



Original research article

Effects of dietary threonine:lysine ratios and sanitary conditions on performance, plasma urea nitrogen, plasma-free threonine and lysine of weaned pigs

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ARTICLE INFO

Article history:

Received 4 September 2015

Accepted 15 September 2015

Available online 25 September 2015

Keywords:

Threonine:lysine ratio

Sanitation

Weaned pigs

Growth performance

ABSTRACT

Two 21 d-experiments were conducted to determine the optimum standardized ileal digestible (SID) threonine:lysine ratio (Thr:Lys) for weaned piglets reared under clean (Exp. 1) or unclean (Exp. 2) sanitary conditions and fed antibiotic-free diets. In each experiment, 90 mixed-sex pigs (Duroc × [Yorkshire × Landrace]; initial BW 7.2 ± 0.3 kg) were randomly assigned to 5 dietary treatments each with 6 replicates (3 pigs per pen). The dietary treatments were 5 graded levels of SID Thr:Lys (55, 59, 63, 67 and 71%). Diets were corn-wheat-soybean meal-based with a constant SID Lys of 1.18% that was set to be second limiting amino acid. In Exp. 1 and Exp. 2, plasma-free Thr increased ($P = 0.05$) with increasing dietary SID Thr:Lys. In Exp. 1, the SID Thr:Lys for gain-to-feed ratio (G:F) was optimized at 65%. In Exp. 2, the estimated optimal SID Thr:Lys for overall G:F was 66.5%. In conclusion, an average optimal SID Thr:Lys of 65 and 66.5% could be used to optimize feed efficiency for weaned pigs under clean and unclean sanitary conditions, respectively.

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1. Introduction

Threonine (Thr) is the second-limiting amino acid (AA) after lysine (Lys) in pigs when fed wheat and barley-based diets and the third-limiting AA in corn-based diets (Adeola et al., 1994; Saldana et al., 1994). Besides protein synthesis, the major functions of Thr include maintenance of gut integrity and immunity (Ruth and Field, 2013). Hence, the requirement for Thr is likely to vary according to the importance of each of its functions. The NRC (2012) recommends a diet with a standardized ileal digestible (SID) threonine-to-lysine ratio (Thr:Lys) of 59% for pigs between 7 and 25 kg, however, it does not consider the health status of the pig. These levels might not be sufficient for pigs reared in commercial

production conditions, where the chances of clinical and sub-clinical infections occur.

Weaned pigs reared under unclean sanitary conditions was used as a model to provoke a low-grade inflammatory response (Le Floch et al., 2009; Kahindi et al., 2014a) and those pigs had reduced growth performance (Kahindi et al., 2014a) and activated immune system (Williams et al., 1997) that would interfere with growth because of competition between protein deposition in structural tissues and immune function (Le Floch et al., 2009).

A current interest in the nutritional management of nursery pigs is to utilize nutritional programs without in-feed antibiotic growth promoter (AGP) (Nyachoti et al., 2006; de Lange et al., 2010), which may alter the Thr:Lys requirement for optimal performance, especially when piglets are exposed to an immunological challenge as is often the case under unsanitary conditions. For example, it has been shown that metabolism and requirement for Thr changes in pigs during an immune challenge (Le Floch and Seve, 2004; Ren et al., 2014). Furthermore, exposing piglets to unclean conditions is considered as a predisposing factor for weaning disorders (Williams et al., 1997).

Information about Thr:Lys for piglets fed AGP-free diets and subjected to an immunological challenge is limited. Thus, it was

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



hypothesized that the Thr:Lys required to optimize piglet performance is higher when subjected to unclean sanitary conditions. The objective of this study was to determine the optimum SID Thr:Lys for weaned piglets reared in clean or unclean sanitary conditions.

2. Materials and methods

2.1. Animal care

The experimental protocol (F10-041/1/2) was reviewed and approved by the Animal Care Committee of University of Manitoba and pigs were cared for in accordance with the guidelines of Canadian Council on Animal Care (2009).

2.2. Experimental diets

Diets were corn (*Zea mays*), wheat (*Triticum aestivum*) and soybean (*Glycine max*) meal-based with a constant SID Lys of 1.18% that was set to be second limiting AA (Table 1). Ingredients contributing AA (corn, wheat and soybean meal) were analyzed for AA composition and the values were used in diet formulation. The diets contained graded levels of SID Thr:Lys (55, 59, 63, 67 and 71%). All other nutrients were provided in quantities meeting or exceeding National Research Council (2012) recommendations for a 6–10 kg pig. All diets were fed in mash form and did not contain any AGP.

