

## The Effects of L-Carnitine and Different Levels of Animal Fat on Performance, Carcass Characteristics, some Blood Parameters and Immune Response in Broiler Chicks

Research Article

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### ABSTRACT

This study was conducted to investigate the effects of L-carnitine and different levels of animal fat on performance, carcass characteristics and some blood parameters of broiler chickens. Two hundred one-day old male broiler chicks were allotted to 5 treatments in 4 replicates and 10 birds in each replicate. Dietary treatments consisted of: 1) diet with 5% vegetable oil (T1), 2) diet with 5% vegetable oil + 300 mg/kg L-carnitine (T2), 3) diet with 4% animal fat + 300 mg/kg L-carnitine (T3), 4) diet with 5% animal fat + 300 mg/kg L-carnitine (T4) and 5) diet with 6% animal fat + 300 mg/kg L-carnitine (T5). The dietary treatments had not significant effect on body weight gain in days 1-21 ( $P>0.05$ ). The body weight of chicks fed T4 diet was increased in days 22-42 and whole period of the experiment ( $P<0.05$ ). The diet with L-carnitine and 5% animal fat had a significant effect on feed conversion ratio ( $P<0.05$ ). L-carnitine increased both thigh meat ( $P<0.05$ ) and breast muscle percentages ( $P<0.01$ ). The diets T3 and T4 reduced abdominal fat percentage ( $P<0.05$ ). Diet T4 had a significant effect on fabricious bursa weight ( $P<0.05$ ). The T3 and T5 diets had a significant effect on spleen weight ( $P<0.05$ ). The diets with L-carnitine had a significant effect on heart weight ( $P<0.05$ ). The results showed that adding L-carnitine in diet had no significant effect on blood parameters ( $P>0.05$ ). Adding L-carnitine had a significant effect on Newcastle disease antibody titer at day 32 ( $P<0.05$ ) but it had no significant effect on Newcastle disease antibody titer at day 42 ( $P>0.05$ ).

**KEY WORDS** animal fat, blood metabolites, broiler performance, immune response, L-carnitine.

### INTRODUCTION

L-carnitine (B-hydroxy-Y-N-trimethyl aminobutyrate) is a water-soluble quaternary amine that occurs naturally in microorganisms, plants and animals (Bremer, 1983). L-carnitine is biosynthesized in the kidneys and liver from lysine and methionine in the presence of ferrous ions and three vitamins, ascorbate, niacin and pyridoxine (Leibetseder, 1995; Rabie and Szilágyi, 1998; Rebouche, 1992; Rebouche, 1991). L-carnitine is applied as feed additive in poultry diets to increase yield and to improve feed

efficiency (Bell *et al.* 1987). L-carnitine is needed for transfer of fatty acids across the mitochondrial membrane and to subsequently facilitate  $\beta$ -oxidation of long-chain fatty acids for energy production (Casillas and Newburgh, 1969). This process provides muscular energy by burning fat, and also prevents fatty build-up around vital organs. In addition to producing energy, L-carnitine removes toxic accumulations of fatty acids from mitochondria, keeping these organelles healthy and functioning at their best performance (Mayes, 2003). The effect of L-carnitine supplementation in broiler diets on performance at different energy levels is still un-

clear. Studies with broiler chickens have shown that supplementing a diet with L-carnitine leads to increased body weight gain (BWG), improved feed conversion ratio (FCR) and decreased abdominal fat content in broiler chickens (Rabie *et al.* 1997a; Rabie and Szilágyi, 1998). Additionally, L-carnitine may modulate immune function as evidenced by enhanced antibody response in L-carnitine supplemented broiler chickens (Mast *et al.* 2000) and pigeons (Janssens *et al.* 2000). This study was conducted because of the limitation that exist on published results concerning the effects of L-carnitine and different types of fat on performance, carcass characteristics, some blood parameters and immune responses in broiler chicks.

