Effects of Dietary L-Carnitine and Protein Level on Plasma Carnitine, Energy and Carnitine Balance, and Carnitine Biosynthesis of 20 kg Pigs[2,3,4]

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ABSTRACT : Growing pigs (N=25; 18 kg) were used to study effects of L-carnitine and protein intake on plasma carnitine, energy and carnitine balance, and carnitine biosynthesis. Corn-soybean meal basal diets containing low or high protein (13.6% or 18%) were formulated so that protein accretion would be limited by metabolizable energy (ME). Each basal diet was supplemented with 0 or 500 mg/kg L-carnitine and limit fed to pigs for 10 d in a balance trial. Final carnitine concentration was compared with weight/age matched pigs measured on d 0 to calculate carnitine retention rates. Supplementation of carnitine increased ($p<0.01$) plasma free carnitine (by 250%), short-chain (by 160%) and long-chain acyl-carnitine concentrations (by 80%) irrespective of blood sampling time ($p<0.01$). The proportion of long-chain carnitine esters decreased by 40% ($p<0.01$) by carnitine supplementation; whereas, the proportion of short-chain acyl-carnitine concentration was not changed ($p>0.10$). All criteria of energy balance were unaffected by L-carnitine ($p>0.10$). Total body carnitine retention was increased by 450% over unsupplemented controls ($p<0.01$). Carnitine biosynthesis rates in pigs fed diets without L-carnitine were estimated at 6.71 and 10.63 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ in low protein and high protein groups, respectively. In supplemented pigs, L-carnitine absorption and degradation in the intestinal tract was estimated at 30-40% and 60-70% of L-carnitine intake, respectively. High protein feeding effect did not affected plasma carnitine concentrations, carnitine biosynthesis or carnitine retention ($p>0.10$). We conclude that endogenous carnitine biosynthesis may be adequate to maintain sufficient tissue levels during growth, but that supplemental dietary carnitine (at 500 ppm) sufficiently increased plasma acyl-carnitine and total body carnitine. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 11 : 1568-1575*)

Key Words : Pigs, Plasma Carnitine, Energy Balance, Carnitine Balance, Carnitine Biosynthesis

INTRODUCTION

Biosynthesis of carnitine in the liver and kidney is generally considered sufficient for all metabolic needs in mammalian adults (Rebouche and Seim, 1998). On the other hand, dietary carnitine is necessary to maintain normal carnitine concentration in the newborn (Borum, 1983). Furthermore, it was demonstrated that the capacity of fatty acid oxidation depends on L-carnitine supply in neonatal pigs (Coffey et al., 1991; Kempen and Odle, 1993, 1995). But it is not known how well weaning or growing pigs can synthesize carnitine *de novo* nor how dependant they are on carnitine supplied by the diet. Previous studies have revealed apparent controversy as to whether supplemental dietary L-carnitine effects pig growth performance (Hoffman et al., 1993; Owen et al., 1996); however, high dietary carnitine tended to reduce carcass fat accretion without affecting protein accretion (Owen et al., 1996). These results may lead to the suggestion that endogenous carnitine synthesis is sufficient to maintain optimal growth performance, but that added carnitine might alter nutrient partitioning and thus body composition. Even though researchers have reported that L-carnitine is degraded in the gastrointestinal tract of rats and humans by indigenous flora (Rebouche et al., 1984; Rebouche and Chenard, 1991), the rates of absorption and microbial degradation of dietary L-carnitine have not been investigated in growing pigs. If L-carnitine availability in pigs were extremely poor, even at high doses, pigs might not accumulate enough L-carnitine to alter metabolism. To further examine these issues in growing pigs, we chose to study pigs at about 7-8 wks of age based on the supposition that removal of dietary carnitine sources (animal products) might occur while pigs were not fully competent with respect to *de novo* carnitine biosynthesis, and supplemented carnitine into diets which varied in lysine to energy ratio to study the effects on plasma carnitine, energy and carnitine balance including carnitine biosynthesis

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[4] Abbreviations used: BR, body retention of carnitine; BS, rate of carnitine biosynthesis; CP, crude protein; FE, fecal excretion of carnitine; MD, microbial degradation of carnitine; ME, metabolizable energy; N, nitrogen; UE, urinary excretion of carnitine.
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and microbial degradation. Comparison of carnitine intake with excretion and body accumulation rates allowed for estimation of carnitine biosynthesis and microbial degradation rates as well. Furthermore, as these data were collected from pigs on a larger study (Heo et al., 2000a) examining the influence of dietary variables (i.e., carnitine and protein) on nitrogen balance and nutrient partitioning, the data reported herein will show that dietary carnitine may sufficiently increase plasma acyl-carnitine and total body carnitine to support the overall hypothesis that L-carnitine may alter nutrient partitioning and thus body composition of 20-kg pigs (Heo et al., 2000a).

