Effect of L-Carnitine and Source of Dietary Fat on Growth Performance and Serum Biochemical Parameters of Piglets Weaned at 35 Days of Age

Defa Li*, Q. Qiao, E. W. Johnson, J. Jiang, F. Wang, R. Blum[1] and G. Allee[2]
Ministry of Agriculture Feed Industry Center, China Agricultural University
No. 2. Yuanmingyuan West Road, Haidian Beijing, China 100094

ABSTRACT : The effects of carnitine in diets with or without added fat (5% lard or soybean oil) were evaluated in 72 Large White×Landrace×Duroc pigs weaned at 35 days of age. Pigs were fed a 1.30% lysine corn-soybean basal diet+15% dried whey+4% fish meal with carnitine at 0 or 50 mg/kg and either 0% added fat, 5% soybean oil or 5% lard for 6 weeks in a 2×3 factorial trial (6 treatments, 3 pens per treatment, 4 pigs per pen). Addition of carnitine increased average daily gain (ADG) and average daily feed intake (ADFI) in the second two weeks of the six-week trial and overall, but had no significant effect on feed per gain (F/G). Lard alone depressed ADG ($p<0.05$) in the last two weeks of the trial and overall, but the ADG for pigs fed lard+carnitine was similar to the control. Lard reduced feed intake in the first two weeks of the trial ($p<0.05$). Carnitine reduced the percentage of pigs with poor (ADG<375 g/d) growth (15 vs 40%; $p<0.05$). The greater uniformity of growth was most evident in low-weaning-weight pigs in the second period (16 vs 62%, $p<0.005$). Addition of fat did not produce any positive effect on uniformity and had no interaction with carnitine on uniformity. Carnitine addition increased serum total carnitione and short-chain acyl-carnitine levels ($p<0.05$), but did not modify free carnitine levels. Serum carnitine levels were lower at weaning than at 14, 28 or 39 days after weaning ($p<0.05$). Carnitine increased serum protein levels on day 14 ($p<0.05$). Addition of fat in the form of soybean oil or lard did not improve piglet growth performance. Addition of 50 mg/kg of carnitine to the diet of weanling pigs enhanced postweaning performance. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 8 : 1263-1272*)

Key Words : Carnitine, Fat, Weaning, Pig, Growth

INTRODUCTION

Many studies have examined the effects of supplementing pigs with the vitamin-like substance L-carnitine. β-oxidation of fatty acids is dramatically increased by infusion or addition of carnitine in colostrum-deprived newborn piglets and in liver and muscle tissue cultures obtained from colostrum-deprived piglets (Honeyfield and Froseth, 1990; Ven Kempen and Odle, 1993, 1995; Odle et al., 1995). Piglets fed carnitine-free diets for two to three weeks displayed decreased blood, urine and muscle carnitine content, vesicular fat deposition in skeletal muscle and decreased in vitro hepatocyte β-oxidation of palmitate. *In vitro* β-oxidation of palmitate by hepatocytes from carnitine deficient piglets was improved by addition of carnitine (Penn et al., 1996).

Several European researchers have demonstrated improvements in weaning pig growth performance with addition of carnitine at the rate of 20 to 80 ppm (LONZA, 1996b; Weeden et al., 1990) Neither carnitine nor soybean oil resulted in any effect on growth performance during the first two weeks after weaning. Soybean oil improved ADG and carnitine improved F/G in phase 2, while carnitine tended to improve soybean oil utilization in phase 2. A similar effect of carnitine on feed conversion ratio and a similar effect of soybean oil on ADG in the period 3 to 5 weeks after weaning was reported by Owen et al. (1996).

Soybean oil and animal fats are frequently used in swine diets to enhance dietary energy density. Research on utilization of dietary fat by the weaned pig and differences in utilization of vegetable vs animal fats have yielded variable results (Cera et al., 1990; Dove, 1993; Tokach et al., 1995). Carnitine has been shown to have significant effects on energy metabolism, particularly metabolism of fatty acids; however, there is little data published on the effect of carnitine on the utilization of saturated fatty acid (lard) and unsaturated fatty acid (soybean oil) in piglets. The present studies were designed to evaluate the effect of two different fat sources, soybean oil and lard, in postweaning piglets fed diets with and without carnitine.

MATERIALS AND METHODS

Experimental design and dietary treatments

A 2×3 factorial trial was conducted using corn-soybean-whey-fishmeal based diets containing 1.30% lysine supplemented with L-carnitine (Lonza, Basel, Switzerland) at levels of 0 and 50 mg/kg combined with 0% added fat, 5% soybean oil or 5% lard (table 1).

* Address reprint request to Defa Li. Tel: 86-10-62893588, Fax: 86-10-62893688, E-mail: Defali@public2.bta.net.cn or Defali@mafic.ac.cn.
[1] Lonza LTD, Switzwerland.
[2] University of Missouri-Columbia.
Received April 8, 1999; Accepted June 10, 1999

Table 1. Composition of experimental diets

	Control	Soybean oil	White grease
Ingredients :			
Corn	50.11	45.02	45.02
Soybean meal	27.5	27.5	27.5
Whey powder	15.0	15.0	15.0
Fish meal (Peruvian)	4.0	4.0	4.0
Soybean oil	0.0	5.0	0.0
Lard	0.0	0.0	5.0
Lysine	0.25	0.26	0.26
Methionine	0.12	0.13	0.13
Threonine	0.02	0.04	0.04
Monocalcium phosphate	1.0	1.05	1.05
Limestone	1.0	1.0	1.0
Vitamin-mineral premix[1]	1.0	1.0	1.0
Antioxidant (BHT)	10.0 mg/kg	10.0 mg/kg	10.0 mg/kg
Chemical composition (%, as fed)[2] :			
Digestible energy (mcal/kg)	3.13	3.36	3.36
Crude protein	19.69	19.68	19.68
Fat	2.03	6.83	6.83
Calcium	0.85	0.85	0.85
Phosphorus	0.70	0.70	0.70
Lysine	1.30	1.30	1.30

[1] Vit-Min premix provide ed kilogram weight of basal diet : Vitamin A, 5512 IU; Vitamin D, 3551 IU; Vitamin B_{12}, 27.6 μg; Vitamin K, 2.2 mg; riboflavin, 5.5 mg; d-pantothenate, 13.8 mg; niacin, 30.3 mg; choline chloride, 551 mg; Mn, 100 mg; Fe, 100 mg; Zn, 100 mg, Cu, 250 mg; I, 0.3 mg; Co, 1 mg; Se, 0.3 mg.