2.3. Animals and experimental design

A total of 180 piglets (Duroc × [Yorkshire × Landrace]; mixed sex) were used for the two experiments. In each of the two experiments, 90 pigs (weaned at 21 ± 1 d of age) were fed a corn-

soybean meal-based starter diet (20% CP; 59% SID Thr:Lys) for 6 d adaptation period and then, pigs (initial BW 7.36 ± 0.2 kg) were randomly assigned to 1 of 5 dietary treatments with SID Thr:Lys (55, 59, 63, 67 and 71%) each with 6 replicates (3 pigs per pen). In Exp. 1, 90 piglets were housed in a clean room that had been cleaned and disinfected before the arrival of the piglets and the room was cleaned once weekly. In Exp. 2, 90 piglets were housed in the same room which was not cleaned and disinfected at the end of wk 3 from the first batch to allow the build-up of manure. Moreover, manure from swine herd was added (5 kg per pen) to the pens on d 0 and 7 of the Exp. 2. In both studies, pigs were provided ad libitum access to feed and water. Body weight and pen feed disappearance were recorded weekly to determine ADG, ADFI and gain-to-feed ratio (G:F) calculated on per pen basis. Blood was collected on d 0 and 14 and analyzed for plasma urea nitrogen (PUN) and plasma-free Thr and Lys. Faecal consistency scoring (0 = normal, 1 = soft faeces, 2 = mild diarrhoea, and 3 = severe diarrhoea) was done as described by Marquardt et al. (1999) by 2 independent trained individuals with no prior knowledge of the treatment allocation.

2.4. Sample preparation and laboratory analyses

Diet samples were ground through a 1-mm mesh screen. The DM content was determined according to AOAC (2000). The N content of the diets was determined with a gas combustion method using a Leco FP-2000 Nitrogen analyzer (Leco Corp., St. Joseph, MI). Amino acid analyses were carried out at the lab of Evonik Industries AG, Hanau-Wolfgang, Germany. Dietary concentrations of all essential and non-essential AA, except for tryptophan and tyrosine, were determined by ion-exchange chromatography with post-column derivatization with ninhydrin. Amino acids were oxidized with performic acid, which was neutralized with Na metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were liberated from the protein by hydrolysis with 6 mol/L HCl for 24 h at 110°C and quantified with the internal standard 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 (Commission Directive, 2000). Tyrosine was not determined.

2.5. Plasma urea nitrogen

Blood sampling and PUN analysis were performed according to Nyachoti et al. (2006). Briefly, on d 0 and 14, a 10-mL blood sample was collected from one pig per pen via jugular venipuncture into heparinized vacutainer tube (Becton Dickinson, Rutherford, US) and stored on ice for 20 min before being centrifuged at 2,000 × g for 10 min at 4°C to recover plasma. Plasma samples were stored at –20°C until used for further analysis. Plasma samples were thawed and then analyzed for PUN using a Nova Stat Profile M blood gas and electrolyte analyzer (Nova Biomedical Corporation, Waltham, MA).

2.6. Plasma-free threonine and lysine

Plasma-free Thr and Lys concentrations were determined using amino acid analyzer (Skykam Amino Acid Analyzer, Germany) after being deproteinized with 4% sulfosalicylic acid.

2.7. Statistical analysis

Data were subjected to ANOVA using the Proc mixed procedure of SAS 9.2 (SAS Inst. Cary, NC). The data were analyzed as

Table 1
Ingredient and nutrient composition of experimental diets (as-is basis).

