Effects of L-Carnitine on Growth Performance, Carcass Composition, and Metabolism of Lipids in Male Broilers

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ABSTRACT We studied the effects of L-carnitine on growth performance, carcass composition, and lipid metabolism in male broilers. Six hundred male commercial broilers were allotted to five groups, each of which included three replicates (40 birds per replicate). The groups received the same basal diet supplemented with 0, 25, 50, 75, or 100 mg/kg L-carnitine, respectively. The feeding trial showed that L-carnitine had no significant effect on daily gain or feed conversion. Supplementation with L-carnitine (above 25 mg/kg) in the diet increased breast muscle yield ($P < 0.05$) and crude fat content of the muscles and decreased abdominal fat content ($P < 0.05$). Addition of 50, 75, or 100 mg/kg L-carnitine to the diet decreased total activities of glucose-6-phosphate dehydrogenase, malic dehydrogenase, isocitrate dehydrogenase, and lipoprotein lipase ($P < 0.05$) in the subcutaneous fat and total activity of carnitine palmitoyltransferase-I ($P < 0.05$) in breast muscles. The results of this study indicate that L-carnitine could reduce the deposit of subcutaneous fat by decreasing total activities of enzymes in the fat and enhance intramuscular fat by decreasing the activity of carnitine palmitoyltransferase-I in breast muscles.

(*Key words*: broiler, carcass composition, L-carnitine, lipid metabolism)

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INTRODUCTION

Production of broiler chickens containing excess body fat is a problem in the poultry industry. Several factors, such as nutrients and genetics, contribute to the tendency for broilers to accumulate excess body fat. Therefore, improving carcass composition with additives has become a main focus of nutrition research. L-Carnitine, a zwitterionic compound synthesized in vivo from lysine and methionine, is essential for the transport of long-chain fatty acids across the inner mitichondrial membrane for β-oxidation (Borum, 1983). Carnitine supplementation of diets could be used to augment carnitine supply for use in metabolism, thereby facilitating fatty acid oxidation and reducing the amount of long-chain fatty acids available for storage in adipose tissue. Results of research with pigs (Owen, 1994; Kachura et al., 1995; Kudo et al., 1995) and sea bass (Burtle and Liu, 1994) indicate that L-carnitine supplementation to diets alters fat metabolism and reduces body fat.

Several studies have been done to determine whether dietary L-carnitine influences the carcass composition of broiler chickens, but the results obtained are not in agreement. Rabie et al. (1997) and Rabie and Szilagyi (1998) reported that supplementing 50 mg L-carnitine/kg diet to broiler chickens increased breast muscle yield and leg meat yield and content of fat in breast muscle ($P < 0.05$). Cartwright (1986) observed no effects of L-carnitine fed at 50 mg/kg of diet from 5 to 7 wk of age on abdominal fat weight and body fat. The experiment reported herein was conducted to determine the effects of dietary L-carnitine on metabolism of fat in broiler chickens.

MATERIALS AND METHODS

Six hundred 1-d-old male broiler chickens (Arbor Acres) obtained from a commercial hatchery were weighted and allotted to five treatment groups, each of which included three replicates of 40 birds. Broiler chickens were offered the same basal diet that was supplemented with 0 (control), 25, 50, 75, or 100 mg L-carnitine/kg diet. Diets were fed from 1 to 49 d and included starter (1 to 21 d), grower (22 to 36 d), and finisher (37 to 49 d). Nutrient levels of the diets were based on the National Research Council (1994) recommended nutrient requirements of broiler chickens (Table 1). At the 49 d of age, 18 broilers per treatment group (six birds per replicate) were killed for carcass analyses; 90 broilers were killed

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Abbreviation Key: CoA = coenzyme A; CPT-I = carnitine palmitoyltransferase I; FFA = free fatty acid; G-6-PD = glucose-6-phosphate dehydrogenase; HSL = hormone-sensitive lipase; ICD = isocitrate dehydrogenase; LPL = lipoprotein lipase; MDH = malic dehydrogenase; TG = triglyceride.

