Effects of Supplemental Synthetic Amino Acids to the Low Protein Diets on the Performance of Growing Pigs

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ABSTRACT : A total of 120 pigs (L × LW × D) averaged 14.16 kg of body weight were reared under six dietary treatments to evaluate the effects of amino acid supplementation on their performances. Treatments were 1) 18% CP diet (control); 2) 15% CP+0.28% Lys (B, 15L); 3) B+0.06% Met (15LM); 4) B+0.13% Thr (15LT); 5) B+0.06% Met+0.13% Thr (15LMT); 6) B+0.06% Met+0.13% Thr+0.05% Trp (15LMTT). Each treatment had 4 replicates with 5 pigs per replicate. The daily weight gains were statistically similar in 15LMT, 15LMTT and control groups. Threonine and methionine supplementation resulted in improved growth performance and nutrient digestibilities, while tryptophan supplementation had little beneficial effect. However, the best feed conversion was found in the control group. Dry matter and CP digestibilities were improved in the 15LMT and 15LMTT groups. Gross energy, crude fat and phosphorus digestibilities were not affected by the treatment. Among the 15% CP groups, nitrogen digestibility showed the tendency to be increased as the more synthetic amino acids were added. Dry matter and nitrogen excretions were significantly reduced by feeding low protein, amino acid fortified diets, while phosphorus excretion was not influenced. Essential amino acids digestibility was higher in 15LT, 15LMT and 15LMTT groups and lower in the control. The 15LT group showed the best lysine digestibility, and methionine digestibilities were higher in all treated groups than control one. Threonine digestibilities higher in 15LT, 15LMT, and 15LMTT than the control. The result shows that threonine and methionine should be added to the diet containing 15% CP diet, fortified with lysine to get same performance of 18% CP diet.
(**Key Words**: Pigs, Protein, Lysine, Methionine, Threonine, Tryptophan, Nutrient Excretion)

INTRODUCTION

In most cereal based diets lysine and methionine are the first or second limiting amino acid for monogastric animal. Tryptophan and threonine are often more limiting than methionine in some swine diets (Cunha, 1977). Supplementation of these diets with synthetic amino acids or good quality protein (e.g. fish meal) may be desirable. For efficient use, supplemental amino acids must be fed at the proper level, at the proper time, and with the proper level of all other essential amino acids, because individual amino acids are not stored and excesses are usually catabolized quickly.

A grain-soybean meal based diet formulated to meet the NRC (1988) lysine requirements of the 10-20 kg pig typically contains 18% to 21% crude protein (CP). Reducing dietary CP content below these levels lowers pigs' performance due to an essential amino acid deficiency. Increased production and lowered cost of synthetic amino acids such as lysine (Lys), methionine (Met), threonine (Thr), and tryptophan (Trp) may allow the economical use of low-protein, amino acid fortified, grain-soybean meal based diets for the young pigs.

Feed conversion ratio and growth rate of pigs fed to appetite are not significantly affected by dietary protein or nonessential nitrogen content when essential amino acids are maintained adequate to meet the requirements (Wahlstrom and Libal, 1974; Bereskin et al., 1976; Sharda et al., 1976; Easter and Baker, 1980; Noblet et al., 1987; Stahly et al., 1981; Asche et al., 1985). In corn-soybean meal based diets, CP was reduced to 14% and performance equivalent to that obtained on an 18% CP diet was obtained when Lys and Trp were added; a 12% CP diet caused inferior performance regardless of Lys, Met, Thr, and Trp additions (Corley and Easter 1980, 1983). Yen and Veum (1982) reported equal performance for starting pigs fed a 15% CP, corn-soybean meal based diet fortified with Lys or an 18% CP diet.

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To reduce porcine N excretion, it is clear that the protein/amino acid balance in the diet must be checked (Lenis, 1989; Gatel and Grosjean, 1992).

