

Effects of Isoleucine Supplementation of a Low Protein, Corn-Soybean Meal Diet on the Performance and Immune Function of Weanling Pigs**

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ABSTRACT : This experiment was conducted to investigate the effects of crystalline isoleucine supplementation of a low protein, corn-soybean meal diet on the performance and immune function of weanling pigs. Forty-five crossbred (Duroc × Landrace × Large White) piglets, weighing an average of 11.00 ± 0.07 kg, were assigned to either a control diet containing 20% crude protein (0.64% isoleucine), a 16% crude protein diet without isoleucine supplementation (0.41% isoleucine) or a 16% crude protein diet supplemented with isoleucine (0.64% isoleucine). Reducing the crude protein content of the diet from 20 to 16% significantly ($p < 0.05$) reduced both average daily gain and feed intake. Feed conversion also tended ($p = 0.07$) to be poorer for a low protein diet without isoleucine supplementation. Isoleucine supplementation of the 16% crude protein diet increased both gain and feed intake to a level similar to that obtained by pigs fed the 20% crude protein diet ($p > 0.05$). Blood urea nitrogen, serum total protein and serum globulin were significantly ($p < 0.05$) higher for pigs fed the unsupplemented 16% crude protein diet than for pigs fed the isoleucine-supplemented diet or the control. Egg albumin antibody titre decreased significantly ($p < 0.05$) in pigs fed the diet with isoleucine supplementation, whereas the antibody titre of pigs fed the low protein and low isoleucine diet was similar to that of pigs fed the diet containing 20% crude protein and 0.64% isoleucine. It was suggested that crystalline isoleucine supplementation of a low protein and low isoleucine diet improved pig performance but suppressed humoral immune function. (*Asian-Aust. J. Anim. Sci.* 2001. Vol. 14, No. 1 : 70-76)

Key Words : Isoleucine, Pig, Performance, Immune Function, Protein Deficiency

INTRODUCTION

In the early 1940's, Cannon (1942) suggested that antibody production was dependent upon the intake of amino acids and could be impaired by an inadequate intake of dietary protein. As a consequence, a considerable amount of research has been conducted on the role of dietary protein in regulating immune function in many species (for reviews see Chandra and Newberne, 1977; Gershwin et al., 1985; Chandra, 1992).

In recent years, diets containing lower levels of crude protein have been used increasingly by the swine industry in an attempt to save valuable protein feed resources, reduce feed costs and to minimize environmental pollution caused by nitrogen excretion in feces and urine (Zheng et al., 1999). Crystalline lysine and methionine are widely used in diet formulation while tryptophan and threonine are also used by some

feed manufacturers (Lenis and Jongbloed, 1999). This reliance on synthetic amino acids might result in a lack of branched-chain amino acids, particularly isoleucine and valine, in the diet (Zheng and Li, 1999).

There have been very few studies examining the role of individual amino acid in immune function (Austic et al., 1991). However, dietary leucine overload has been shown to suppress the immune system in rats (Chevalier and Aschkenasy, 1977) while this suppression was prevented by supplementing isoleucine and valine (Aschkenasy, 1979). In addition, several reports have shown that a deficiency of dietary isoleucine reduced the production of white blood cells including lymphocytes (Aschkenasy, 1964, 1966, 1975). These findings suggest a role for isoleucine as a modulator of the immune system. To date, no studies have been conducted to determine if isoleucine has any effect on immune function in pigs. Therefore, this study was conducted to investigate the effects of crystalline isoleucine supplementation of a low protein, corn-soybean meal diet on the performance and immune function of weanling pigs.

MATERIALS AND METHODS

Animals and diets

Forty-five crossbred (Duroc × Landrace × Large White) piglets, weighing an average of 11.00 ± 0.07 kg, were assigned on the basis of sex and weight to

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one of three dietary treatments during a 28 day growth trial (table 1). The treatments consisted of a control diet containing 20% crude protein (0.64% isoleucine), a 16% crude protein diet without isoleucine supplementation (0.41% isoleucine) or a 16% crude protein diet supplemented with isoleucine (0.64% isoleucine). Ajinomoto Animal Nutrition (Tokyo, Japan) provided the crystalline isoleucine.

