Effects of L-Carnitine with Different Lysine Levels on Growth and Nutrient Digestibility in Pigs Weaned at 21 Days of Age

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ABSTRACT : This study was conducted to investigate the effects of L-carnitine with different levels of lysine on performance of pigs weaned at 21 days of age. A total of 120 pigs were alloted into a 3×2 factorial design with three different levels of lysine (1.40%, 1.60% and 1.80%) and two levels of L-carnitine (0 and 1,000 ppm). Each treatment had 4 replications with 5 pigs per replicate. Pigs of 22±1 days (5.9 kg of body weight) were grouped into a completely randomized block design. Treatments were 1) 1.4-Crt; 1.40% of lysine with 1,000 ppm of L-carnitine, 2) 1.4-N; 1.40% of lysine without L-carnitine, 3) 1.6-Crt; 1.60% of lysine with 1,000 ppm of L-carnitine, 4) 1.6-N; 1.60% of lysine without L-carnitine, 5) 1.8-Crt; 1.80% of lysine with 1,000 ppm of L-carnitine and 6) 1.8-N; 1.80% of lysine without L-carnitine. Growth performance was optimized in pigs fed 1.6% lysine regardless of carnitine addition. For the first 7 days of the experimental period, the best ADG and F/G were found in pigs within the 1.6-Crt group. During the 14~21 day period, the 1.4-Crt group showed similar performance with the 1.6-Crt group. Carnitine significantly improved ($p<0.05$) ADG of pigs when the lysine level in the diet was 1.6%. Only in the third week carnitine had a significant influence on growth performance of pigs. A lysine-sparing effect of L-carnitine was not detected in this study. The 1.6-Crt group showed the best proximate nutrient digestibility, and the crude fat and gross energy digestibility were higher when the L-carnitine was added in the diet. Lysine level significantly affected the digestibilities of DM ($p<0.001$), GE ($p<0.001$), CP ($p<0.01$) and C. fat ($p<0.05$). Carnitine also significantly improved digestibility of nutrients. Lysine level as well as carnitine level affected the amino acids digestibility, however, in 1.8% lysine diet carnitine did not influence on amino acids digestibility. Plasma carnitine content was significant higher ($p<0.05$) in pigs fed L-carnitine. This indicates the increased biological availability of carnitine within the body. L-carnitine supplementation tended to improve feed utilization during the third week ($p<0.10$) and during the entire period ($p=0.10$). Lysine level significantly affected feed utilization of pigs during the third week and entire period ($p<0.05$). As pigs grew, the lysine requirement was reduced. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 5 : 799-805*)

Key Words : L-carnitine, Lysine, Pig, Growth Performance, Nutrient Digestibility

INTRODUCTION

Carnitine has been known as a naturally occurring vitamin-like compound, and its primary function is to facilitate the transport of long chain fatty acids into mitochondria for energy production (Fritz and Yue, 1963; Bray and Briggs, 1980). In general, carnitine is low in food ingredients of plant origin and high in animal sources (Owen et al., 1997). Previous studies have indicated that a dietary requirement for carnitine exists in neonatal mammals because carnitine is not synthesized in sufficient amounts (Borum et al., 1979; Borum, 1981; Hahn, 1982; Warshaw and Curry, 1980).

Recently, the importance of L-carnitine in the nutrition of neonatal and young pigs has received an intensive interest. Numerous studies with neonatal pigs (Baltzell et al., 1987; Coffey et al., 1991; Honeyfield and Froseth, 1991; Kempen and Odle, 1993, 1995) and weaned pigs (Weeden et al., 1990; Newton and Burtle, 1992; Owen et al., 1994, 1996a,b) have showed that L-carnitine might be beneficial when added to the diets for neonatal and weaned pigs. In constrast, Hoffman et al. (1993) reported that L-carnitine did not affect ADG and energy utilization in neonatal and young pigs. Also, Honeyfield and Froseth (1991) suggested that L-carnitine did not seem to be the limiting factor of piglet energy metabolism in their experiments with neonatal pigs. They emphasized the presence of fat in the sow diet as well as adequate lysine for the piglet for more efficient utilization of energy. Furthermore, Newton and Burtle (1992) suggested that high level of dietary lysine (1.5% total lysine) to be detrimental to growth performance when carnitine was added to the diets.