There have been a lot of efforts to reduce excessive CP in swine diets (Chae et al., 1988; Lewis et al., 1979; Kephart and Sherritt, 1990; Han et al., 1978, 1995; Hansen et al., 1993a,b; Page et al., 1993; Brudevold and Southern, 1994). A decrease in CP in the diet will decrease N loss to the environment. Lenis (1989) showed that lowering the CP level by 1% in diets for growing-finishing pigs decreased the N excretion by 8.5%.

The purposes of this research were to determine the limiting amino acid in 15% CP corn-soybean meal based diets and to investigate the effect of feeding low protein diet, fortified with essential amino acids, on growth performance, amino acid digestibility and excretion of nitrogen and phosphorus.

MATERIALS AND METHODS

Table 1. Composition of the experimental diets

Treatments	CP 18%	CP 15% +Lys	CP 15% +Lys +Met	CP 15% +Lys +Thr	CP 15% +Lys +Met +Thr	CP 15% +Lys +Met +Thr +Trp
Ingredients (%)						
Corn	65.65	73.58	73.51	73.42	73.35	73.27
Soybean meal	28.23	19.87	19.88	19.90	19.92	19.93
Corn oil	3.00	3.00	3.00	3.00	3.00	3.00
Mono calcium phosphate	1.30	1.50	1.50	1.50	1.50	1.50
Limestone	1.05	1.00	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Vit. Min.[1]	0.25	0.25	0.25	0.25	0.25	0.25
Antibiotics	0.22	0.22	0.22	0.22	0.22	0.22
Lysine	–	0.28	0.28	0.28	0.28	0.28
Methionine	–	–	0.06	–	0.06	0.06
Threonine	–	–	–	0.13	0.13	0.13
Tryptophan	–	–	–	–	–	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00
Chemical composition[2]						
ME (Mcal/kg)	3.38	3.38	3.38	3.37	3.37	3.37
CP (%)	18.00	15.00	15.00	15.00	15.00	15.00
	(18.13)[3]	(15.04)	(15.09)	(15.08)	(15.90)	(15.11)
Ca (%)	0.80	0.80	0.80	0.80	0.80	0.80
P (%)	0.70	0.70	0.70	0.70	0.70	0.70
	(0.63)	(0.66)	(0.63)	(0.64)	(0.66)	(0.64)
Lysine (%)	0.95	0.95	0.95	0.95	0.95	0.95
	(0.96)	(0.94)	(0.94)	(0.95)	(0.96)	(0.95)
Met (%)	0.30	0.24	0.30	0.24	0.30	0.30
	(0.31)	(0.25)	(0.31)	(0.25)	(0.31)	(0.31)
Threonine (%)	0.73	0.60	0.60	0.73	0.73	0.73
	(0.74)	(0.60)	(0.62)	(0.74)	(0.73)	(0.75)
Tryptophan (%)	0.24	0.19	0.19	0.19	0.19	0.24

[1] Vit. min.-mixture contains per kg: Vitamin A, 2,000,000 IU; Vitamin D_3, 400,000 IU; Vitamin E, 250 IU; Vitamin K_3, 200 mg; Vitamin B_1, 20 mg; Vitamin B_2, 700 mg; Pantothenic acid, 3,000 mg; Choline chloride, 30,000 mg; Niacin, 8,000 mg; Vitamin B_{12}, 13 mg; Mn, 12,000 mg; Zn, 15,000 mg; Co, 100 mg; Cu, 500 mg; Fe, 4,000 mg; Folic acid, 40 mg; BHT, 5,000 mg; sucrose to make 1 kg vit.-min. mixture.

[2] Calculated value.

[3] Analyzed value.

Three way crossbreed (Landrace × Large White × Duroc) barrows were used as experimental animals. A total of 120 pigs (6 weeks of age) averaged 14.16 kg in body weight were chosen and alloted to a completely randomized block design. The treatments were 1) 18% CP diet; 2) 15% CP+0.28% Lys (B); 3) B+0.06% Met; 4) B+0.13% Thr; 5) B+0.06% Met+0.13% Thr; 6) B +0.06% Met+0.13% Thr+0.05% Trp. Each treatment had 4 replicates with 5 pigs per replicate.