## MATERIALS AND METHODS

Two hundred one-day old broiler chicks (Ross-308) were randomly allocated to 5 dietary treatment groups with 4 replicates of 10 birds each. Feed and water were provided *ad libitum* through the experimental period. Treatments involved with the study were: 1) diet with 5% vegetable oil (T1); 2) diet with 5% vegetable oil + 300 mg/kg L-carnitine (T2); 3) diet with 4% animal fat + 300 mg/kg L-carnitine (T3); 4) diet with 5% animal fat + 300 mg/kg L-carnitine (T4) and 5) diet with 6% animal fat + 300 mg/kg L-carnitine (T5). The feed formula and diet composition are shown in table 1. Diets (Table 1) were offered to the chicks from d 1 to 42 days and included starter (1-10 days), grower (11-24 days) and finisher period (25-42 days). Feed consumption and body weight (BW) (by pen) were recorded at 3 weeks intervals. Feed conversion ratio (FCR) was calculated as the unit of eaten feed per unit of body weight gain. On day 32, one chicken was selected randomly from each replicate and blood samples were taken from the wing vein. These chickens were marked till end of the experiment (day 42). Marked chickens from each replicate were selected and blood samples were taken from the wing vein and then were slaughtered. Carcass composition and abdominal fat (the fat around the cloaca and gizzard) were measured. Serum was separated (centrifuged 15 min; 3000y) and stored in a freezer at -20 °C till analysis. Concentration of triglyceride, cholesterol, high-density Lipoproteins (HDL), glucose, low-density lipoproteins (LDL), total protein, albumin, globulin were measured by an enzymatic method with diagnostic kits (Parsazmoon® produced by an Iranian company) and Newcastle titer was measured by HI (haemagglutination inhibition) test. The data were subjected to statistical analysis according to a completely randomized design. Data were analyzed as a one-way ANOVA using the GLM procedure of SAS (1996) with pen serving as the experimental unit for performance parameters and bird as the experimental unit for blood pa-

rameters. Significant differences between treatments means were determined using Duncan multiple range test (5% probability or error).

## RESULTS AND DISCUSSION

The effects of L-carnitine supplementation and different levels of animal fat on body weight gain, feed intake and feed conversion ratio and carcass characteristics of broiler chicks are summarized in Table 2. The diet with L-carnitine and animal fat had a significant ( $P < 0.01$ ) effect on body weight gain (BWG). Results showed that experimental diets had no significant ( $P > 0.01$ ) effect on body weight gain in period from 1-21 of age. Diet with 5% animal fat + 300 mg/kg L-carnitine increased body weight of chickens. Diet with 5% animal fat + 300 mg/kg L-carnitine (T4) increased significantly ( $P < 0.01$ ) the body weight gain of broiler chicks during the period from 22 to 42 days of age and the overall period but there was no significant difference in comparison with diet with 5% vegetable oil + 300 mg/kg L-carnitine. Diet with levels of animal fat + 300 mg/kg L-carnitine, had a significant effects on feed conversion ratio (FCR) ( $P < 0.01$ ). The chicks fed on diets of T4 and T5 recorded significantly the lowest amounts of feed intake (FI) while those fed on T4 recorded an improvement in (FCR) during the whole periods ( $P < 0.01$ ).

In conclusion, results of the present experiment show that diet with L-carnitine and 5% animal fat improve body weight, feed conversion ratio, reduced abdominal fat percentage and also improved immune response system of broiler chicken. The effect of diets on some blood parameters is presented in table 4 and 5. Results of the experiment showed that there were no significant ( $P > 0.05$ ) effect on glucose, HDL, LDL, cholesterol and triglyceride levels. While the diet with 4% animal fat + 300 mg/kg L-carnitine reduced triglyceride and cholesterol. Results in Table 5 showed that treatments had no significant ( $P > 0.05$ ) effect on total protein, albumin and globulin. The data obtained also showed that adding L-carnitine had no significant effect on Newcastle antibody titer on day 32 ( $P > 0.05$ ), but it had a significant effect on Newcastle antibody titer on day 42 ( $P < 0.05$ ). Diet with 5% vegetable oil + 300 mg/kg L-carnitine increased Newcastle antibody titer on day 32 and also diet with 5% vegetable oil + 300 mg/kg L-carnitine and diet with either 5% or 6% animal fat + 300 mg/kg L-carnitine increased Newcastle antibody titer on day 42. Diets with L-carnitine had a significant effect on body weight gain (BWG), carcass weight, leg meat and breast muscle ( $P < 0.05$ ). Bozkurt, (2008) reported that adding animal or vegetable fat at the 5% level in broiler breeder hens and males diet had no significant effect on performance at 22, 34, 46 and 58 week of age.