TABLE 1. Ingredient composition and nutrient content of diets

	Starter	Grower	Finisher
Ingredients (%)			
Corn	45.00	53.50	59.84
Soybean meal	36.00	33.00	27.00
Middlings	10.00	5.00	5.00
Corn oil	5.00	5.00	5.00
Calcium phosphate	1.50	1.10	0.80
Limestone	1.20	1.20	1.20
Salt	0.40	0.40	0.40
Methionine	0.20	0.10	0.06
Vitamin-mineral premix[1]	0.70	0.70	0.70
Total	100.00	100.00	100.00
Chemical composition			
ME,[2] (Mcal/kg)	3.19	3.21	3.24
Crude protein, %	22.12	20.79	18.52
Lysine, %	1.17	1.08	0.93
Methionine + cysteine, %	0.94	0.71	0.63
Calcium, %	1.05	0.92	0.82
Total phosphorus, %	0.74	0.62	0.53

[1]Supplied per kilogram of diet: riboflavin, 8.0 mg; niacin, 50 mg; pantothenic acid, 15 mg; 50% choline-chloride, 1,000 mg; cobalamin, 15 μg; cholecalciferol, 82.5 μg; vitamin E (DL-α-tocophery acetate), 25 IU; vitamin A (*trans*-retinyl acetate), 10,000 IU; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4 \cdot 7H_2O$, 300 mg; MnO, 100 mg; $CuSO_4 \cdot 5H_2O$, 20 mg; $ZnSO_4 \cdot H_2O$, 150 mg; $NaSeO_3$, 0.15 mg; KI, 0.5 mg; ethoxyquin, 100 mg; avoparcin, 15 mg.

[2]Values were calculated from data provided by Feed Database in China (1999).

in total. Each of these birds was deprived of feed for 12 h and individually weighed just prior to slaughter. The birds were bled via cardiac puncture for serum sample and then slaughtered and dissected by a trained team. The abdominal fat pad was dissected and weighed, and deboned breasts and leg muscles were also obtained and weighed.

In this experiment, broiler chickens were maintained in 2.3 × 1.9 m pens, equipped with nipple drinkers and hanging tuber feeders. Temperature was maintained at 32 C for the first 5 d and then gradually reduced according to normal management practices, until a temperature of 22 C was reached. Continuous lighting was maintained.

Serum, breast muscle, liver, and subcutaneous fat were collected and snap-frozen in liquid nitrogen. Frozen tissues were stored at –70 C prior to analysis. The concentrations of free carnitine in liver, breast muscles, and serum were determined according to the method of Wieland (1985). The activity of carnitine palmitoyltransferase I (CPT-I) in breast muscle was analyzed according to Grantham and Zammit (1988). The activities of hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), glucose-6-phosphate dehydrogenase (G-6-PD), malic dehydrogenase (MDH), and isocitrate dehydrogenase (ICD) in subcutaneous fat were determined according to the methods of Harry (1988), Taskinen (1980), Clock and Mclean (1953), Ochoa (1955), and Plant (1962), respectively. Serum triglyceride (TG) and free fatty acid (FFA) were analyzed with a biochemical analyzer.[2]

Data were analyzed by Student's *t*-test. The statistical analysis was accomplished using the general linear models procedure of SAS software (SAS Institute, 1996). A $P < 0.05$ was considered statistically significant. Pen was considered as the experimental unit for the entire index determined. Numbers (n) used for statistics is noted in the tables.

RESULTS

Growth Performance

No differences in weight gain, feed intake, or feed conversion efficiency were observed in male broilers fed the carnitine-supplemented diets (Table 2).

Carcass Composition

Supplementation with L-carnitine had little effect on leg muscle yield (Table 3). Breast muscle yield was in-

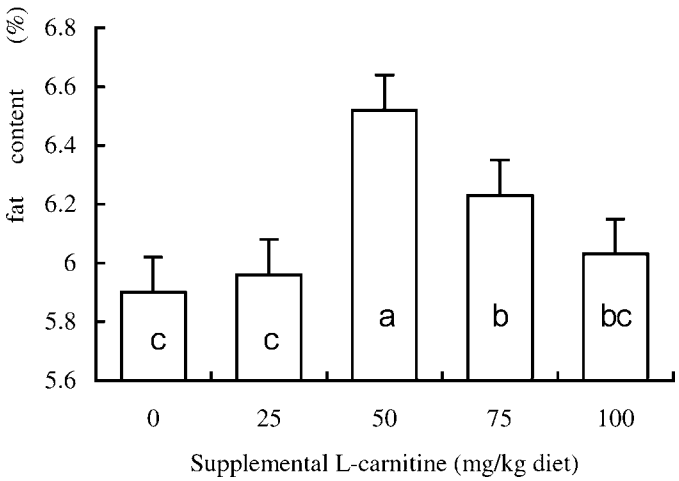

FIGURE 1. Content of fat in breast muscles of male broilers. [a–c]Bars without common letter differ ($P < 0.05$).