The experimental diets were based on corn and soybean meal and contained spray dried animal blood cells which are low in isoleucine (NRC, 1998). The low protein diets were supplemented with synthetic lysine, methionine, threonine and tryptophan so that they provided a similar balance of amino acids as did the 20% control diet. Sufficient vitamins and minerals were added to meet the recommended levels of the National Research Council (NRC, 1998). All diets were fed in mash form.

The experimental animals had been weaned at 35 days old and were 42 days old at the beginning of the experiment. They were housed in groups of three in 170 cm × 175 cm steel wire floored pens equipped with self-feeders. Five pens were assigned to each dietary treatment comprised of three pens that housed gilts and two pens that housed castrates. The pens were located in an environmentally controlled building with the temperature in the pig barn maintained at 23-27°C. The pigs had ad libitum access to feed and water throughout the experiment.

Experimental procedures

On day 14 of the trial, two pigs were randomly selected from each pen and injected subcutaneously immediately behind the lower ear with egg albumin at a dose of 1 mg/kg body weight. Twenty-four hours before the end of the trial, the remaining pig from each pen was injected with 0.5 ml phytohemagglutinin (PHA) (400 mg/ml) in the right flank and 0.5 ml physiological salt in the left flank. Double skinfold thickness at both flanks was measured with a constant pressure dial micrometer twenty-four hours after injection of egg albumin (Blecha et al., 1983).

At the end of the trial, a blood sample was taken from the anterior vena cava of each pig using an uncoated vacutainer tube. Blood samples were centrifuged at 3500 rpm for 15 min. The serum was then separated and stored at -20°C until required for analysis.

Chemical analysis of diets

The crude protein, calcium and phosphorus content of the diets were determined using the methods of the AOAC (1990). Crude protein was analyzed using the Kjeldahl method (AOAC method 988.05), calcium by titration with 0.1 N KMnO₄ (AOAC method 927.02) and total phosphorus was

determined colorimetrically using a molybdovanadate reagent (AOAC method 965.05). Samples of the diets were hydrolyzed with 6 M HCl at 110°C for 24 hours and analyzed for amino acids using an amino acid auto analyzer (Hitachi L-8800, Tokyo, Japan). Methionine and cysteine were determined using formic acid (9 parts of 88% formic acid plus 1 part 30% hydrogen peroxide) protection before acid hydrolysis.

Determination of serum constituents

Free amino acid concentrations in serum were measured with an amino acid analyzer (Hitachi L-8800, Tokyo, Japan) after being deproteinized with

Table 1. Ingredient levels and chemical composition of diets fed to determine the effects of isoleucine supplementation on performance and immune function of weanling pigs

	20% crude protein	16% crude protein	16% crude protein + isoleucine
Ingredients (%)			
Corn	62.70	76.10	76.10
Soybean meal	22.60	7.40	7.40
Soybean oil	1.00	0.50	0.50
Spray dried animal blood cells	4.50	6.00	6.00
Dried whey	6.00	6.00	6.00
Limestone	1.00	1.05	1.05
Dicalcium phosphate	1.10	1.15	1.15
Vitamin-mineral premix ¹	1.00	1.00	1.00
Lysine	0.04	0.30	0.30
Methionine	0.06	0.20	0.20
Threonine	-	0.13	0.13
Tryptophan	-	0.07	0.07
Isoleucine	-	-	0.23
Chemical analysis (% as fed)			
Crude protein	20.03	16.03	16.10
Calcium	0.75	0.72	0.76
Total phosphorus	0.62	0.59	0.60
Lysine	1.22	1.23	1.25
Methionine+cysteine	0.69	0.67	0.64
Threonine	0.77	0.73	0.74
Isoleucine	0.64	0.41	0.65
Leucine	2.21	2.02	1.99
Valine	1.22	1.08	1.14

¹ Premix provided the following per kg of complete diet: vitamin A, 5512 IU; vitamin D₃, 2200 IU; vitamin E, 64 IU; vitamin K, 2.2 mg; riboflavin, 5.5 mg; D-pantothenic acid, 13.8 mg; niacin, 30.3 mg; choline chloride, 551 mg; vitamin B₁₂, 27.6 µg; Mn, 100 mg; Fe, 100 mg; Zn, 100 mg; Cu, 250 mg; I, 0.3 mg; Se, 0.3 mg.

