Effects of Crystalline Lysine, Threonine and Tryptophan Supplementation of Diets Containing Reduced Protein Levels on Performance of Growing Pigs

D. F. Li and C. T. Xiao
China Agricultural University, Beijing 100094, China
and
J. H. Kim, W. T. Cho and In K. Han[1]
Department of Animal science and Technology, College of Agriculture and Life Sciences,
Seoul National University, Suweon 441-744, Korea

ABSTRACT : Three hundred and fifty-two growing pigs were utilized in three growth trials to investigate the effects of crystalline amino acid supplementation on growing pigs' growth performance and feed consumption. In Exp. 1, diets were formulated on the basis of digestible amino acids. Utilization of rapeseed meal plus cottonseed meal (R + C) in diet supported similar average daily gain (ADG), feed/gain (F/G) and cost per gain ($p > 0.05$) to group of soybean meal (SBM). In Exp. 2, 14% CP diets containing equal amount of cottonseed meal and rapeseed meal were formulated with the addition of threonine and/or tryptophan. Supplementation with threonine improved ADG and F/G ($p < 0.05$). Supplementation with only tryptophan made no benefits for ADG of growing pigs. In Exp. 3, diets with different threonine level were formulated. Increasing dietary threonine to 0.54% improved ($p < 0.05$) ADG and F/G compared to 0.45% dietary threonine. Increase in dietary threonine did not improve ADG ($p > 0.05$) but decreased F/G ($p < 0.05$) because of a decrease in average daily feed intake. Pigs fed diet with dietary threonine/lysine ratio of 67.5% supported optimum growth performance.
(Key Words: Growing Pigs, Lysine, Threonine, Tryptophan)

INTRODUCTION

There are great shortages of protein resources in China as well as in the world. This has been the limiting factor for development of feed industry and animal production. Moreover, pigs' diets are frequently supplied a large amount of protein in comparing to the requirements of animals. Scientists have been seeking alternatives for soybean meal to make use of unconventional protein sources and to lower the feed cost. Cottonseed meal (36.8-41.7% CP) and rapeseed meal (38% CP) have high protein contents (NRC, 1988) and are abundant in China. They are expected to provide protein and amino acids for livestock.

Two major factors restrict the utilization of cottonseed meal and rapeseed meal. First, they both have toxins or anti-nutritional factors which present poor palatability and may do harm to animal. Second, because of the low digestibility, their amino acids can not be utilized by livestock so efficiently as those of soybean meal. Only a limited amount of cottonseed meal or rapeseed meal can be used in case of toxication. If diets are formulated on the basis of digestible amino acids, the imbalance of amino acids due to low digestibility of cottonseed meal and rapeseed meal might have to be modified (Tanksley et al., 1984). Formulating diets on basis of available/digestible amino acids depends on the knowledge of accurate digestibility of each ingredient (Batterham et al., 1993) and the supplementation of crystalline amino acids, especially when a low protein diet is formulated.

Amino acid supplementation has been well studied when growing pig diets included barley (Fuller et al., 1979a; 1979b) or sorghum (Hansen et al., 1993a; 1993b; Brudevold and Southern, 1994). When a low-protein diet is formulated, certain amino acid(s) will be limiting for growth (Russell et al., 1987; Sharda et al., 1976), thus crystalline amino acid(s) supplementation is necessary (Russell et al., 1983; 1986).

This study was conducted to evaluate the effectiveness of supplementing crystalline lysine, threonine, and/or tryptophan to cottonseed meal and/or rapeseed meal-added diets formulated on digestible amino acid basis for growing pigs.

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[1] Address reprint requests to In K. Han.
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MATERIALS AND METHODS

General

This study was conducted in Yuanda Pig Farm, Beijing suburb, and in Monogastric Animal Nutrition Laboratory, College of Animal Science and Technology, China Agricultural University. A total of 352 growing pigs (Duroc × Landrace × Beijing black) were used in three growth trials. In each treatment, pigs were randomly allotted into 4 dietary treatments with 4 pens per treatment according to the initial weight and sex of pigs. All pigs were housed on the ground and had *ad libitum* access to feed and water during all trial period. Body weight and feed consumption were determined every 4 weeks in Exp. 1 and every 2 weeks in Exp. 2 and 3. Average daily gain (ADG), average daily feed intake (ADFI) and feed over gain (F/G) were calculated. Feed cost was also included in Exp. 1.

