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Effects of Different Levels of L-carnitine Supplementation on Egg Quality and Blood Parameters of Laying Japanese Quail

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Abstract: The objective of present study was to investigate the effects of various levels of L-carnitine on performance, egg quality and blood parameters of laying Japanese quail. This experiment was carried out using 128 quail in a completely randomized design with four levels of L-carnitine (0, 125, 250 and 500 mg/kg). Four replicates with 8 quails were allocated to each experimental treatment and birds were reared from 35-70 days. The results showed that there were no significant differences in feed intake and egg production among experimental treatments ($p>0.05$). The effect of L-carnitine on feed conversion ratio was significant ($p<0.05$). The quails were fed with rations containing L-carnitine had lower feed conversion ratio ($p<0.05$). Adding of L-carnitine supplementation (125 and 250 mg/kg) significantly reduced egg yolk cholesterol and triglyceride ($p<0.05$). Dietary L-carnitine supplementation had no significant effect on other egg quality parameters ($p>0.05$). The quails were fed with ration containing L-carnitine supplementation (125 mg/kg) had lower blood triglyceride ($p<0.05$). Furthermore, L-carnitine at levels of 125 and 500 mg/kg significantly reduced blood cholesterol in comparison with control group ($p<0.05$). Based on current results, it can be concluded that supplementing diet with L-carnitine will reduce blood triglyceride, cholesterol and improve egg quality in laying Japanese quail.

Key words: Japanese quail, L-carnitine, egg quality, cholesterol

INTRODUCTION

The chemical structure of L-carnitine (ß-hydroxy γ-trimethyl amino butyrate) was elucidated in 1927 (Tomita and Sendju, 1927). The major metabolic role of L-carnitine appears to be the transport of long-chain fatty acids into the mitochondria for ß-oxidation (Coulter, 1995). Consequently, L-carnitine supplementation in diets reduces the amount of long-chain fatty acids availability for esterification to triacylglycerols and storage in adipose tissue (Xu *et al*., 2003). Furthermore, increased import of fatty acids into the mitochondria for oxidation has the potential to spare the catabolism of proteins for energy. Thus, animals fed diets with elevated L-carnitine contents may have more protein energy available for growth (Dikel *et al*., 2010). Poultry diets are composed mainly of maize and soyabean and plant products are low in carnitine, while animal-derived feedstuffs are rich in L-carnitine. Poultry feeds contain high percentages of cereals and this situation may lead to a deficiency of carnitine (Baumgartner and Blum, 1997). Consequently, L-carnitine supplementation in diet or in drinking water would be useful for poultry (Arslan *et al*., 2004).

Studies on the effect of L-carnitine supplementation to quail diets are spare. Studies on broiler chickens have shown that supplemental dietary L-carnitine increases body weight gain, improves feed conversion ratio and reduces abdominal fat content (Rabie *et al*., 1997; Rabie and Szilagyi, 1998). However, there are contradictory studies in which dietary L-carnitine supplementation did not affect growth performance and some internal organs weight (Corduk *et al*., 2007; Rezaei *et al*., 2007; Lien and Horng, 2001). There are limited papers about the effects of carnitine supplementation of laying quail diets on performance and egg quality. Therefore, in this study, L-carnitine was added to laying Japanese quail diets and the effects of the supplementation on egg quality and blood parameters were investigated.

MATERIALS AND METHODS

This experiment was carried out using 128 quails in a completely randomized design with four levels of L-carnitine (0, 125, 250 and 500 mg/kg). Four replicates with 8 quails were allocated in each experimental treatment and birds were reared from 35-70 days. Water was provided via nipple drinkers and feed was provided via trough feeders. The experimental diets were formulated to meet minimum nutrient requirements of laying Japanese quail, as established by the National Research Council (NRC, 1994). The composition and calculated nutrient content of the experimental diets are presented in Table 1. Experimental diets (in mash form) and water were provided ad libitum. House temperature was maintained at 33°C and reduced 2°C weekly thereafter. The photoperiod was 16 h light and 8 h dark during the experiment.