Item	Dietary SID Thr:Lys, %				
	55	59	63	67	71
Ingredients, %					
Corn	46.20	46.20	46.20	46.20	46.20
Wheat	15.00	15.00	15.00	15.00	15.00
Soybean meal	30.00	30.00	30.00	30.00	30.00
Vegetable oil	3.85	3.85	3.85	3.85	3.85
Corn starch	0.50	0.45	0.40	0.36	0.31
Monocalcium phosphate	1.50	1.50	1.50	1.50	1.50
Limestone	1.08	1.08	1.08	1.08	1.08
Salt	0.30	0.30	0.30	0.30	0.30
Min-Vit premix ¹	1.00	1.00	1.00	1.00	1.00
L-Lys HCl	0.35	0.35	0.35	0.35	0.35
L-Trp	0.05	0.05	0.05	0.05	0.05
DL-Met	0.14	0.14	0.14	0.14	0.14
L-Thr	–	0.05	0.09	0.14	0.19
Calculated nutrient composition, %²					
NE, Kcal/kg	2,484	2,484	2,484	2,484	2,484
SID Lys	1.18	1.18	1.18	1.18	1.18
SID Met	0.49	0.49	0.49	0.49	0.49
SID Cys	0.23	0.23	0.23	0.23	0.23
SID Met + Cys	0.72	0.72	0.72	0.72	0.72
SID Trp	0.26	0.26	0.26	0.26	0.26
SID Thr	0.65	0.70	0.75	0.79	0.84
SID Ile	0.76	0.76	0.76	0.76	0.76
SID Val	0.85	0.85	0.85	0.85	0.85

SID = standardized ileal digestible; Thr:Lys = threonine-to-lysine ratio.

¹ Supplied the following per kg of diet: vitamin A, 8,250 IU; vitamin D₃, 835 IU; vitamin E, 40 IU; vitamin B₁₂, 25 µg; vitamin K, 4 mg; niacin, 25 µg; choline, 600 mg; riboflavin, 12 mg; biotin, 200 µg; pyridoxine, 4.5 mg; folic acid, 4 mg; thiamin, 2 mg; Mn, 50 mg; Zn, 150 mg; Fe, 120 mg; Cu, 25 mg; Se, 0.35 mg; I, 0.4 mg.

² The calculated total contents of Lys, Thr, Met + Cys and Trp in the basal diet were 1.32, 0.77, 0.82, and 0.30%, respectively.

completely randomized design. Orthogonal polynomial contrasts were used to determine the linear and quadratic effects of increasing levels of SID Thr:Lys. Statistical significance was accepted at $P < 0.05$ and $0.05 < P < 0.10$ was considered a trend. To determine the optimal SID Thr:Lys level, data were subjected to broken-line analysis (Robbins et al., 2006) using the Proc NLIN of SAS (SAS Inst. Inc. Cary, NC). Each pen was considered as an experimental unit.

3. Results

The analyzed AA and crude protein contents of the experimental diets were presented in Table 2. The SID Thr:Lys were then corrected based on the analyzed contents using the following formula: corrected SID Thr:Lys = (calculated SID Thr:Lys × analyzed total Thr:Lys)/calculated total Thr:Lys. The corrected SID Thr:Lys in the diets were 61, 64, 67, 69 and 72% which were used for the regression analysis.

Table 2
Analyzed crude protein and amino acid composition of experimental diets.

Item	Dietary SID Thr:Lys, % ¹				
	55	59	63	67	71
Crude protein	21.29	22.19	21.68	22.01	22.26
Lys	1.26	1.29	1.30	1.29	1.31
Thr	0.77	0.82	0.87	0.89	0.94
Met + Cys	0.77	0.79	0.80	0.80	0.81
Ile	0.89	0.91	0.91	0.90	0.92
Val	0.99	1.01	1.01	0.99	1.01
Trp	0.28	0.29	0.29	0.29	0.29
SID Thr:Lys ²	61.00	64.00	67.00	69.00	72.00

SID = standardized ileal digestible; Thr:Lys = threonine-to-lysine ratio.

¹ Calculated dietary SID Thr:Lys.

² Corrected dietary SID Thr:Lys = (calculated SID Thr:Lys × analyzed total Thr:Lys)/calculated total Thr:Lys.

3.1. Experiment 1

All animals remained healthy throughout the experimental period. No signs of diarrhea or any other clinical illness were observed for piglets. The initial and final BW (Table 3) were similar ($P > 0.10$) for the pigs fed the different dietary SID Thr:Lys. During wk 1, there was an increase ($P < 0.05$) in G:F, with increasing SID Thr:Lys, whereas there was no effect ($P > 0.10$) on ADG and feed intake (Table 3). On d 14, dietary SID Thr:Lys did not influence ($P > 0.10$) PUN and plasma-free Lys concentrations (Table 4), whereas there was an increase ($P = 0.05$; linear) in plasma-free Thr concentration with increasing dietary SID Thr:Lys. Based on overall G:F, the SID Thr:Lys was optimized at 65% using broken line quadratic (BLQ) model (Fig. 1).