Experiment 1. Effect of formulating diets on basis of digestible amino acids on growth performance of growing and finishing pigs

Exp. 1 was conducted to investigate the effect of formulating diets on the basis of digestible amino acids on performance of growing pigs. Corn-based diets were formulated for each group including three growth phases according to pig body weight. A total of 224 pigs with initial weight of 22.0 ± 1.0 kg were assigned into one of 4 dietary treatments. All diets for each growth phase had same metabolizable energy and digestible lysine level, but the protein sources for each group were not the same. Treatment 1 (SBM) used soybean as the sole protein source, treatment 2 (RSM) and 3 (CSM) used rapeseed meal or cottonseed meal, respectively. Equal amount of

Table 1. Percentage composition of experimental diets (Exp. 1)[a]

Ingredient	20 to 35 kg phase				35 to 60 kg phase				60 to 90 kg phase			
	SBM	RSM	CSM	R + C	SBM	RSM	CSM	R + C	SBM	RSM	CSM	R + C
Corn	65.21	69.54	64.24	63.23	65.33	61.96	64.37	63.13	64.18	61.09	63.14	62.07
Wheat bran	10.00	10.00	10.00	10.00	13.00	13.00	13.00	13.00	18.00	18.00	18.00	18.00
Soybean meal	16.60	15.40	11.50	12.60	14.50	11.00	8.50	9.70	10.90	7.20	4.80	6.00
Cottonseed meal	–	–	6.00	3.00	–	–	7.00	3.50	–	–	7.00	3.50
Rapeseed meal	–	6.00	–	3.00	–	7.00	–	3.50	–	7.00	–	3.50
PSC yeast	4.00	4.00	4.00	4.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.88	0.88	0.90	0.93	0.86	1.00	0.89	0.90	0.93	1.05	0.91	1.00
Dicalcium phosphate	1.80	1.65	1.75	1.65	1.85	1.50	1.65	1.71	1.57	1.25	1.60	1.40
Premix[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine · HCl	0.21	0.25	0.29	0.28	0.16	0.23	0.27	0.25	0.12	0.20	0.23	0.22
DL-methionine	–	0.01	0.02	0.01	–	0.01	0.02	0.01	–	0.01	0.02	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis												
ME (Mcal/kg)	3.1	3.1	3.1	3.1	3.0	3.0	3.0	3.0	2.96	2.95	2.96	2.96
CP (%)	16.1	16.1	16.2	16.2	14.4	14.7	14.6	14.6	13.1	13.5	13.3	13.4
Ca (%)	0.8	0.8	0.8	0.8	0.72	0.72	0.72	0.72	0.65	0.65	0.65	0.65
P (%)	0.7	0.7	0.7	0.7	0.63	0.63	0.63	0.63	0.60	0.60	0.60	0.60
Ileal digestible AA												
Lysine (%)	0.78	0.79	0.79	0.81	0.72	0.73	0.72	0.72	0.62	0.65	0.62	0.64
Met + Cys (%)	0.44	0.47	0.45	0.46	0.40	0.45	0.43	0.43	0.40	0.41	0.40	0.41
Threonine (%)	0.45	0.45	0.43	0.44	0.43	0.45	0.43	0.45	0.41	0.41	0.40	0.41
Tryptophan (%)	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.11	0.11	0.11	0.11

[a] As-fed basis.

[b] Provided the following per kilogram of diet: vitamin A, 3,800 IU; vitamin D_3, 380 IU; vitamin E, 18 IU; vitamin K, 5 mg; riboflavin, 3.2 mg; D-calcium pantothenate, 16.5 mg; niacin, 20 mg; choline chloride, 254 mg; vitamin B_{12}, 22 μg; Fe, 90 mg; Zn, 90 mg; Mn, 20 mg; Cu, 20 mg; I, 0.6 mg; and Se, 0.2 mg.

rapeseed meal and cottonseed meal was used in group 4 (R + C). L-lysine · HCl was supplemented to make equal digestible lysine level for each dietary treatment (table 1).

On day 28, before feeding in the morning, one pig from each pen was sampled via jugular vein using vacuum container to obtain blood samples. All blood samples were centrifuged for 15 minutes at 3,000 rpm to obtain serum. The serum samples were divided into two parts. One was stored at −20℃ for analysis of blood urea nitrogen (BUN) using diacetyl monoxime, the other was deproteined and centrifuged at low temperature for analysis of free amino acid concentration by HPLC.