Though, carnitine has received intensive interest in newly weaned piglet nutrition, there are scare information available on the lysine sparing effect of carnitine in young pigs. Lee (1976) reported that carnitine had a lysine sparing effect in rat and because both lysine and methionine are required for the

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biosynthesis of L-carnitine (Cox and Hoppel, 1973a,b). Therefore, this study was designed to investigate the effect of dietary L-carnitine with different lysine levels for optimum growth of weaned pigs.

MATERIALS AND METHODS

All procedure approved by the IACVC committee of North Carolina State University. A total of 120 weaned piglets (Landrace×Yorkshire×PIC #326) were obtained from the Lake Whealer Field Laboratory of North Carolina State University. Pigs averaged 21 days of age and 5.9 kg of body weight were weaned and housed in 90×160 cm^2 pens with iron slat flooring. Room temperature was maintained at 29±1℃ with a gas heater and the air ventilation was controlled with an electric controller and fan simultaneously during the entire period. On the first day of the pre-experimental period, pigs were fed commercial creep feed for adaptation. Pigs of 22±1 days (5.9 kg of body weight) were grouped into a completely randomized block design with 4 replications, 5 pigs per pen.

Treatments consisted of three different lysine levels (1.40%, 1.60%, 1.80%) and two levels of carnitine (0 ppm and 1,000 ppm). Experimental diets were formulated to contain 3.3 Mcal ME/kg, 21% of crude protein and adequate amounts of vitamins and minerals as suggested by the NRC (1988). Chromic oxide was added as an indigestible marker to allow digestibility determinations.

Pigs were allowed *ad libitum* access to water and diets during the 21 day growth trial, and all diets were provided in mash form. Body weights and feed intakes were measured on days 7, 14 and 21 from the beginning of experiment. Average daily gain (ADG), average daily feed intake (ADFI) and feed/gain (F/G) were calculated on a group basis. Fecal samples were collected on 19~20, dried immediately at 60℃ in air forced drying oven, ground with a 1 mm mesh Wiley mill, and stored in a refrigerator for chemical analysis.

Proximate composition of diets and feces were analyzed according to the methods of AOAC (1990), and Cr was measured by an atomic absorption spectrophotometer (Shimadzu, AA-6410F, Japan). Amino acid contents were determined following acid hydrolysis with 6N HCl for 24 hours, using an automatic amino acid analyzer (Biochrom 20, Pharmacia Biotech., England).

Upon termination of the experiment, blood plasma samples from 1 pig per pen were collected for analyzing the carnitine contents. Carnitine was measured by the method of Bhuiyan et al. (1992). Internal standard hexadecanoyl-[DH_3.^{3}H]carnitine (100 Bq) was added to 100~200 μl plasma followed by 200 μl of ice-cold $HClO_4$ (1 mol/l).

Statistical analysis of data was carried out by comparing means according to LSD (least significant difference) test, using the GLM (general linear model) of the procedure of SAS (1985). Lysine level and carnitine level were used as main effects and their interaction was also examined.

Table 1. Formula and chemical composition of basal diet (%)[1]

Lysine levels (%)	1.40	1.60	1.80
Ingredients :			
Corn	48.68	48.68	48.68
SBM (48%)	23.00	23.00	23.00
Dried skim milk	17.16	17.16	17.16
Soy oil	4.00	4.00	4.00
Corn starch	2.72	2.52	2.32
Monocalciumphosphate	1.64	1.64	1.64
Limestone	0.65	0.65	0.65
Antibiotics	1.00	1.00	1.00
Salt	0.25	0.25	0.25
Vit-min. mixture[2]	0.35	0.35	0.35
Lysine (78%)	0.17	0.37	0.57
Methionine (99%)	0.22	0.22	0.22
Threonine (99%)	0.15	0.15	0.15
Total	100.00	100.00	100.00
Chemical composition[3] :			
ME (mcal/kg)	3.30	3.30	3.30
Crude protein, %	21.00	21.00	21.00
Lysine, %	1.40	1.60	1.80
Met.+Cys., %	0.96	0.96	0.96
Threonine, %	1.04	1.04	1.04
Ca, %	0.90	0.90	0.90
Total P, %	0.80	0.80	0.80

[1] L-carnitine (Lonza, Fair Lawn, NJ) was added to the three basal diets at 1,000 ppm to produce a 3×2 factorial arrangement of treatments.