[2] All the above nutrient data were the analyzed results expect DE, which was derived from the China Feed Data Base (1995).

Animals

Seventy-two crossbred (Large White × Landrace × Druoc) pigs, weaned at 35 days of age, were randomly assigned to 6 dietary treatments in 1.5 × 1.5 m pens of 4 pigs (2 barrows and 2 gilts), each with 3 pens per dietary treatment. Pigs had *ad libitum* access to feed and nipple waterers and were housed in a thermal neutral environment in a well ventilated pig house on slatted steel rod flooring. Pigs were individually identified with an ear tag. They were weighed at the beginning of the trial, at 14 days, 28 days and the termination of the trial (39 days). Feed intake was recorded at 14 days, 28 days and 39 days. Blood was drawn from two healthy pigs (one barrow and one gilt selected randomly in each pen at 14 days, 28 days and 39 days. The serum and plasma were separated and stored at -40℃ pending analysis.

Biochemical analysis

Serum was analyzed for total protein, glucose, triglycerides, cholesterol and blood urea nitrogen (BUN) using a Technicon RA-1000 Autoanalyzer. Total protein was analyzed by the biuret method (Skaggs and Hochstrasser, 1964). Glucose was analyzed by a hexokinase method (Leon et al., 1997). BUN was analyzed by the enzymatic method of Tiffany et al. (1976). Cholesterol was identified by an enzymatic method for glycerol after hydrolysis with lipoprotein lipase, using glycerol-phosphate-oxidase, which releases H_2O_2 (Fossati and Precipe, 1982). Test reagents for total protein, glucose, cholesterol, triglycerides and BUN were manufactured by Beijing Zhongsheng Hightech Bioengineering Co., Beijing, China.

Serum was analyzed for free carnitine after perchloric acid (PCA) extraction and for total acid soluble carnitine after alkaline hydrolysis of the PCA extract. Carnitine was measured by an enzymatic method using carnitine acetyltansferase (CAT) to transfer acetyl from acetyl-CoA to carnitine, resulting in formation of acetylcarnitine and reduced CoA (Co ASH). 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was used as an indicator and the absorbance of the yellow DTNB-CoASH complex measured with a spectrophotometer at 412 nm. Short-chain acyl-carnitines were calculated as the difference between the total and the free serum carnitine concentrations.

Gas chromatography

Fatty acid methyl esters were prepared from serum according to the method of Sukhija and Palmquist (1988) Fatty acids of chain length 16 to 18 carbons

were separated in a HP6890 gas chromatograph (Hewlett-Packard, Wilmington, DE) with a 30 m×0.32 mm×0.02 mm Innowax column. Peaks were identified, integrated and quantitated with the HP Chemstation software.

Statistics

Data were analyzed as a 2×3 factorial and by treatment group using analysis of variande (ANOVA). When ANOVA indicated a significant P. means were separated using Duncan's multiple range test. The daily gains were dichotomized into range groups according to weaning weight and analyzed by a T-test. The daily gains were further dichotomized into range groups according to weaning weight and growth rate and analysis of four-fold tables was performed with carnitine inclusion rate as the risk factor using the EpIInfo6 epidemiological statistical analysis software (Center for Disease Control and WHO, 1997).

RESULTS AND DISCUSSION

Addition of carnitine to diets with and without added fat increased ADG and feed intake ($p<0.05$) during the second period, from 15 to 28 days after weaning (table 2). During this period, the ADG of pigs fed the basal diet was increased to 0.42 kg/d, while the ADG of pigs consuming the basal diet plus 50 mg/kg carnitine was increased to 0.52 kg/d ($p<0.05$). Addition of fat in the form of soybean oil or lard did not increase ADG significantly. Addition of carnitine to the diet containing soybean oil increased ADG significantly. Addition of carnitine to the diet containing soybean oil increased ADG ($p<0.05$) from 0.46 kg/d to 0.58 kg/d; addition of carnitine to the diet containing lard resulted in a smaller ($p>0.05$) increase, from 0.46 g/d to 0.50 kg/d. Addition of carnitine increased feed intake during the period 15 to 28 days after weaning ($p>0.05$), but dietary fat did not significantly modify intake during the second period (table 2).

During the first two weeks of the trial, carnitine addition produced no significant effects on ADG, ADFI or F/G (table 2). Addition of fat during this period reduced ADFI ($p<0.05$). Addition of lard depressed in feed intake more than did soybean oil ($p<0.05$), but numerically improved F/G ($p>0.05$). There were no significant carnitine×fat interactions in the periods 0 to 14 days and 15 to 28 days.

In the third period from 28 to 39 days post-weaning, addition of carnitine numerically improved ADG and F/G, Addition of soybean oil or lard significantly reduced intake during this period ($p<0.05$) (table 2); however, addition of carnitine to the lard diet resulted in increased feed intake as compared to the diet containing lard alone ($p<0.05$).

