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K Q. Owen

H Ji

C V. Maxwell

See next page for additional authors

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Abstract

Thirty six Yorkshire gilts (initially 123 lb BW) were used to investigate the effect of dietary carnitine on growth performance, carcass characteristics, fatty acid oxidation, and enzyme kinetics. Dietary carnitine reduced fat deposition in favor of protein deposition, stimulated fatty acid oxidation, induced the expression of pyruvate carboxylase, increased the capacity of pyruvate carboxylase flux, and decreased the capacity of branch chain keto-dehydrogenase.; Swine Day, Manhattan, KS, November 21, 1996

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Authors

K Q. Owen, H Ji, C V. Maxwell, G C. Tremblay, S I. Koo, S A. Blum, Jim L. Nelssen, Robert D. Goodband, and Michael D. Tokach

EFFECT OF DIETARY L-CARNITINE ON GROWTH, CARCASS CHARACTERISTICS, AND METABOLISM OF SWINE[1]

K. Q. Owen, H. Ji[2], C. V. Maxwell[3], J. L. Nelssen, R. D. Goodband, M. D. Tokach, G. C. Tremblay[2], S. I. Koo[4], and S. A. Blum[5]

Summary

Thirty six Yorkshire gilts (initially 123 lb BW) were used to investigate the effect of dietary carnitine on growth performance, carcass characteristics, fatty acid oxidation, and enzyme kinetics. Dietary carnitine reduced fat deposition in favor of protein deposition, stimulated fatty acid oxidation, induced the expression of pyruvate carboxylase, increased the capacity of pyruvate carboxylase flux, and decreased the capacity of branch chain keto-dehydrogenase.

(Key Words: Carnitine, Feed Efficiency, Carcass.)

Introduction

The primary role of carnitine in intermediary metabolism is to transport long chain fatty acids across the mitochondrial membrane into the mitochondrial matrix, where they are broken down through β-oxidation. Results from Kansas State University has shown that feeding L-carnitine to pigs during the growing-finishing phase resulted in small increases in longissimus muscle area and decreases in backfat thickness and lipid accretion rates. However, the mode of action by which carnitine elicits these responses has not been investigated in swine. Therefore, the objective of our study was to evaluate the influence of dietary carnitine on growth performance, carcass characteristics, fatty acid oxidation rates, and enzyme kinetics in finishing swine.

Procedures

Animals. All research was conducted at Oklahoma State University, Stillwater, OK. Thirty-six Yorkshire gilts (initially 123 lb BW) were blocked by weight and sire group in a randomized complete block design. Three pigs were housed per pen (7 ft × 8 ft) in an environmentally regulated finishing barn with total slatted concrete flooring. There were four replicate pens per treatment. Each pen contained a single-hole self-feeder and a nipple waterer to provide ad libitum access to feed and water. Pig weights and feed disappearance were recorded every 14 d to determine ADG, ADFI, and F/G.

A basal diet based on corn and soybean meal (Table 1), was formulated to contain .85% lysine and 2.5% soy oil and was fed in meal form. L-carnitine replaced corn to provide added dietary carnitine levels of 50

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[2]Department of Biochemistry, Microbiology, and Molecular Genetics, University of Rhode Island, Kingston.

[3]Department of Animal Science, Oklahoma State University, Stillwater.

[4]Department of Foods and Nutrition.

[5]Lonza Inc., Fair Lawn, NJ.

and 125 ppm. All other nutrients either met or exceeded NRC (1988) estimates for pigs between 110 and 240 lb.

Table 1. Diet Composition[a]

Ingredient	Percent
Corn[b]	73.80
Soybean meal, (48% CP)	21.32
Soybean oil	2.50
Dicalcium phosphate, (18% P)	.85
Limestone	.78
Salt	.35
Copper sulfate	.10
Antibiotic[c]	.05
Vitamin and mineral premix	.25
Total	100.00

[a]Formulated to contain .85% lysine, and .60% Ca and .50% P.
[b]L-carnitine replaced corn to provide dietary carnitine levels of 50 and 125 ppm. Analyzed values were 9, 49, and 123 ppm.
[c]Provided 18 mg/kg tylosin.

Carcass Characteristics. Food was withheld from pigs for 18 to 24 hrs prior to slaughter, after which all pigs were slaughtered and standard carcass measurements were recorded. The heart, liver, kidney, and kidney fat were removed from each carcass following slaughter and weighed. Three samples per muscle (25 to 50 g) were taken from the longissimus muscle (a three rib sampie taken between the 9th and 11th rib), the biceps femoris and semitendinosus muscles of the ham, and the liver. After the liver and muscles were dissected, they were ground and subsampled for proximate analysis and measurement of tissue carnitine and free amino acid concentrations.