[2]ERBA CHEM-5, Beijing Biochemical Instrument Company, Beijing, China.

TABLE 2. Effect of L-carnitine on growth performance of male broilers

	Supplemental L-carnitine (mg/kg diet)						
	0	25	50	75	100	SEM	n
Weight gain (g/bird/d)	49.42	50.11	50.23	50.18	50.14	0.250	3
Feed intake (g/bird/d)	108.12	108.37	110.21	109.73	109.14	0.871	3
Feed: gain (g:g)	2.49	2.43	2.32	2.37	2.35	0.063	3

creased ($P < 0.05$) by supplementing with 50 or 75 mg/kg L-carnitine (Table 3). Abdominal fat content expressed as a percentage of body weight was decreased ($P < 0.05$) in male broilers fed the carnitine-supplemented diets (Table 3).

Carnitine Contents in Liver, Breast Muscle, and Serum

Free carnitine in liver increased ($P < 0.05$) by treatment with carnitine (Table 4). Supplementation of 75 or 100 mg L-carnitine/kg diet elevated ($P < 0.05$) free-carnitine contents in breast muscles (Table 4). Free carnitine in serum increased ($P < 0.05$) by supplementing of 50, 75, or 100 mg L-carnitine/kg diet (Table 4).

Fat Content in Breast Muscle

The content of fat in breast muscle of male broilers was increased ($P < 0.05$) by supplementing with 50 or 75 mg L-carnitine/kg diet (Figure 1).

Activities of Enzymes in Subcutaneous Fat

No difference was observed in the activity of HSL in subcutaneous fat (Table 5), whereas the activities of LPL, G-6-PD, MDH, ICD were decreased ($P < 0.05$) in subcutaneous fat of male broilers fed the carnitine-supplemented diets (Table 5).

Activity of CPT-I in Breast Muscles

The activity of CPT-I in breast muscles was decreased by supplementing with 50, 75 or 100 mg L-carnitine/kg diet (Figure 2).

TG and FFA in Serum

Serum FFA concentration was increased, whereas serum TG concentration was decreased by treatment with L-carnitine (Table 6).

DISCUSSION

In the study presented here, no differences in weight gain, feed intake, or feed conversion were observed in male broilers fed the carnitine-supplemented diets. Barker and Sell (1994) found that L-carnitine had no effect on BW or feed consumption in broilers when fed at 50 or 100 mg/kg. Supplementing of L-carnitine to starter pigs at 0 or 1,000 mg/kg showed a linear improvement in feed efficiency of young pigs with increasing dietary carnitine concentration (Owen et al., 1996). Owen et al. (1996) also noted an improvement in average daily gain of pigs fed 250 or 500 mg carnitine/kg diet. Future evaluations of carnitine on growing broilers probably should use greater dietary concentration of carnitine, especially because of limited intestinal absorptive capacity of carnitine and its considerable microbial degradation in the intestine.

We found an increase ($P < 0.05$) in the breast muscle percentage of body weight and fat content in breast muscles and a decrease ($P < 0.05$) in the abdominal fat percentage of body weight by adding L-carnitine in diet (above 25 mg/kg).The abdominal fat percentage of body weight was reduced ($P < 0.05$) at all levels of L-carnitine supplementation. Rabie et al. (1997, 1998) reported that supplementation with 50 mg/kg L-carnitine to broiler chicken diets increased breast muscle and leg meat yields, whereas quantity and percentage of abdominal fat were reduced by L-carnitine.