**Table 1** Ingredients and chemical compositions of the experimental diets (g/100 g)

Ingredients	Broiler diet (g/100 g)										
	Starter (0-10 d)	Grower (11-24 d)					Finisher (25-42 d)				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	
Yellow corn	57	54.21	54.18	55.74	54.18	52.63	59.06	59.03	60.58	59.03	57.46
Soybean meal (44%)	38.6	37.06	37.06	36.49	37.06	37.62	32.34	32.34	31.78	32.34	32.91
Soybean oil	-	5.00	5.00	-	-	-	5.00	5.00	-	-	-
Animal fat	-	-	-	4.00	5.00	6.00	-	-	4.00	5.00	6.00
Limestone	1.18	1.01	1.01	1.01	1.01	1.01	1	1	1	1	1
Dicalcium phosphate	1.85	1.56	1.56	1.56	1.56	1.56	1.49	1.49	1.49	1.49	1.49
Salt	0.16	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.200	0.20
Sodium bicarbonate	0.23	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix <sup>2</sup>	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix <sup>3</sup>	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.19	0.20	0.20	0.20	0.20	0.20	0.16	0.16	0.16	0.16	0.17
L-lysine	0.29	0.06	0.06	0.07	0.06	0.05	0.05	0.05	0.06	0.05	0.04
L-carnitine	-	-	0.03	0.03	0.03	0.03	-	0.03	0.03	0.03	0.03
Nutrient composition											
ME (kcal/kg)	2850			2950			3050				
Crude protein (%)	22.00			20.60			19.00				
ME/CP	129.54			143.20			160.52				
Calcium (%)	1.00			0.85			0.82				
Available phosphor (%)	0.50			0.42			0.41				
Methionine (%)	0.52			0.42			0.40				
Methionine + cysteine (%)	0.92			0.90			0.82				
Arginine (%)	0.40			1.20			1.10				
L-lysine (%)	1.38			1.17			1.04				

(1) diet with 5% Vegetable oil (T1); 2) diet with 5% vegetable oil + 300 mg/kg L-carnitine (T2); 3) diet with 4% animal fat + 300 mg/kg L-carnitine (T3); 4) diet with 5% animal fat + 300 mg/kg L-carnitine (T4) and 5) diet with 6% animal fat + 300 mg/kg L-carnitine (T5).

<sup>2</sup> Vitamin A: 400 IU; vitamin D<sub>3</sub>: 250 IU; vitamin E: 30 mg; vitamin K<sub>3</sub>: 13 mg; vitamin B<sub>1</sub>: 10 mg; vitamin B<sub>2</sub>: 16 mg; vitamin B<sub>6</sub>: 12 mg; vitamin B<sub>12</sub>: 0/1 mg; Niacin 83 mg; Calcium D-pantothenate: 60 mg; Folic acid: 0/2 mg and Choline chloride: 105 mg.

<sup>3</sup> Co: 0.4 mg; Zn: 3/7 mg; I: 0/5 mg; Mn: 80 mg; Mg: 108 mg; Cu: 62 mg; Fe: 42 mg; Ca: 11 mg; Na: 390 mg; Cl: 671 mg and K: 78 mg.

**Table 2** Effects of different levels of animal fat and L-carnitine supplementation on growth performance of broiler chickens

Treatment	BWG (g)			FI			FCR <sup>d</sup>		
	(1-3 w)	(4-6 w)	(1-6 w)	(1-3 w)	(4-6 w)	(1-6 w)	(1-3 w)	(4-6 w)	(1-6 w)
T1	5822.5	12805 <sup>b</sup>	18627 <sup>c</sup>	869 <sup>a</sup>	3193 <sup>a</sup>	4062 <sup>a</sup>	1.49 <sup>ab</sup>	2.49 <sup>ab</sup>	1.8 <sup>b</sup>
T2	6233.5	14595 <sup>ab</sup>	20828 <sup>b</sup>	836 <sup>ab</sup>	3212 <sup>a</sup>	4048 <sup>a</sup>	1.37 <sup>b</sup>	2.23 <sup>b</sup>	1.94 <sup>b</sup>
T3	5615	13240 <sup>b</sup>	18855 <sup>c</sup>	506 <sup>b</sup>	2928 <sup>b</sup>	3434 <sup>b</sup>	1.74 <sup>a</sup>	2.21 <sup>b</sup>	1.82 <sup>b</sup>
T4	6400	16262 <sup>a</sup>	22662 <sup>a</sup>	860 <sup>a</sup>	2743 <sup>c</sup>	3603 <sup>b</sup>	1.35 <sup>b</sup>	1.68 <sup>c</sup>	1.59 <sup>c</sup>
T5	5252	10550 <sup>c</sup>	15802 <sup>d</sup>	786 <sup>ab</sup>	2758 <sup>c</sup>	3544 <sup>b</sup>	1.49 <sup>ab</sup>	2.61 <sup>a</sup>	2.24 <sup>a</sup>
SEM	18.1	29.4	20	54.1	18.7	23.5	0.04	0.045	0.027
P-value	0.217	0.0001	0.0001	0.15	0.0001	0.005	0.03	0.0001	0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

(1) diet with 5% Vegetable oil (T1); 2) diet with 5% vegetable oil + 300 mg/kg L-carnitine (T2); 3) diet with 4% animal fat + 300 mg/kg L-carnitine (T3); 4) diet with 5% animal fat + 300 mg/kg L-carnitine (T4) and 5) diet with 6% animal fat + 300 mg/kg L-carnitine (T5).