Blood Samples. Blood samples were collected via vena cava puncture between 1 and 2 h after pigs were removed from pens. Plasma was harvested and stored until analysis for plasma carnitine, IGF-1, IGF-2, insulin, and other blood metabolites.

Isolation of Mitochondria. As the gilts were slaughtered, a 5 to 10 g sample of liver and muscle was excised and mitochondria were isolated by centrifugation. The sample was taken from the semitendinosus muscle from the ham.

Isolation of Hepatocytes. A 20 to 30 g sample of the liver was excised and used to isolate hepatocytes by collagenase digestion. Viability of the hepatocytes was assessed by exclusion of trypan blue and was routinely higher than 95%. Protein content of the hepatocyte suspension was determined by the biuret method, using bovine albumin as the standard.

Palmitate Oxidation in Isolated Mitochondria. Oxidation of fatty acids was assayed using [1-^{14}C] palmitate. The sum of radioactivity from CO_2 trapped in a center well and radioactivity in the acid-soluble fraction of a reaction mixture was used to calculate the oxidation of palmitate.

Pyruvate Carboxyase Flux in Isolated Mitochondria. Pyruvate carboxylase flux was assayed by measuring pyruvate-dependent incorporation of [^{14}C] $KHCO_3$ into acid-stable radiolabeled products during 10 min of incubation at 98.6°F.

Branch Chain Keto-Dehydrogenase Flux in Isolated Liver Mitochondria. Flux through branch chain keto-dehydrogenase was assayed in the same reaction mixture used for measuring pyruvate carboxylase flux except [1-^{14}C]Na isocaproate was used in place of pyruvate and $KHCO_3$ was used instead of [^{14}C]$KHCO_3$.

Pyruvate Carboxylase Activity in Particle-Free Extracts of Liver Mitochondria. Particle-free extracts of mitochondria were prepared by homogenizing mitochondria (~20 mg protein) in 1 mL of detergent solution containing: deoxycholate (0.1%), Tris buffer (0.1 mol/L, pH 7.2), and glutathione (1 mmol/L). Enzymatic activity was assayed by coupling with excess malate dehydrogenase and spectrophotometric measurement of the resultant pyruvate-dependent

oxidation of NADH at 339 nm during 5 min incubation at 77°F.

Branch Chain Keto-Dehydrogenase Flux in Isolated Muscle Mitochondria. Flux through branch chain keto-dehydrogenase in muscle mitochondria was assayed as described for liver mitochondria.

Palmitate Oxidation in Isolated Hepatocytes. Palmitate oxidation by hepatocytes was assayed similar to palmitate oxidation with mitochondria (see above).

Protein Synthesis in Isolated Hepatocytes. Protein synthesis was assayed by measuring incorporation of [^{35}S]methionine into TCA precipitate.

Statistical Analysis. The pen was the experimental unit for analyses of performance and carcass data, and the pig was the experimental unit for analyses of liver and muscle assays. Means were separated using linear and quadratic polynomials. Carcass data and organ weights were analyzed using cold carcass weight as a covariate.

Results and Discussion

Increasing dietary L-carnitine increased carnitine concentrations found in plasma, liver, longissimus muscle, and bicep femoris muscle (linear, $P<.01$; Table 3). This indicates increased biological availability of carnitine within the body. Despite increased concentrations of carnitine in plasma, liver, and muscle tissues in response to dietary carnitine supplements, adding L-carnitine at levels up to 125 ppm had no effect on growth performance ($P>.10$; Table 3). However, supplemental L-carnitine reduced 10th and average backfat thickness (linear, $P<.10$ and $P<.05$, respectively) and increased percentage lean and muscle (linear, $P<.05$); Table 3), which suggests that providing L-carnitine reduces fat accretion. Also, visual scores for carcass muscling, longissimus marbling, and firmness were not affected ($P>.10$) by dietary treatment (Table 3). However, increasing L-carnitine increased visual scores for longissimus color (quadratic, $P<.05$).

The second objective of our study was to evaluate a metabolic model to determined carnitine's influence on intermediary metabolism in finishing swine. This metabolic model anticipated that added dietary L-carnitine could promote the breakdown of fatty acids, thereby increasing the rate of acetyl CoA formation and thus, the energy charge (ATP/ADP ratio) of the cell. By increasing the breakdown of fatty acids, the activity of enzymes such as pyruvate carboxylase (rate-limiting enzyme in gluconeogenesis) and branch chain keto-dehydrogenase (rate-limiting enzyme in branch chain amino acid breakdown) also should be altered. Activation of pyruvate carboxylase should favor gluconeogenesis and the use of carbon chains of pyruvate for the production of amino acids. Also, enhanced fatty acid oxidation should inhibit branch chain keto-dehydrogenase by elevating concentrations of acetyl CoA, NADH, and ATP and, thereby, reducing the breakdown of branch chain amino acids. In turn, this should reduce the breakdown of other amino acids and ultimately promote protein synthesis.