MATERIALS AND METHODS

Animals and diets

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of North Carolina State University. Animals used in this study were part of a larger research project determining effects of dietary carnitine and protein level on nutrient partitioning and body composition in young pigs (Heo et al., 2000a). Pigs fed corn-soybean meal diets (0.9% or 1.2% lysine) containing either 0 ppm or 500 ppm added L-carnitine (2×2 factorial by randomized complete block design). Diets were formulated to contain 14.24 MJ ME/kg diet and 4% supplemental soy oil, and to exceed requirements for vitamins and minerals (NRC, 1988) as shown in table 1. The low protein diet was marginally adequate in protein containing 0.63 g lysine/MJ ME. The high protein diet contained 0.84 g lysine/MJ ME. The diets were supplemented with crystalline amino acids to provide the same optimal ratios of essential amino acids (Chung and Baker, 1992) to that of lysine.

Carnitine and energy balance

Animals were placed into metabolism cages (0.77 m, 1.27 m, and 0.85 m in width, depth and height, respectively). After five days of adaptation to the 1.2% lysine diet without L-carnitine, animals (ca. 54 d of age) were allocated to experimental diets and a 10 d carnitine and energy balance trial was conducted. Detailed procedures of the balance trial were reported previously (Heo et al., 2000a). Fecal and urine samples were analyzed for gross energy (GE) by adiabatic oxygen bomb calorimeter (Parr Instrument, Moline, IL; AOAC, 1990), and for total carnitine by a radioenzymatic method (given below). Apparent balance (of carnitine and energy) was computed as the difference between consumption and excretion. To calculate body carnitine retention, a total of 25 pigs in five identical trials were killed by electrocution. Four treatment pigs/trial were killed for final body carnitine values (pig age=64 d), and one pig/trial was selected randomly and killed to calculate the initial empty body carnitine value of treatment pigs at the beginning of each metabolic trial (pig age=54 d). Detailed procedures of body retention were reported in Heo et al. (2000a). Net energy for gain (NEg) was calculated by the difference between initial and final total body

Table 1. Formula and chemical composition of low and high protein basal diets, as fed basis[1]

Protein level	Low	High
Ingredients (%)		
Corn	77.52	66.79
Soybean meal (48% CP)	13.63	24.47
Soy oil	4.00	4.00
L-Lysine-HCl	0.36	0.34
DL-Methionine	0.06	0.11
L-Threonine	0.05	0.08
Dicalcium phosphate	2.12	1.91
Limestone	0.57	0.63
Salt	0.35	0.35
Vitamin-mineral premix[2]	0.33	0.33
Antibiotic[3]	1.00	1.00
Chemical composition[4]		
CP, %[5]	13.60	18.00
Fat, %[6]	6.91	6.63
Ca, %	0.80	0.80
P, %	0.70	0.70
Lysine, %	0.90	1.20
Methionine, %	0.30	0.40
Threonine, %	0.59	0.78
ME, MJ/kg diet	14.24	14.24

[1] Each basal diet was fed with or without supplemental L-carnitine at 500 mg/kg diet. Low protein diets were formulated to contain 0.63 g lysine/MJ ME, and high protein diets were formulated to contain 0.84 g lysine/MJ ME. Analyzed L-carnitine (mg/kg) values (mean±SEM) in five trials were 3±1, 464±10, 6±1 and 483±14 for the low protein+0 mg/kg (LP-0), low protein+500 mg/kg (LP-500), high protein+0 mg/kg (HP-0) and high protein+500 mg/kg diet L-carnitine diet (HP-500), respectively.

