PROTEIN SPARING EFFECT AND AMINO ACID DIGESTIBILITIES OF SUPPLEMENTAL LYSINE AND METHIONINE IN WEANLING PIGS

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Summary

Experiments were conducted to evaluate the nutritive values of supplemental L-lysine, liquid and powder type, and DL-methionine in weanling pigs. For feeding trial, 165 weanling pigs were treated in 2 controls; 18 and 16% CP, 6 supplementations of lysine alone to 16% CP diets; 0.1, 0.2 and 0.4% of liquid and powder type each, and 3 supplementations of lysine + methionine to 15% CP diets; 0.05 + 0.025, 0.1 + 0.05 and 0.2 + 0.1%. Pigs were fed for 5 week to investigate the protein sparing effect of supplemental amino acid, and the optimal supplemental level. A metabolic trial included the measurements of digestibilities of dry matter, crude protein, crude fat, crude fiber, energy, phosphorus and amino acids. The liver acinar cell culture was conducted for the protein synthesis activity of the pigs fed each experimental diet. Supplementation of both type of L-lysine in 16% CP diet showed improved daily weight gain and feed efficiency which were compatible with those of pigs fed 18% CP diet. Groups fed liquid lysine did not differ from those fed powder type in growth performance. Supplementation of lysine and methionine to 15% CP diet did not improve growth performance of pigs to the extent that 18% CP diet was fed. In nutrient digestibility, 16% CP control diet showed significantly ($p < 0.05$) lower crude protein digestibility than any other treatments. Digestibilities of 16% CP diets with lysine supplementation were equal to that of 18% CP control, while digestibilities of 15% CP diets with the supplementation of lysine + methionine was inferior to that of 18% CP control. Supplementation of lysine alone reduced the nitrogen excretion compared to the none supplemented control groups. However, addition of lysine + methionine excreted more nitrogen than controls. Pigs fed diet supplemented with lysine alone, or lysine + methionine excreted less fecal phosphorus than those fed none supplemetation. Retained protein from liver tissue of pigs fed 18% CP diet was significantly ($p < 0.05$) greater than those fed 16% CP diet. A significant difference ($p < 0.05$) was observed in physical type of lysine. Feeding of powder type showed less secreted protein and greater retained protein in the culture of liver acinar cell. It is concluded that supplementation of lysine at the level of 0.1 to 0.2% can spare 2% of dietary protein and reduce nitrogen excretion by 19.3%. Also, no difference in nutritional values was observed between liquid and powder lysine in weanling pigs.
(**Key Words**: Protein Sparing Effect, Amino Acid Digestibility, L-lysine, DL-Methionine, Nitrogen Excretion, Phoshorus Excretion, Weanling Pigs, Protein Synthesis)

Introduction

Lysine and methionine are the most common synthetic amino acids which replace or supplement natural protein source of them. The purpose of using synthetic amino acids in animal diets is to reduce dietary protein level and production cost of animal feed without sacrificing animal performance. The use of supplemental lysine and methionine depends upon not only nutritional interest, but also economic and environmental importance.

When the protein level of the corn-soybean meal diet is reduced by 2%, normal growth rate and feed efficiency in pigs can be obtained by supplementing the diet with lysine (Bowland, 1962; Sharda et al., 1976; Han et al., 1978; Chae et al., 1988). Further reduction of protein level results in a deficiency of amino acids other than lysine in

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Received November 28, 1994
Accepted April 20, 1995

young pigs (Yen and Veum, 1982: Russell et al., 1986; Grosbach et al., 1985; Hansen et al., 1993a,b). A low protein diet for pig is limited by lysine first, then by methionine, threonine or tryptophan. Research findings and field experiences at present time clearly demonstrated the economics of using synthetic L-lysine and DL-methionine in low protein diets for monogastric animals (Meade et al., 1966; Balogun and Fetuga, 1981a,b; Daghir, 1983; Han et al., 1991).

The last stage for L-lysine production involve condensation, crystallization and drying. Liquid type of lysine has no last stage in recovery process. Since production cost lowers by omission of the last stage of the process, liquid lysine could be rather profitable to feed manufacturers.

Lowering dietary protein level for pigs reduces nitrogen excretion. Likewise, phytic phosphorous decreases in feed as dietary protein decreases. Perhaps, 30 % of nitrogen and 40% of phosphorus excreted from pig farms can be reduced by synthetic amino acids in the following ten years (Lenis, 1989; Gatel and Grosjean, 1992; Jongbloed and Lenis, 1992).