[2] Vit-min mixture contained per kilogram : Vitamin A, 2,200,000 IU; Vitamin D_3, 500,000 IU; Vitamin E, 10,000 mg; Vitamin K_3, 800 mg; Vitamin B_1, 250 mg; Vitamin B_2, 1,760 mg; Niacin, 8,800 mg; Pantothenic acid, 6,500 mg; Pyridoxine, 440 mg, Choline 90,000 mg; Folic acid, 330 mg; Biotin 23 mg; Vitamin B_{12}, 9 mg; Ca, 67,800 mg; Zn, 42,600 mg; Se, 100 mg; Mn, 19,500 mg; Fe, 32,000 mg; 7,700 mg; I, 450 mg.

[3] Calculated values.

RESULTS AND DISCUSSIONS

1. Growth performance

Growth performance was optimized in pigs fed 1.6% lysine regardless of carnitine addition during entire period (table 2). For the first 7 days of the experimental period, the best ADG and F/G were found in pigs within the 1.6-Crt group. Though, there was no significant difference during the 7~14 day

Table 2. Effects of L-carnitine with different lysine levels on growth performance in pigs weaned at 21 days of age[1]

Lysine, %	1.40		1.60		1.80		SE[2]
L-carnitine, ppm	1,000	0	1,000	0	1,000	0	
0~7 day							
ADG, g	152[b]	148[b]	174[a]	167[ab]	161[ab]	166[ab]	5.1
ADFI, g	194	192	217	213	209	218	6.8
F/G	1.27[ab]	1.31[ab]	1.25[b]	1.27[ab]	1.29[ab]	1.32[a]	0.009
7~14 day							
ADG, g	297	276	311	299	296	293	6.9
ADFI, g	454	436	472	461	465	457	10.1
F/G	1.54	1.58	1.52	1.54	1.57	1.56	0.009
14~21 day							
ADG, g	499[ab]	485[bc]	502[a]	487[bc]	490[abc]	479[c]	4.0
ADFI, g	881	886	882	879	915	904	7.28
F/G	1.77[c]	1.83[abc]	1.76[c]	1.81[bc]	1.86[ab]	1.89[a]	0.015
0~21 day							
ADG, g	316[ab]	303[b]	326[a]	317[ab]	316[ab]	313[ab]	4.4
ADFI, g	510	505	524	517	529	527	6.3
F/G	1.62[bc]	1.67[abc]	1.61[c]	1.63[abc]	1.68[ab]	1.69[a]	0.001

Interaction	Among lysine levels	Between L-carnitine	Lysine × L-carnitine
0~7 day			
ADG, g	0.2994	0.8228	0.8969
ADFI, g	0.4144	0.9458	0.9159
F/G	0.1524	0.1647	0.9502
7~14 day			
ADG, g	0.5925	0.4215	0.8935
ADFI, g	0.7162	0.5989	0.9824
F/G	0.2832	0.2945	0.5220
14~21 day			
ADG, g	0.6283	0.1237	0.9800
ADFI, g	0.2470	0.8431	0.9223
F/G	0.0112	0.0848	0.8377
0~21 day			
ADG, g	0.5692	0.3800	0.9093
ADFI, g	0.4810	0.7340	0.9920
F/G	0.0263	0.1036	0.6157

[1] Average initial body weight was 5.9 kg and the final body weight was 12.5 kg. [2] Standard error.
[a,b,c] Means with different superscripts are significantly different ($p<0.05$).

period, the 1.6-Crt group showed the best performance. During the 14~21 day period, the 1.4-Crt group showed similar performance with the 1.6-Crt group. For the pigs fed the 1.6% lysine diet, carnitine significantly improved ADG. For the overall period, there was no significant difference among treatments except for ADG between 1.4-N and 1.6-Crt groups. For the pigs fed 1.8% lysine, a similar growth performance was found during the entire period.