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Table 1: Composition of experimental diets

Ingredients %	Diet
Corn	50.00
Soybean meal	34.75
Soybean oil	5.00
Wheet bran	2.65
Dicalcium phosphate	1.00
Limestone	5.71
Salt	0.30
Mineral premix	0.25
Vitamin premix	0.25
DL-methionine	0.09
L-carnitine	-
Calculated composition	
Metabolizable energy (kcal/kg)	2900.00
Crude protein (%)	20.00
Calcium (%)	2.50
Available phosphorus (%)	0.35
Sodium (%)	0.13
Lysine (%)	1.00
Methionine (%)	0.45
Methionine + Cystine (%)	0.70

Each kg of vitamin premix contained: Vitamin A, 3,500,000 IU; Vitamin D_3, 1,000,000 IU; Vitamin E, 9000 IU; Vitamin K_3, 1000 mg; Vitamin B_1, 900 mg; Vitamin B_2, 3,300 mg; Vitamin B_3, 5,000 mg; Vitamin B_5, 15,000 mg; Vitamin B_6, 150 mg; Vitamin B_9, 500 mg; Vitamin B_{12}, 7.5 mg; Biotin, 500 mg; Choline chloride, 250,000 mg and each kg of mineral premix contained: Mn, 50,000 mg; Fe, 25,000 mg; Zn, 50,000 mg; Cu, 5,000 mg; I, 500 mg; Se, 100 mg

Data collection: The performance of laying Japanese quail was evaluated by recording feed intake, Feed Conversion Ratio (FCR) and egg production. Feed intake for each cage was recorded weekly. Feed conversion ratio was calculated as kg of feed intake for kg of egg production at the end of the trial. Eggs were collected daily and egg production was calculated on a quail-day basis. Egg quality parameters were measured weekly during the experiment. The eggs were weighed and yolks were separated using an egg separator and weighed. Albumen weight was calculated by subtracting yolk and shell weight from total egg weight. The shells were rinsed with tap water, dried overnight at 60°C, cooled and weighed. Albumen height was documented and Haugh unit was calculated as follows:

$$\text{Haugh unit} = 100 \times \log (T - 1.7 \times W^{0.37} + 7.57)$$

Shell thickness (without inner and outer shell membranes which were manually removed) was measured at three areas (broad end, middle portion and narrow end of the shell), by using a micrometer (Mitutuyo Corporation, 0.01-20 mm, Kawasaki, Japan) (Wells, 1968). The egg shell breaking strength was measured using a cantilever system by applying increased pressure to the broad pole of the shell (Balnave and Muheereza, 1997). Six yolks per treatment were separated and frozen. After extraction of total lipid (Folch *et al*., 1957), cholesterol content of the yolk determined in 6 samples per treatment using a commertial diagnostic kit (Zlatkis *et al*., 1953). Egg yolk triglyceride was analyzed by commertial kit in spectrophotometer.

Table 2: Effects of L-carnitine on performance of laying Japanese quail

Main effect	FI (g/quail per day)	FCR (kg of feed/kg of egg)	EP (%)
L-carnitine (mg/kg)			
0	41.97	3.58[a]	82.18
125	37.79	3.08[b]	82.60
250	39.08	3.17[b]	80.56
500	34.63	3.10[b]	79.99
SEM	2.63	0.11	1.08
p-value	0.34	0.04	0.36

[a,b]Mean values in the same column with different superscript letters were significantly different ($p<0.05$). FI = Feed Intake (g/quail per day), FCR = Feed Conversion Ratio (kg of feed/kg of egg), EP = Egg Production (%)

Blood samples were taken from jugular vein from 8 birds in each treatment at the end of experiment, then serum separated by centrifugation at 3000 g, for 10 min at room temperature and then labeled and stored in a deep freezer (-20°C) until analysis. Serum glucose, Triglyceride (TG), cholesterol and HDL were determined using an enzyme kit with an autoanalyser (Roche, Switzerland). VLDL cholesterol was calculated from triglyceride by dividing the factor 5. The LDL cholesterol was calculated using following formula:

LDL cholesterol = Total cholesterol - HDL cholesterol - VLDL cholesterol

Statistical analysis: The data obtained from the experiment were analyzed using GLM procedure of SAS (SAS Institute, 1999). Significant differences among treatment means were evaluated using Duncan's multiple range test (Duncan, 1955).

RESULTS

Laying Japanese quail performance: Effects of L-carnitine on laying Japanese quail performance (feed intake, feed conversion ratio and egg production) are presented in Table 2. Feeding with ration containing L-carnitine supplementation significantly ($p<0.05$) decreased feed conversion ratio compared to control group. The best feed conversion ratio was found with ration containing L-carnitine supplementation at levels of 125 mg/kg. Dietary carnitine supplementation had no effect on feed intake and egg production ($p>0.05$).

Egg quality of laying Japanese quail: The effects of dietary L-carnitine on egg quality (egg weight, yolk weight, albumen weight, yolk height, albumen height, yolk index and haugh unit) of laying Japanese quail are shown in Table 3. Dietary L-carnitine supplementation had no significant effect on egg quality parameters ($p>0.05$).