4% sulfosalicylic acid. Ninhydrin was used as a detection reagent. Serum urea nitrogen, glucose, total protein, and albumin were measured using a Technicon RA-1000 auto analyzer using reagents supplied by the Zhongsheng High-Tech Bioengineering Company (Beijing, China). Serum insulin and cortisol concentrations were determined by radioimmunoassay. The kits were supplied by Furui Bioengineering Company (Beijing, China). Enzyme linked immunosorbent assay (ELISA) optical density values for egg albumin antibody titers were tested following the procedures of Yang (1996) using a 511 ELISA Reader (Third Analytical Equipment Factory, Shanghai, China).

Statistics

Data were analyzed using a one way analysis of variance (SPSS 6.0). Where appropriate, differences among treatment means were compared using Duncan's multiple range test. The differences in double skinfold thickness before and after injection of phytohemagglutinin were analyzed according to paired sample *t* tests.

RESULTS

Pig performance

Reducing the protein content of the diet from 20 to 16% significantly ($p < 0.05$) reduced both average daily gain and feed intake (table 2). Feed conversion also tended ($p = 0.07$) to be poorer for pigs fed the lower protein diet. Supplementation of the 16% crude protein diet with isoleucine increased both gain and intake to a level similar to that obtained by pigs fed

the 20% crude protein diet ($p > 0.05$). Pig performance did not differ between male and female pigs.

Urea nitrogen, glucose, insulin and cortisol in serum

Blood urea nitrogen for pigs fed the unsupplemented 16% crude protein diet was 36% higher ($p < 0.05$) than that for pigs fed the 20% crude protein diet and 27% ($p < 0.05$) higher than that for pigs fed the isoleucine-supplemented 16% crude protein diet (table 3). The serum glucose concentration of pigs fed the unsupplemented 16% crude protein diet was 10% ($p < 0.05$) and 26% ($p < 0.05$) higher than for the 16% crude protein diet supplemented with isoleucine and the control. Serum insulin and cortisol concentrations did not differ between treatments ($p > 0.05$).

Free amino acids in serum

The serum isoleucine levels for pigs fed the low protein, isoleucine-supplemented diet were 63% ($p < 0.05$) and 56% ($p < 0.05$) lower than that of pigs fed the 20% crude protein diet and the unsupplemented 16% crude protein diet, respectively (table 4). The concentrations of total essential amino acids, total non-essential amino acids, and total amino acids in serum were not significantly affected by treatment ($p > 0.05$).

Serum proteins

Serum total protein and serum globulin were significantly ($p < 0.05$) higher for pigs fed the unsupplemented 16% crude protein diet than for pigs fed the 20% crude protein diet and the isoleucine-

Table 2. Effects of isoleucine supplementation of the diet on weanling pig performance from 11 to 24 kg¹

	20% Crude protein	16% Crude protein	16% Crude protein + Isoleucine	SEM ²	p value
Daily gain (kg)	0.47 ^a	0.27 ^b	0.49 ^a	0.026	0.0001
Daily feed intake (kg)	0.97 ^a	0.68 ^b	1.01 ^a	0.033	0.0001
Feed conversion	2.13	2.52	2.05	0.14	0.07

¹ Means in the same row followed by different superscripts differ at the *p* values indicated.

² Standard error of the mean.

Table 3. Effects of isoleucine supplementation of the diet on the concentrations of blood urea nitrogen, glucose, insulin, and cortisol in the serum of weanling pigs¹

	20% Crude protein	16% Crude protein	16% Crude protein + Isoleucine	SEM ²	p value
Blood urea nitrogen (mg/100 mL)	5.5 ^a	7.5 ^b	5.9 ^a	0.55	0.03
Glucose (mg/100 mL)	77.3 ^a	97.6 ^b	89.1 ^b	5.0	0.03
Insulin (IU/mL)	9.6	7.9	8.7	0.56	0.11
Cortisol (ng/mL)	105	98	101	8	0.82

¹ Means in the same row followed by the different superscripts differ at the *p* values indicated.

² Standard error of the mean.