BWG: body weight gain= average for 10 chicks in each replicate and FI: feed intake= one chick during period.

SEM: standard error of the means.

FCR: feed conversion ratio.

**Table 3** Effects of different levels of animal fat and L-carnitine supplementation on carcass characteristics of broiler chickens

Treatment	Heart (%)	Liver (%)	Spleen (%)	Fabricious bursa (%)	Carcass weight (%)	Thigh meat (%)	Breast muscle (%)	Abdominal fat (%)
T1	0.48 <sup>b</sup>	3.2	0.08 <sup>b</sup>	0.18 <sup>b</sup>	71.74ab	24.40b	34.79c	2.53a
T2	0.54 <sup>ab</sup>	2.82	0.09 <sup>b</sup>	0.2 <sup>b</sup>	73.06ab	23.47b	31.25b	1.46bc
T3	0.61 <sup>ab</sup>	2.8	0.15 <sup>a</sup>	0.19 <sup>b</sup>	73.73ab	24.99	36.60ab	1.2c
T4	0.58 <sup>ab</sup>	2.61	0.11 <sup>ab</sup>	0.2 <sup>b</sup>	75.29a	27.54a	38.47a	1.03c
T5	0.71 <sup>a</sup>	3	0.15 <sup>a</sup>	0.26 <sup>a</sup>	69.93b	25.81ab	33.46bc	2.02ab
SEM	0.12	0.02	0.005	0.005	0.65	0.04	0.5	0.09
P-value	0.098	0.587	0.013	0.001	0.093	0.028	0.002	0.0002

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

(1) diet with 5% Vegetable oil (T1); 2) diet with 5% vegetable oil + 300 mg/kg L-carnitine (T2); 3) diet with 4% animal fat + 300 mg/kg L-carnitine (T3); 4) diet with 5% animal fat + 300 mg/kg L-carnitine (T4) and 5) diet with 6% animal fat + 300 mg/kg L-carnitine (T5).

SEM: standard error of the means.

**Table 4** Effects of different levels of animal fat and L-carnitine supplementation on blood parameters of broiler chicks

Treatment	Glucose (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
T1	228.7	46	53.1	120.2	105.7
T2	235.2	38.5	70.2	135.5	128.7
T3	240.2	37.5	61	116.2	88.75
T4	236.2	46	56.9	123	100.2
T5	245.2	44.5	54	120.2	110.2
SEM	4.1	1.6	3.25	4.22	35.2
P-value	0.7	0.204	0.378	0.621	0.606

(1) diet with 5% Vegetable oil (T1); 2) diet with 5% vegetable oil + 300 mg/kg L-carnitine (T2); 3) diet with 4% animal fat + 300 mg/kg L-carnitine (T3); 4) diet with 5% animal fat + 300 mg/kg L-carnitine (T4) and 5) diet with 6% animal fat + 300 mg/kg L-carnitine (T5).

HDL: high-density lipoprotein and LDL: low-density lipoprotein.

SEM: standard error of the means.

**Table 5** Effects of different levels of animal fat and L-carnitine supplementation on blood parameters of broiler chickens

Treatment	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Newcastle (32 day)	Newcastle (42 day)
T1	3.97	1.57	2.4	4.5 <sup>a</sup>	4.5
T2	4.17	1.62	2.55	5.25 <sup>a</sup>	5.5
T3	4.15	1.55	2.6	0.75 <sup>b</sup>	4
T4	4.15	1.47	2.67	3.75 <sup>a</sup>	5.25
T5	4	1.42	2.57	1.75 <sup>b</sup>	5.5
SEM	0.3	0.047	0.067	0.3	0.27
P-value	0.196	0.614	0.719	0.246	0.0007

The means within the same column with at least one common letter, do not have significant difference ( $P > 0.05$ ).

(1) diet with 5% Vegetable oil (T1); 2) diet with 5% vegetable oil + 300 mg/kg L-carnitine (T2); 3) diet with 4% animal fat + 300 mg/kg L-carnitine (T3); 4) diet with 5% animal fat + 300 mg/kg L-carnitine (T4) and 5) diet with 6% animal fat + 300 mg/kg L-carnitine (T5).