[2] Provided the following (mg per kg of the complete diet): Retinol, 2.2; Cholecalciferol, 0.042; α-tocopherol, 22.1; menadione, 2.6; riboflavin, 5.8; niacin, 29; choline, 308; biotin, 0.08; pyridoxine, 1.45; folic acid, 1.13; d-pantothenic acid, 22; vitamin B12, 0.029; Mn, 64; Fe, 104; Zn, 141; Cu, 25; I, 1.6; Se, 0.3.

[3] Provided 55 mg of carbadox per kilogram of complete diet.

[4] Calculated values.

[5] Analyzed CP (%) values (mean±SEM) in five trials were 13.72±0.12, 13.74±0.03, 17.48±0.16 and 17.72±0.14 for the Low-0, Low-500, High-0 and High-500 diets, respectively.

[6] Analyzed fat (%) values in pooled five trials were 6.51, 6.66, 6.44 and 6.44 for the Low-0, Low-500, High-0 and High-500 diets, respectively.

energy using the respective bomb values obtained.

Carnitine biosynthesis, microbial degradation and retention

Because previous studies demonstrated that carnitine can be degraded by the microflora within the gastrointestinal tract (Rebouche et al., 1984; Rebouche and Chenard, 1991), a simple balance equation (intake+biosynthesis=body retention+excretion) could not be used in this study. We speculated that negative carnitine balance computed using the simple equation was due to microbial degradation of L-carnitine in the gastrointestinal tract caused by low efficiency of carnitine absorption from the small intestine when supplemented at 500 ppm (Rebouche and Chenard, 1991). On the other hand, endogenous carnitine degradation by the pig was excluded in our revised equation given that carnitine is not degraded by any known mammalian enzyme (Rebouche et al., 1984). Thus, the revised carnitine balance equation used in our computations was :

DC+BS=BR+UE+FE+MD

Where DC=dietary L-carnitine intake;
BS=rate of carnitine biosynthesis;
BR=body retention of carnitine;
UE=urinary carnitine excretion;
FE=fecal carnitine excretion;
MD=microbial degradation of carnitine; each expressed as μmol·kg^{-1} empty BW·d^{-1}.

The main purpose of this equation was to estimate theoretical limits of carnitine biosynthesis rate and microbial degradation rate without using radioisotope-labeled diets. Because two estimated variables (i.e., BS+MD) were present in one equation, only the maximum and minimum rates could be calculated. Even though the ranges from maximum to minimum of those rates (instead of absolute values) were estimated from this equation, they can help to explain unknown aspects of carnitine metabolism in pigs. Firstly, the biosynthesis rate of L-carnitine was estimated from pigs fed diets with 0% added L-carnitine (assuming negligible microbial degradation under these conditions). The minimum biosynthesis rate of L-carnitine was estimated assuming MD=0, [i.e., BS=BR+UE+FE-DC]. Similarly, the maximum biosynthesis rate of L-carnitine was estimated assuming MD=DC-FE, [i.e., BS=BR+UE]. Secondly, dietary L-carnitine degraded in the GI tract was estimated from pigs fed the diets containing 500 ppm L-carnitine. The minimum microbial degradation rate of dietary L-carnitine was estimated assuming BS=0 under these conditions, [i.e., MD=DC-BR-UE-FE]. The maximum microbial degradation rate of dietary L-carnitine was then estimated assuming BS=maximum BS of pigs fed the same protein diet without carnitine, [i.e., MD=DC+BSmax-BR-UE-FE]. Thirdly, dietary L-carnitine absorbed from the GI tract was estimated from pigs fed diets with 500 ppm L-carnitine. The minimum absorption of L-carnitine was estimated assuming BS=maximum BS of pigs fed the same protein diet without carnitine, [i.e., absorbed L-carnitine=BR+UE-BSmax]. The maximum absorption of L-carnitine was estimated assuming BS=0, [i.e., absorbed L-carnitine=BR+UE]. The above calculations were based on the assumptions that L-carnitine-carbon excretion into expired air (less than 0.1% of dose), and reabsorption from blood back into the GI tract (less than 0.5% of blood carnitine) were negligible based on the findings of Rebouche et al. (1984). To reduce unmeasured variables, we felt justified to exclude these factors from the balance equation.