Table 4. Effects of isoleucine supplementation of the diet on concentrations of free amino acids in the serum of pigs¹

Amino acids	20% Crude protein	16% Crude protein	16% Crude protein + Isoleucine	SEM ²	p value
Arginine	2.72	3.04	2.38	0.34	0.439
Histidine	2.41	2.52	2.20	0.26	0.722
Isoleucine	1.28 ^a	1.08 ^a	0.47 ^b	0.16	0.012
Leucine	3.86	3.53	2.95	0.38	0.303
Lysine	5.22	5.84	3.83	0.63	0.133
Methionine	0.86 ^a	1.07 ^{ab}	1.26 ^b	0.10	0.045
Phenylalanine	2.32 ^a	3.14 ^b	4.29 ^c	0.25	0.001
Threonine	4.79	4.67	6.00	0.69	0.436
Valine	5.11	4.81	4.64	0.43	0.721
Total essential amino acids ³	28.57	29.71	28.01	2.17	0.84
Alanine	6.30	6.47	5.77	0.47	0.626
Aspartic acid	1.87	1.93	1.92	0.17	0.945
Cysteine	0.91	1.00	0.72	0.11	0.278
Glutamic acid	4.81 ^a	5.13 ^a	3.28 ^b	0.43	0.032
Proline	3.24	3.68	3.34	0.31	0.455
Glycine	8.21	7.64	7.12	0.89	0.706
Serine	2.55	2.60	2.70	0.23	0.915
Tyrosine	1.77 ^a	2.44 ^b	2.31 ^{ab}	0.20	0.023
Total non-essential amino acids	29.66	30.88	27.16	1.81	0.421
Total amino acids ³	58.23	60.59	55.17	3.60	0.610

¹ Means in the same row followed by different superscripts differ at the p values indicated.² Standard error of the mean.³ Tryptophan was not included in the calculation of total essential amino acids or total amino acids.**Table 5.** Effects of isoleucine supplementation of the diet on serum proteins of weanling pigs¹

	20% Crude protein	16% Crude protein	16% Crude protein + Isoleucine	SEM ²	p value
Serum total protein (g/100 ml)	5.46 ^a	6.89 ^b	5.60 ^a	0.40	0.034
Serum albumin (g/100 ml)	2.74	3.43	2.55	0.28	0.076
Serum globulin (g/100 ml)	2.72 ^a	3.46 ^b	3.05 ^{ab}	0.18	0.028
Globulin/albumin ratio	1.02	0.98	0.86	0.08	0.350

¹ Means in the same row followed by different superscripts differ at the p values indicated.² Standard error of the mean.

supplemented 16% crude protein diet, respectively (table 5). Serum albumin and the globulin/albumin ratio were unaffected by dietary treatment.

Double skinfold thickness and egg albumin antibody titre

According to paired sample t tests, injection of phytohemagglutinin significantly increased double skinfold thickness at the flank compared with injection of saline ($p < 0.05$, table 6). Egg albumin antibody titers were significantly lower ($p < 0.05$) for pigs fed the low protein, isoleucine supplemented diet than for pigs fed either the unsupplemented 16% crude protein diet or the control.

DISCUSSION

There is little literature reporting on the effects of crystalline isoleucine supplementation of swine diets on pig performance (Brinegar et al., 1950; Becker et al., 1957; Mitchell et al., 1968b; Lewis et al., 1979). However, the available literature has established that the isoleucine requirement of 10-20 kg pigs is 0.63% (NRC, 1998).

In a corn-based diet, the order in which amino acids become limiting for the pig growth is lysine, tryptophan, threonine and then isoleucine (Grosbach et al., 1985). For the present experiment, the 16% crude protein diet without isoleucine was supplemented with

Table 6. Effects of isoleucine supplementation of the diet on double skinfold thickness after phytohemagglutinin injection and egg albumin antibody titre¹

Double skinfold thickness (mm)	20% Crude protein	16% Crude protein	16% Crude protein + Isoleucine	SEM ²	p value
Before injection					
Left flank	1.77	1.59	1.66	0.127	0.63
Right flank	1.76	1.61	1.78	0.121	0.56
After injection					
Left flank	1.91	1.71	1.81	0.133	0.60
Right flank	3.40	3.32	3.20	0.294	0.89
Increase					
Left flank	0.14	0.12	0.15	0.026	0.68
Right flank	1.64	1.71	1.42	0.249	0.70
Difference between left and right flank	1.50	1.59	1.27	0.258	0.67
Egg albumin antibody titer	1.07 ^a	0.94 ^a	0.59 ^b	0.09	0.003

¹ Means in the same row followed by different superscripts differ at the p values indicated.

² Standard error of the mean.

sufficient amino acids to ensure that isoleucine became the limiting amino acid. The improvement in pig performance obtained when isoleucine was added to the diet was a typical the results of many other studies showing improved pig performance as a result of supplementation of an unbalanced ration with the limiting amino acid (Lewis et al., 1981; Hansen et al., 1993; Li et al., 1999).