Corn-soybean meal based diets were used for the experiment. Each diet was formulated to contain 3.37-3.38 Mcal ME/kg, 0.8% Ca, and 0.7% P. Composition of the experimental diets is presented in table 1.

The pigs were kept in concrete-floored pens, and feed and water were provided *ad libitum* during the entire experimental period of 3 weeks. Body weight and feed intake were recorded weekly to calculate average daily weight gain (ADG) and average daily feed intake (ADFI).

For determination of the digestibilities of dietary nutrients, pigs were fed diets containing 0.25% Cr_2O_3 during the second week of the experimental period and feces were collected three days and three times (08:00, 14:00, 20:00) a day after four days of adjustment period. Fecal samples were collected and then dried in an air-forced drying oven at 60℃ for 72 hours and ground with 1 mm mesh Wiley mill for chemical analysis.

Feed and fecal samples were analyzed for proximate analysis and mineral composition (AOAC, 1990). Chromium was measured using Atomic Absorption Spectrophotometer (Shimadzu, AA6145F, Japan). For energy utilization, the gross energy values of feed and feces samples were measured by Adiabatic Oxygen Bomb Calorimeter (Model 1241, Parr Instrument Co., Molin, IL) and amino acid contents were measured by Automatic Amino Acid Analyzer (Model 4150 alpha, LKB, UK) following acid hydrolysis with 6N HCl at 110℃ for 16 hours.

All data were analyzed according to a completely randomized block design. The GLM procedure of SAS package (1985) was used for all statistical analyses, and treatment means were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth Performance:

Growth performances of pigs fed the experimental diets are presented in table 2. For the first week, 15LMTT group showed the best average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR), while 15L group showed the worst. There was no significant difference among the control, 15LMT and 15LMTT in ADG and ADFI. The 15LT group showed significantly better FCR ($p<0.05$) and numerically better ADG than 15LM group. During the second week, 15LMT group showed the best ADG and ADFI, while 15L showed the worst ($p<0.05$). The best FC was found in the control group ($p<0.05$). Between 15LM and 15LT groups, 15LT group showed better performance in all parameters evaluated than 15LM group ($p<0.05$). 15LMT and 15LMTT showed equivalent ADG to the control, but slightly worse FC than the control group. For the third week, 15LMTT showed the best performance, while 15L group showed the worst ($p<0.05$). The effect of supplementing synthetic amino acids was in order of 15LMTT, 15LMT, 15LT, 15LM and 15L. The effect of supplementing synthetic amino acids seemed to be decreased as the pigs aged.

For the overall period of 3 weeks, 15LMT, 15LMTT and the control were not significantly different in ADG. However, the best FC was found in the control group. FC was improved ($p<0.05$) by supplementation of the 15% CP diet with lysine, methionine, threonine and tryptophan, but it remained inferior to that of control group. This might be due to the fact that a reduction in the protein content below recommended levels may limit nonessential nitrogen (Corley and Easter, 1980; Easter and Baker, 1980). From the results obtained in this study, in the corn-soybean meal based diet containing 15% CP the second limiting amino acid appeared to be threonine. In this study, threonine supplementation resulted in positive effect on the growth performance, while tryptophan supplementation showed little beneficial effect. Hansen et al. (1993b) reported that threonine supplementation elicit positive effect on the growth performance of weaned pigs fed 17% CP diet, while tryptophan supplementation showed no significant improvement. Cohen and Tanksley (1976) reported similar results when they examined the effect of synthetic amino acids supplementation to the 12% CP sorghum-soybean meal based diets. La Rue (1985) also reported that threonine and methionine supplementation had a positive effect on pig growth but methionine and tryptophan supplementation alone did not improve the growth performance of pigs. In the low protein lysine fortified diet, it was found that threonine had more weight than methionine, and these two amino acids should be supplemented in combination. Although lysine supplementation to grain alone generally improved performance with reducing dietary CP, addition of other amino acids, especially threonine, has been shown to be beneficial (Aw-Young and Beames, 1975; Hansen et al., 1993b). Tryptophan was not found to be the limiting amino acid