Experiment 2. Effects of lysine, threonine and/or tryptophan supplementation on growth performance of growing pigs

Exp. 2 was conducted to investigate whether the threonine and/or tryptophan in a 14% CP growing pig diet was adequate for maximum performance. Sixty four pigs with initial weight of 45.0 ± 1.0 kg were utilized in a 28 day growth trial. Corn-soybean-based diet with equal amount (3%) of rapeseed meal and cottonseed meal was formulated as a control treatment (CTRL). Each diet was supplemented with L-lysine · HCl to get 0.8% digestible lysine. In groups 2 (THR), 3 (TRP), and 4 (THR + TRP),

Table 2. Percentage composition of experimental diets (Exp. 2)[a]

Treatment	CTRL	THR	TRP	THR + TRP
Corn (%)	72.72	70.88	70.86	70.82
Soybean meal (%)	11.51	12.03	11.00	11.00
Wheat bran (%)	3.50	6.00	6.00	6.00
Cottonseed meal (%)	4.50	4.00	5.00	5.00
Rapeseed meal (%)	4.50	4.00	4.00	4.00
Limestone (%)	1.36	1.10	1.19	1.19
Dicalcium phosphate (%)	0.36	0.41	0.40	0.40
Salt (%)	0.30	0.30	0.30	0.30
Premix[b] (%)	1.00	1.00	1.00	1.00
L-lysine · HCl (%)	0.22	0.22	0.22	0.22
DL-methionine (%)	0.03	0.03	0.03	0.03
L-threonine (%)	–	0.03	–	0.03
L-tryptophan (%)	–	–	0.01	0.01
Total (%)	100.00	100.00	100.00	100.00
Calculated analysis				
ME (Mcal/kg)	3.0	3.0	3.0	3.0
CP (%)	14.0	14.0	14.0	14.0
Ca (%)	0.6	0.6	0.6	0.6
P (%)	0.5	0.5	0.5	0.5
Ileal digestible AA				
Lysine (%)	0.8	0.8	0.8	0.8
Met + Cys (%)	0.5	0.5	0.5	0.5
Threonine (%)	0.48	0.59	0.48	0.59
Tryptophan (%)	0.16	0.16	0.17	0.17

[a] As-fed basis.

[b] Provided the following per kilogram of diet: vitamin A, 3,800 IU; vitamin D_3, 380 IU; vitamin E, 18 IU; vitamin K, 5 mg; riboflavin, 3.2 mg; D-calcium pantothenate, 16.5 mg; niacin, 20 mg; choline chloride, 254 mg; vitamin B_{12}, 22 μg; Fe, 90 mg; Zn, 90 mg; Mn, 20 mg; Cu, 20 mg; I, 0.6 mg; and Se, 0.2 mg.

0.03% L-threonine and/or 0.01% L-tryptophan were supplemented, respectively (table 2).

Experiment 3. Effect of threonine supplementation on growth performance of growing pigs.

Exp. 3 was conducted to determine whether the addition of threonine in a 16% CP growing pig diet was adequate for maximum performance. Sixty four pigs with initial weight of 17.0 ± 1.0 kg were utilized in a 28 day growth trial. Corn-soybean-based diet with equal amount (3%) of rapeseed meal and cottonseed meal was formulated as a control (CTRL). L-lysine · HCl was supplemented into each diet to achieve 0.9% digestible lysine. L-threonine was supplemented to treatment 2, 3 and 4 to get graded dietary threonine levels. L-tryptophan was also added in each diet in case of deficiency (table 3).

Statistical analysis

All performance data collected from each of the three experiments were analyzed as a randomized complete block design. Blocks were based on initial weight. The GLM procedures of SAS (1985) was used for all statistical analyses.

Table 3. Percentage composition of experimental diets (Exp. 3)[a]

Threonine (%)	0.45	0.54	0.64	0.74
Corn	65.23	65.15	65.05	64.95
Soybean meal	14.60	14.60	14.60	14.60
Wheat bran	10.28	10.28	10.28	10.28
Cottonseed meal	3.00	3.00	3.00	3.00
Rapeseed meal	3.00	3.00	3.00	3.00
Limestone	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.30	1.30	1.30	1.30
Salt	0.30	0.30	0.30	0.30
Premix[b]	1.00	1.00	1.00	1.00
L-lysine · HCl	0.21	0.21	0.21	0.21
L-methionine	0.05	0.05	0.05	0.05
L-threonine	–	0.08	0.18	0.28
L-tryptophan	0.03	0.03	0.03	0.03
Total	100.00	100.00	100.00	100.00
Calculated analysis				
ME (Mcal/kg)	3.05	3.05	3.05	3.05
CP (%)	16.0	16.1	16.1	16.2
Ca (%)	0.84	0.84	0.84	0.84
P (%)	0.74	0.74	0.74	0.74
Ileal Digestible AA				
Lysine (%)	0.8	0.8	0.8	0.8
Met + Cys (%)	0.5	0.5	0.5	0.5
Threonine (%)	0.45	0.54	0.64	0.74
Tryptophan (%)	0.17	0.17	0.17	0.17

[a] As-fed basis.