The objectives of this study were to investigate; 1) protein sparing effect and optimal level of supplementation of lysine and methionine, 2) nutrient digestibilities of diets added with lysine and methionine, 3) amounts of nitrogen and phosphorus, 4) activity of *in vitro* protein synthesis in the liver acinar cell culture, and 5) nutritive values of liquid lysine comparing with powder lysine in weanling pigs.

Materials and Methods

Purebred pigs (Landrace) were used as experimental animals. At 35 days of age, a total of 165 pigs averaged 9.5 kg of body weight were chosen and assigned in a Completely Randomized Design (11 treatments × 3 replications; 5 heads per replication) for a feeding trial. Eleven treatments consisted of 2 control groups; CP 18% (C1) and CP 16% (C2), 3 levels of liquid lysine; C2 + lysine 0.1% (L1), C2 + lysine 0.2% (L2), and C2 + lysine 0.4% (L3), 3 levels of powder lysine; C2 + lysine 0.1% (P1), C2 + lysine 0.2% (P2), and C2 + lysine 0.4% (P3), and 3 levels of lysine and methionine combination; CP 15% + lysine 0.05% + methionine 0.025% (M1), CP 15% + lysine 0.1% + methionine 0.05% (M2), and CP 15% + lysine 0.2% + methionine 0.1% (M3). The pigs were kept in concrete-floored pens, and feed and water were provided *ad libitum* during the entire experimental period of 5 weeks. Body weight and feed intake were recorded at 3 and 5 weeks. Experimental diets were presented in table 1.

For digestibilities of experimental diets, a metabolic trial was conducted before the feeding trial was terminated. Four pigs from each treatment were selected, caged individually, and then allowed *ad libitum* access to feed and water. Experimental diets with 0.25% marker (chromium oxide) were fed to pigs for 7 days. After the first 4 days of adjustment period, feces were collected twice a day at 12-h intervals for the last 3 days.

Fecal samples were dried in an air-forced drying oven at 60℃ for 48 or 72 hours (Nongyao et al., 1990) and ground with 1 mm mesh Wiley mill for chemical analysis. Liquid lysine, feed and fecal samples were analyzed for proximate analysis and mineral composition by AOAC methods (1990). Chromium was measured by Atomic Absorption Spectrophotometer (Shimadzu, AA625, 423 nm). For energy utilization, energy values of feed and feces were measured by Adiabatic Oxygen Bomb Calorimeter (Model 1241, Parr Instrument Co., Molin, IL). Amino acid contents were measured following acid hydrolysis with 6N HCl at 110℃ for 16 hours (Mason, 1984), using Amino Acid Analyzer (Model; 4150 alpha, LKB). Amino acid contents of experimental diets are presented in table 3.

Protein synthesis activity of the liver acinar cell was measured by using the methods of Choi et al. (1992).

All statistical data were analyzed by analysis of variance using General Linear Model (GLM) Procedure of SAS program (SAS, 1985). Collected data were analyzed by two part; 1) diets supplemented with lysine alone included Treatment C1, C2, L1, L2, L3, P1, P2, and P3, 2) diets supplemented with lysine and methionine included C2, M1, M2, and M3. The differences of means between treatment were compared by Duncan's multiple range test (Duncan, 1955).

Results and Discussion

Chemical analysis of liquid lysine

Chemical analysis of liquid lysine was conducted to show that 54% of moisture, 43% of crude protein and 32 % of lysine. The content of crude ash was 0.69% with no noticeable toxic substances detected.

Feeding trial

1) diets supplemented with lysine alone

Table 2 summarized effect of different levels of dietary CP and lysine (liquid/powder) on average daily gain (ADG), average daily feed intake and feed efficiency (FE) for the experimental period of 5 weeks which was 6 to 10 weeks of age. Among controls, the group fed C1

TABLE 1. INGREDIENT AND CHEMICAL COMPOSITION OF THE EXPERIMENTAL DIETS (%)