Table 2. Effects of supplemental methionine, threonine and tryptophan on performance of pigs fed 15% protein, lysine-fortified diet

Parameters (days)	CP 18% Control	CP 15% +Lys	CP 15% +Lys +Met	CP 15% +Lys +Thr	CP 15% +Lys +Met +Thr	CP 15% +Lys +Met +Thr +Trp	SE[1]
0 to 7							
ADG (g)	593.5[a]	529.8[c]	535.0[b]	564.8[b]	592.3[a]	600.3[a]	7.25
ADFI (g)	1,100.0[a]	1,075.5[c]	1,075.5[c]	1,093.5[b]	1,115.5[a]	1,123.5[a]	10.05
FCR	1.85[d]	2.03[a]	2.01[a]	1.83[b]	1.88[c]	1.87[cd]	0.02
7 to 14							
ADG (g)	650.1[a]	592.0[d]	604.5[c]	627.0[b]	652.8[a]	650.8[a]	5.41
ADFI (g)	1,264.3[c]	1,247.5[d]	1,259.8[cd]	1,272.5[bc]	1,287.5[a]	1,285.0[ab]	8.36
FCR	1.94[e]	2.10[a]	2.08[b]	2.03[d]	1.97[d]	1.97[d]	0.01
0 to 14							
ADG (g)	621.8[a]	561.0[d]	569.8[c]	596.0[b]	622.5[a]	625.5[a]	4.93
ADFI (g)	1,182.3[b]	1,161.5[c]	1,167.8[c]	1,183.0[b]	1,201.8[a]	1,204.5[a]	5.18
FCR	1.90[e]	2.06[a]	2.05[b]	1.98[c]	1.93[d]	1.93[d]	0.01
14 to 21							
ADG (g)	695.0[b]	648.8[e]	661.5[d]	680.3[c]	697.3[ab]	699.8[a]	2.90
ADFI (g)	.1,433.5[b]	1,419.8[c]	1,428.8[bc]	1,437.0[b]	1,449.5[a]	1,451.8[a]	7.84
FCR	2.06[e]	2.18[a]	2.16[b]	2.11[c]	2.08[d]	2.07[d]	0.01
0 to 21							
ADG (g)	646.3[a]	590.3[d]	600.3[c]	624.0[b]	647.5[a]	650.0[a]	3.04
ADFI (g)	1,266.0[b]	1,248.0[d]	1,254.8[c]	1,267.8[b]	1,284.5[a]	1,286.8[a]	2.73
FCR	1.96[e]	2.11[a]	2.09[b]	2.03[c]	1.98[d]	1.98[d]	0.01

[1] Pooled standard error, n=24.

[a,b,c,d,e] Figures at the same row without the same superscript are significantly different at $p<0.05$.

ADG: Average daily weight gain.

ADFI: Average daily feed intake.

FCR: Feed conversion ratio.

in this study. However, it should be noted that tryptophan could be a limiting amino acid under other conditions like growing stage (Shrada et al., 1976), composition of feed ingredients (Wahlstrom et al., 1985) and the extent of decrease in CP (Russel et al., 1986). Shrada et al. (1976) suggested that lysine and tryptophan should be added in the diet of 12% CP to get equal performance to the performance of finishing pigs fed 16% CP diet. Wahlstrom et al. (1985) reported that tryptophan and threonine was the most limiting amino acids for growing pigs in a lysine-supplemented, 12% CP, corn-sunflower meal based diet.

Nutrient digestibilities and excretion:

Nutrient digestibilities of experimental diets are summarized in table 3. Dry matter and nitrogen digestibilities were improved in the 15LMT and 15LMTT groups ($p<0.05$). Gross energy, crude fat and phosphorus digestibilities were not affected by the treatments. Similar results were reported by Han et al. (1995). Greeley et al. (1964) and Just (1982a) reported a big difference in energy digestibility, while Kerr and Easter (1995) found no big difference in energy digestibility. However, as suggested by Kerr and Easter (1995), in this study, the range of CP was not wide, thus energy digestibility might not be affected by the treatments.