Table 3: Effects of L-carnitine on egg quality of laying Japanese quail

Main effect	Egg weight (g)	Yolk weight (g)	Albumen weight (g)	Yolk height (mm)	Albumen height (mm)	Yolk index	Haugh unit
L-carnitine (mg/kg)							
0	11.91	3.70	6.18	11.67	3.96	47.65	92.88
125	11.55	3.54	5.99	12.19	4.10	50.16	93.75
250	12.28	3.81	6.37	11.32	3.89	46.72	92.33
500	11.44	3.55	5.93	11.38	4.05	46.98	93.49
SEM	0.41	0.11	0.20	0.32	0.17	1.21	0.86
p-value	0.49	0.36	0.49	0.35	0.87	0.35	0.80

[a,b]Mean values in the same column with different superscript letters were significantly different (p<0.05)

Table 4: Effect of L-carnitine on egg yolk cholesterol and teriglyceride

Main effect	Yolk cholesterol (mg/g yolk)	Yolk teriglyceride (mg/g yolk)
L-carnitine (mg/kg)		
0	32.48[a]	11.87[a]
125	27.52[b]	9.81[b]
250	29.50[ab]	10.69[ab]
500	27.60[b]	10.16[ab]
SEM	1.30	0.45
p-value	0.04	0.04

[a,b]Mean values in the same column with different superscript letters were significantly different (p<0.05)

Table 5: Effects of L-carnitine on egg shell quality

Main effect	Shell weight (g)	Shell thickness (mm)	Shell strength (kg/cm^2)
L-carnitine (mg/kg)			
0	1.04	0.19	0.90
125	1.01	0.20	0.94
250	0.99	0.19	1.07
500	0.97	0.20	0.81
SEM	0.02	0.01	0.08
p-value	0.72	0.65	0.35

[a,b]Mean values in the same column with different superscript letters were significantly different (p<0.05)

Egg yolk cholesterol and teriglyceride: Effects of L-carnitine on egg yolk cholesterol and teriglyceride are shown in Table 4. Results showed that using of L-carnitine in laying Japanese quail feeding significantly (p<0.05) dercreased the levels of egg yolk cholesterol and teriglyceride. The lowest egg yolk cholesterol and teriglyceride level were obtained with using of 125 mg/kg L-carnitine and the highest egg yolk cholesterol and teriglyceride level were obtained in control group.

Egg shell quality: Effects of L-carnitine on egg shell quality of quails (shell weight, shell thickness and shell strength) are presented in Table 5. Dietary carnitine supplementation had no effect on egg shell quality parameters (p>0.05).

Blood parameters: Effects of L-carnitine on blood parameters of quails are presented in Table 6. The quails were fed with ration containing L-carnitine supplementation (125 mg/kg) had lower triglyceride in comparison with control group (p<0.05). Furthermore, L-carnitine at levels of 125 and 500 mg/kg significantly reduced blood cholesterol in comparison with control group (p<0.05). The lowest cholesterol level was obtained with using of 125 mg/kg L-carnitine. The effect of L-carnitine on other blood parameters including, glucose, HDL, VLDL and LDL were not significant (p>0.05).

Table 6: Effects of L-carnitine on blood parameters (mg/dl) of laying Japanese quail

Main effect	Glucose	Teriglyceride	Cholesterol
L-carnitine (mg/kg)			
0	274.07	154.62[a]	209.97[a]
125	275.69	146.15[b]	200.60[b]
250	281.07	148.07[ab]	205.40[ab]
500	281.07	149.80[ab]	202.03[b]
SEM	2.67	2.10	2.71
p-value	0.12	0.04	0.04
Main effect	HDL	VLDL	LDL
L-carnitine (mg/kg)			
0	127.70	30.92	51.35
125	123.80	29.23	47.57
250	125.61	29.61	50.17
500	126.55	29.96	45.49
SEM	2.05	0.42	3.07
p-value	0.23	0.08	0.16

[a,b]Mean values in the same column with different superscript letters were significantly different (p<0.05)

DISCUSSION

L-carnitine plays an important role in lipid metabolism and it has the potential to induce some desirable modifications in poultry performance and products. In present study, using L-carnitine did not affect feed intake of laying Japanese quails that in agreement with Corduk *et al.* (2007), Daskiran *et al.* (2009) and Sarica *et al.* (2007). Sarica *et al.* (2007) observed that various levels of L-carnitine did not affect body weight gain and feed intake of quails over the 28 d of the experimental period. Diet L-carnitine density did not cause any significant changes in feed intake. This may be because poultry are able to compensate their feed intake according to the energy density of the diet and in this research, the rations had similar energy. Thus, similar feed intake should be maintained over a range of dietary L-carnitine levels. Likewise, Rodehutscord *et al.* (2002) showed that L-carnitine supplementation is not linked to energy or protein utilization. Buyse *et al.* (2001) pointed out that performance data is not the only criteria from which the effectiveness of L-carnitine as a feed supplement should be evaluated.