Palmitate oxidation in isolated liver mitochondria (linear, $P<.01$) and hepatocytes (linear, $P<.01$) was increased in carnitine-fed pigs (Table 4 and 5). This clearly demonstrates that carnitine increases breakdown of fatty acids. These results are consistent with the reduction in backfat thickness in pigs fed L-carnitine.

Flux through pyruvate carboxylase in mitochondria (linear, $P<.01$) and pyruvate carboxylase activity (linear, $P<.01$) in liver mitochondria extract was increased for pigs fed added dietary L-carnitine (Table 4). Although neither the concentration of acetyl CoA or the energy charge (ATP/ADP ratios) were measured in this study, we could speculate that the increase in pyruvate carboxylase flux was caused by these effectors.

Flux through branch chain keto-dehydrogenase in liver (linear, $P<.01$) and muscle (linear, $P<.01$) mitochondria was reduced in pigs fed increasing L-carnitine (Table 4.). As in the case of pyruvate carboxylase flux, we could hypothesize that increased concen-

trations of acetyl CoA, NADH, and ATP from carnitine's influence on β-oxidation were responsible for the inhibitory effect on branch chain keto-dehydrogenase flux. By enhancing fatty acid breakdown, branch chain keto-dehydrogenase flux should be inhibited by elevations of acetyl CoA, NADH, and ATP levels, thereby, reducing the breakdown of branch chain amino acids.

Increasing dietary L-carnitine increased the content of some amino acids in liver and muscle tissues (Tables 6, 7, and 8). Among these amino acids, glutamine; proline; and the branch chain amino acids (leucine, isoleucine, and valine) were elevated consistently. Because the branch chain keto-dehydrogenase flux was inhibited, we would expect increases in the contents of branch chain amino acid, which were increased consistently. Other amino acids that were increased included: aspartic acid, threonine, cysteine, methionine, tyrosine, phenylalanine, histidine, and lysine. Most of these are essential amino acids and cannot be synthesized in vivo but possibly can be spared.

Increased protein synthesis (linear, $P<.01$; Table 5) in the isolated hepatocytes of pigs fed increasing L-carnitine and an increase in percentage muscle are consistent with the effect that branch chain amino acids have on protein synthesis. Therefore, these results indicate that dietary carnitine reduced fat deposition in favor of protein deposition in finishing gilts. However, more research is needed to document the effect L-carnitine has on amino acid metabolism.

Increasing dietary L-carnitine increased insulin (linear, $P<.05$) concentrations taken after a 1 hr fast. Also, albumin and lactate dehydrogenase concentrations were decreased (linear, $P<.01$) by increasing levels of dietary L-carnitine. This is the first research to show these effects on insulin and blood metabolites from L-carnitine. Current research is ongoing to validate these responses.

In summary, dietary carnitine reduced fat deposition in favor of protein deposition, stimulated fatty acid oxidation, and positively influenced amino acid metabolism. Because of carnitine's role in fat and amino acid metabolism, research is being conducted to determine if carnitine can be used to spare dietary energy and amino acids. This possibly could allow producers to reduce diet cost and still obtain added benefits in carcass merits. Results from this experiment provide evidence of carnitine's role in intermediary metabolism and insight into the different mechanisms involved with its utilization.

Table 2. Tissue Carnitine Concentrations Found in Liver, Longissimus Muscle, Biceps Femoris, and Plasma[a]

	L-Carnitine, ppm			
Item	0	50	125	CV
Whole tissue, nmol/g				
Liver[b]	93.4	123.9	155.1	28.5
Longissimus muscle[b]	864.6	1316.5	1569.4	13.5
Biceps femoris[b]	838.7	1239.8	1640.2	9.8
Plasma Carnitine, μM/L				
Total[b]	6.2	10.7	14.3	26.3
Free[b]	5.0	8.4	12.0	27.9
Esters[c]	1.2	2.3	2.2	61.6

[a]A total of 24 pigs, eight pigs/treatment.
[b]Linear effect of dietary L-carnitine ($P<.01$).
[c]Linear effect of dietary L-carnitine ($P<.10$).