Table 2. Factorial analysis of performance of 35 day weaned pigs fed diets containing 0 or 50 mg/kg L-carnitine and either 0% added fat or 5% added fat in the form of soybean oil or lard

Period	Added L-carnitine level				Added fat level					Carnitine×Fat Interaction p
	0 mg/kg	50 mg/kg	SEM[1]	P	0	5% Soybean oil	5% Lard	SEM	P	
Day 0-14										
Daily feed intake[2]	0.45	0.46	0.02	0.86	0.51[c]	0.46[d]	0.40[e]	0.02	>0.01	0.34
Daily gain	0.22	0.22	0.02	0.85	0.23	0.21	0.22	0.02	0.84	0.63
Feed per gain	2.35	2.39	0.16	0.87	2.52	2.55	2.02	0.19	0.12	0.78
Day 15-28										
Daily feed intake	0.75[a]	0.99[b]	0.04	0.00	0.86	0.94	0.83	0.05	0.18	0.42
Daily gain	0.44[a]	0.53[b]	0.02	0.00	0.49	0.52	0.48	0.02	0.23	0.59
Feed per gain	1.74	1.90	0.08	0.22	1.79	1.94	1.74	0.11	0.46	0.27
Day 29-39										
Daily feed intake	1.11	1.12	0.03	0.70	1.20[c]	1.10[d]	1.05[d]	0.03	>0.01	>0.01[3]
Daily gain	0.52	0.57	0.03	0.14	0.58	0.54	0.53	0.03	0.29	0.12
Feed per gain	2.22	2.00	0.08	0.06	2.10	2.22	2.03	0.11	0.40	0.48
Day 0-39										
Daily feed intake[2]	0.79[a]	0.83[b]	0.01	0.03	0.84[c]	0.82[c]	0.73[d]	0.03	0.01	0.38
Daily gain	0.39[a]	0.43[b]	0.01	0.05	0.43	0.42	0.39	0.02	0.17	0.14
Feed per gain	1.96	1.96	0.06	0.96	1.95	2.00	1.93	0.06	0.80	0.45

[1] Standard error of mean.
[2] Means with different superscripts (a, b or c, d, e) are significantly different ($p<0.05$).
[3] Daily feed intake for Lard + Carnitine greater than for Lard ($p<0.05$).

Table 3. Average daily gain of pigs weaned at 35 days of age with low and high weaning weights fed diets containing 0 and 50 mg/kg carnitine

Carnitine (mg/kg)	Weaning weight group[1]										
	Low (0-50 percentile)				High (50-100 percentile)				p-values		
	0	50	SEM[3]	P	0	50	SEM	P	Carn[2]	WW[2]	C×WW[2]
0-14 days	0.16	0.16	0.03	0.56	0.28	0.22	0.03	0.67	0.22	0.01	0.04
15-28 days	0.38	0.51	0.02	>0.01	0.53	0.52	0.02	0.74	0.01	0.01	0.04
29-39 days	0.48	0.53	0.03	0.14	0.55	0.61	0.04	0.42	0.08	0.09	0.67
0-39 days	0.35	0.40	0.01	0.03	0.46	0.46	0.03	0.81	0.09	0.01	0.32

[1] Median weight at 35 days weaning was 10.3 kg.
Low=below median weaning weight (0 to 50 percentile); High = above median weaning weight.
[2] Carn=L-Carnitine; WW = Weaning Weight; C×WW = Carnitine×Weaning Weight Interactions.
[3] SEM=Standard error of mean.

Table 4. Tabular analysis of rapid or slow growth of pigs weaned at 35 days of age with low or high weaning weights fed diets containing 0 or 50 mg/kg carnitine

Carnitine (mg/kg)	Weaning weight group[1]					
	Low		High		All	
	0	50	0	50	0	50
Days 0-14[2]						
% Rapid growth pigs	25	21	83	64	44	42
% Slow growth pigs	75	79	17	36	56	58
MLE of odds ratio[6]		0.80		0.38		0.89
p-Value for MLE[7]		0.527		0.250		0.500
Days 15-28[3]						
% Rapid growth pigs	38	84	83	88	53	86
% Slow growth pigs	62	16	17	12	47	14
MLE of odds ratio		8.40		1.48		5.53
p-Value for MLE		0.002		0.556		0.002
Days 29-39[4]						
% Rapid growth pigs	50	52	83	94	53	86
% Slow growth pigs	50	48	17	6	47	14
MLE of odds ratio		1.12		2.88		1.53
p-Value for MLE		0.556		0.384		0.290
Days 0-39[5]						
% Rapid growth pigs	38	76	91	93	60	85
% Slow growth pigs	62	34	9	7	40	15
MLE of odds ratio		4.85		1.35		3.65
p-Value for MLE		0.027		0.680		0.025

[1] Median Weight at 35 day weaning was 10.3 kg.
Low = below median weaning weight (0 to 50 percentile); High = above median (50 to 100 percentile).
[2] Median overall (all groups) growth rate ~200 g/d. Rapid growth = above median (>200 g/day).
[3] Median overall (all groups) growth rate ~450 g/d. Rapid growth = above median (>450 g/day).
[4] Median overall (all groups) growth rate ~500 g/d. Rapid growth = above median (>500 g/day).
[5] Median overall (all groups) growth rate ~375 g/d. Rapid growth = above median (>375 g/day).
[6] Maximum Likelyhood Estimate (MLE) of Odds Ratio, odds of a pig displaying rapid growth as result of carnitine supplementation.
[7] p-Values for MLE greater than 0.05 indicate no significant effect of carnitine supplementation.

