to increasing standardized ileal digestible (SID) Trp:Lys ratios in growing pigs (Exp. 2). The optimum SID Trp:Lys ratio (breakpoint of the 2 straight lines) was 15.09% ($P < 0.001$). The asymptote of the quadratic curve was 15.86% ($P < 0.001$).

Figure 6. Linear and quadratic broken-line analysis of the plasma urea nitrogen (PUN) response to increasing standardized ileal digestible (SID) Trp:Lys ratios in growing pigs (Exp. 2). The optimum SID Trp:Lys ratio (breakpoint of the 2 straight lines) was 14.54% ($P < 0.001$). The asymptote of the quadratic curve was 14.59% ($P < 0.001$).

Figure 8. Linear and quadratic broken-line analysis of the G:F response to increasing standardized ileal digestible (SID) Trp:Lys ratios in growing pigs (Exp. 2). The optimum SID Trp:Lys ratio (breakpoint of the 2 straight lines) was 15.30% ($P < 0.001$). The asymptote of the quadratic curve was 16.42% ($P < 0.001$).

and nonnormal growth performance results. As SID Trp:Lys ratios increased, ADG for the 21-d period pigs fed the diets with or without antibiotics increased linearly ($P < 0.001$) and quadratically ($P < 0.001$), respectively. However, there were no effects of the antibiotic treatment on ADG, ADFI, G:F, or PUN concentrations. In addition, there were no interactions ($P > 0.29$) between antibiotics and Trp:Lys ratios in ADG, ADFI, G:F, or PUN concentrations. When the data for all pigs were pooled for the various Trp:Lys ratios, the optimum SID Trp:Lys for ADG based on linear broken-line analysis was 14.58% ($P < 0.001$; Fig. 5). These results were supported by the PUN response, which decreased linearly ($P < 0.001$) and quadratically ($P < 0.001$) as SID Trp:Lys ratios increased. The breakpoint from linear broken-line analysis revealed an optimum SID Trp:Lys ratio for PUN of 14.54% ($P < 0.001$; Fig. 6). Average daily feed intake and G:F increased linearly ($P < 0.001$) and quadratically ($P < 0.001$), respectively, as SID Trp:Lys ratios increased (Table 2). Linear broken-line analysis yielded an optimum SID Trp:Lys ratio of 15.09% for ADFI ($P < 0.001$; Fig. 7) and 15.30% for G:F ($P < 0.001$; Fig. 8). In general, based on the mean of the determined optima for the ADG and PUN responses from linear broken-line analysis, the optimum SID Trp:Lys ratio for growing pigs was 14.56%. The mean of the optimum ADG and PUN responses from quadratic broken-line analysis revealed a higher SID Trp:Lys ratio of 15.37%.

DISCUSSION

Both experiments demonstrated that ADG, ADFI, and G:F increased, whereas PUN concentrations decreased with increasing SID Trp:Lys ratios, regardless of antibiotic treatment, which agrees with previous studies (Quant et al., 2012; Salyer et al., 2013; Ma et al., 2015). In Exp. 2, the results indicate that the response in growth performance (ADG, ADFI, and G:F) of 30- to 50-kg pigs to the antibiotic was not different at the same SID Trp:Lys ratio, and there was no interaction between the antibiotic and Trp:Lys ratios, indicating that dietary antibiotic inclusion did not impact the SID Trp:Lys ratio. In contrast, Becker et al. (1955) demonstrated that the Trp requirement of lighter pigs (BW: 14.52 to 32.34 kg) than used in the current experiments was higher when they were fed diets without antibiotics compared with antibiotic-supplemented diets, whereas Bikker and Dirkzwager (2003) and Bikker et al. (2007) reported that the withdrawal of antibiotics increased the EAA requirements and the optimum dietary SID Thr:Lys ratio for maximum growth performance in growing-finishing pigs. The main reasons for the different results may be due to use of in-feed antibiotics (the particular antibiotic chosen and its dosage), animal health, and physical stage. For example, if pigs

had suffered from inflammation, plasma Trp concentration would decline, with Trp use for immune functions increasing at the expense of growth performance (Melchior et al., 2004). Additionally, the sanitary conditions of these experiments were high; van der Meer et al. (2016) demonstrated that AA requirements depend on the sanitary conditions, being greater for 1 or more of Met, Thr, and Trp under practical or poor sanitary conditions. Thus, it is possible that the requirement of Trp or dietary Trp:Lys ratio is greater for pigs fed diets without antibiotics compared to pigs fed the diets with antibiotics. Further study needs to be made on all of the potential factors.

In the current study, the optimum SID Trp:Lys ratios of 17.05% (Exp. 1) and 14.56% (Exp. 2) were established for 30- to 50-kg pigs fed diets with and without antibiotics, respectively. These values are similar to the Trp:Lys ratio of 15.71% indicated by Quant et al. (2012), lie in the optimum range of Trp:Lys ratios of 14.5% to 17% as reported by Kendall et al. (2007), and are close to the value of 16.5% reported by Salyer et al. (2013). The Trp:Lys ratio is also marginally lower in Exp. 2 than the Trp:Lys ratio of 17% as summarized by Susenbeth (2006) and 18% from ideal AA pattern studies reported by others (Wang and Fuller, 1989; Chung and Baker, 1992), as well as the calculated Trp:Lys of 18% reported by NRC (1998) for growing pigs. To some degree, the main factors that may have attributed to these variations existing among the published literature include statistical philosophy (Quant et al., 2012; Ma et al., 2015), dietary CP and large neutral AA levels (Jansman et al., 2000), genotypes and sex (Friesen et al., 1994; Kendall et al., 2007), and health and sanitary conditions (Melchior et al., 2004, 2005; Le Floch et al., 2006, 2009; Jayaraman et al., 2016). Although ADFI and G:F were not primary responses, the optimum Trp:Lys ratios determined with them did, in fact, strongly support the ADG and PUN results.

Because of many contributing factors to the variation, it is important that animal nutritionists use consistent guidelines or standardization of experimental procedures for AA ratio experiments in pigs. Further studies on the SID Trp:Lys ratios in pigs would provide more precise data if the experiments used identical procedures. However, the present results support the viewpoint that 30- to 50-kg pigs do not require a SID Trp:Lys ratio above the recommendation by NRC (1998) as long as dietary AA are formulated to an SID basis (Quant et al., 2012).

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