Ingredient	Control		Liquid lysine			Powder lysine			Powder lysine + methionine		
	C1	C2	L1	L2	L3	P1	P2	P3	M1	M2	M3
Corn	60.70	66.45	66.12	65.79	65.13	66.35	66.25	65.05	69.40	69.30	69.10
Soybean meal	19.40	16.40	16.40	16.40	16.40	16.40	16.40	16.40	14.35	14.35	14.35
Fish meal	3.00	1.00	1.00	0.79	0.75	1.00	1.00	1.00	0.40	0.40	0.40
Tallow	2.50	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.80	1.80	1.80
Milk replacer	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	2.40	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.80	1.80	1.80
Liquid L-lysine			0.33	0.66	1.32						
Powder L-lysine						0.10	0.20	0.40	0.05	0.10	0.20
DL-Methionine (50%)									0.05	0.10	0.20
Limestone	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
TCP	0.65	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit.-min mix.[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Antibiotics	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Chemical composition[2]											
ME (kcal/kg)	3,246	3,247	3,250	3,225	3,224	3,244	3,241	3,234	3,248	3,245	3,238
CP (%)	18.00	16.01	16.12	16.23	16.46	16.08	16.16	16.31	15.09	15.17	15.32
Lysine (%)	0.93	0.79	0.88	0.98	1.18	0.86	0.94	1.10	0.76	0.80	0.88
Methionine (%)	0.33	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.32	0.37
Ca (%)	0.70	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.65	0.65	0.65
P (%)	0.59	0.56	0.56	0.56	0.56	0.57	0.57	0.57	0.56	0.56	0.56

[1] Vit-min mixture contains per kg of diet: 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; Riboflavin, 10,000 mg; Pantothenic calcium, 3,000 mg; Choline chloride, 30,000 mg; Niacin, 8,000 mg; Folacin, 200 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; Co, 100 mg.

[2] Calculated values.

diet showed better ADG and FE than the group fed C2 diet, significantly only for FE. No difference was observed among physical types of lysine in all growth performances. Among lysine supplemented levels, the groups fed 0.1% lysine supplemented diets showed better ADG and FE than any other groups. It was found out that L1 and P1 diets contained less lysine than NRC requirement when amino acid composition of the diets were measured (table 3). These data indicated that diets containing 16% CP supplemented with lysine improved performance to the extent which is equal or superior to 18 % CP diet with no lysine supplementation.

Results reported by Yen and Veum (1982) were in agreement with our results, where performance of starting pigs fed a 15% CP, corn-soybean meal diet with lysine supplementation were similar to that of pigs fed an 18% CP diet.

2) Diets supplemented with lysine and methionine

The effects of different levels of dietary CP and lysine in combination with methionine on ADG and FE during 6-10 weeks of age are presented in table 4.

The pigs fed 15% CP diets fortified with lysine and methionine showed significantly ($p < 0.05$) poor ADG and FE comparing with pigs fed C1 diet. Although lysine and methionine supplementation contributed to improve performance to some extent these diets failed to achieve the performance of pigs fed the 18% CP diet.

Reducing CP from 18 to 16% did not affect ADG and FE when the diets were fortified with lysine, whereas a further reduction to 15% CP did affect the growth performance of pigs fed lysine and methionine-fortified diets. Diets of M1, M2, and M3 were defficient in not only lysine but other essential amino acids (table 3).

TABLE 2. EFFECTS OF DIFFERENT LEVELS OF DIETARY CP AND LYSINE (LIQUID OR POWDER) ON ADG, ADFI AND FEED EFFICIENCY DURING 6-10 WEEKS OF AGE

Treatment		Initial body wt. (kg)	Final body wt. (kg)	ADG (kg)	ADFI (kg)	FE
C1		9.6	25.7	0.460	0.916	1.99[bc]
C2		9.1	23.8	0.420	0.923	2.20[a]
L1		9.6	25.9	0.466	0.923	1.98[bc]
L2		9.2	25.4	0.460	0.919	2.00[bc]
L3		9.5	25.6	0.460	0.931	2.02[b]
P1		9.4	26.3	0.480	0.932	1.94[c]
P2		9.3	25.0	0.448	0.890	1.99[bc]
P3		9.5	26.1	0.475	0.947	2.00[bc]
Physical type	Liquid	9.4	25.6	0.462	0.925	2.00
	Powder	9.4	25.8	0.468	0.923	1.97
Lysine level	0.1	9.5	26.1	0.473	0.928	1.96[b]
	0.2	9.3	25.2	0.454	0.905	1.99[ab]
	0.4	9.5	25.8	0.467	0.939	2.01[a]
Probability (P)						
Physical type		0.9261	0.7262	0.6789	0.9623	0.1026
Lysine level		0.6105	0.3516	0.5004	0.5884	0.0531
Type × level		0.8725	0.7764	0.6527	0.7654	0.7498

[abc] Mean values with different superscripts within the same column in each factor are significantly different ($p < 0.05$).