Table 3. Performance and Carcass Characteristics of Pigs Fed L-Carnitine from 123 to 267 lb[a]

	Added Carnitine, ppm			
Item	0	50	125	CV
Growth performance				
ADG, lb	1.96	2.01	1.94	6.2
ADFI, lb	6.26	6.45	6.17	5.8
F/G	3.20	3.22	3.17	6.3
Carcass characteristics				
Live wt, lb	267	264	260	3.2
Dressing percentage	75.51	74.53	74.62	3.0
Average BF, in[b]	1.20	1.16	1.14	9.7
10th rib BF, in[c]	.95	.88	.85	15.7
Longissimus muscle, in^2	5.78	5.87	6.31	12.0
Percentage lean[cd]	50.03	50.91	52.08	4.2
Percentage muscle[ce]	54.19	54.86	55.48	4.6
Organ weights				
Liver, g	1680	1666	1710	12.7
Heart, g	411	419	388	16.2
Kidney, g	378	370	353	12.1
Kidney fat, g	1160	1114	1044	23.9
Quality				
Muscle score[f]	2.40	2.64	2.68	17.8
Color[gh]	2.64	3.00	2.75	15.8
Firmness	3.18	3.07	2.92	19.8
Marbling	2.59	2.63	2.50	47.2

[a]A total of 36 pigs, three pigs/pen, four replicate pens/treatment.
[b]Linear effect of dietary L-carnitine ($P < .10$).
[c]Linear effect of dietary L-carnitine ($P < .05$).
[d]Percentage lean was calculated from NPPC (1991) equation for percentage lean with 5% fat.
[e]Percentage muscle was calculated from NPPC (1991) equation for percentage muscle with 10% fat.
[f]Carcasses were evaluated on a 3-point scale ranging from thin muscling (1) to extremely thick muscling (3).
[g]Quadratic effect of dietary L-carnitine ($P < .05$).
[h]Loins were evaluated on a 5-point scale according to NPPC (1991) procedures with 1 = light and 5 = dark.

Table 4. Effect of Dietary L-Carnitine on Palmitate Oxidation, Pyruvate Carboxylase, and Branch Chain Keto-Dehydrogenase in Liver Mitochondria; Branch Chain Keto-Dehydrogenase in Muscle Mitochondria; and Pyruvate Carboxylase Activity in Liver Extracts[a]

	L-Carnitine, ppm			
Item	0	50	125	CV
Liver mitochondria				
Palmitate oxidation, nmol/mg protein/hr[b]	10.6	11.9	15.3	13.8
Pyruvate carboxylase flux, nmol/mg protein/hr[b]	20.4	30.9	44.2	16.5
Branch chain keto-dehydrogenase flux, nmol/mg protein/hr[bc]	82.2	60.4	54.1	13.2
Muscle mitochondria				
Branch chain keto-dehydrogenase flux, nmol/mg protein/hr[bcd]	108.8	110.1	86.5	9.2
Liver mitochondria extract				
Pyruvate carboxylase activity, nmol product/mg protein/min[bc]	.09	.15	.26	17.2

[a]A total of 24 pigs, eight pigs/treatment.
[b]Linear effect of dietary L-carnitine ($P<.01$).
[c]Quadratic effect of dietary L-carnitine ($P<.10$).
[d]Quadratic effect of dietary L-carnitine ($P<.01$).

Table 5. Effect of L-Carnitine on Palmitate Oxidation and Protein Synthesis in Isolated Hepatocytes[a]

	L-Carnitine, ppm			
Item	0	50	125	CV
Palmitate oxidation, nmol/mg protein/hr[b]	.94	1.56	2.38	24.8
Protein synthesis, nmol/mg protein/hr[b]	.95	1.25	1.75	10.0

[a]A total of 24 pigs, eight pigs/treatment.
[b]Linear effect of dietary L-carnitine ($P<.01$).

Table 6. Amino Acid Concentrations in Longissimus Muscle (Samples from 9th, 10th and 11th rib)[a]

	L-Carnitine, ppm			
Item, μmol/g	0	50	125	CV
Aspartic acid[b]	7.09	7.49	7.70	7.0
Threonine[c]	3.44	3.66	3.77	7.7
Glutamic acid[c]	10.99	11.89	12.12	8.9
Proline[c]	2.82	2.99	3.16	10.2
Alanine[b]	4.38	4.60	4.73	6.3
Cysteine[c]	.90	.98	.97	6.4
Valine	3.90	4.11	4.10	7.4
Methionine[c]	2.15	2.35	2.36	7.3
Isoleucine	3.59	3.82	3.79	7.8
Leucine[b]	6.26	6.66	6.80	6.2
Tyrosine[b]	2.74	2.94	2.98	6.5
Phenylalanine[b]	3.15	3.37	3.42	5.6
Histidine[cd]	3.37	3.69	3.66	8.4
Lysine[c]	6.89	7.31	7.49	6.8
Arginine[c]	5.01	5.31	5.42	6.9
Tryptophan[c]	.95	1.07	1.05	8.6