After 28 days of the trial, it was observed that the pigs fed diets containing carnitine appeared more uniform and had a lower incidence of runts and poor-doing pigs than the other groups. To test this hypothesis, an odds ration calculation was performed. The average growth rate of the pigs during each period was calculated and pigs were assigned to either rapid or slow growth rate categories. If a pig grew at or faster than the median growth rate, it was tabulated with the rapid growth group. Pigs growing slower than

the median growth rate were tabulated with the slow growth group. A Chi-square analysis was conducted using carnitine inclusion as the risk factor. This yielded to results presented the right hand column ("All") in table 4. For the period from 14 to 28 days, an odds ratio of 5.53 ($p=0.002$) indicated a highly significant effect of carnitine in reducing the incidence of slow growing pigs. The data set was next further subdivided into weaning weight groups. The median weaning weight for this study was 10.3 kg. Pigs were divided into low and high weaning weight groups based upon their weaning weights. This yielded the data presented in the left-hand portion of table 4. This analysis revealed that the main effect of carnitine in reducing the incidence of slow growing pigs occurred in pigs with lower weaning weights (odds ration 8.4; $p=0.002$), and that there was little effect (odds ratio-1.48; $p=0.556$) in the heavier pigs. The ADG of each subgroup was calculated, and it was found that the low weaning weight pigs supplemented with carnitine grew at a rate similar to that of the higher weaning weight pigs (0.51 kg/d), while the unsupplemented group grew much more slowly (0.38 kg/d). The effect was highly significant ($p=0.0005$). Factorial ANOVA using fat inclusion as a factor and stratified tabular analysis revealed no significant effect of fat supplementation and no significant carnitine $\times$ fat interactions ($p>0.35$).

At the conclusion of the trial, the data were dichotomized by growth rate and weaning weight for each period of the trial (tables 3 and 4). The results showed that carnitine had no effect in the first two weeks after weaning, but there was a highly significant effect in increasing growth in the period 14 to 28 days after weaning (table 3). This effect occurred in the "bottom end" pigs with lower weaning weights and resulted in an overall improvement in growth rate among the small pigs that lessened in the last period of the trial (tables 3 and 4). Lower weaning weight pigs that received carnitine in this study gained an average of 47 grams per day faster than their counterparts that did not receive Carnitine ($p=0.03$) (table 3), which amounted to over 1800 grams more for the overall trial period. The average weaning-weights of carnitine supplemented ($n=25$) and-unsupplemented ($n=30$) lower weight pigs were similar ($p>0.78$), 9.58 ± 4.7 kg and 9.58 ± 5.5 kg, respectively. Larger weaning weight pigs grew more rapidly than lower weaning weight pigs for the overall period, whether or not carnitine was supplemented (table 3).

The data from serum carnitine analysis indicated lower free carnitine levels at weaning than at 14, 28 or 39 days after weaning. This indicates the presence of some endogenous production of carnitine in the post-weaning period, as the free carnitine levels were higher after weaning than at weaning time in both supplemented and unsupplemented pigs (tables 6 and 7). Supplementation of carnitine did not increase free carnitine levels, but short-chain acyeated carnitine levels were higher at day 14 and 28 in pigs that received supplemental carnitine ($p<0.10$).

Biochemical data indicated that at 14 days after weaning, serum protein levels were significantly elevated ($p<0.05$) by supplemental carnitine (table 5). There was a tendancy toward elevated serum protein in the pigs that received dietary fat ($p=0.12$). Serum glucose levels at day 14 were numerically higher in pigs receiving either fat or carnitine, but there was no significant carnitine $\times$ fat interaction. Serum cholesterol levels on day 14 tended to be elevated when carnitine or soybean oil was supplemented and were significantly elevated when lard was added. Addition of dietary fat resulted in reduced blood urea nitrogen, the effect being significant during the second period ($p<0.05$). Addition of dietary fat on day 39 increased serum cholesterol levels significantly. Serum cholesterol was lower in the pigs receiving lard during the second period, but was higher when both lard and carnitine were supplemented than when lard alone was provided in the diet. Triglycerides were increased by the addition of carnitine during the second period, the effect approaching significance at $p=0.06$.

Gas chromatographic analysis of serum fatty acids (table 8) indicated that at the end of the first two weeks after weaning at 35 days, fatty acids C16, C18:0 and C18:1 were significantly elevated ($p<0.10$) in the groups receiving carnitine. C18:2 and total 16-18 carbon chains were numerically increased by addition of carnitine. Addition of lard elevated C18:2 ($p<0.10$) and numerically increased total C16-18 fatty acids at 14 days post weaning, whereas soybean oil feeding did not result in modified C16-18 fatty acid levels. At day 28, C16-18 fatty acid levels in pigs receiving carnitine did not differ from those not receiving carnitine. Addition of lard resulted in significantly lower levels ($p<0.10$) of all C16-18 fatty acids except C18:1. Addition of soybean oil elevated C18:1 levels ($p<0.10$), but did not alter the levels of other fatty acids. At day 39, carnitine addition resulted in lowered C18:2 levels ($p<0.10$). Addition of fat did not modify C16-18 levels at day 39. There were no significant interactions of carnitine and dietary fat.