TABLE 3. AMINO ACID COMPOSITION OF THE EXPERIMENTAL DIETS (%)

Amino acid	C1	C2	L1	L2	L3	P1	P2	P3	M1	M2	M3
Arginine	0.94	0.81	0.83	0.87	0.84	0.82	0.80	0.82	0.81	0.79	0.77
Histidine	0.69	0.42	0.43	0.39	0.37	0.40	0.41	0.41	0.36	0.35	0.35
Isoleucine	0.56	0.50	0.46	0.45	0.45	0.47	0.46	0.48	0.39	0.43	0.37
Leucine	1.13	1.03	1.06	1.03	1.08	1.06	1.08	1.07	1.07	1.04	1.04
Lysine	0.92	0.78	0.87	0.97	1.16	0.86	0.94	1.11	0.75	0.80	0.87
Methionine	0.29	0.24	0.24	0.23	0.24	0.25	0.23	0.24	0.24	0.27	0.32
Phenylalanine	0.74	0.64	0.62	0.61	0.62	0.63	0.64	0.63	0.55	0.56	0.55
Threonine	0.56	0.50	0.52	0.50	0.51	0.50	0.48	0.49	0.45	0.47	0.45
Valine	0.62	0.53	0.58	0.53	0.56	0.58	0.54	0.56	0.43	0.45	0.43
Alanine	0.64	0.59	0.58	0.61	0.62	0.57	0.61	0.59	0.56	0.53	0.62
Aspartic acid	1.14	0.94	0.93	0.93	0.96	0.92	0.93	0.95	0.93	0.93	0.92
Glutamic acid	2.56	2.29	2.33	2.43	2.41	2.35	2.30	2.32	2.33	2.35	2.33
Glycine	1.08	0.94	0.86	0.89	0.89	0.85	0.87	0.89	0.82	0.79	0.76
Proline	0.85	0.78	0.80	0.82	0.78	0.80	0.79	0.81	0.75	0.76	0.77
Serine	0.63	0.55	0.54	0.57	0.56	0.55	0.56	0.56	0.54	0.55	0.57
Tyrosine	0.45	0.40	0.38	0.37	0.38	0.37	0.40	0.39	0.37	0.31	0.34
Total	13.82	11.97	12.05	12.19	12.46	12.01	12.14	12.36	11.36	11.38	11.61

TABLE 4. EFFECTS OF DIFFERENT LEVELS OF DIETARY CP, LYSINE AND METHIONINE ON ADG, ADFI AND FEED EFFICIENCY DURING 6-10 WEEKS OF AGE

Treatment	Initial body wt. (kg)	Final body wt. (kg)	ADG (g)	ADFI (g)	FE
C1	9.6	25.7[a]	0.460[a]	0.916	1.99[c]
M1	9.2	21.7[b]	0.357[b]	0.816	2.28[a]
M2	9.1	22.0[b]	0.369[b]	0.805	2.18[b]
M3	9.2	23.3[b]	0.405[ab]	0.873	2.16[b]

[abc] Mean values with different superscripts within the same column are significantly different ($p < 0.05$).

The data suggest that lysine is the first-limiting amino acid in 16% CP, whereas methionine is not first-limiting amino acid in 15% CP plus lysine diets. Excluding lysine and methionine, threonine, tryptophan or isoleucine would be the next limiting amino acid in a 15% CP diet, based on amino acid contents comparing to NRC nutrient requirements of swine (1988).

According to Hansen et al. (1993b), threonine and methionine are limiting in 15% CP sorghum-soybean meal diets fortified with lysine in growing pigs. Especially, threonine is often more limiting than methionine in some swine diets (Cunha, 1977). Although lysine supplementation to grain alone generally improves performance with reducing dietary CP (Beames et al., 1968) addition of other amino acids, particularly threonine, has been shown to be beneficial (Muller et al., 1967a,b; Aw-Young and Beames, 1975; Hansen et al., 1993a)

Nutrient digestibilities

1) Diet supplemented with lysine alone

Effects of different levels of dietary CP and lysine (liquid or powder) on the digestibilities of the dry matter (DM), crude protein (CP), crude fat (CF), crude ash (CA), total carbohydrate (CHO), phosphorus (P) and energy (E) are summarized in table 5. The lowest crude protein digestibility was found for C2 diet. As a whole lysine supplemented diets were better in DM, CF, CA, CHO, and E digestibilities than no lysine supplemented controls.

