

Effect of Supplementation of L-Carnitine in Diet Containing Animal Fat on Serum Biochemical Parameters, Minerals and Lipid Peroxidation Level in Broiler Chicken

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Abstract

An experiment was conducted to examine the effects of L-carnitine supplementation on blood biochemical parameters, minerals and lipid peroxidation level in broiler chicken fed with diet containing animal fat. Eighty-day old commercial broiler chicks (Vencobb) were randomly grouped into two treatment groups (T1 and T2) with four replications of ten chicks each and the rations were prepared with five per cent animal fat. The T2 group was supplemented with 900 mg of L-carnitine/kg of feed. L-carnitine supplementation did not show any effect on serum protein, albumin and minerals concentrations between treatment groups, whereas, serum glucose and iron contents were significantly increased. The serum Malondialdehyde, an indicator of oxidative stress, was significantly reduced in T2 compared to T1 group. From the results of present study, it is concluded that supplementation of L-carnitine in diet containing animal fat reduced the lipid peroxidation level in broiler chicken.

Keywords: Broiler Chicks, L-carnitine, Malondialdehyde, Serum Biochemical Parameters and Serum Minerals

Introduction

Feed cost plays a major part on fixing the market prices for meat and eggs, which in turn directly related the cost of ingredients for the preparation of ration. The energy source level can be altered to reduce the production cost, by partially replacing with the cheaper energy source which gives more body growth rate at per unit cost (Fouad and El-Senousey, 2014). Animal fat is available at cheaper rates as an energy source, which is widely used in commercial broiler rations due to its higher energy content and extra calorific effect. However, addition of animal fat in diet causes fat deposition in birds, which is undesirable to the consumers and also susceptibility to lipid oxidation in meat products (Murali *et al.*, 2015). In order to overcome these problems, feed additives can be used to manipulate the diets with animal fat (Al-Kassie, 2009; Deng *et al.*, 2012; El-Senousey *et al.*, 2013; Fouad *et al.*, 2013).

Carnitine (β -hydroxy γ -trimethylaminobutyrate) is a quaternary amine compound, which is synthesized endogenously from the essential amino acids, lysine and methionine (Arslan, 2006). L-Carnitine helps in the transfer of long chain fatty acids from cytoplasm to mitochondria for β -oxidation and energy production (Cakir and Yalcin, 2007; Kucukersan *et al.*, 2011). Thereby, reduces long chain fatty acid availability for esterification to triacylglycerols and storage in the adipose tissue. L-carnitine acts as an antioxidant by increasing the level of antioxidant enzymes like reduced glutathione and superoxide dismutase. In addition, it controls biological process like gluconeogenesis, metabolism of triglycerides, ketones, branched chain amino acids and cholesterol (Murali *et al.*, 2015). In view of key role of L-carnitine in energy metabolism and antioxidative effect, the objective of this present study was to evaluate the effects of L-carnitine on serum biochemical parameters, minerals and lipid peroxidation of birds fed with animal fat containing diet. As the type and level of fat in the diet will influence the biochemical parameters of blood, measurement of blood parameters could be useful to detect some metabolic effects of L-carnitine and health status of broilers.

Materials and Methods

This experiment was conducted at Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy, Trichur, Kerala using eighty-day old (Vencobb) broiler chicks, randomly allotted into two treatment groups (T₁ and T₂) with four replicates of ten chicks each. The dietary rations were prepared as per BIS (1992) with 5 per cent animal fat. The second treatment group (T₂) was supplemented with L-carnitine at 900 mg/kg diet. Broiler starter ration was fed to birds up to 4 weeks of age and finisher ration up to 6 weeks of age. The birds were maintained under deep litter system. Throughout the experimental period, *ad libitum* feed and clean drinking water were provided and birds were reared under identical management conditions. The ingredient composition of the two different broiler starter and finisher rations are presented in Table 1.