It has been shown that carnitine can improve fatty acid oxidation in the pig in vivo and in vitro (Honeyfield and Froseth, 1990; Van Kempen and Odle, 1993, 1995; Odle et al., 1995; Penn et al., 1996). The biochemical data in the present experiment indicates that pigs fed under practical conditions may demonstrate significant modifications in fat and energy metabolism when carnitine is supplemented. Interestingly, most of the biochemical changes appeared in

Table 5. Biochemical data of 35 days weaned pigs fed 0 or 50 mg/kg L-carnitine and either 9% or 5% added fat in the form of soybean oil or lard

Day	Added L-carnitine level				Added fat level					Carnitine × Fat Interaction p
	0 mg/kg	50 mg/kg	SEM[1]	P	0	5% Soybean oil	5% Lard	SEM	P	
Blood urea nitrogen (mmol/l)										
14	4.8	4.7	0.57	0.99	5.1	4.7	4.5	0.5	0.78	0.73
28	3.8	4.4	0.80	0.56	6.0[a]	3.4[b]	3.3[b]	0.5	0.01	0.58
39	5.3	5.2	0.89	0.75	6.5	5.0	4.3	0.7	0.18	0.49
Cholesterol (mg/dl)										
14	90	114	13	0.10	77[a]	107[ab]	117[b]	5	0.05	0.50
28	76	86	17	0.64	101[a]	92[a]	50[b]	5	0.01	0.02
39	109	109	13	0.94	82[a]	119[a]	119[b]	6	0.04	0.74
Glucose (mg/dl)										
14	103	121	9	0.11	102	110	122	8	0.23	0.49
28	87	86	8	0.87	85	89	86	5	0.93	0.74
39	101	92	7	0.32	90	97	102	6	0.48	0.71
Triglycerides (mg/dl)										
14	68	69	24	0.99	55	88	58	21	0.52	0.66
28	49	49	10	0.06	49	39	31	10	0.52	0.06
39	41	41	7	0.66	33	48	33	8	0.22	0.53
Total protein (g/dl)										
14	6.3[a]	7.3[b]	0.4	0.04	6.1	7.0	7.2	0.4	0.12	0.71
28	5.9	5.9	0.4	0.81	6.4	5.8	5.6	0.4	0.45	0.60
39	7.0	7.2	0.4	0.69	6.8	7.3	7.1	0.4	0.80	0.51

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

Table 6. Serum carnitine concentrations (μm/ml) by time (all treatments)[1]

Carnitine fraction	Day post-weaning					
	0	14	28	39	SEM[1]	P
Total carnitine[2]	0.265	0.268	0.272	0.284	0.004	0.41
Free carnitine	0.193[a]	0.214[b]	0.221[b]	0.212[b]	0.002	0.04
Short chain acyl carnitine	0.073	0.054	0.051	0.071	0.008	0.24

[1] Standard error of mean.
[2] Means with different superscripts (a, b) are significantly different ($p<0.10$).

Table 7. Serum carnitine concentrations (μm/ml) by treatment[1]

Carnitine fraction	Carnitine supplementation			
	0 mg/kg	50 mg/kg	SEM[1]	P
Day 14				
Total carnitine[2]	0.252[a]	0.278[b]	0.008	0.01
Free carnitine	0.206	0.210	0.008	0.62
Short-chain acyl carnitine	0.054[a]	0.082[b]	0.010	0.03
Day 28				
Total carnitine	0.265	0.287	0.016	0.20
Free carnitine	0.223	0.210	0.013	0.33
Short-chain acyl carnitine	0.042[a]	0.077[b]	0.017	0.07
Day 39				
Total carnitine	0.286	0.272	0.011	0.24
Free carnitine	0.206	0.222	0.013	0.27
Short-chain acyl carnitine	0.080	0.050	0.018	0.14

[1] Standard error of mean.
[2] Means with different superscripts (a, b) are significantly different ($p<0.10$).

Table 8. Fatty acids (C16-18) in serum of 35 day weaned pigs fed 0 or 50 mg/kg L-Carnitine and 0 or 5 % added fat in the form of lard or soybean oil (G/L)

Fatty acid	Added L-carnitine level				Added fat level					
	0 mg/kg	50 mg/kg	SEM	P	0	5% Soybean oil	5% Lard	SEM	P	Carnitine × Fat Interaction p
Day 14										
C16	76	270	83	0.08	93	150	260	100	0.51	0.38
C18:0	54	170	40	0.05	75	98	161	60	0.28	0.62
C18:1	160	790	164	0.02	380	388	670	330	0.66	0.65
C18:2	1700	3400	1300	0.26	1400	2000	4300	840	0.03	0.81
Total	2000	4600	1600	0.17	2000	2600	5400	1400	0.14	0.76
Day 29										
C16	200	230	99	0.78	280	290	77	17	0.01	0.96
C18:0	150	210	71	0.48	260	200	89	32	0.07	0.98
C18:1	330	450	270	0.67	200	760	210	73	0.02	0.86
C18:2	4200	3100	1940	0.61	4400	5500	1100	740	0.05	0.99
Total	4900	4000	2260	0.72	5200	6700	1400	670	0.02	0.99
Day 39										
C16	320	370	22	0.07	370	360	320	27	0.47	0.83
C18:0	220	230	20	0.62	210	250	210	11	0.17	0.58
C18:1	620	860	67	0.02	660	810	750	120	0.73	0.28
C18:2	7400	5100	260	0.01	6100	6400	6100	1100	0.98	0.10
Total	8500	6600	290	0.01	7400	7800	7400	7376	0.94	0.22

the first two weeks after weaning, when there were no apparent effects on carnitine or fat on growth performance, while there was little modification in the biochemical parameters during the period from 14 to 28 days, during which the effects on growth performance were observed. Serum glucose and protein levels were significantly modified by the addition of carnitine, which can have positive effects on the growth, health and well-being of the pig.