Among lysine supplemented groups, addition of 0.2% lysine was better than 0.1% lysine addition in CA digestibility. Except for CA, there was no significant difference in nutrient digestibility on physical types and supplemented lysine levels. Also, no interaction between physical type and lysine supplemented level was observed in nutrient digestibilities.

2) Diets supplemented with lysine and methionine

The significant ($p < 0.05$) effects of different levels of dietary CP and lysine in combination with methionine on digestibilities of CP and CF are shown in table 6. C1 diet was significantly ($p < 0.05$) greater CP digestibility than M1 or M2. Also, crude fat digestibility of C1 diet was significantly ($p < 0.05$) greater than 15% CP diets with amino acid supplementation.

Digestibilities of DM, CF, CHO, P, E was not affected by the different levels of dietary CP and lysine in combination with methionine.

Amino acid digestibilities

1) Diets supplemented with lysine alone

As shown in table 7, the digestibilities of amino acids were affected by the level of dietary crude protein and lysine supplementation. C1 diet showed slightly greater amino acid digestibility than C2 diet. These differences seemed to be caused by the relatively higher content of soybean meal in C1 diet than C2 diet (19.4 vs 16.4%). Soybean meal has more digestible amino acids (Knabe et al., 1989) than those of corn (Lin et al., 1987). Since synthetic lysine could be completely absorbed in the small intestine of pigs, the addition of lysine-HCl increased the digestibility of lysine from 75.9 to 86.6% (no lysine supplemented group vs 0.2% lysine supplemented group).

Digestibilities of leucine and valine increased as lysine level increased. Other amino acids showed no significant differences in physical type and lysine level. Also, no interaction in physical type and lysine supplemented level was found on amino acid digestibilities.

2) Diets supplemented with lysine and methionine

C1 diet was greater amino acid digestibility than M1, M2, and M3 diets (table 8). These differences were likely to be caused by amino acid unbalance or deficiency in 15

TABLE 5. EFFECTS OF DIFFERENT LEVELS OF DIETARY CP AND LYSINE (LIQUID OR POWDER) ON NUTRIENT DIGESTIBILITY (%)

Treatment	Dry matter	Crude protein	Crude fat	Crude ash	Total carbo-hydrate	Energy	P
C1	72.66[b]	66.66[a]	55.14	39.57[c]	80.02[c]	71.06[c]	49.40
C2	73.36[b]	59.78[b]	54.09	40.40[c]	82.13[bc]	71.67[bc]	50.68
L1	76.92[a]	65.24[a]	59.42	42.34[bc]	85.11[ab]	74.56[abc]	56.44
L2	78.97[a]	69.83[a]	57.89	52.55[a]	86.04[a]	77.29[a]	57.66
L3	76.84[a]	66.47[a]	59.47	50.45[ab]	83.99[ab]	75.70[a]	58.04
P1	77.47[a]	67.80[a]	54.75	46.17[abc]	85.38[ab]	76.10[a]	53.45
P2	78.64[a]	68.76[a]	57.79	51.54[a]	86.02[a]	76.77[a]	58.70
P3	77.35[a]	66.84[a]	53.00	46.89[abc]	85.67[a]	74.87[ab]	53.04
Physical type							
Liquid	77.58	67.18	58.92	48.45	85.05	75.85	57.38
Powder	77.82	67.80	55.18	48.20	85.69	75.91	55.06
Lysine level							
0.1	77.20	66.52	57.09	44.26[b]	85.25	75.33	54.94
0.2	78.80	69.29	57.84	52.04[a]	86.03	77.03	58.18
0.4	77.10	66.05	56.24	48.67[ab]	84.83	75.29	55.54
Probability (P);							
Physi. type	0.7934	0.6508	0.2460	0.9224	0.4584	0.9504	0.4808
Lys level	0.2646	0.1970	0.9163	0.0611	0.5154	0.2592	0.6869
Type × level	0.9084	0.5558	0.6969	0.4831	0.6845	0.5503	0.7406

[abc] Mean values with different superscripts within the same column in each factor are significantly different ($p < 0.05$).