Table 3. Effects of synthetic amino acid supplementation to the low protein diet on nutrient digestibilities in growing pigs (%)

Treatments	GE	Dry matter	Nitrogen	Crude fat	Crude ash	Phosphorous
18 C (Control)	72.29	78.02[c]	75.18[b]	78.97	59.83[a]	37.72
15 L	77.27	78.34[c]	75.61[b]	78.58	55.93[c]	36.29
15 LM	76.93	79.11[bc]	76.17[ab]	78.37	58.01[b]	37.59
15 LT	77.36	80.56[abc]	77.26[ab]	78.26	59.39[a]	37.75
15 LMT	77.71	81.13[a]	78.17[a]	78.83	58.03[b]	38.10
15 LMTT	76.46	81.67[a]	77.88[a]	78.55	59.81[a]	38.94
SE[1]	0.69	0.40	0.36	0.29	0.31	0.50

[1] Pooled standard error, n=24.

[a,b,c] Figures at the same column superscript without the sam are significantly different at $p<0.05$.

Only in 15LMT and 15LMTT, nitrogen digestibility was better than the control group ($p<0.05$). The lower nitrogen digestibility of the control group was not expected, because numerous researchers (Just, 1982a,b; Noblet et al., 1987; Kephart and Sherritt, 1990; Han et al., 1995) reported an increase in nitrogen digestibility as CP levels increased. This is likely associated with the less negative effect of endogenous nitrogen at high nitrogen intakes (Just, 1982b), higher digestibility of feedstuffs used to obtain higher CP levels (Just, 1982b; Sato et al., 1987). Just (1982b) reported that the digestibility of CP increased with increasing percentages in the diet when they examined the range of CP from 13.2% CP to 29.4% crude protein. However, in this study, the difference in CP level was not as big as in the study by Just (1982b), thus CP digestibility was not much affected by the CP levels. Kerr and Easter (1995) found an increased nitrogen digestibility with supplemented lysine, threonine, tryptophan and dispensable amino acids to the low protein diet.

Among the 15% CP groups, CP digestibility tended to increase as the more synthetic amino acids were added. This increased digestibility may be due to the greater digestibility of synthetic amino acids (Kerr and Easter, 1995) and the improvement in amino acid balance by adding some essential amino acids. Improved nitrogen retention when lysine and tryptophan were added was also observed by Shrada et al. (1976).

Dry matter and nitrogen excretion (table 4) significantly reduced by feeding low protein, amino acids supplemented diets ($p<0.05$), while phosphorus excretion (table 4) was not affected ($p>0.05$) by the treatments. In 15LMTT group, pigs excreted approximately 20% less nitrogen than those fed 18% CP (control) diet. This decrease in dry matter and nitrogen excretion seemed to be resulted from the higher digestibilities as described above. It is well known that the excretion of nitrogen can be reduced by feeding low protein, amino acids supplemented diet (Chae et al., 1988; Han et al., 1978, 1995; Kerr and Easter, 1995, Paik et al., 1996). Kerr and Easter (1995) suggested that for each unit reduction in dietary CP combined with amino acids supplementation, total nitrogen losses can be reduced by approximately 8%. Paik et al. (1996) reviewed previous studies and summarized that 20 to 25% reduction in nitrogen losses can be achieved by feeding low protein, amino acids fortified diets. Our results support these previous findings.

Table 4. Effects of synthetic amino acid supplementation to the low protein diet on nutrient excretion in growing pigs (g/1,000 g weight gain)

Treatments	Dry matter	Nitrogen	Phosphorus
18 C (Control)	455.33[c]	13.81[d]	5.03
15 L	454.73[c]	12.33[c]	5.11
15 LM	450.26[c]	12.21[c]	5.06
15 LT	433.87[b]	11.76[b]	4.96
15 LMT	422.06[a]	11.44[a]	5.00
15 LMTT	419.43[a]	11.37[a]	5.00
SE[1]	4.02	0.18	0.04

[1] Pooled standard error, n=24.

[a,b,c] Figures at the same column superscripts without the same are significantly different at $p<0.05$.