Contrary to our findings, Russell et al. (1983) reported that the addition of isoleucine to a low protein (12%) corn-soybean meal diet fortified with lysine, methionine, threonine and tryptophan did not affect growth rate and feed efficiency in 18-35 kg pigs. However, in that experiment, the isoleucine level (0.61%) of the negative control diet, with no isoleucine supplementation, exceeded the value (0.51%) recommended for growing pigs (20~50 kg) by NRC (1998) and the levels of dietary lysine, threonine and tryptophan were lower than the values recommended by NRC (1998). In other words, isoleucine was not the limiting factor in the diet. Therefore, it is not surprising that an improvement in pig performance was not observed.

Blood urea nitrogen concentration has been suggested to be a useful rapid response criterion to determine amino acid requirements (Brown and Cline, 1974; Coma et al., 1995). It has been shown that the addition of an amino acid to a basal diet first-limiting in that amino acid will result in a decrease in blood urea nitrogen (Coma et al., 1995). Our finding of a reduction in blood urea nitrogen, as a consequence of isoleucine addition to the diet, provides support for the concept that isoleucine was the limiting factor in the low protein diet without isoleucine supplementation.

The assay for serum amino acids produced results contrary to our expectations. Most experiments have

shown that when a diet that is limiting in an amino acid is supplemented with that amino acid, the serum concentrations of the limiting amino acid increase with increasing levels of supplementation (Mitchell et al., 1968a; Lewis et al., 1981). In the present experiment, when the 16% crude protein diet was supplemented with isoleucine, serum isoleucine levels declined. We have no explanation for this anomaly. However, to ensure that an error had not occurred, this portion of the experiment was repeated and similar results were obtained.

Since the growth rate of pigs fed the unsupplemented 16% crude protein diet was significantly lower than that for pigs fed the other two treatments, their use of amino acids for muscle tissue synthesis would also be expected to be lower. As a consequence, more amino acids may have been available for synthesis of serum proteins, which would account for the higher levels of serum total protein and globulin for this treatment.

The intradermal response to the mitogen phytohemagglutinin was used as an indicator of in vivo cellular immunity. The increase in double skinfold thickness at the pigs right flank, where it had been injected with phytohemagglutinin, was significantly greater than at its left flank, where it had received an injection of physiological salt. This indicates that the phytohemagglutinin was stimulatory. However, the magnitude of the increase in double skinfold thickness was unaffected by dietary treatment indicating that isoleucine deficiency does not appear to affect cellular immunity.

Egg albumin antibody titres in the pigs which were fed the low protein, isoleucine-supplemented diet were significantly lower than those of pig's fed the low protein, unsupplemented diet indicating that isoleucine

has a depressing effect on humoral immunity. This result was unexpected as isoleucine supplementation had previously been shown to be beneficial in overcoming a depression in the immune system of rats caused by dietary leucine overload (Aschkenasy, 1979). It is unclear if this discrepancy is due to species differences or due to the fact that leucine was not present in excess levels in the present experiment. In addition, we cannot speculate on any potential mechanisms through which supplementation with crystalline isoleucine could negatively impact on immune function.

Although crystalline isoleucine supplementation suppressed immune function, pig performance was significantly improved as a result of isoleucine supplementation. These results are not necessarily in conflict as previous experiments (Williams et al., 1997a, b, c; Stahly, 1996) have shown an interaction between immune function and amino acid nutrition in pigs. Immune system activation changes metabolic processes within the body such that nutrients are deviated from the growth process and directed towards support of the immune system (Beisel, 1977). It is possible that more nutrients will be available for growth instead of being utilized for the immune system, if immune function is suppressed.

To our knowledge, our study is the first showing an effect of isoleucine on immune function in pigs. However, other amino acids have been implicated in immune function both in pigs and in other species. For example, in swine, excess leucine has been shown to reduce immune function (Gatnau et al., 1995) while dietary threonine was stimulatory (Li et al., 1999). Excess leucine has also been shown to adversely affect immune function in sheep (Kuhlman et al., 1988) and rats (Aschkenasy, 1979). In the rat, excess methionine has been shown to suppress humoral immune function while a deficiency of tryptophan or phenylalanine decreased antibody production (Gershoff et al., 1968). Additional studies on the role of amino acids in immune function have been reviewed by Austic et al. (1991).