TABLE 6. EFFECTS OF DIFFERENT LEVELS OF DIETARY CP, LYSINE AND METHIONINE ON NUTRIENT DIGESTIBILITY (%)

Treatment	Dry matter	Crude protein	Crude fat	Crude ash	Total carbo-hydrate	Energy	P
C1	72.66	66.66[a]	55.14[a]	39.57	80.02	71.06	49.40
M1	72.16	54.80[b]	36.20[b]	41.86	82.74	69.60	48.21
M2	73.35	55.85[b]	39.73[b]	41.80	83.93	71.21	51.52
M3	71.74	59.70[ab]	34.70[b]	42.59	81.21	70.46	50.31

[ab] Mean values with different superscripts within the same column are significantly different ($p < 0.05$).

% CP lysine and methionine supplemented diet. Threonine content in 15% CP diets were lower than NRC requirement (0.47% vs 0.56%). While lysine and methionine content in 15% CP diets were increased by synthetic amino acid supplementation. Digestibilities of lysine and methionine were significantly ($p < 0.05$) improved by supplemented lysine and methionine levels.

Amounts of fecal nitrogen and phosphorus

1) Diets supplemented with lysine alone

Table 9 shows the significant ($p < 0.05$) effects of different levels of dietary CP and lysine in combination with methionine on nitrogen excretion. The highest nitrogen and phosphorous excretion were found for a 16% CP basal diet and 18% CP control diet, respectively.

TABLE 7. EFFECTS OF DIFFERENT LEVELS OF DIETARY CP AND LYSINE ON AMINO ACID DIGESTIBILITY (%)

Treatment	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	VAL	Sub-mean*	Mean**
C1	81.0	71.6	75.4^{ab}	74.2	77.2^{b}	69.2^{ab}	77.9	70.1^{b}	73.2^{ab}	74.4^{ab}	74.3^{ab}
C2	84.2	60.3	74.7^{ab}	73.8	75.9^{b}	65.4^{b}	78.5	70.6^{b}	70.7^{ab}	72.7^{ab}	73.2^{b}
L1	88.1	61.0	72.2^{ab}	76.3	81.0^{ab}	68.7^{ab}	76.5	75.6^{ab}	73.7^{ab}	74.8^{ab}	75.3^{ab}
L2	88.2	60.4	80.0^{a}	80.8	89.5^{a}	75.1^{a}	83.3	82.1^{a}	79.4^{a}	79.8^{a}	80.6^{a}
L3	85.7	58.9	76.7^{ab}	77.8	81.3^{ab}	69.3^{ab}	79.8	75.7^{ab}	78.0^{a}	75.9^{ab}	76.2^{ab}
P1	85.2	62.1	67.0^{a}	73.7	77.5^{b}	70.0^{ab}	75.6	73.0^{ab}	68.3^{b}	72.5^{b}	73.5^{ab}
P2	82.9	64.4	74.9^{ab}	78.4	83.6^{ab}	72.8^{ab}	82.3	79.6^{ab}	75.4^{ab}	77.1^{ab}	76.8^{ab}
P3	87.5	63.3	78.2^{a}	79.5	87.6^{a}	74.8^{ab}	82.8	78.0^{ab}	77.4^{ab}	78.8^{ab}	79.2^{ab}
Physical type											
Liquid	87.3	60.1	76.3	78.3	84.0	71.0	79.9	77.8	77.0	76.8	77.4
Powder	85.2	63.4	73.9	77.5	83.4	72.7	80.7	77.2	74.2	76.5	76.8
Lysine level											
0.1	86.8	61.5	70.0^{b}	75.2	79.5^{b}	69.3	76.1^{b}	74.5	71.4^{b}	73.8	74.5
0.2	85.5	62.4	77.4^{a}	79.6	86.6^{a}	73.9	82.8^{a}	80.9	77.4^{a}	78.5	78.7
0.4	86.6	61.1	77.5^{a}	78.7	84.5^{ab}	72.0	81.3^{ab}	76.8	77.7^{a}	77.3	77.7
Probability (P);											
Physi. type	0.4768	0.4293	0.2413	0.5597	0.6800	0.5617	0.8805	0.7375	0.1453	0.7010	0.6691
Lysine level	0.9424	0.9646	0.0278	0.1441	0.7498	0.3649	0.1002	0.1961	0.0387	0.1107	0.1999
type × level	0.6013	0.9387	0.4374	0.5684	0.1165	0.4420	0.7467	0.7269	0.6502	0.3806	0.3434

* Mean digestibility of essential amino acids except for tryptophan.