Blood sampling and carnitine analysis

Blood samples were obtained in heparinized tubes by vena cava puncture, 2 to 3 h after feeding on the last morning of each balance trial, and also on the following morning before pigs were killed (24 h after previous meal). Blood samples were centrifuged at 2,300 g for 25 min within 1 h of collection. Plasma samples were stored at -20℃ and analyzed for three carnitine fractions by a radioenzymatic method. Detailed procedures of carnitine analysis were reported previously (Heo et al., 2000a).

Statistical analysis

All data were analyzed as a randomized complete block design with a 2×2 factorial (L-carnitine× protein level) arrangement of treatments using the GLM procedure of SAS (1989). In addition, daily excretion data (figure 1) were analyzed as above with an additional split-plot in time (Steel et al., 1997). The relationship between plasma free carnitine carnitine and urinary free carnitine was determined by regression analysis. Significant relationships were accepted at $p<0.05$.

RESULTS

Plasma carnitine concentrations and proportions

L-carnitine supplementation increased plasma free carnitine concentration at 2 h and 24 h post feeding by 290% and 210%, respectively ($p<0.01$, table 2). Short-chain and long-chain acyl-carnitine concentrations were increased by 160% and 80%, respectively, with dietary L-carnitine supplementation irrespective of blood sampling time ($p<0.01$). The proportions of free carnitine (irrespective of sampling time) was increased by L-carnitine supplementation (16%, $p<0.01$, table 3); whereas, the proportion of long-chain carnitine esters

Table 2. Effects of L-carnitine and protein level on plasma carnitine amount of 20 kg pigs

Protein level	Low		High		
L-carnitine, mg/kg diet	0	500	0	500	SE[1]
2 h fasting, μmol/L					
Free[a]	3.75	14.49	4.63	18.22	1.67
Short chain[a]	2.97	6.53	2.77	8.77	1.14
Long chain[a]	1.00	1.65	0.87	1.79	0.13
Total[a]	7.73	22.67	8.27	28.77	2.63
Acylated[a]	3.97	8.18	3.64	10.55	1.22
24 h fasting, μmol/L					
Free[a]	4.45	12.65	3.96	14.12	1.12
Short chain[a]	2.45	6.14	2.96	7.64	0.54
Long chain[a]	0.87	1.33	0.93	1.76	0.15
Total[a]	7.78	20.12	7.84	23.51	1.53
Acylated[a]	3.32	7.47	3.88	9.39	0.57

[1] Pooled standard error.
[a] L-Carnitine effect ($p<0.01$).

Table 3. Effects of L-carnitine and protein level on plasma carnitine proportion of 20 kg pigs

Protein level	Low		High		
L-carnitine, mg/kg diet	0	500	0	500	SE[1]
2 h fasting, %					
Free[a]	48.96	63.52	57.62	62.76	3.19
Short chain	37.88	29.02	31.86	30.46	3.42
Long chain[a]	13.14	7.46	10.56	6.74	1.28
Acylated[a]	51.04	36.48	42.38	37.24	3.19
24 h fasting, %					
Free[b]	57.16	62.33	50.52	59.98	2.49
Short chain	30.88	30.58	37.78	32.52	2.89
Long chain[a]	11.93	7.13	11.70	7.50	1.36
Acylated[b]	42.83	37.68	49.48	40.02	2.48

[1] Pooled standard error.
[a,b] L -Carnitine effect ($p<0.01$, $p<0.05$, respectively).

decreased by 40% ($p<0.01$). However, the proportion of short-chain acyl-carnitine concentration was not changed by carnitine supplementation ($p>0.10$). Neither fasting for 24 h nor high protein feeding affected plasma carnitine concentrations and their relative compositions ($p>0.10$). All criteria of energy balance were unaffected by L-carnitine ($p>0.10$, table 4). However, as expected, high protein feeding increased net energy for gain (NEg, $p<0.10$). No interactions were found between L-carnitine and protein level ($p>0.10$).