The best known is to facilitate the transport of long-chain fatty acids across the inner mitochondrial membrane. Schumacher *et al.* (1993) reported that L-carnitine had an effective influence in improving bodyweight gain, mainly in groups fed with diets marginally in lysine and methionine plus cystine content, respectively. Dietary supplementation with L-carnitine from 20 to 60 mg/kg tended to improve growth performance of broiler chickens (Lettner *et al.* 1992). The improvement in body weight gain caused by dietary L-carnitine supplementation may be partially explained by an increase in the concentration of plasma insulin-like growth factor-I, which consists of 70 amino acids and has the potency to stimulate body weight gain (Kita *et al.* 2002). Xu *et al.* (2003) reported that supplementation of diet with L-carnitine had a little effect on leg and breast muscle yield. Leg and breast muscle yield was increased by supplementing with 50 or 75 mg/kg L-carnitine. L-carnitine has two major functions. The best known is to facilitate the transport of long-chain fatty acids across the inner mitochondrial membrane. L-carnitine also facilitates the removal of short and medium-chain fatty acids from the mitochondria that accumulate as a result of normal and abnormal metabolism (Rabie *et al.* 1997a; Rabie and Szilagyi, 1998; Matalliotakis *et al.* 2000; Xu *et al.* 2003). Thus, dietary L-carnitine supplementation promotes the  $\beta$ -oxidation of these fatty acids in order to generate adenosine triphosphate (ATP) energy and improve energy utilisation (Rabie *et al.* 1997b; Neuman *et al.* 2002).

Consequently, L-carnitine supplementation in diets reduces the amount of long-chain fatty acids availability for esterification to triacylglycerols and storage in the adipose tissue (Barker and Sell, 1994; Xu *et al.* 2003). Diet with levels of animal fat + 300 mg/kg L-carnitine had a significant effects on feed intake (FI) and feed conversion ratio (FCR) ( $P < 0.05$ ). Used diet with 5% animal fat + 300 mg/kg L-carnitine reduced (FI) and improved (FCR) in whole period ( $P < 0.05$ ). So the results of this study is agreement with results of previous studies (Lien and Horng, 2001; Xu *et al.* 2003; Rabie *et al.* 1997a). Supplementation of L-carnitine in pigs diet improves feed efficiency (Owen *et al.* 1996). Also, Bayram *et al.* (1999) detected significant decreases in feed intake and in feed efficiency in quails supplemented with 500 mg/kg diet L-carnitine. On the other hand, Barker and Sell, (1994) found that L-carnitine had no effect on feed consumption in broilers when fed at 50 or 100 mg/kg. Diets with 4% animal fat + 300mg/kg L-carnitine and Diet with 5% animal fat + 300mg/kg L-carnitine, had a significant effect on abdominal fat but it had no significant effect on liver and heart weight. Adding 25 or 50 ppm L-carnitine has been shown to decrease abdominal fat pads (Rabie *et al.* 1997a; Rabie *et al.* 1997b; Rabie and Szilagyi, 1998; Xu *et al.* 2003). Burtle and Liu, (1994) indicated that L-carnitine supplementation to diets alters fat metabolism and reduces body fat. In the present study, L-carnitine supplemented diets had no significant effect on glucose' cholesterol' triglyceride' HDL and LDL parameters ( $P > 0.05$ ), but adiet

with 4% animal fat + 300 mg/kg L-carnitine reduced triglyceride and cholesterol. Findings in this study are consistent with Arslan *et al.* (2004) who reported that L-carnitine administration via drinking water did not influence serum total cholesterol, total lipid and triglyceride of Japanese quail. Several studies indicated that L-carnitine supplementation decreased serum cholesterol, total lipid and triglyceride levels (Bell *et al.* 1987; Mondola *et al.* 1992). Parsaeimehr *et al.* (2012) reported that adding L-carnitine with animal fat in broiler diet has a significant effect on triglyceride and also causes reduced blood cholesterol and LDL. Elmansy (2006) reported that the higher level of energy induced a higher level of triglyceride and cholesterol. Also, the present study shows that use of L-carnitine had a significant effect on Fabricious bursa and spleen weight but it had no significant effect on total protein, albumin and globulin. The mechanism(s) accounting for the positive effect of L-carnitine on antibody production is currently not clear. Mast *et al.* (2000) reported that adding 100 mg L-carnitine/kg had a significant effect on immune response by increasing the total Ig and Ig G levels in 2 to 6 week old animals.

## CONCLUSION

In conclusion, results of the present experiment show that diet with L-carnitine and 5% animal fat improve body weight, feed conversion ratio, reduced abdominal fat percentage and also improved immune response system of broiler chicken.

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