[b] Provided the following per kilogram of diet: vitamin A, 3,800 IU; vitamin D_3, 380 IU; vitamin E, 18 IU; vitamin K, 5 mg; riboflavin, 3.2 mg; D-calcium pantothenate, 16.5 mg; niacin, 20 mg; choline chloride, 254 mg; vitamin B_{12}, 22 μg; Fe, 90 mg; Zn, 90 mg; Mn, 20 mg; Cu, 20 mg; I, 0.6 mg; and Se, 0.2 mg.

RESULTS AND DISCUSSION

Experiment 1. Effects of formulating diets on the basis of digestible amino acids on growth performance of growing and finishing pigs

Data of growth performance and cost of gain are presented in table 4. In each phase and overall period, pigs in group SBM had relatively better ADG, ADFI and F/G than pigs in other three groups, but no statistical significance were detected ($p > 0.05$). Pigs in group RSM

Table 4. Effects of formulating diets on basis digestible amino acids on growth performance and cost per gain of growing and finishing pigs (Exp. 1)

Treatment	SBM	RSM	CSM	R + C	SEM
Initial weight (kg)	22.20	22.35	22.32	22.17	0.05
Phase I					
Terminal weight (kg)	35.6	34.3	34.1	34.4	0.08
ADG (g/d)	480	423	421	437	16.1
ADFI (g/d)	1,157	1,088	1,068	1,070	17.2
F/G	2.41	2.56	2.54	2.45	0.06
Phase II					
Terminal weight (kg)	52.4	50.0	49.4	49.9	0.89
ADG (g/d)	597	564	546	554	18.8
ADFI (g/d)	1,707	1,658	1,628	1,635	19.4
F/G	2.86	2.94	2.98	2.95	0.12
Phase III					
Terminal weight (kg)	72.5	69.4	68.4	69.7	0.92
ADG (g/d)	720	692	679	706	21.2
ADFI (g/d)	2,311	2,360	2,296	2,308	22.6
F/G	3.21	3.41	3.38	3.27	0.09
Overall					
ADG (g/d)	599	560	549	565	18.9
ADFI (g/d)	1,725	1,702	1,664	1,671	19.6
F/G	2.88	3.04	3.03	2.95	0.08
Cost per gain (Yuan RMB/kg)	7.40	7.69	7.83	7.39	

Note: No significant differences were detected.

and CSM had similar growth rates and feed conversion ratio. Differences between ADFI of SBM group and other groups may be due to the poorer palatability or anti-nutritional factors of RSM and CSM than that of SBM. During overall period, among groups CSM, RSM and R + C, pigs in group R + C had numerically higher ADG and better F/G. ADG and F/G were similar between SBM and R + C ($p > 0.05$).

Formulating diets on the basis of digestible amino acids needs more accurate data of amino acid digestibility and crystalline amino acid supplementation (Tanksley et al., 1984). Because of different feed processing such as heating, protein sources may have different digestibility (Bjarnason and Carpenter, 1969; Ford et al., 1971; Hurrell et al., 1976). Concentration of blood urea nitrogen (BUN) and free lysine supported this explanation (table 5). Eggum (1970) found an inverse relationship between plasma urea N and dietary amino acid balance. This relationship has been used to determine amino acid requirements in pigs (Brown and Cline, 1974). Blood free amino acids have been used to determine the adequacy of amino acids in animals (Lewis, 1992). In this experiment, BUN and blood lysine of groups SBM and R + C were almost the same. This can be an indication that these two diets have similar digestible amino acid profile and balance status. This was in agreement with the data of growth performance.

Amino acid content and digestibility of certain ingredient may vary with different processing, production location, etc. Meanwhile, different ingredients have different amino acid profiles. This kind of amino acid variation makes it difficult to formulate an accurately

Table 5. Effects of formulating diets on basis digestible amino acids on urea nitrogen and free lysine concentration in blood of growing pigs (Exp. 1)

Treatment	SBM	RSM	CSM	R + C	SEM
Blood Urea N (mg/dl)	8.74	7.02	6.35	7.46	0.98
Blood Lysine (mg/dl)	2.26	2.47	2.72	2.34	0.22

Note: No significant differences detected.

balanced diet.