Amino acid digestibilities:

Essential amino acid digestibility was the highest in 15LT, 15LMT and 15LMTT groups and the lowest in the control group. The 15LT group showed the best lysine digestibility, and methionine digestibility were all high in treated groups than control group. Threonine digestibility were higher in 15LT, 15LMT, and 15LMTT than the control. Amino acid digestibilities of the low protein, amino acid fortified diets have not been intensively studied so far. Han et al. (1995) observed the improved lysine digestibility when they added 0.2% synthetic lysine alone and lysine and methionine in combination to the basal diet. The increased lysine, methionine and threonine digestibility observed in this study may be due to the high digestibility of synthetic amino acids.

Table 5. Effects of synthetic amino acid supplementation to the low protein diet on amino acids digestibilities in growing pigs (%)

AA	18 C (Control)	15 L	15 LM	15 LT	15 LMT	15 LMTT	SE[1]
THR	76.14^{c}	79.01^{abc}	78.36^{bc}	83.75^{a}	80.28^{abc}	81.60^{ab}	0.80
VAL	75.70^{b}	75.63^{b}	77.72^{ab}	79.83^{a}	79.46^{ab}	80.53^{a}	0.64
MET	75.57^{b}	81.19^{a}	84.27^{a}	82.80^{a}	81.70^{a}	84.46^{a}	1.46
ILE	78.55^{bc}	71.69^{c}	86.50^{a}	78.63^{bc}	85.65^{ab}	83.39^{ab}	1.35
LEU	87.34^{a}	83.15^{ab}	88.53^{a}	80.64^{b}	89.23^{a}	84.45^{ab}	0.96
PHE	73.57^{ab}	80.15^{a}	78.27^{ab}	78.89^{a}	79.51^{a}	76.58^{a}	1.93
HIS	73.91^{b}	78.87^{ab}	77.17^{ab}	80.35^{ab}	84.61^{a}	81.19^{ab}	1.13
LYS	83.44^{ab}	81.11^{b}	83.86^{ab}	85.48^{a}	84.26^{ab}	84.76^{a}	0.49
ARG	83.38^{b}	88.37^{a}	86.48^{ab}	86.15^{ab}	87.77^{a}	87.37^{a}	0.53
EAA:	78.07^{c}	79.91^{bc}	80.57^{abc}	81.54^{ab}	83.61^{a}	82.71^{ab}	0.58
ASP	80.27^{c}	82.02^{bc}	80.75^{c}	84.92^{ab}	84.13^{ab}	85.23^{a}	0.59
SER	83.85	83.59	82.82	86.21	84.77	85.77	0.53
GLU	82.77	83.30	84.02	88.18	84.80	86.93	0.83
PRO	80.49^{b}	81.92^{ab}	82.61^{ab}	87.78^{a}	84.56^{ab}	86.08^{ab}	1.00
GLY	80.65^{a}	74.42^{b}	74.33^{b}	78.46^{ab}	81.09^{a}	80.62^{a}	0.84
ALA	72.64^{b}	74.77^{ab}	75.57^{ab}	76.19^{ab}	74.67^{ab}	77.31^{a}	0.58
TYR	82.10	74.83	88.23	73.49	82.65	77.70	2.16
NEAA:	80.40^{ab}	79.27^{b}	81.19^{ab}	82.18^{ab}	82.38^{a}	82.81^{a}	0.50
Total	79.09^{c}	76.63^{bc}	80.84^{abc}	81.98^{ab}	83.07^{a}	82.75^{a}	0.42

[1] Pooled standard error, n=24.

[a,b,c] Figures with different superscript at the same row are significantly different at $p<0.05$.

IMPLICATION

Threonine and methionine should be added to the diet containing 15% CP, lysine fortified diet to get equivalent performance to 18% control diet. Threonine and methionine supplementation resulted in improved growth performance and nutrient digestibilities, while tryptophan supplementation had little beneficial effect. Based on the present results it was clear that threonine was the most likely limiting amino acid and tryptophan was not likely the limiting in the diet containing 15% CP, lysine fortified diet.

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