Despite the observation of a negative effect of isoleucine on immune function, it is important to remember that swine diets that meet normally recommended nutrient levels, formulated using conventional feedstuffs, will not lack isoleucine. With the exception of blood meal (and other blood-based products), which has a high leucine and low isoleucine concentration, the content of branched-chain amino acids is relatively high in ingredients such as fishmeal, meat and bone meal, soybean meal, rapeseed meal and cottonseed meal (NRC, 1998). Therefore, the likelihood that negative effects on the immune system will occur as a result of isoleucine supplementation, under most

commercial situations, is exceedingly remote.

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REFERENCES

- AOAC. 1990. Official Methods of Analysis. 15th edn. Association of Official Analytical Chemists, Arlington, Virginia.
- Aschkenasy, A. 1964. Poid des organes lymphoides et des surrenales dans diverses carences en acides amines essentiels chez le rat male. *C. R. Soc. Biol. (Paris)*. 158:479-483.
- Aschkenasy, A. 1966. Effets de divers melanges d'acides amines sur la regeneration des leucocytes chez des rats carences en proteines. II. Lymphocytes. *C. R. Soc. Biol. (Paris)*. 160:1787-1792.
- Aschkenasy, A. 1975. Dietary proteins and amino acids in leucopoiesis: Recent hematological and immunological data. *World Rev. Nutr.* 21:151-197.
- Aschkenasy, A. 1979. Prevention of the immunodepressive effects of excess dietary leucine by isoleucine and valine in the rat. *J. Nutr.* 109:1214-1222.
- Austic, R. E., R. R. Dietert, Y. L. Sung and R. L. Taylor. 1991. Amino acids in immune function. *Proc. Cornell Nutr. Conf.* pp. 109-114.
- Becker, D. E., A. H. Jensen, S. W. Terrill, I. D. Smith and H. W. Norton. 1957. The isoleucine requirement of weanling swine fed two protein levels. *J. Anim. Sci.* 16:26-34.
- Beisel, W. R. 1977. Metabolic and nutritional consequences of infection. In: *Advances in Nutritional Research*. Vol. 1 (Ed. H. H. Draper). Plenum, New York, NY. p. 125.
- Blencha, F. D. S. Pollmann and D. A. Nichols. 1983. Weaning pigs at an early age decreases cellular immunity. *J. Anim. Sci.* 56:397-400.
- Brinegar, M. J., J. K. Loosli, L. A. Maynard and H. Williams. 1950. The isoleucine requirement for growth of swine. *J. Nutr.* 42:619-624.
- Brown, J. A. and T. R. Cline. 1974. Urea excretion in the pig: An indicator of protein quality and amino acid requirements. *J. Nutr.* 104:542-545.
- Brudevold, A. B. and L. L. Southern. 1994. Low protein, crystalline amino acid supplemented, sorghum-soybean meal diets for the 10 to 20 kilogram pig. *J. Anim. Sci.* 72:635-647.
- Cannon, P. R. 1942. Antibodies and the protein reserves. *J. Immunol.* 44:107-114.
- Chandra, R. K. and P. M. Newberne. 1977. Nutrition, Immunity and Infection: Mechanisms of Interactions. Plenum Press, New York, New York. p. 246.
- Chandra, R. K. 1992. Protein-energy malnutrition and immunological responses. *J. Nutr.* 122:597-600.
- Chevalier, P. and A. Aschkenasy. 1977. Hematological and immunological effects of excess dietary leucine in the young rat. *Am. J. Clin. Nutr.* 10:1645-1654.
- Corn, J., D. Carrion and D. R. Zimmerman. 1995. Use of