Ewan (1987) reported that the addition of 700 ppm of L-carnitine to diets fed to pigs weaned at 22 days of age did not improve growth performance. Hoffman et al. (1993) also reported that L-carnitine did not affect ADG and energy utilization in neonatal and young pigs. In that study, total lysine level was 1.65% or 1.85% from day 0 to 21 postweaning which was similar to our study, however, their diets were based on soy-protein and the amounts of soybean oil were 1.2% and 12.3%. The content of oil differed by more than 10% with our study, and they added 800 ppm of carnitine in the diet with factorial design. Also, recent research reported by Tokach et al. (1995) showed that added fat does not improve growth performance from day 0 to 14 postweaning, and weanling pigs require an adjustment period to utilize fat.

On the other hand, Owen et al. (1996a) reported that from d 0 to 14 postweaning, L-carnitine increased ADG ($p<0.08$) and ADFI ($p<0.02$) using diets containing 20% dried whey and the same amount of dried skim milk in pigs weighing 5.6 kg of body weight (19±2 days of age). And the lysine level was 1.45% in the diet. Results from our study showed the best performance of pigs fed 1.60% of lysine with 1,000 ppm of L-carnitine during the first week. Thereafter, pigs fed 1.40% of lysine with 1,000 ppm of L-carnitine showed similar performance with pigs fed 1.6% lysine. Lysine requirement of pigs weaned at 21 days of age was found to be 1.55% during the first week and 1.45% for second and third week postweaning (Jin et al., 1998). However, Newton and Burtle (1992) found high levels of dietary lysine (1.5% total lysine) to be detrimental to growth performance when supplemental L-carnitine was fed to nursery pigs (28 to 42 days of age). In this study, carnitine did not show any effect on the performance of pigs fed 1.8% lysine diet and it might be explained by the report of Newton and Burtle (1992). It seemed that 1.4% lysine for the first week and 1.8% lysine for the entire period might not be adequate for pigs weaned at 21 days of age. Newton and Haydon (1988) reported that improved performance of pigs fed carnitine might be related to lysine intake and the age or size of pigs.

Only in third week postweaning, carnitine had a significant influence on growth performance of pigs in this study. In some reports (Weeden et al., 1990 and 1991; Galvez et al., 1996), carnitine appeared to improve the growth performance of pigs after three to four weeks postweaning. This is in contrast to the assumption that carnitine might improve the growth performance of pigs right after weaning. Galvez et al. (1996) suggested that this might be due to the fact that under certain conditions, carnitine from sow milk during suckling period and biosynthesis by the piglets after weaning together could be sufficient to meet the metabolic need for carnitine.

Lee (1976) suggested that carnitine had a sparing effect on lysine. Because both lysine and methionine are required for the biosynthesis of L-carnitine (Cox and Hoppel, 1973ab), increasing the level of dietary L-carnitine might be expected to spare both lysine and methionine. Considering these results, slightly improved growth rate was found in pigs fed 1.4% lysine after day 7 with 1,000 ppm of L-carnitine. L-carnitine also showed numerically improved growth performance of pigs fed 1.6% lysine diet, however, it is hard to confirm the response of our study as a sparing effect.

Based on growth performance, supplementation of L-carnitine tended to improve the feed utilization during the third week ($p<0.10$) and tended to improve the feed utilization during the entire period ($p=0.10$). Lysine level significantly affected the feed utilization of pigs during the third week and entire period ($p<0.05$). As pigs grew, the lysine requirement was shown to be reduced. The 1.40% of lysine might be too low for the pigs weaned at 21 days of age. However, the performance was improved during 7~21 days.

2. Proximate nutrient digestibility

Proximate nutrient digestibility was improved with increased level of lysine and supplementation of L-carnitine (table 3). The 1.6-Crt group showed the best proximate nutrient digestibility, and the crude fat and gross energy digestibility were higher when the L-carnitine was added in the diet. It can be assumed that L-carnitine improved the fat utilization, thus affecting energy utilization for optimum growth. Since the digestibilities were determined in the third week, improved ADG and F/G of pigs fed 1.6-Crt or 1.4-Crt in the third week could be explained by higher nutrient digestibilities.