** Mean digestibility of all amino acids except for tryptophan.

^{ab} Mean values with different superscripts within the same column in each factor are significantly different ($p < 0.05$).

TABLE 8. EFFECTS OF DIFFERENT LEVELS OF DIETARY CP, LYSINE AND METHIONINE ON AMINO ACID DIGESTIBILITY (%)

Treatment	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	VAL	Sub-mean*	Mean**
C1	81.0	71.6^{a}	75.4^{a}	74.2^{a}	77.2^{a}	69.2^{a}	77.9^{a}	70.1	73.2^{a}	74.4^{a}	74.3^{a}
M1	83.7	54.7^{b}	59.4^{b}	68.4^{b}	67.3^{c}	52.1^{b}	67.6^{b}	63.9	53.8^{b}	63.4^{b}	64.2^{b}
M2	79.9	54.4^{b}	65.2^{b}	66.8^{b}	69.8^{bc}	64.8^{a}	66.9^{b}	62.4	57.6^{b}	65.3^{b}	65.7^{b}
M3	83.1	51.3^{b}	65.8^{b}	67.1^{b}	75.2^{ab}	65.9^{a}	68.2^{b}	68.2	53.5^{b}	66.5^{b}	66.6^{b}

* Mean digestibility of essential amino acids except for tryptophan.

** Mean digestibility of all amino acids except for tryptophan.

^{ab} Mean values with different superscripts within the same column are significantly different ($p < 0.05$).

Lysine supplemented diets were lower in nitrogen excretion (8.38 g vs 9.60 g) and phosphorous excretion (2. 94 g vs 3.54 g) than C1 control diets. Lowering dietary crude protein level by 2% reduced nitrogen excretion up to 19.3%. This value agreed with 20% reduction in nitrogen output reported by Schutte et al. (1990).

2) Diets supplemented with lysine and methionine

Nitrogen excretion of pigs fed C1 diet was less than

TABLE 9. EFFECTS OF DIFFERENT LEVELS OF DIETARY CP AND LYSINE (LIQUID OR POWDER) ON FECAL NITROGEN AND PHOSPHOROUS AMOUNTS AND RELATIVE PERCENTAGE PER KG DIET

Treatment	Nitrogen		Phosphorus	
	(g)	(%)	(g)	(%)
C1	9.60[ab]	100.0	3.54	100.0
C2	10.30[a]	107.2	3.30	93.3
L1	8.90[bc]	92.7	2.92	82.4
L2	7.75[c]	80.7	2.84	80.1
L3	8.68[bc]	90.4	2.81	79.4
P1	8.25[bc]	86.0	3.12	88.1
P2	8.04[c]	83.7	2.77	78.1
P3	8.60[bc]	89.6	3.15	88.8
Physical type				
Liquid	8.45	88.0	2.86	80.8
Powder	8.30	86.5	3.01	85.0
Lysine level				
0.1	8.58	89.4	3.02	85.3
0.2	7.90	82.3	2.80	79.1
0.4	8.64	90.0	2.98	84.2
Probability (P);				
Physi. type	0.6768		0.4799	
Lys level	0.1800		0.6867	
Type × level	0.5537		0.7404	

[abc] Mean values with different superscripts within the same column in each factor are significantly different ($p < 0.05$).

TABLE 10. EFFECTS OF DIFFERENT LEVELS OF DIETARY CP, LYSINE AND METHIONINE ON FECAL NITROGEN AND PHOSPHOROUS AMOUNTS AND RELATIVE PERCENTAGE PER KG DIET

Treatment	Nitrogen		Phosphorus	
	(g)	(%)	(g)	(%)
C1	9.60	100.0	3.54	100.0
M1	10.91	113.7	3.42	96.5
M2	10.71	111.5	3.20	90.3
M3	9.86	102.7	3.28	92.6

those fed 15% diets (9.60 g vs 10.49 g, table 10). The reason might be because amino acid digestibilities were relatively poor for 15% CP diets compared with a 18% CP control diet. Phosphorous excretion of pigs C1 control diet was greater than those of 15% CP diets (3.54 g vs 3.30 g).