Table 4. Effects of L-carnitine and protein level on energy balance of 20 kg pigs[1,2]

Protein level	Low		High		
L-carnitine, mg/kg diet	0	500	0	500	SE[1]
GE consumed, MJ/d	14.20	14.16	14.15	14.33	0.08
DE consumed, MJ/d	12.75	12.62	12.73	12.90	0.09
ME consumed, MJ/d	12.54	12.43	12.50	12.70	0.08
eat production, MJ/d	9.04	8.62	8.31	8.49	0.27
NEg, MJ/d[e]	3.50	3.81	4.19	4.20	0.27
Energy content, MJ/kg EBW[4,5]	8.48	8.72	8.56	8.40	0.15
DE/GE, %	89.80	89.14	89.98	90.03	0.38
ME/GE, %	88.34	87.80	88.37	88.58	0.34
HP/GE, %	63.68	60.86	58.84	59.29	1.86
NEg/GE, %	24.67	26.90	29.53	29.29	2.07

[1] Values are means for five pigs per treatment.
[2] A total of 25 pigs in five identical trials, four pigs/trial were used for final body energy values, and one pig/trial was used to calculate the initial empty body energy value of treatment pigs for energy balance.
[3] Pooled standard error.
[4] Whole body energy except for the contents of the gastrointestinal tract and urine contained within the bladder.
[5] Initial energy content of five littermate pigs was 8.20± 0.15 MJ/kg EBW.
[a] Effect of protein level ($p<0.10$).
Abbreviations: GE, gross energy; DE, digestible energy; ME, metabolizable energy; HP, heat production, NEg, net energy gain; EBW, average empty body weight.

Daily carnitine excretion and correlation analysis

Carnitine supplementation increased daily urinary carnitine excretion ($p<0.01$) linearly over time, but had little affect on daily fecal carnitine excretion (figure 1). High protein feeding had negligible effects on urinary or fecal daily carnitine excretions. There were positive correlations between plasma free carnitine and urinary carnitine excretion. Plasma free carnitine was positively correlated to urinary carnitine excretion ($r^2=0.474$, $p<0.001$, figure 2). The renal threshold for carnitine was not detected during the balance trial in that excretion continued to increase linearly ($p<0.001$) with increasing plasma concentration.

Carnitine balance, biosynthesis and retention

Carnitine supplementation increased carnitine retention in the body ($p<0.01$, table 5), urinary excretion ($p<0.01$) and fecal excretion ($p<0.05$). The range in biosynthesis rate of L-carnitine in pigs fed 0.9% lysine+0 ppm L-carnitine diet was 6.71 to 7.23 μmol $\cdot$ kg^{-1} EBW $\cdot$ d^{-1}, and that in pigs fed 1.2% lysine diet+0 ppm L-carnitine was 10.13 to 11.62 μ mol $\cdot$ kg^{-1} EBW $\cdot$ d^{-1} (table 5). In the 500 ppm