Lysine level significantly affected the digestibilities of DM ($p<0.001$), GE ($p<0.001$), CP ($p<0.01$) and C. fat ($p<0.05$), respectively. Carnitine also significantly improved digestibility of nutrients.

Previous studies (Broquist, 1982; Borum, 1983; Bremer, 1983; Rebouche and Paulson, 1986), showed

Table 3. Effects of L-carnitine with different lysine levels on nutrient digestibility in pigs weaned at 21 days of age

Lysine, %	1.40 %		1.60 %		1.80 %		SE[1]
L-carnitine, ppm	1,000	0	1,000	0	1,000	0	
Dry matter	82.58[b]	79.86[e]	83.16[a]	80.57[d]	81.69[c]	79.76[e]	0.272
Crude protein	78.10[ab]	75.79[bc]	79.86[a]	76.15[c]	77.18[bc]	74.18[d]	0.429
Crude fat	68.91[ab]	64.23[bc]	73.32[a]	67.43[b]	63.77[bc]	60.95[c]	1.064
Phosphorus	46.77[a]	36.08[d]	49.11[a]	40.23[cd]	46.14[ab]	41.38[bc]	1.179
Gross energy	80.14[b]	77.94[c]	81.68[a]	78.62[c]	79.84[b]	78.20[c]	0.287

[a,b,c,d,e] Means with different superscripts are significantly different ($p<0.05$). [1] Standard error.

Table 4. Effects of L-carnitine with different lysine levels on essential amino acids digestibility in pigs weaned at 21 days of age

Lysine, %	1.40 %		1.60 %		1.80 %		SE[1]
L-carnitine, ppm	1,000	0	1,000	0	1,000	0	
Threonine	82.00^{b}	80.80^{b}	84.84^{a}	81.25^{b}	79.40^{b}	80.44^{b}	0.474
Valine	72.38^{b}	68.22^{c}	78.53^{a}	73.41^{b}	73.95^{b}	75.19^{ab}	0.791
Methionine	80.21^{b}	81.71^{ab}	81.69^{ab}	76.68^{c}	82.51^{ab}	83.36^{a}	0.547
Isoleucine	76.25^{b}	70.47^{c}	83.07^{a}	78.92^{b}	78.59^{b}	83.01^{a}	0.970
Leucine	80.61^{a}	78.49^{ab}	80.91^{a}	79.20^{a}	75.78^{c}	76.09^{bc}	0.514
Phenylalanine	75.36^{c}	65.57^{d}	78.98^{bc}	76.04^{c}	80.44^{ab}	84.10^{a}	1.317
Lysine	80.32^{bc}	78.89^{c}	85.46^{a}	81.67^{b}	87.12^{a}	81.24^{bc}	0.671
Histidine	85.78^{ab}	82.41^{c}	87.16^{a}	84.91^{b}	85.57^{ab}	80.67^{c}	0.515
Argine	88.57^{ab}	87.18^{ab}	90.57^{a}	86.23^{ab}	84.24^{b}	78.56^{c}	0.932
Essential AA	80.12^{b}	77.39^{c}	83.55^{a}	79.95^{b}	80.82^{b}	80.06^{b}	0.472

[a,b,c] Means with different superscripts are significantly different ($p<0.05$). [1] Standard error.

Table 5. Effects of L-carnitine with different lysine levels on non-essential amino acids digestibility in pigs weaned at 21 days of age