Studies on the effects of dietary carnitine on laying Japanese quail have been scare. In current study, dietary L-carnitine did not affect egg production, egg weight, yolk weight, albumen weight, shell weight and shell thickness which is in agreement with the findings of Rabie *et al.* (1997) and Celik *et al.* (2004). Rabie *et al.* (1997) reported that supplementation of 50, 100, or 500 ppm of dietary L-carnitine did not affect egg production, egg weight, shell weight and shell thickness during the late laying period from 65 to 73 wk in a Hungarian brown hybrid line. These investigators also reported a lower yolk percentage in eggs of hens consuming dietary L-carnitine. Celik *et al.* (2004) reported that egg weight, yolk weight, shell weight and shell thickness were not affected by supplementation of 50 ppm of L-carnitine in the drinking water of 47-wk-old laying hens for 8 wk. Zhai *et al.* (2008) found, supplementation of L-carnitine to hen diets starting at hatch did not affect egg production, egg weight, yolk weight, shell weight and shell thickness, but increased yolk L-carnitine concentration, decreased yolk sac weight and yolk sac lipid content and altered fatty acid composition. In current study, dietary L-carnitine at 125 and 500 mg/kg significantly decreased egg yolk cholesterol and triglyceride as compared with control group, which is in agreement with the finding of Golzar Adabi *et al.* (2006). An additional possibility is that the presence of carnitine in the yolk may have affected the efficiency of enzymes involved in fatty acid metabolism. L-carnitine plays a well established role in lipid metabolism, so it may induce some favorable modifications in poultry products, particularly eggs and meat (Golzar Adabi *et al.*, 2006). During embryonic development, approximately 50% of the initial yolk lipid is oxidized for energy production, the other 50% is incorporated into the body tissue and residual yolk of hatchlings. Therefore, L-carnitine supplementation of quail diets improve embryo yolk lipid mobilization. The mobilized yolk lipid may be used to produce energy or be incorporated into body tissues (Lin *et al.*, 1991).

In present study, using L-carnitine significantly decreased the amount of blood cholesterol and triglyceride. Findings in this study are consistent with Rezaei *et al.* (2007) who reported adding L-carnitine to diet significantly decreased the level of serum triglyceride in broilers. Also, in research done by Zhang *et al.* (2010), total cholesterol, triglyceride, LDL cholesterol and lipoprotein lipase decreased and free fatty acid and lipase in serum increased with increased carnitine in diet. Decreasing the level of serum TG in birds fed diets supplemented with L-carnitine probably related to increasing oxidation of fatty acids. With increasing the transportation capacity of fatty acids to inner mitochondrial membrane, the serum TG level reduces. L-carnitine supplementation to diets containing high level of fat, increases oxidation of fatty acids and reduces the secretion of VLDL in liver, thus the level of serum VLDL reduces (Lien and Horng, 2001). Feeding carnitine increased activity of lipase and decrease activity of lipoprotein lipase, 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 (Zhang *et al.*, 2010). Lipoprotein lipase, which catalyzes the conversion of TG to glycerol and fatty acids, showed a decrease in activity which signified an increased hydrolysis of VLDL. VLDL play a major role in regulating fat deposition, leading to minimization of subcutaneous fat deposition (Griffin and Whitehead, 1982). Our data suggest that L-carnitine supplementation is beneficial to lipid metabolism. Contrary to these finding, Arslan *et al.* (2004) observed that L-carnitine administration via drinking water did not influence serum total cholesterol, total lipid and triglyceride of Japanese quail. The discrepancies between studies may result from different levels of L-carnitine supplementation, basic carnitine levels in the raw ingredients, the supply or absence of essential amino acids (Rodehutscord *et al.*, 2002), the possible effects of enzymatic breakdown of branched-chain amino acids (Owen *et al.*, 1996), sparing effects of carnitine with regard to its precursors (lysine and methionine), limited intestinal absortive capacity of carnitine and its considerable microbial degradation in the intestine (Xu *et al.*, 2003), interspecies differences, age, sex, feeding program and the managerial or environmental conditions of the animals (Celik *et al.*, 2003).

Conclusion: It is concluded that the supplementation of diet with L-carnitine has positive effects on egg and blood by reducing triglyceride and cholesterol amount in laying Japanese quail. But because of there are few research in laying Japanese quail, further investigation are required to identify the role of L-carnitine in lipid metabolism in laying Japanese quail.

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