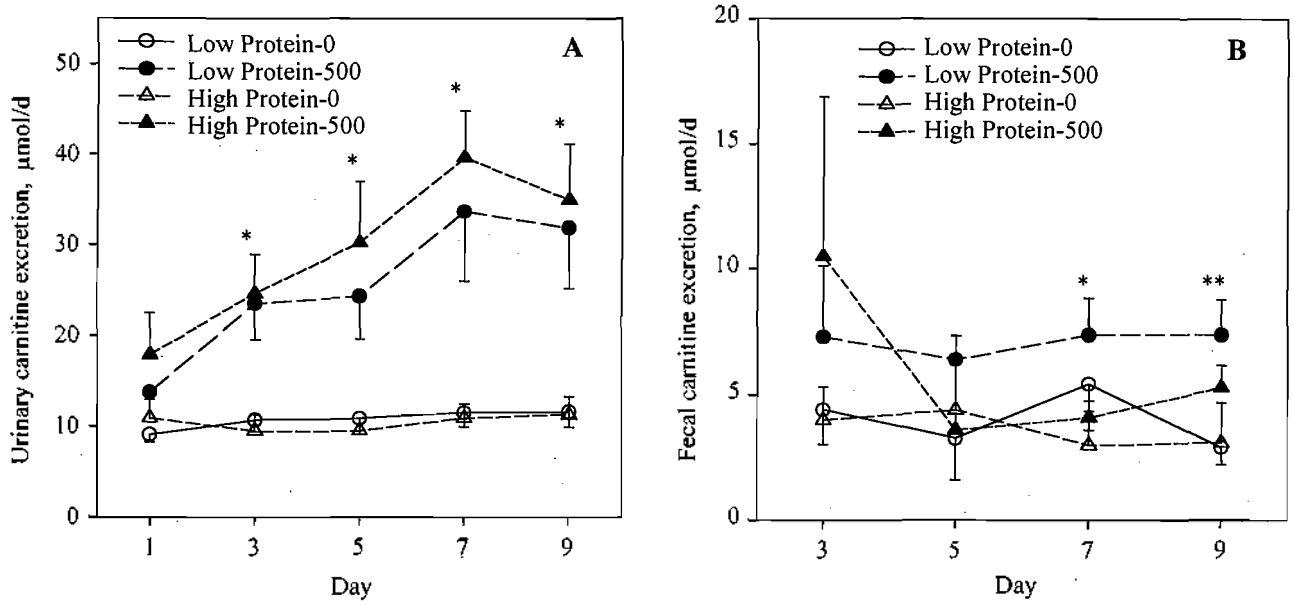

Figure 1. Effect of dietary L-carnitine and protein levels on daily carnitine excretion of 20 kg pigs. Open and closed symbols refer to 0 and 500 ppm dietary carnitine, respectively. Each value represents the mean±SEM of 5 pigs. A, urinary excretion. L-Carnitine main effect ($p<0.01$). Time main effect ($p<0.01$). L-Carnitine × time interaction ($p<0.01$). * L-Carnitine effect within each respective day ($p<0.01$). B, fecal excretion. L-Carnitine main effect ($p<0.05$). * Protein effect on d 7 ($p<0.05$). ** L-Carnitine effect on d 9 ($p<0.01$).

L-carnitine group, approximately 30 to 40% of dietary L-carnitine was absorbed while 60-70% was degraded in the intestinal tract, and thus negligible amounts were excreted in the feces. High protein feeding tended to increase the maximum biosynthesis rate of L-carnitine (μmol · kg^{-1} EBW · d^{-1}) by 61% in pigs fed diets without L-carnitine ($p<0.10$). However, the estimated amounts or percentages of dietary L-carnitine either degraded or absorbed from the GI tract in pigs fed diets with 500 ppm L-carnitine were not affected by protein.

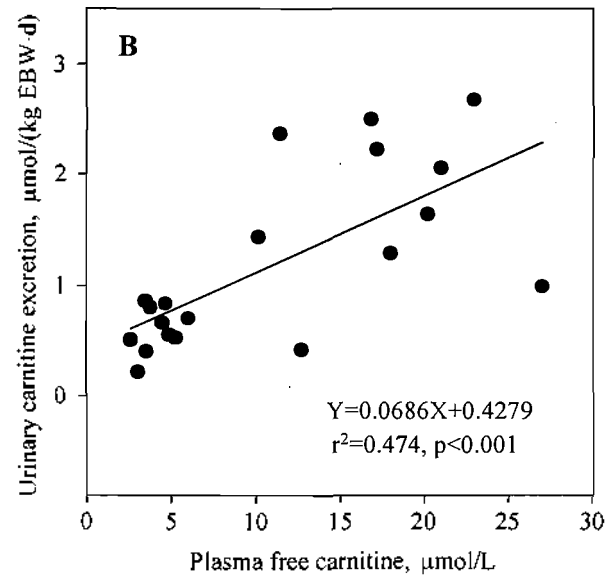

Figure 2. Linear relationships between plasma free carnitine and urinary carnitine excretion. Plasma free carnitine was measured 2 h after previous meal (on d 10).

DISCUSSION

Plasma carnitine concentrations and proportions

Plasma free, short-chain and long-chain carnitine concentrations were increased by dietary carnitine at both sampling times. The observed increase in concentrations of plasma short-chain carnitine may result from increased production of acetyl-CoA (via carnitine acetyltransferase), as a product of -oxidation of long-chain acyl-CoA which also could have been accelerated by elevated tissue carnitine concentration (Brass and Hoppel, 1980). Increased plasma short-chain and long-chain carnitine concentrations reported herein may result from increased tissue short-chain and long-chain carnitine concentrations (Heo et al., 2000b).