We observed that total activities of G-6-PD, MDH, and ICD in subcutaneous fat were decreased ($P < 0.05$)

TABLE 3. Effect of L-carnitine on carcass composition of male broilers

	Supplemental L-carnitine (mg/kg diet)						
	0	25	50	75	100	SEM	n
SBWT[1] (g)	2,035.67	2,022.83	2,035.83	2,039.67	2,026.33	6.210	3
PDB[2] (%)	16.99[b]	17.06[b]	17.97[a]	17.79[a]	17.27[b]	0.162	3
PDL[3] (%)	22.48	22.97	23.17	23.43	23.12	0.407	3
PDAT[4] (%)	1.17[a]	1.11[b]	0.98[c]	1.00[c]	1.03[c]	0.017	3

[a–c]Means within a line without a common superscript differ ($P < 0.05$).

[1]SBWT = weight just prior to slaughter.

[2]PDB = breast muscle weight (without bone and skin)/SBWT.

[3]PDL = leg muscle weight (without bone and skin)/SBWT.

[4]PDAT = abdominal fat weight/SBWT.

TABLE 4. The contents of carnitine in liver, breast muscle, and serum of male broilers

Free carnitine in tissue (μmol/g)	Supplemental L-carnitine (mg/kg diet) 0	25	50	75	100	SEM	n
Liver	0.46^{c}	0.55^{b}	0.60^{ab}	0.61^{a}	0.62^{a}	0.018	3
Breast	0.96^{b}	0.99^{b}	1.02^{b}	1.12^{a}	1.15^{a}	0.026	3
Serum	0.83^{c}	0.86^{c}	0.95^{b}	1.02^{ab}	1.03^{a}	0.025	3

$^{a-c}$Means within a line without a common superscript differ ($P < 0.05$).

TABLE 5. The activity of enzyme in subcutaneous fat of male broilers

	Supplemental L-carnitine (mg/kg diet) 0	25	50	75	100	SEM	n
HSL[1] (U/g)	22.39	22.72	23.65	23.43	22.63	0.813	3
LPL[2] (U/g)	20.37^{a}	17.68^{b}	13.00^{c}	13.06^{c}	13.02^{c}	0.515	3
G-6-PD[3] (U/g)	6.36^{a}	5.23^{b}	3.77^{c}	4.08^{c}	4.09^{c}	0.204	3
MDH[4] (U/g)	7.21^{a}	7.11^{a}	6.59^{b}	6.36^{c}	6.32^{c}	0.041	3
ICD[5] (U/g)	19.96^{a}	18.83^{b}	17.76^{c}	17.98^{c}	17.97^{c}	0.215	3

$^{a-c}$Means within a line without a common superscript differ ($P < 0.05$).

[1]HSL = hormone sensitive lipase.

[2]LPL = lipoprotein lipase.

[3]G-6-PD = glucose-6-phosphate dehydrogenase.

[4]MDH = malic dehydrogenase.

[5]ICD = isocitrate dehydrogenase.

TABLE 6. Effect of L-carnitine on triglyceride (TG) and free fatty acid levels in serum of male broilers

	Supplemental L-carnitine (mg/kg diet) 0	25	50	75	100	SEM	n
TG (mg/dL)	70.04^{a}	68.86^{ab}	65.57^{c}	66.39^{bc}	67.03^{bc}	0.872	3
Free fatty acid (μmol/L)	109.13^{c}	127.38^{b}	150.24^{a}	143.93^{a}	140.16^{ab}	4.960	3

$^{a-c}$Means within a line without a common superscript differ ($P < 0.05$).

by adding 50, 75, or 100 mg/kg L-carnitine to diets. G-6-PD, MDH, and ICD are NADPH-generating enzymes. NADPH is the necessary hydrogen provider and is generated by G-6-PD, MDH, and ICD in cytosol. The activity of G-6-PD, MDH, and ICD directly influence the synthesis of fatty acids. With decreased NADPH-generating enzymes in subcutaneous fat, the content of the necessary hydrogen provider—NADPH, which is essential for prolonging of carbon chain—might be decreased, and the synthesis of fatty acid in subcutaneous fat would accordingly be decreased.