Dietary fat produced significant modifications in biochemical parameters but no significant effects on growth. The diets fed contained amino acid levels equal to or higher than those currently in use for 10-20 kg pigs in China (Li, 1996), but the lower BUN levels might indicate that the pigs fed the diets containing supplemental fat could have profited from slightly higher amino acid levels. Yen et al. (1986) observed lower BUN levels associated with feeding of amino acid levels below the nutritive requirement of the pig. The relationship of dietary energy and lysine levels has been well established (Kyriazakis and Emmans, 1992; Quiniou et al., 1995). The issue of whether or not to add fat to diets for weaning pigs is problematic, as many studies show little or no benefit of supplemental fat in the diet of the young pig, and many hypotheses for the lack of effect have been offered (NRC, 1998). In the present study, dietary fat produced no significant effect on piglet performance and there was no significant interaction between carnitine and dietary fat on piglet performance. It has been postulated that low lipase activity in the intestine of the weaned pig is responsible for the lack of response to dietary fat observed in some studies (Weeden et al., 1990). The data in the present study seems to indicate that fat absorption was occurring during the first two weeks of the study, as evidenced by elevations in serum cholesterol, glucose, triglycerides and linoleic acid levels in pigs receiving fat supplementation. The decreased supply of nutrients relative to energy would seem to be a factor, as feed intake was significantly decreased by addition of fat.

Supplemental carnitine improved piglet growth performance in the period 14 to 28 days after 35 day weaning at 35 days but had no effect on feed conversion ration. Weeden et al. (1990) and Owen et al. (1996) found no effect of carnitine in the two week period immediately following weaning. Both Weeden and Owen reported a F/G response to carnitine in the second phase after weaning, but no such effect was found in the present study. Neither Weeden nor Owen reported an increase in ADG due to carnitine in the second phase, but both reported a growth response to soybean oil, and Weeden found that carnitine improved soybean oil phase 2. Hoffman et al. (1983) reported that addition of carnitine produced no effect on ADG, Gain per megacalorie ME, energy metabolizability or nitrogen retention in pigs weaned at 3 days of age and fed diets that contained isolated soy protein, corn syrup solids and dextrose and either 1.2 or 12% soybean oil. In the

present study, although the group that received both cornitine and soybean oil had the numerically highest daily gains among treatments in phase 2, the daily gains were not significantly different from the those of the group that received carnitine alone. The present study is the first to report a weaning weight dependent effect of carnitine. Added fat in the form of soybean oil or lard did not improve piglet performance. There were no significant interactions of carnitine and fat on growth performance, but carnitine did restore a portion in feed intake associated with the feeding of lard.

At present, the major metabolic functions of carnitine in mammalian systems are generally considered to be those functions related to transport and transient stocking of activated fatty acids in the cell. These functions include acceptance of long-to medium-chain acyls across the mitochondrial membrane, receipt of acetyl from acetyl-CoA, transfer of acetyl among mitochondria, cells and organs, and receipt and transport of potentially toxic acids out of mitochondria (Siliprandi et al., 1989; Angelini et al., 1992). In addition, a variety of other metabolic functions are attributed to carnitine, including membrane regeneration and stabilization, maintenance of immune competency, intracellular pH buffering, and neuroprotective neurotransmitter (possibly cholinergic) effects (Ramsay and Arduini, 1993; Uhlenbruck, 1996).

The function of carnitine in acyl group transfer involves the esterification of carnitine to acyl groups at the β-hydroxy position of carnitine. This esterification is modulated by a group of enzymes known as carnitine acyltransferases. Carnitine acyl-transferases have been found associated not only with mitochondria, but with other cellular organelles, including the plasma membrane, endoplasmic reticulum, sarcoplasmic reticulum, and peroxisomes, The basic carnitine acyltransferase reaction, acyl-CoA + L-carnitine $\leftrightarrow$CoASH + acyl-L-carnitine, serves and important function, in that the limited supply of CoA is regenerated and acyls are safely stored. Accumulated acyl-CoA leading to a high acyl-CoA/CoA ratio inhibits the activity of many enzymes, including pyruvate dehydrogenase, essential for metabolism of glucose into energy, and acyl-CoA: lysophospholipid acyltransferase, which functions in membrane repair. The cellular pool of acyl-carnitine not only frees up CoA for metabolic processes, but serves as a readily available acetyl energy reserve (Ramasy and Arduini, 1993; Angelini et al., 1992).

Carnitine may be obtained exogenously from dietary sources or endogenously synthesized. Plant source feedstuffs used in livestock feeds, such as grains and oilseed meals, are low in carnitine cantent, whereas animal source feedstuffs and proteins, such as fishmeal and milk products, are rather high in carnitine content. Notably, dried plasma protein is low in carnitine (Neumann, 1996; LONZA, 1996a).

In endogenous carnitine synthesis, lysine is incorpor ated into lysine-rich protein, which is methylated to ϵ -N-trimethyllysine using methionine as a methyl group donor. The ϵ-N-trimethyllysine is released from the protein by a hydrolase and is converted to β-hydroxy-ϵ-trimethyllysine via a gydroxylation requring ascorbic acid. The β-hydroxy-ϵ-trimethyllysine is converted in several steps to γ-butyrobetaine. Most of the reactions leading the production of γ-butyrobetaine take place in muscle tissue. γ-Butyrobetaine, also known as deoxycarnitine, is exchanged for carnitine by the muscle cells. Deoxycarnitine is hydroxylated in the liver and kidney to carnitine by butyrobetaine hydroxylase (Siliprandi et al., 1989; Rebouche, 1996). Butyrobetaine hydroxylase activity in the liver of human infants less than 3 months of age is less than 12% of adult activity (Atkins and Clandinin, 1990). This hydroxylation reaction requires ascorbic acid (Yang and Zhang, 1992). Guinea pigs, which cannot synthesize ascorbate and require a dietary source of ascobate, display decreased tissue carnitine levels as a result of carnitine deficiency. Resupplementation of ascorbate *in vitro* using cultured guinea pig hepatocytes and in vivo results in improved carnitine status and increased β-oxidation of fatty acids (Ha et al., 1994).