Energy utilization

Energy-balance data reported in the present paper are in agreement with the results of Hoffman et al. (1993) in that L-carnitine did not alter overall energy balance/accretion. On the other hand, we suggest that energy limitation in our study using the same pigs (i.e., high lysine/ME treatment and 85% ad libitum feeding) amplified energy utilization from dietary fat,

Table 5. Effect of L-carnitine and protein level on L-carnitine balance, biosynthesis, absorption and microbial degradation in 20 kg pigs[1,2]

Protein level	Low		High		
L-carnitine, mg/kg diet	0	500	0	500	SEM
Carnitine balance, μmol · kg^{-1} EBW · d^{-1}					
Intakea	0.72	129.30	1.67	131.05	2.60
Body Retention[a]	6.67	46.03	11.12	51.10	2.82
Urine excretion[a]	0.55	1.35	0.49	1.50	0.12
Fecal excretion[b]	0.20	0.41	0.19	0.31	0.06
Limits of biosynthesis in body, μmol · kg^{-1} EBW · d^{-1}					
Calculated minimum[3]	6.71		10.13		1.26
Calculated maximum[4,c]	7.23		11.62		1.40
Dietary L-carnitine absorbed, μmol · kg^{-1} EBW · d^{-1}					
Calculated minimum[5]		40.12		40.98	2.83
Calculated maximum[6]		47.38		52.60	1.79
Dietary L-carnitine degraded in GI tract, μmol · kg^{-1} EBW · d^{-1}					
Calculated minimum[7]		81.50		78.13	2.32
Calculated maximum[8]		88.73		89.75	3.09
Dietary L-carnitine absorbed/intake, %					
Calculated minimum		31.20		31.83	2.22
Calculated maximum		36.76		40.67	1.52
Dietary L-carnitine degraded in GI tract/intake, %					
Calculated minimum		62.92		59.08	1.55
Calculated maximum		68.48		67.93	2.24

[1] Values are means for five pigs per treatment. A total of 25 pigs in five identical trials, four pigs/trial for treatments, and one pig/trial for initial body composition were used. Average empty body weight (EBW) between initial and final EBW was used for the kg EBW unit. Initial EBW was calculated using the EBW percentage of the littermate pig in each trial. Final EBW was weighed except for contents of the gastrointestinal tract and urine contained within the bladder.

[2] Carnitine balance equation used was [dietary L-carnitine (DC)+biosynthesis (BS)=body retention (BR)+urinary excretion (UE)+fecal excretion (FE)+microbial degradation (MD)].

[3] Values were estimated assuming MD=0, [i.e., BS=BR+UE+FE-DC].

[4] Values were estimated assuming MD=DC-FE, [i.e., BS=BR+UE].

[5] Values were estimated assuming BS=maximum BS of the same protein diet without carnitine supplementation, [i.e., absorbed carnitine=BR+UE-maximum BS].

[6] Values were estimated assuming BS=0, [i.e., absorbed carnitine=BR+UE].

[7] Values were estimated assuming BS=0, [i.e., MD=DC-BR-UE-FE].

[8] Values were estimated assuming BS=maximum BS of the same protein diet without carnitine supplementation, [i.e., MD= DC+maximum BS-BR-UE-FE].

[a,b] Effect of L-Carnitine ($p<0.01$, $p<0.05$, respectively).

[c] Effect of protein level ($p<0.10$).

and thus N utilization was impacted (Heo et al., 2000a). Collectively these observations show that carnitine improved nitrogen utilization under ME-limited conditions, without a net impact on overall energy balance. The most likely explanation for the lack of additional energy gain is that energy was diverted from fat storage and used for body protein synthesis. Another possibility is that the maintenance energy cost in the L-carnitine group was higher, because muscle tissue is higher than adipose tissue in the basal metabolic rate (Ramsey et al., 1998; Whittemore, 1983).

Daily carnitine excretions and correlations

The trend in daily urinary carnitine excretion was similar to that observed by Baker et al. (1993) who administrated one 2,500 mg L-carnitine tablet per day to adult humans and investigated plasma and urinary carnitine status over a 10-d period. These observations are in general agreement with renal adaptation to elevated carnitine intake via reduction in efficiency of carnitine reabsorption (Rebouche et al., 1993). Penn et

al. (1997) reported that the renal carnitine threshold was between 15 and 35 μmol/L of plasma free carnitine in neonatal pigs. However, a threshold was not detected in our study (up to 25 μmol/L of plasma free carnitine). In addition, the apparent renal threshold of plasma free carnitine was estimated to be 46.4 $\pm$ 2.0 μmol/L using continuous blood and urine sampling techniques in newborn pigs (Heo, 2000).