3.2. Experiment 2

The initial and final BW was similar ($P > 0.10$) for the pigs from the different dietary SID Thr:Lys. During the overall experimental period, dietary SID Thr:Lys increased ($P < 0.05$; quadratic) G:F, whereas there was no difference ($P > 0.10$) in ADG and feed intake (Table 3). During wk 1, growth performance was not affected ($P > 0.10$) by different SID Thr:Lys. During wk 2 and 3, there was an increase ($P < 0.05$; quadratic) in G:F with increasing dietary SID Thr:Lys, however, there was no difference ($P > 0.10$) in ADG. During wk 3, feed intake increased ($P < 0.05$; quadratic) due to different SID Thr:Lys. On d 14, plasma-free Thr concentration increased

($P < 0.05$; linear) with increasing SID Thr:Lys, whereas PUN and plasma Lys concentrations were not affected ($P > 0.10$) by dietary treatments (Table 4). Based on overall G:F, the SID Thr:Lys was optimized at 66.5% using BLQ model (Fig. 2).

4. Discussion

The goal of this study was to determine the optimal SID Thr:Lys for weaned pigs reared under clean or unclean sanitary conditions using growth rate and PUN as response criteria. In this study, diets were corn-wheat-soybean meal-based with a constant SID Lys content of 1.18% was set to be 10% lower than the requirement level that was established in our lab (Kahindi et al., 2014b).

In Exp. 1, increasing the SID Thr:Lys improved the overall G:F but had no effect on ADG during wk 1, which is in agreement with Fernandez and Strathe (2009). The specific role of Thr could have been directed to intestinal integrity and function compared to protein accretion, which might be the reason for not showing significant difference in daily weight gain (Fernandez and Strathe, 2009). Based on overall feed efficiency, SID Thr:Lys of 65% was optimized using BLQ model (Fig. 1), which is consistent with previous studies (James et al., 2003; Lenehan et al., 2004).

Weaned pigs subjected to unclean sanitary condition was used as a model of moderate immune system stimulation (Le Floch et al., 2006), which was in turn anticipated to have effect on partitioning of AA between lean tissue deposition and supporting the immune system (Williams et al., 1997). In Exp. 2, piglets raised under unclean sanitary conditions had reduced growth performance compared with the values reported by other studies (335 g, NRC, 2012; 500 g, Zhang and Kim, 2014). This suggests that the sanitation model of immune challenge was effective, which is consistent with the findings of others (Lee et al., 2005; Le Floch et al., 2006; Kahindi et al., 2014a) that showed decreased growth performance when piglets subjected to poor sanitary conditions.

Under unclean sanitary conditions, based on G:F, SID Thr:Lys of 66.5% was optimized using BLQ model (Fig. 2). The optimal SID Thr:Lys estimated in this study was in agreement with previous studies (Baker, 2000; Trevisi et al., 2011) using feed efficiency as response criteria. The estimated SID Thr:Lys requirement for weaned pigs in the current study is higher (11%) than NRC (2012) recommendations (59% SID Thr:Lys) for the 7 to 25 kg pigs, which implies that during general immune challenge conditions, such as unclean sanitary conditions, Thr requirements might be increased for weaned pigs.

Plasma urea nitrogen has been often used as a response criterion for determining AA requirements since PUN can be used as an indicator of protein utilization efficiency (Coma et al., 1995). When there is an excess AA, PUN is known to increase because excess AA cannot be stored and therefore they are degraded with the production of urea (Heo et al., 2009; Waguespack et al., 2011). If there is decrease in PUN, it would indicate that either an increase in nitrogen use efficiency or decrease in protein breakdown, (Shen et al., 2012). In this study, both under clean and unclean sanitary conditions, dietary Thr:Lys did not affect PUN concentration. In this study, the optimal SID Thr:Lys could not be determined using PUN as response criteria which is contradictory to previous studies (Defa et al., 1999; Wang et al., 2006; Zhang et al., 2013) reporting that by increasing Thr levels in the diet, there was a decrease in PUN.

Under both clean and unclean sanitary conditions, increasing levels of dietary SID Thr:Lys increased concentration of plasma-free Thr. This result is consistent with the previous studies (Defa et al., 1999; Wang et al., 2006) who also reported that increasing levels of dietary Thr increased serum free Thr. The increase in plasma-free Thr could be due to increasing levels of dietary SID Thr:Lys which is in agreement with Zhang et al. (2013).