The liver is the primary site for fatty acid synthesis in poultry. Whether supplemental carnitine has an effect on hepatic fat synthesis should further be studied. We also observed in our results that supplementation of 50, 75, or 100 mg/kg L-carnitine decreased ($P < 0.05$) the total activity of LPL, which catalyzes the conversion of TG to glycerol and fatty acids. With the decrease of its activity, LPL increases hydrolysis of very-low-density lipoproteins, which have been suggested to play a major role in regulating the deposition of fat in animal body and, thus, minimize the deposition of fat in subcutaneous fat (Griffin and Whitehead, 1982). Whereas the mechanism by which L-carnitine influences the activity of G-6-PD, MDH and ICD are not clearly understood. The results of the experiment implicated that L-carnitine reduced deposition of fat in animal bodies by decreasing the formation of fat.

In our experiment, the activity of CPT-I was decreased ($P < 0.05$) by adding L-carnitine (above 25 mg/kg). CPT-I catalyzes the formation of long-chain acyL-carnitine from activated fatty acids and free carnitine, thus committing fatty acids to oxidation. By virtue of its inhibition by malonyl-coenzyme (CoA), CPT-I controls the rate of β-oxidation and regulates the deposition or oxidation of fatty acids (Zammit, 1999). Carboxylation of acetyl-CoA in muscle and liver forms malonyl-CoA. An increase in dietary L-carnitine resulted in increased carnitine concentrations in muscle and liver, which led to increased activity of carnitine acetyltransferase and accelerate the transportation of acetyl-CoA from mitochondria to cytosol. The rate of synthesis of malonyl-CoA increases with the increase of acetyl-CoA concentration in cytosol (Dyck et al., 1998). Furthermore, L-carnitine has the ability to decrease the ratio of adenosine diphosphate to adenosine triphosphate (Ji and

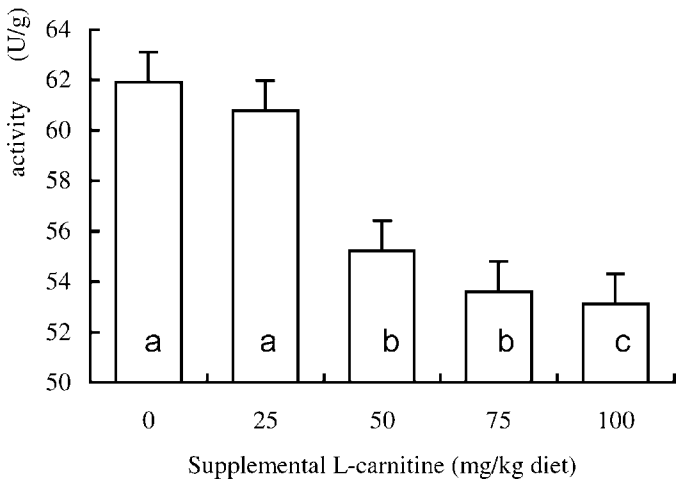

FIGURE 2. The activity of carnitine palmitoyltransferase I in breast muscle of male broilers. [a–c]Bars without common letter differ ($P < 0.05$).

Bradley, 1993), subsequently decreasing the adenosine monophosphate to adenosine triphosphate ratio. This decrease would lead to dephosphorylation and inactivation of adenosine monophosphate kinase, which plays a central role in regulating fat metabolism (Winder and Hardie, 1996; Velasco et al., 1997), and to an increase in the concentration of malonyl-CoA. By virtue of inhibiting of malonyl-CoA in cells, the activity of CPT-I in muscles decreases (Velasco et al., 1997, 1998) and results in a lower rate of β-oxidation of fatty acids (Kudo et al., 1995; Winder and Hardie, 1996; Velasco et al., 1997), thus enhancing fat content of muscles.

The level of FFA in plasma is an important indicator of fat metabolism. Higher concentrations of FFA in serum may enhance the deposition of fatty acids in muscles. In the current experiment, an increase ($P < 0.05$) in FFA in serum was observed. Feeding L-carnitine increased activity of HSL and decreased ($P < 0.05$) activity of LPL, thereby leading to a higher concentration of fatty acid in serum by accelerating hydrolysis of TG to glycerol and fatty acid, while reducing the concentration of TG in serum.

The results obtained from this study implicate that L-carnitine could decrease abdominal fat content expressed as a percentage of body weight by decreasing the activities of G-6-PD, MDH, ICD, and LPL and increase crude fat content in breast muscles by increasing the activity of CPT-I.

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