Biosynthesis, microbial degradation and absorption of L-carnitine

Because corn and soybean meal are devoid of carnitine (Mitchell, 1978; Borum, 1983), corn-soybean meal diets were used in this study to minimize basal diet levels of carnitine. Only a trace amount of carnitine was measured in the corn-soybean basal diets, and those values (3-5 ppm) in the 0 ppm L-carnitine diets were included in the balance equation calculations. Considerable evidence from rat and human studies has delineated the metabolic pathway and the efficiency of dietary L-carnitine utilization in mammals. Gross and Henderson (1984) found that L-carnitine concentration in the intestinal lumen was negatively correlated to L-carnitine uptake efficiency into intestinal tissue in rats. Uptake by the intestinal tissue in 30 min was reduced from 78% to 6% when the dose of L-carnitine injected into the lumen was increased from 0.002 μmol to 288 μmol. According to this result, intestinal tissues can absorb more than 70% of intestinal carnitine when it is present in trace amounts. Thus, biosynthesis rates in our study of 20 kg pigs fed diets with no added L-carnitine could be close to the minimum calculated rates (6.71 and 10.63 μmol $\cdot$ kg^{-1} $\cdot$ d^{-1} of low protein and high protein group, respectively). Rebouche and Chenard (1991) estimated endogenous carnitine synthesis rate in adult humans consuming low- and high-carnitine (1.79 vs. 9.89 μmol $\cdot$ kg^{-1} BW $\cdot$ d^{-1}) by administering a tracer dose of [methyl-^{3}H] L-carnitine orally with a meal. The high-carnitine diet decreased endogenous carnitine synthesis rate from 2.47 to 0.48 μmol $\cdot$ kg^{-1} $\cdot$ d^{-1} in their research. Because L-carnitine intake ($\sim$130 μmol $\cdot$ kg^{-1} EBW $\cdot$ d^{-1}) in our 500 ppm carnitine group was 13 times higher than in their study, we felt justified in our assumption that the biosynthesis rate would approach zero in this group. With these collective findings and assumptions, we estimated that the dietary L-carnitine absorbed from the GI tract of 20 kg pigs fed diets with 500 ppm L-carnitine could be near to a maximum rate of 47.4 and 52.6 μmol $\cdot$ kg^{-1} EBW $\cdot$ d^{-1} for the low protein and high protein groups, respectively. The efficiency of carnitine absorption is less than that of amino acids (active transport mechanism; Thwaites et al., 1993, 1994), and L-carnitine intake is negatively correlated with the efficiency of carnitine absorption (Gross and Henderson, 1984). For example, the efficiency of carnitine absorption in humans (65 to 75%) who consumed 1.8 μmol $\cdot$ kg^{-1} $\cdot$ d^{-1} of L-carnitine (Rebouche and Chenard, 1991) was greater than that in pigs (31 to 41%) which were fed 130 μmol $\cdot$ kg^{-1} $\cdot$ d^{-1} of L-carnitine in our study. The estimated rate of carnitine biosynthesis of growing pigs from this study was greater than that of adult humans ($\sim$9 vs. 2.47 μmol $\cdot$ kg^{-1} $\cdot$ d^{-1}, Rebouche et al., 1991). Furthermore, the maintenance of constant body carnitine concentration between d 54 and 64 in pigs fed 0 ppm L-carnitine suggested that growing animals could synthesize sufficient L-carnitine *de novo* to match their daily body weight gain.

We conclude that endogenous carnitine biosynthesis may be adequate to maintain sufficient tissue levels during growth, but that supplemental dietary carnitine (at 500 ppm) may be sufficiently retained to support the overall hypothesis that L-carnitine may alter nutrient partitioning and thus body composition of 20-kg pigs (Heo et al., 2000a). Furthermore, increased plasma acyl-carnitine concentration reported herein may support underlying hypothesis that dietary carnitine may increase in vivo fatty acid oxidation in growing pigs. To strengthen this underlying hypothesis, the free carnitine concentrations in liver and muscle were compared with the corresponding Km's for carnitine of carnitine palmitoyltransferase-I in growing pigs (Heo et al., 2000b). This enzyme kinetic result also suggests a metabolic need for supplemental carnitine in these pigs to ensure that the activity of CPT-I *in vivo* is not constrained by carnitine availability.

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