Table 3
Effects of different standardized ileal digestible threonine:lysine ratios (Thr:Lys) on growth performance of weaned pigs reared under clean and unclean sanitary conditions.¹

Item	Clean sanitary condition ²					SEM	P-value ⁴			Unclean sanitary condition ³					SEM	P-value		
	Corrected SID Thr:Lys, %						Thr	Lin	Quad	Corrected SID Thr:Lys, %						Thr	Lin	Quad
	61	64	67	69	72					61	64	67	69	72				
Initial BW, kg	7.37	7.16	7.33	7.40	7.30	0.39				7.18	7.03	7.05	7.11	7.12	0.33			
Final BW, kg	17.5	17.3	17.7	17.6	18.0	0.34	0.598	0.166	0.508	15.7	16.0	15.1	15.7	15.8	0.63	0.896	0.940	0.637
wk 1 (d 0 to 7)																		
ADG, g	299	320	313	317	354	24.0	0.582	0.171	0.638	209	214	221	234	197	18.5	0.714	0.947	0.283
ADFI, g	449	447	430	444	432	31.4	0.987	0.712	0.922	322	349	303	344	299	26.0	0.558	0.533	0.576
G:F	0.66	0.73	0.73	0.73	0.82	0.04	0.178	0.033	0.835	0.66	0.65	0.73	0.69	0.65	0.05	0.738	0.879	0.343
wk 2 (d 8 to 14)																		
ADG, g	501	442	459	512	507	27.7	0.317	0.352	0.166	411	415	403	439	381	19.4	0.35	0.555	0.313
ADFI, g	828	749	797	835	808	44.9	0.714	0.750	0.575	580	537	535	599	568	25.3	0.354	0.655	0.358
G:F	0.60	0.59	0.58	0.62	0.62	0.03	0.464	0.487	0.710	0.71	0.78	0.75	0.73	0.67	0.03	0.263	0.247	0.066
wk 3 (d 15 to 21)																		
ADG, g	665	707	736	664	687	33.4	0.509	0.997	0.265	544	570	574	583	596	26.9	0.731	0.184	0.842
ADFI, g	1,002	980	1,011	981	964	39.9	0.921	0.556	0.732	876	804	774	808	878	31.4	0.102	0.929	0.007
G:F	0.67	0.72	0.73	0.68	0.71	0.03	0.409	0.581	0.283	0.63	0.71	0.75	0.72	0.68	0.04	0.170	0.322	0.024
Overall (d 0 to 21)																		
ADG, g	487	487	500	499	521	16.9	0.640	0.154	0.651	388	399	400	423	387	15.9	0.508	0.651	0.233
ADFI, g	758	720	741	757	744	28.8	0.899	0.928	0.684	592	564	538	594	571	20.8	0.340	0.870	0.245
G:F	0.64	0.68	0.68	0.67	0.70	0.02	0.407	0.138	0.822	0.66	0.71	0.75	0.72	0.70	0.02	0.071	0.440	0.006

SID = standardized ileal digestible; SEM = standard error of mean.

¹ Values are least square means; *n* = 6 per treatment.

² Clean sanitary condition: piglets were housed in cleaned and disinfected rooms and fed antibiotic-free diets.

³ Unclean sanitary conditions: piglets were housed in a room where cleaning and disinfection was not done, moreover, manure from swine herd was added (5 kg per pen) to the pens on d 0 and 7 of the experiment.

⁴ Probability values of linear (Lin) and quadratic (Quad) effects for dietary SID Thr:Lys (%).

Table 4
Effects of different standardized ileal digestible threonine:lysine ratios (Thr:Lys) on plasma threonine, lysine and urea nitrogen concentrations (mmol/L) of weaned pigs reared under clean and unclean sanitary conditions.¹

Item	Clean sanitary condition ²					SEM	P-value ⁴			Unclean sanitary condition ³					SEM	P-value		
	Analyzed SID Thr:Lys, %						Thr	Lin	Quad	Analyzed SID Thr:Lys, %						Thr	Lin	Quad
	61	64	67	69	72					61	64	67	69	72				
Plasma-free Thr	523	617	622	652	634	38.4	0.199	0.050	0.185	415	517	629	592	639	55.4	0.070	0.010	0.241
Plasma-free Lys	138	117	127	119	123	15.0	0.896	0.570	0.581	199	200	168	171	209	33.0	0.848	0.938	0.399
Plasma urea nitrogen	3.55	3.73	2.92	2.64	3.37	0.33	0.110	0.226	0.207	3.74	4.10	3.40	3.99	3.81	0.33	0.616	0.980	0.871

SID = standardized ileal digestible; SEM = standard error of mean.