Table 1: Ingredient composition of broiler starter and finisher ration, %

Ingredients	Broiler Starter Rations, %		Broiler Finisher Rations, %	
	T1	T2	T1	T2
Maize	40	40	48.5	48.5
Soybean meal	41.4	41.4	32.89	32.89
Wheat bran	9	9	9	9
Animal fat	5	5	5	5
Dicalcium phosphate	2	2	2.1	2.1
Calcite	1.79	1.79	1.8	1.8
DL-methionine	0.14	0.14	0.04	0.04
Choline chloride	0.1	0.1	0.1	0.1
Trace mineral mixture*	0.01	0.01	0.01	0.01
Supplements**	0.31	0.31	0.31	0.31
Salt	0.25	0.25	0.25	0.25
Total	100	100	100	100
To 100kg of the above mixture following are added				
L-Carnitine (mg/kg)	-	900	-	900

*Trace mineral mixture containing Manganese sulphate-60 g, Zinc sulphate-50 g, Ferrous sulphate-40 g, Iodide-2 g, Copper-5 g, Cobalt-2 g and Selenium-0.3 g. **Supplements containing B complex vitamins, Vitamin AB₂D₃K, Toxin binder, Coccidiostat and Liver supplement, Carniking® (Lonza Group Ltd, Muenchensteinerstrasse, Switzerland) containing lab grade L-carnitine.

The chemical composition of experimental rations were determined as per the standard procedures (AOAC, 2012). At the end of the experimental period, five birds from each treatment were fasted overnight, slaughtered and dressed as per the standard procedures (BIS, 1973). Serum Ca, Mg, Cu and Fe concentration were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer AAS Model 400) while the serum inorganic P (P_i) level was analyzed using blood analyser (Phosphomolybdate method). The serum biochemical parameters like total proteins (Biuret method), albumin (Bromocresol green method) and glucose (GOD-POD method) were analyzed using the kits supplied by Agappe diagnostics, Kerala. The serum lipid peroxidation level was determined by the method of Fraga *et al.* (1988). Data collected on various parameters were statistically analyzed (Snedecor and Cochran, 1994) using SPSS 16.0 (2008). Means were compared by Independent Samples t Test.

Results and Discussion

The chemical compositions of broiler rations were presented in Table 2. The crude protein and metabolisable energy content of starter and finisher rations were 23.25 and 20.14%, 2805 and 2900 kcal/kg, respectively.

Table 2: Chemical composition of broiler starter and finisher rations*

Parameters	Broiler Starter Ration	Broiler Finisher Ration
Dry matter, %	86.83	87.1
Crude protein, %	23.25	20.14
Ether extract, %	5.48	5.73
Crude fibre, %	4.38	4.16
Nitrogen free extract, %	57.47	62.09
Total ash, %	9.42	7.88
Acid insoluble ash, %	1.9	1.25
Mineral Composition		
Calcium, %	1.41	1.37
Total phosphorus, %	1.23	1.13
Magnesium, %	0.37	0.35
Iron, ppm	90.82	81.8
Copper, ppm	19.92	16.65
Calculated Values		
Metabolisable energy, kcal/kg	2805	2900
Lysine, %	1.27	1.07
Methionine, %	0.34	0.31

*On dry matter basis

The serum biochemical parameters such as total protein (g/dl), albumin concentration (g/dl) and glucose concentration (mg/dl) of experimental birds maintained on two dietary treatments are shown in Table 3. The statistical analysis showed that L-carnitine supplementation did not show any significant effect ($p > 0.05$) on the serum total protein and albumin concentration and levels were within the normal range for the species as mentioned by Benjamin (1978) and Silva *et al.* (2007), respectively. In agreement with the present study, no effect on total protein concentrations by L-carnitine supplementation was reported in ducks (Arslan *et al.*, 2003), in laying hens (Yalcin *et al.*, 2006) and in broilers (Cakir and Yalcin, 2007 and Koksall *et al.*, 2011). On contrary, Hassan *et al.* (2011) observed higher plasma total protein concentrations in Golden Montazah laying hens as a result of supplement of L-carnitine at 100 and 200 ppm. Similarly, a higher serum album concentration were observed in broilers supplemented with L-carnitine at the level of 100 ppm (Cakir and Yalcin, 2007).

The serum glucose concentration was significantly increased ($p < 0.05$) in L-carnitine supplement group compared to control group. An increase in blood glucose concentration by L-carnitine supplementation at 100 ppm in Japanese quails (Arslan *et al.*, 2004) and at 60 ppm in broilers (Kheiri *et al.*, 2011) was reported in previous studies. The increase in serum glucose level in the present experiment should have been triggered by the secretion of some of the adrenal glands, which stimulated the process, which leading to increase in the serum glucose level in broiler chicken (Ihsanullah *et al.*, 2017). While Ringseis *et al.* (2018) stated about the role of L-carnitine in glucose metabolism is by enhancing the mitochondrial oxidation of long chain acyl CoA, otherwise which would lead to

insulin resistance in muscle and heart and by modulating the intramitochondrial acetyl-CoA/CoA ratio and the activity of the pyruvate dehydrogenase complex and also alters the expression of glycolytic and gluconeogenic enzymes. In contrast to the present findings, serum glucose level was not affected by L-carnitine supplementation at 100mg/kg broiler diet containing different levels of sunflower oil (Corduk *et al.*, 2007). Likewise, in ducks (Arslan *et al.*, 2003), in laying hens (Du *et al.*, 2005) and in broilers (Parsaeimehr *et al.*, 2012).