Weaning piglets may be deficient in ascorbic acid (Mahan et al., 1994) which is essential for endogenous carnitine synthesis. It has been shown in humans that carnitine synthesis can be modified by the adequacy of the diet in providing lysine and methionine (Rebouche et al., 1989). Improved management and/or genetics may result in increased need for dietary lysine and methionine (Stahly et al., 1994; Williams and Stahly, 1995). Schuhmacher et al. (1993) concluded that the effect of carnitine on growth rate and feed conversion was more pronounced when lysine or sulfur amino acids were marginal than when these amino acids were supplied to requirements. Cho et al. (1998) found that 21-d-old piglets fed diets containing 1,000 mg/kg carnitine with 1.6% lysine level improved growth performance, indicating that the age of piglets and the dose of dietary carnitine may be factors influencing the efficacy of carnitine on pig growth performance.

The present study found that serum acylcarnitine levels were significantly elevated in pigs receiving supplemental carnitine. This would seem to indicate that endogenous carnitine production in the first 4 weeks after weaning does not provide sufficient carnitine for receipt of acyl groups in fatty acid metabolism and processes such as glycolysis that results in the production of acetyl and amino acid degradations that yield other acyl groups such as the valproyl and benzoyl which result from degradation of

valine, tyrosine and phenslalanise. This information, combined with the growth rate data, suggests supplementation of carnitine promoted growth by mechanisms which involve acyl metabolism. Endogenous carnitine production may be more limiting in pigs at the lower end of the weight distribution. The results of the present study show that carnitine can produce a significant growth response in the weaned piglet but that the effect may be mainly in the smaller piglets. It is postulated that in the smaller piglets, the endogenous synthesis of carnitine may be inadequate to support optimum growth and that carnitine supplementaion may relieve one factor restricting growth.

IMPLICATIONS

Variation in weight at weaning is constantly a problem in swine production and is the result of many factors, including genetics, sow age, litter size, nutrition, disease, and management factors. Finding ways to reduce variation and better manage pig variability could reduce costs and improve pig health and variability. Further work should be done to determine the factors associated with endogenous carnitine synthesis and to determine the conditions under which carnitine supplementation is useful and necessary. There is a relationship between carnitine and energy metabolism. Further work should be done to determine the effects of carnitine in combination with different dietary energy levels and various energy sources and to determine the effects of carnitine on immune function in the pig.

ACKNOWLEDGMENTS

We would like to thank LONZA of Basel, Switzerland for their financial and technical support in this project and the staff and leadership of Sijiqing Feed Mill and Xishan Pig Farm in Beijing for their assistance in, and tolerance of our research activities.

REFERENCES

Allain, L. C., L. S. Poon, C. S. Chan, W. Richmond and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470-475.

Angelini, C., L. Vergani and A. Martinuzzi. 1992. Clinical and biochemical aspects of carnitine deficiency and insufficiency: transport defects and inborn errors of b-oxidation. Crit. Rev. Clin. Lab. Sci. 29:217-242.

Atkins, J. and M. Clandinin. 1990. Nutritional significance of factors affecting carnitine dependent transport of fatty acids in neonates. A review. Nutr. Res. 10:117-128.

Center for Disease Control, USA and world Health Organization, Geneva. 1997. EpiInfo 6.04b (software).

Cera, K., D. Mahan and G. Reinhart. 1990. Evaluation of various extracted vegetable oils, roasted soybeans, medium-chain triglyceride and an animal-vegetable fat blend postweaning swine. J. Anim. Sci. 68:2756-2765.

China Feed. 1995. tables of feed composition and nutritive value in China : 1995 edition feed database in China. China Feed. 22:40-45.

Cho, W. T., J. H. Kim, In K. Han. and Y. K. Han. 1998. Effects of L-carnitine with different lysine levels on growth and nutrient digestibility in pigs weaned at 21 days of age. Asian-Aus. J. Anim. Sci. 12(5):799-805.

Dove, C. R. 1993. The effect of adding copper and various fat sources to the diets of weanling swine on growth performance and serum fatty acid profiles. J. Anim. Sci. 71:2187-2193.

Fossati, P. and L. Prencipe. 1982. Serum triglycerides determined colotimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28(10):2077.

Ha, T. Y., M. Otsuka and N. Atakawa. 1994. Ascorbate indirectly stimulates fatty acid utilization in primary cultured guinea pigs hepatocytes by enhancing carnitine synthesis. J. Nutr. 124:732-737.

Hoffman, L., D. Ivers, M. Ellersieck and T. Veum. 1993. The effect of L-carnitine and soybean oil on performance and nitrogen and energy utilization by neonatal and young pigs. J. Anim. Sci. 71:132-138.

Honeyfield, D. and J. Froseth. 1990. Evaluation of energy sources with and without carnitine in newborn pig heart and liver. J. Nutr. 121:1117-1122.

Junge, W. K., K. Leybold and B. Kraack. 1983. Influence of colipase on the turbidimetric determination of pancreatic lipase catalytic activity. J. Clin. Chem. Biochem. 21:445-451.

Kyriazakis, I. and G. C. Emmans. 1992. The effects of varying protein and energy intakes on the growth and body composition of pigs. Br. J. Nutr. 68:603-625.

Leon, L. P., D. K. Chu, R. D. Stasiw and L. R. Snyder, 1976. New more specific methods for the SMA 12/60 multichannel biochemical analyzer. In Advances in Automated Analysis, Vol 1. Technicon International Congress. Tarrytown, Ny. Mediad, Inc, pp. 152-156.

Li, D. F. 1996. Swine Nutrition. (Chinese) China Agricultural University Press, Beijing. pp. 25-70.

LONZA. 1996a. L-Carnitine in animal nutrition. LONZA Ltd., Basle.

LONZA. 1996b. L-Carnitine in feedstuffs. Research report. LONZA Ltd., Basle.