¹ Values are least square means; *n* = 6 per treatment.

² Clean sanitary condition: piglets were housed in cleaned and disinfected rooms and fed antibiotic-free diets.

³ Unclean sanitary conditions: piglets were housed in a room where cleaning and disinfection was not done, moreover, manure from swine herd was added (5 kg per pen) to the pens on d 0 and 7 of the experiment.

⁴ Probability values of linear (Lin) and quadratic (Quad) effects for dietary SID Thr:Lys (%).

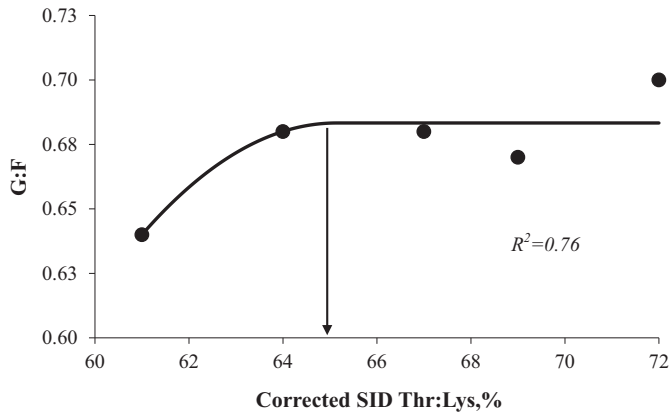


Fig. 1. The optimal dietary standardized ileal digestible (SID) threonine:lysine ratio (Thr:Lys) for gain-to-feed ratio (G:F) in weaned pigs reared under clean sanitary conditions (Exp. 1) determined using quadratic broken-line analysis was 65% [$Y = 0.68 - 0.0025(65 - x)^2$]. Data points (●) represent least square means of dietary treatments ($n = 6$).

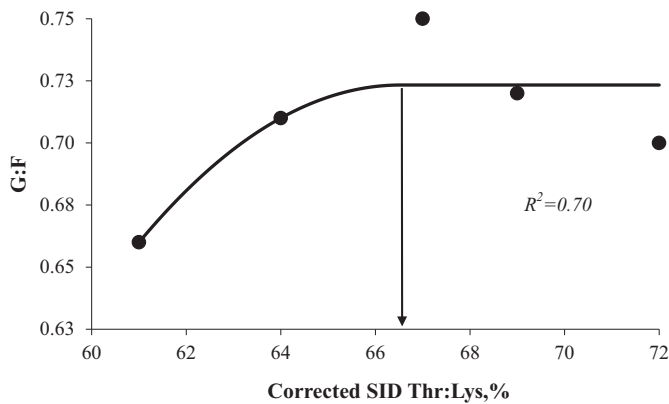


Fig. 2. The optimal dietary standardized ileal digestible (SID) threonine:lysine ratio (Thr:Lys) for gain-to-feed ratio (G:F) in weaned pigs reared under unclean sanitary conditions (Exp. 1) determined using quadratic broken-line analysis was 66.5% [$Y = 0.72 - 0.0021(66.5 - x)^2$]. Data points (●) represent least square means of dietary treatments ($n = 6$).

5. Conclusions

In this study, under clean sanitary conditions, the optimal SID Thr:Lys based on G:F was 65% using BLQ model. Under unclean sanitary conditions, the estimated optimal SID Thr:Lys for overall G:F using BLQ model was 66.5%. In conclusion, an average optimal SID Thr:Lys of 65 and 66.5% could be used to optimize feed efficiency for weaned pigs under clean and unclean sanitary conditions, respectively.

Conflict of interest

The authors declare that there are no conflict of interest.

Acknowledgments

Funding for this study from Evonik Industries and Natural Science and Engineering Research Council is greatly appreciated. The authors would like to thank Robert Stuski, TK Cheung Animal Science Research Unit for physical assistance.

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