Lysine, %	1.40 %		1.60 %		1.80 %		SE[1]
L-carnitine, ppm	1,000	0	1,000	0	1,000	0	
Aspartic acid	82.52^{ab}	78.80^{cd}	83.99^{a}	80.48^{bc}	76.08^{de}	73.41^{e}	0.857
Serine	82.91^{ab}	79.58^{cd}	84.15^{a}	80.95^{bc}	77.62^{d}	77.32^{d}	0.627
Glutamic acid	87.10^{a}	84.98^{a}	86.22^{a}	84.83^{a}	80.53^{b}	78.08^{b}	0.734
Proline	86.52^{a}	85.18^{a}	83.53^{a}	83.43^{a}	68.57^{c}	75.54^{b}	1.422
Glycine	69.69^{ab}	61.06^{d}	73.62^{a}	66.11^{bcd}	63.14^{cd}	67.14^{bc}	1.072
Alanine	69.44^{ab}	63.93^{c}	73.33^{a}	69.86^{ab}	66.65^{bc}	68.89^{b}	0.768
Cystine	92.15^{ab}	92.64^{a}	91.71^{ab}	84.38^{c}	87.00^{c}	88.02^{bc}	0.846
Tyrosin	81.93^{a}	72.01^{b}	72.12^{b}	64.66^{c}	68.65^{bc}	72.40^{b}	1.247
Non-essential AA	82.85^{a}	79.82^{b}	82.81^{ab}	80.04^{ab}	75.48^{c}	75.21^{c}	0.728
Total AA	81.54^{ab}	78.62^{c}	83.19^{a}	80.00^{bc}	78.35^{c}	77.78^{c}	0.501

[a,b,c,d,e] Means with different superscripts are significantly different ($p<0.05$). [1] Standard error.

that added L-carnitine improved fat utilization, especially fatty acids in β-oxidation in mammals.

3. Amino acid digestibility

Limited research has been conducted concerning effect of dietary L-carnitine on amino acid digestibility. Digestibility of essential amino acids was significantly higher ($p<0.05$) in the 1.6-Crt group than any other groups (table 4). Lysine level as well as carnitine level affected the amino acids digestibility, however, in the 1.8% lysine diet, carnitine did not influence amino acid digestibility.

Lysine digestibility was significantly higher in the 1.6-Crt and 1.8-Crt groups than in any other group. Lysine and methionine digestibilities were higher as the level of lysine was increased, and the L-carnitine supplemented group when the lysine level was 1.40% or 1.60%. Branch-chain amino acids (isoleucine, leucine and valine) digestibility showed a similar trend with those of lysine and methionine. Owen et al. (1996b) discussed about lysine, methionine, isoleucine, leucine and valine concentrations in muscle tissue and suggested that those amino acids were elevated consistently in finishing pigs with carnitine supplementation. They concluded that essential amino acids were not synthesized *in vivo*, thus possibly it seemed a sparing effect. The present study showed a similar effect in pigs fed 1.40% of lysine with 1,000 ppm of L-carnitine. As shown in Table 4 essential amino acid digestibility of the 1.4-Crt group was significantly lower than that of the 1.6-Crt group, however significantly higher than that of 1.4-N group.

Non-essential amino acids digestibility showed a similar trend with essential amino acid digestibility (table 5).

4. Plasma carnitine content

Plasma carnitine content was significantly higher in pigs fed L-carnitine. However, dietary lysine level was not shown to affect the plasma carnitine content. It is agreed with the previous studies (Owen et al., 1996a; Kempen and Odle, 1995) that the absorption and uptake of carnitine within the body was linearly increased by dietary supplementation. This indicate the increased biological availability of carnitine within the body (Owen et al., 1996b).

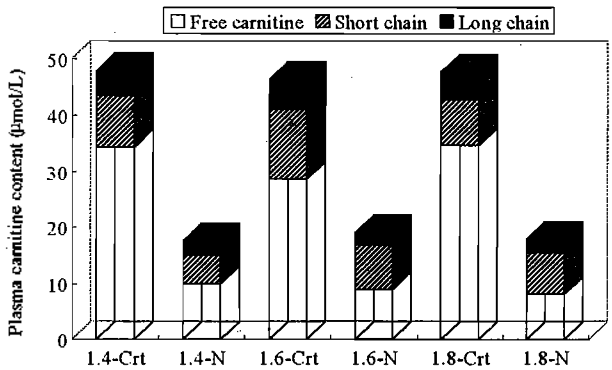

Figure 1. Effects of L-carnitine with different lysine levels on changes of plasma carnitine content in pigs weaned at 21 days of age (μ mol/L)

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