Mahan, D. C., A. Lepine and K. Dabrowski. 1994. Efficacy of magnesium-L-ascorbyl-2-phosphate as a vitamin C source for weanling and growing-finishing swine. J. Anim. Sci. 72:2354-2361.

Neumann, G. 1996 Effect of L-Carnitine on athletic performance. In Carnitine-Pathochemical Basics and Clinical Applications. H. Seim and H. Loster (Ed.). Ponte press, Bochum, pp. 61-72.

Odle, J., X. Lin, T. Van Kempen, J. Drackley and S. Adams. 1995. Carnitine palmitoyltransferase modulation of hepatic acid metabolism and radio-HPLC evidence for low ketogenesis in neonatal pigs. J. Nutr. 125:2441-2549.

Owen, K., J. Nelssen, R. Goodband, R. Weeden and S. Blum. 1996. Effect of L-carnitine and soybean oil on growth performance and body performance of early-

weaned pigs. J. Anim. Sci. 74:1612-1619.

Penn, D., P. Bobrowski, L. Zhang and E. Schmidt-Sommerfield. 1996. Neonatal nutritional carnitine deficiency: a piglet model. In Carnitine-Pathochemical Basics and Clinical Applications. H. Seim and H. Loster (Ed), Ponte Press, Bochum, p262.

Quiniou, N., J. Noblett, J. van Milgen and J. Y. Dourmad. 1995. Effect of energy intake on performance, nutrient and tissue gain and protein and energy utilization in growing boars. Anim. Sci. 61:133-143.

Ramsay, R. and A. Arduini. 1993. The carnitine acyltansferases and their role in modulating acyl-CoA pools. Arch. Biochem. Biophys. 302:307-314.

Rebouche, C. J., E. P. Bosch, C. A. Chenard, K. J. Schabold and S. E. Nelson. 1989. Utilization of dietary percursors for carnitine synthesis in human adults. J. Nutr. 119:1907-1913.

Rotzsch, W. 1996. Carnitine: Historical overview. In Carnitine-Pathochemical Basics and Clinical Applications H. Seim and H. Loster (Ed.). Ponte Press, Bochum, pp. 3-10.

Sardi, L., G. Martelli, M. Dall Olio, P. Parisini. 1996. Use of L-carnitine in feeding heavy pigs. Rivista di Suinocoltura. 37(7):97-101.

Schuhmacher, A., C. Eissner, J. Gropp, G. Flachowsky (Ed.) and R. Schubert. 1993. Carnitine in fish. piglets and wuail. Vitamine und weitere Zusatzstoffe bei Mensch und Tier: 4 Symposium 30.9-01.10.1993 Jena. Friedrich-Schiller-Universitat, Jena, Germany, pp407-412.

Siliprandi, N., L. Sartorelli, M. Ciman and F. DiLisa. 1989. Carnitine: metabolism and clinical chemistry. Clinica Chemica Acta. 183:3-12.

Siliprandi, N., F. DiLisa, G. Pieralisi, P. Ripari, F. Maccari, R. Menabo, M. Giamberardino and L. Vecchiet. 1990. Metabolic changes induced by maximal exercise in human subjects following L-carnitine administration. Biochem. Biophys. Acta. 1043:17-21.

Skaggs, L. T. and H. Hochstrasser. 1964. Multiple automatic sequential analyses. Clin. Chem. 10:918-936.

Stahly, T. S., N. H. Williams and S. Swenson. 1994. Responses of pigs from high and low lean genotypes to dietary lysine levels. J. Anim. Sci. 72(Suppl.1):165 (Abstr.)

Tiffany, T. O., J. M. Jansen, C. A. Burtis, J. B. Overton and C. D. Scott. 1972. Enzymatic kinetic rate and endpoint analysis of substrate. Clin. Chem. 18:829.

Tokach, M., J. Pettigrew, L. Johnston, M. Øverland, J. Rust and S. Cornelius. 1995. Effect of adding fat and (or) milk products to the weanling pig diet on performance in the nursery and subsequent grow-finish stages. J. Anim. Sci. 73:3358-3368.

Van Kempen, T. and J. Odle. 1993. Medium-chain fatty acid oxidation in colostrum deprived newborn piglets: stimulative effect of L-carnitine supplementation. J. Nutr. 123:1531-1537.

Van Kempen, T. and J. Odle. 1995. Carnitine affects octanoate oxidation to carbon dioxide and dicarbozylic acids in colostrum-deprived piglets: In vivo analysis of mechanisms involved based on CoA- and carnitine-ester profiles. J. Nutr. 125:238-250.

Uhlenbruck, G. 1996. L-Carnitine and the immune system: from the mode of metabolism to the modulation of membranes. In Carnitine-Pathochemical Basics and Clinical Applications. H. Seim and H. Loster (Ed.). Ponte Press, Bochum, pp. 47-60.

Williams, N. H. and T. S. Stahly. 1995. Impact of immune system activation on lysine and sulfur amino acid needs of 6-16 kg pigs. J. Anim. Sci. 73(Suppl.1):58(Abstr.)

Weeden, T. L., J. L. Nelssen, R. H. Hines, D. F. Li and J. A. Swanson. 1990. The effect of L-carnitine on the utilization of soybean oil fed to early-weaned pigs. J. Anim. Sci. 68 (Suppl.1):374 (Abstr).

Yang, N. and W. J. Zhang. 1992. Biophysiology and biosynthesis of L-Carnitine. Shengwu Huaxue Yu Shengwu Wuli Jinzhan [Achievements in Biochemistry and Biophysics, Chinese] 19:(2)81-85.

Yen, H. T., D. J. A. Cole and D. Lewis. 1986. Amino acid requirements of growing pigs. Anim. Prod. 43:141-153.