[a]A total of 24 pigs, eight pigs/treatment.
[bc]Linear effect of dietary L-carnitine ($P<.05$, $P<.10$, respectively).
[d]Quadratic effect of dietary L-carnitine ($P<.10$)

Table 7. Amino Acid Concentrations Found in Biceps Femoris Muscle[a]

	L-Carnitine, ppm			
Item, μmol/g	0	50	125	CV
Aspartic acid	5.86	6.13	6.13	8.0
Threonine	2.69	2.93	2.83	9.7
Glutamic acid	8.27	9.76	9.67	18.8
Proline	2.63	2.62	2.69	45.5
Alanine	3.64	3.86	3.93	9.1
Cysteine	1.17	.79	.77	71.3
Valine[b]	2.86	3.26	3.21	17.0
Methionine	1.96	1.85	1.85	20.9
Isoleucine[c]	2.72	3.13	3.19	10.1
Leucine[b]	4.82	5.42	5.45	10.8
Tyrosine	2.65	2.35	2.37	29.9
Phenylalanine[d]	2.55	2.72	2.78	7.5
Histidine	2.29	2.81	2.76	21.5
Lysine[d]	4.83	5.95	6.02	21.8
Arginine	4.48	4.39	4.48	11.6
Tryptophan	1.28	.81	.86	77.9

[a]A total of 24 pigs, eight pigs/treatment.
[bcd]Linear effect of dietary L-carnitine ($P<.05$, $P<.01$, $P<.10$, respectively).

Table 8. Amino Acid Concentrations Found in Liver Tissue[a]

Item, μmol/g	L-Carnitine, ppm 0	50	125	CV
Aspartic acid[b]	5.73	5.99	6.08	4.9
Threonine[c]	2.83	2.92	3.01	4.5
Serine[c]	2.49	2.68	2.88	8.1
Glutamic acid[c]	6.99	7.42	7.88	6.1
Proline[c]	2.78	2.92	3.10	7.1
Alanine[d]	3.66	4.03	4.08	6.5
Cysteine[b]	1.24	1.29	1.29	8.1
Valine	4.06	4.22	4.12	6.9
Methionine[c]	1.46	1.51	1.56	4.0
Isoleucine	3.01	3.06	3.08	6.3
Leucine[b]	6.08	6.46	6.52	6.0
Tyrosine	2.56	2.61	2.64	4.5
Phenylalanine	3.40	3.54	3.54	5.8
Histidine	1.86	1.95	1.90	9.8
Lysine	5.14	5.30	5.32	5.1
Arginine	3.92	4.02	4.06	4.6
Tryptophan	.89	.90	.93	9.9

[a]A total of 24 pigs, eight pigs/treatment.
[bcd]Linear effect of dietary L-carnitine ($P<.05$, $P<.01$, $P<.10$, respectively).

Table 9. Effect of L-Carnitine on IGF-1, IGF-2, IGF1:IGF2 Ratio, Insulin Concentration, and Blood Metabolites after Pigs Were Removed from Feed for at Least One Hour[a]

Item	L-Carnitine, ppm 0	50	125	CV
IGF-1, ng/mL	66.8	72.98	57.64	30.2
IGF-2, ng/mL	139.33	146.39	133.16	12.5
IGF1:IGF2	.47	.50	.43	20.9
Insulin, ng/mL[bd]	.39	.60	.59	27.0
Cholesterol, mg/dL	82.38	86.88	82.73	12.5
Glucose, mg/dL	75.57	81.12	81.69	24.4
Albumin, mg/dL[b]	3.48	3.44	3.05	10.9
Blood urea nitrogen, mg/dL	15.60	15.54	14.34	19.3
Creatine, mg/dL	1.61	1.63	1.54	10.3
Triglycerides, mg/dL	41.99	48.50	42.64	49.8
Lactate dehydrogenase, ug/mL[ce]	1121.7	600.3	670.8	28.9
Aspartate aminotransferase, ug/mL	68.67	35.87	48.30	76.1
Alanine aminotransferase, ug/mL	42.47	42.38	47.23	34.4

[a]Data represents six to eight pigs/treatment.
[bc]Linear effect of dietary L-carnitine ($P<.05$, $P<.01$, respectively).
[de]Quadratic effect of dietary L-carnitine ($P<.10$, $P<.01$, respectively).