Table 3: Serum biochemical and lipid peroxidation level of birds maintained on two dietary treatments[†]

Parameters	Treatments		P value
	T ₁	T ₂	
Total protein (g/dl)	4.16± 0.15	4.02± 0.25	0.62
Albumin (g/dl)	1.18± 0.07	1.22± 0.05	0.69
Glucose (mg/dl)	213.29± 6.03 ^a	238.17± 5.00 ^b	0.01
Serum Lipid Peroxidation Level			
Parameter	T ₁	T ₂	P value
MDA(nmol/ml)	2.13± 0.14 ^a	1.62± 0.17 ^b	0.05

a, b - Means bearing different superscripts within the same row differ significantly ($P < 0.01$) and ($P < 0.05$); [†] Mean of five values with SE

The Serum malondialdehyde (MDA) concentration (nmol/ml) of experimental birds maintained on two dietary treatments are shown in Table 3 and were significantly ($p < 0.05$) reduced in L-carnitine supplemented group. Generally, MDA is the marker for oxidative stress and it indicates the level of lipid peroxidation. The reduction in MDA level is due to the antioxidant effect of L-carnitine. L-carnitine suppressed hydroxyl radical production in the Fenton Reaction, by chelating the iron required for the generation of hydroxyl radicals. In addition, L-carnitine inhibited xanthine oxidase activity, thereby act as a free radical scavenger and stabilizer of cell membranes (Reznick *et al.*, 1992). Also, Derin *et al.* (2004) concluded that L-carnitine reduces the MDA level by increasing the utilisation of long chain fatty acids for energy production, thereby improves the turnover of fatty acids peroxidised by the free oxygen radicals produced during metabolism. Similar to our results, Tan *et al.* (2008) and Yousefi *et al.* (2013) noticed that supplementation of L-carnitine in the diet significantly reduced the plasma MDA level in broiler chicken. The serum mineral concentrations of experimental birds belonging to the two dietary treatments were shown in Table 4 and were within the normal range reported for broiler chicken as mentioned by Silva *et al.* (2007).

Table 4: Serum mineral concentration of birds maintained on two dietary treatments

Parameters	Treatments		P value
	T ₁	T ₂	
Calcium, mg/dl	13.36± 0.50	13.14± 0.49	0.75
Inorganic phosphorus, mg/dl	4.84± 0.07	5.2± 0.19	0.11
Magnesium, mg/dl	2.59± 0.05	2.72± 0.06	0.15
Copper, ppm	0.36± 0.04	0.44± 0.05	0.21
Iron, ppm	3.19± 0.25 ^a	3.81± 0.11 ^b	0.05

a, b - Means bearing different superscripts within the same row differ significantly ($P < 0.05$); [†] Mean of five values with SE

Results indicated that there is no significant difference among treatment groups for serum Ca, P, Mg and Cu. However, serum iron level was significantly higher in T₂ compared to that of T₁. The increase in iron concentration may be due to the increase in stabilization of the erythrocyte membrane fragility by facilitating the lipid uptake and also by increase of Na⁺/K⁺-ATPase activity and reduction of abnormal ATP concentrations (Labonia *et al.* 1987). In contrary to the present findings, Pietruszka *et al.* (2009) observed that supplementation of L-carnitine at 100mg/kg feed could not find any effect on serum iron level in fattening pigs. Whereas, Hassan *et al.* (2011) observed a plasma phosphorus level was increased by supplementing L-carnitine (100 and 200 mg/kg) in laying hens.

Conclusion

As per the results of the present study, it is concluded that L-Carnitine supplementation at the level of 900 mg/kg feed in broiler chicken reduced the oxidative stress of the cells as indicated by a reduction in the MDA concentration. Thereby, L-Carnitine can be used as a feed additive in broiler to reduce the lipid peroxidation and to increase the

quality of meat when fed with a ration with animal fat as energy source.

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Conflict of Interests

There is no conflict of interest.

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