A further substantial reduction in nitrogen excretion can be obtained by reducing the protein level more than 2 %. To do so, the diets should be supplemented with synthetic threonine or tryptophan as well as lysine and methionine.

Protein synthesis activity

1) Diets supplemented with lysine alone

Effects of different levels of dietary CP and lysine on retained protein and secreted protein in liver acinar cell culture are summarized in table 11. Retained protein from

TABLE 11. EFFECTS OF DIFFERENT LEVELS OF DIETARY CP AND LYSINE (LIQUID OR POWER) PROTEIN SYNTHETIC ACTIVITY IN LIVER

Treatment	Secreted protein		Retained protein	
	(dpm / mg)	(%)	(dpm / mg)	(%)
C1	1,446.7[ab]	100.0	2,487.8[abc]	100.0
C2	1,026.9[b]	71.0	1,773.6[d]	71.3
L1	1,528.0[a]	105.6	1,849.1[cd]	74.3
L2	1,402.1[ab]	96.9	1,827.6[cd]	73.5
L3	1,578.2[a]	109.1	1,907.5[bcd]	76.7
P1	1,160.3[ab]	80.2	1,799.9[cd]	72.3
P2	1,261.4[ab]	87.2	2,561.7[ab]	103.0
P3	1,567.3[a]	108.3	3,135.8[a]	126.0
Physical type				
Liquid	1,502.8[a]	103.9	1,861.4[b]	74.8
Powder	1,329.7[b]	91.9	2,499.1[a]	100.5
Lysine level				
0.1	1,344.2	92.9	1,824.5[b]	73.3
0.2	1,331.8	92.1	2,194.7[ab]	88.2
0.4	1,572.8	108.7	2,521.6[a]	101.4
Probability (P);				
Physi. type	0.0303		0.0016	
Lys level	0.2136		0.0109	
Type × level	0.4815		0.0187	

[abcd] Mean values with different superscripts with same column in each factor are significantly different ($p < 0.05$).

liver tissue of pigs fed C1 diet was significantly ($p < 0.05$) higher than those fed C2 diet. Also, lysine supplemented diets and C1 control diet were higher in secreted protein than C2 basal diet, and such trend was consistent in retained protein. Among lysine-supplemented diets, the amount of retained protein was significantly ($p < 0.05$) affected by lysine supplementation.

As for physical type, secreted protein from the cultured tissue of pigs fed liquid type was significantly ($p < 0.05$) higher than those fed powder type. Otherwise, the group fed liquid lysine showed lower retained protein than that fed powder lysine.

Interaction between physical type and lysine supplemented level was found in retained protein. As for powder type, retained protein was increased with lysine supplemented level.

2) Diets supplemented with lysine and methionine

As shown in table 12, protein synthesis in liver acinar cell was affected by dietary CP. Secreted protein from tissues of pigs fed, C1 diet was greater than those fed 15 % CP diets, but retained protein tended to be reversed. Retained protein of tissue of pigs fed C1 control diet was less than those fed M1 while retained protein of tissue with C1 control diet was similar to M2 or M3. Reducing CP from 18 to 16% did not affect secreted protein in tissues treated with lysine-fortified diets.

TABLE 12. EFFECTS OF DIFFERENT LEVELS OF DIETARY CP, LYSINE AND METHIONINE ON PROTEIN SYNTHETIC ACTIVITY IN LIVER

Treatment	Secreted protein		Retained protein	
	(dpm / mg)	(%)	(dpm / mg)	(%)
C1	1,446.7[a]	100.0	2,487.8[bc]	100.0
M1	1,245.9[b]	86.1	4,255.6[a]	171.1
M2	1,388.6[ab]	96.0	3,752.9[ab]	150.9
M3	1,298.3[ab]	89.7	2,247.9[c]	90.4

[abc] Means values with different superscript with same column are significantly different ($p < 0.05$).

The supplementation of amino acids such as lysine and methionine had close relationship with protein synthesis in the liver. Reducing dietary protein level by 3 % with addition of lysine and methionine lowered the quantity of secreted protein to the extent of C1 diet, although increased the amount of retained protein.

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