Although all groups had no significant differences ($p > 0.05$), cost per gain of group SBM and group R + C was similar and they were much lower than those of RSM and CSM. This means that formulating diets on basis of digestible amino acids can support an appropriate use of unconventional protein sources and more ingredients may get more balanced diets. The most interesting conclusion is that the use of digestible amino acids to formulate can save an important amount of protein in the diet of finishing pigs.

Experiment 2. Effects of lysine, threonine and/or tryptophan supplementation on growth performance of growing pigs

When a 14% CP corn-soybean based diet was formulated containing 3% RSM and 3% CSM, fortified with crystalline L-lysine · HCl, ADG was improved ($p < 0.05$) when threonine was supplemented (table 6). Supplementation of tryptophan did not make any further benefits ($p > 0.05$) but decreased feed intake ($p < 0.05$) if threonine was not added in together. This indicated that threonine is the first amino acid needed to be added in lysine-fortified low-protein corn-based growing pig diet provided protein by soybean meal, rapeseed meal and cottonseed meal.

It has been reported that threonine is limiting in lysine-fortified sorghum-soybean meal diets for growing pigs (Hansen et at., 1993a; Cohen and Tanksley, 1976), while tryptophan is not limiting (Lin et al., 1987). Jin et al. (1998) also found that tryptophan addition to low protein diets of growing pigs was not beneficial while threonine might be limiting lysine fortified low protein diet for growing pigs. In this experiment, supplementation in tryptophan alone resulted a decrease in feed intake. This can be explained by dietary amino acid imbalance. Harper and Rogers (1965) reported that an imbalanced diet could lead to lower feed intake, therefore decrease growth performance of animal.

Table 6. Effect of lysine, threonine and tryptophan supplementation on growth performance of growing pigs (Exp. 2)

Treatment	CTRL	THR	TRP	THR + TRP	SEM
Initial weight (kg)	45.5	45.8	45.7	45.5	0.08
Terminal weight (kg)	57.8	59.7	57.9	59.3	1.21
ADG (g/d)	584[a]	665[b]	582[a]	657[b]	18.8
ADFI (g/d)	2,240[ab]	2,204[ab]	1,981[c]	2,268[a]	20.6
F/G	3.84[a]	3.31[b]	3.41[b]	3.30[b]	0.12

Note: Same superscripts within a row mean no significant difference ($p > 0.05$).

Experiment 3. Effects of threonine supplementation on growth performance of growing pigs

Threonine supplementation in a 0.45% threonine diet improved ($p<0.05$) ADG and F/G (table 7). When the dietary threonine was 0.54%, pigs achieved the best growth rate and feed conversion ratio. Increasing dietary threonine further to 0.64% and 0.74% did not support better ADG, but supported better F/G ($p < 0.05$) than pigs in groups of lower dietary threonine. This was due to the decrease of ADFI ($p < 0.05$).

Dietary threonine/lysine ratio in this experiment were 56, 67.5, 81, and 92.5, respectively. The ratio of 0.54% dietary threonine group agrees with that recommended by Wang and Fuller (1990) in ideal amino acid pattern for growing pigs. Other groups had threonine deficiency or surpass which could lead to amino acid imbalance.

Rosell and Zimmerman (1984) reported that a 0.61% dietary threonine was marginally deficient for 15 kg pigs.

Table 7. Effect of threonine supplementation on growth performance of growing pigs

Threonine (%)	0.45	0.54	0.64	0.74	SEM
Initial weight (kg)	17.5	17.8	17.1	17.4	0.42
Terminal weight (kg)	27.6	31.6	29.0	29.5	0.96
ADG (g/d)	361[a]	494[b]	425[cd]	430[cd]	18.80
ADFI (g/d)	1,885[ab]	1,805[ab]	1,609[c]	1,459[d]	21.60
F/G	5.22[a]	3.65[bc]	3.79[bc]	3.39[d]	0.56

Note: same letter within a row mean no significant differences ($p > 0.05$).

In their experiment, only total threonine was taken into account.

IMPLICATIONS

When formulating corn-based diets for growing pigs on the basis of digestible amino acids, with crystalline lysine added in, RSM and CSM can be used and lower protein levels should be recommended to get lower feed cost. Combination of RSM and CSM can make balanced diets for growing pigs. When formulating a 14% CP diet containing RSM and CSM for growing pigs, threonine may be needed to be added in, while tryptophan is adequate. Pigs can get optimum growth performance when dietary threonine/lysine ratio is about 67.5%. It need further studies to investigate if this is also true when other ingredients are involved.

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