- plasma urea nitrogen as a rapid response criterion to determine the lysine requirements of pigs. *J. Anim. Sci.* 73:472-481.
- Gatnau, R., D. R. Zimmerman, S. L. Nissen, M. Wannemuehler and R. C. Ewan. 1995. Effects of excess dietary leucine and leucine catabolites on growth and immune responses in weanling pigs. *J. Anim. Sci.* 73:159-165.
- Gershoff, S. N., T. J. Gill, S. J. Simonian and A. I. Steinberg. 1968. Some effects of amino acid deficiencies on antibody formation in the rat. *J. Nutr.* 95:184-189.
- Gershwin, M. E., R. S. Beach and L. S. Hurley. 1985. *Nutrition and Immunity*. Academic Press, New York, NY. p. 417.
- Grosbach, D. A., A. J. Lewis and E. R. Peo. 1985. An evaluation of threonine and isoleucine as the third and fourth limiting amino acids in corn for growing swine. *J. Anim. Sci.* 60:487-494.
- Hansen, J. A., D. A. Knabe and K. G. Burgoon. 1993. Amino acid supplementation of low-protein sorghum-soybean meal diets for 5-20 kilogram swine. *J. Anim. Sci.* 71:452-458.
- Kuhlman, G., J. A. Roth, P. J. Flakoll, M. J. Vandehaar and S. Nissen. 1988. Effects of dietary leucine, α -ketoisocaproate and isovalerate on antibody production and lymphocyte blastogenesis in growing lambs. *J. Nutr.* 118:1564-1569.
- Lenis, P. C. and A. W. Jongbloed. 1999. New technologies in low pollution swine diets: Diet manipulation and use of synthetic amino acids, phytase and phase feeding for reduction of nitrogen and phosphorus excretion and ammonia emission. *Asian-Aus. J. Anim. Sci.* 12:305-327.
- Lewis, A. J., E. R. Peo, B. D. Moser and T. D. Crenshaw. 1979. Additions of lysine, tryptophan, methionine and isoleucine to all-corn diets for finishing swine. *Nutr. Rep. Int.* 19:533-540.
- Lewis, A. J., E. R. Peo, B. D. Moser and T. D. Crenshaw. 1981. Lysine requirements of pigs weighing 5 to 15 kg fed practical diets with and without added fat. *J. Anim. Sci.* 51:361-366.
- Li, D. F., C. Xiao, S. Qiao, J. Zhang, E. W. Johnson and P. A. Thacker. 1999. Effects of dietary threonine on performance, plasma parameters and immune function of growing pigs. *Anim. Feed Sci. Technol.* 78:179-188.
- Mitchell, J. R., D. E. Becker, A. H. Jensen, B. G. Harmon and H. W. Norton. 1968a. Determination of amino acid needs of the young pig by nitrogen balance and plasma-free amino acids. *J. Anim. Sci.* 27:1327-1331.
- Mitchell, J. R., D. E. Becker, B. G. Harmon, H. W. Norton and A. H. Jensen. 1968b. Some amino acid needs of the young pig fed a semisynthetic diet. *J. Anim. Sci.* 27:1322-1326.
- National Research Council. 1998. *Nutrient requirements of swine*. 10th edn. National Academy Press, Washington, DC.
- Russell, L. E., G. L. Cromwell and T. S. Stahly. 1983. Tryptophan, threonine, isoleucine and methionine supplementation of a 12% protein, lysine-supplemented, corn-soybean meal diet for growing pigs. *J. Anim. Sci.* 56:1115-1123.
- Stahly, T. S. 1996. Impact of immune system on growth and regimens of pigs. In: *Recent Advances in Animal Nutrition* (Ed. P. C. Garnsworthy and D. J. Cole). Nottingham University Press, Nottingham, UK. pp. 197-206.
- Williams, N. H., T. S. Stahly and D. R. Zimmerman. 1997a. Effect of chronic immune system activation on the rate, efficiency and composition of growth and lysine needs of pigs fed from 6 to 27 kg. *J. Anim. Sci.* 75:2463-2471.
- Williams, N. H., T. S. Stahly and D. R. Zimmerman. 1997b. Effect of chronic immune system activation on the body nitrogen retention, partial efficiency of rate, efficiency of lysine needs of pigs. *J. Anim. Sci.* 75:2472-2480.
- Williams, N. H., T. S. Stahly and D. R. Zimmerman. 1997c. Effect of chronic immune system activation on the growth and dietary lysine needs of pigs fed from 6 to 112 kg. *J. Anim. Sci.* 75:2481-2496.
- Yang, Hanchun. 1996. *Veterinary Immunology*. China Agricultural University Press, Beijing, China. p. 210.
- Zheng, C. T., D. F. Li, S. Y. Qiao, J. J. Xing and Y. Chen. 1999. Study on formulation of low protein piglet rations with synthetic amino acids. *Feed Research*. No. 11:4-6.
- Zheng, C. T. and D. F. Li. 1999. Research development in branched-chain amino acids nutrition. In: *Proceedings of the First SNU/CAU Joint Seminar on Recent Advances in Swine Nutrition & Feed Processing Technologies*, Suweon, Korea. pp. 70-95.